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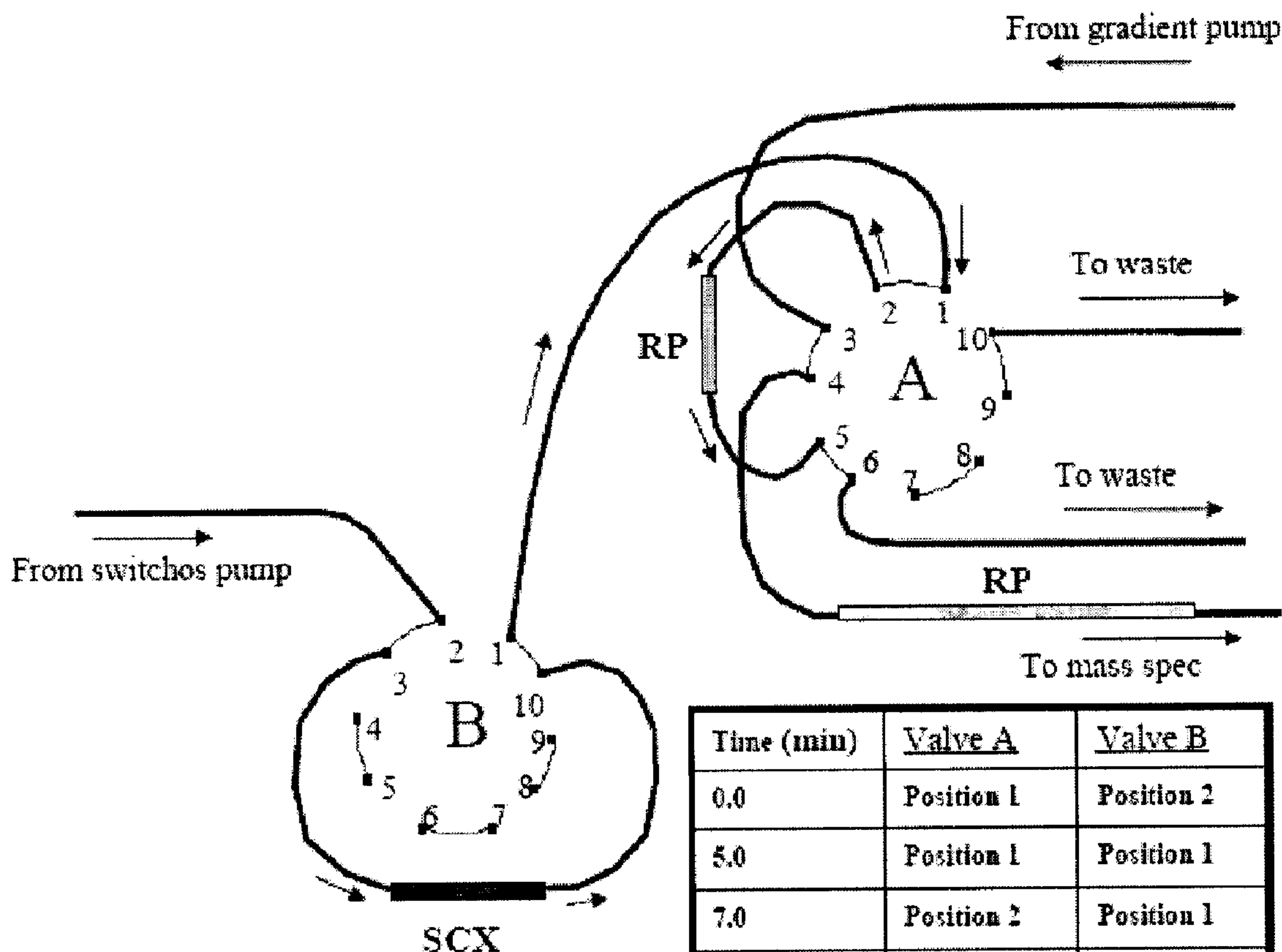
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(54) Titre : BIOMARQUEURS DE CANCER DE LA TETE ET DU COU

(54) Title: HEAD AND NECK CANCER BIOMARKERS



(57) Abrégé/Abstract:

Methods for detecting head and neck cancer or a related disease condition in a subject are described comprising measuring head and neck cancer markers or polynucleotides encoding the markers in a sample from the subject. The invention also provides



(57) **Abrégé(suite)/Abstract(continued):**

localization or imaging methods for head and neck cancers and kits for carrying out the methods of the invention. The invention also contemplates therapeutic applications for head and neck cancers employing head and neck cancer markers, polynucleotides encoding the markers, and/or binding agents for the markers.

ABSTRACT OF THE DISCLOSURE

Methods for detecting head and neck cancer or a related disease condition in a subject are described comprising measuring head and neck cancer markers or polynucleotides encoding
5 the markers in a sample from the subject. The invention also provides localization or imaging methods for head and neck cancers and kits for carrying out the methods of the invention. The invention also contemplates therapeutic applications for head and neck cancers employing head and neck cancer markers, polynucleotides encoding the markers, and/or binding agents for the markers.

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TITLE: Head and Neck Cancer Biomarkers**FIELD OF THE INVENTION**

The invention relates to head and neck cancer markers, methods for assessing the status of head and neck tissue, and methods for the detection, diagnosis, prediction, and therapy of head and neck disease. In one aspect the invention related to biomarkers of head and neck squamous cell carcinoma and the methods for detection, diagnosis predication and therapy for same and related conditions.

BACKGROUND OF THE INVENTION

Differential tagging with isotopic reagents, such as isotope-coded affinity tags (ICAT) (1) or the more recent variation that uses isobaric tagging reagents, iTRAQ (Applied Biosystems, Foster City, CA), followed by multidimensional liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis is a powerful methodology in the search of biomarkers for various disease states.

Annually, about 500,000 cancer-related deaths are estimated in the United States alone; of these approximately 13,000 are attributed to head-and-neck squamous-cell carcinoma (HNSCC), making it the sixth most common cause of cancer deaths (1). A lack of biomarkers for early detection and risk assessment is clearly reflected by the fact that more than 50% of all HNSCC patients have advanced disease at the time of diagnosis (2). The five-year survival rate of HNSCC patients is less than 50%, and the prognosis of advanced HNSCC cases has not changed much over the past three decades (except in a few advanced centers) (2, 3). Conceivably, improvement in understanding of the steps leading to tumorigenesis will provide the ability to identify and predict malignant progression at an earlier stage of HNSCC lesions, in turn leading to more effective treatment and reduction of morbidity and mortality. Currently, there are no clinically established tumor markers available to facilitate the diagnosis or prognosis of head-and-neck cancer. It is expected that identification of novel protein markers or therapeutic targets will ultimately improve patient care and survival. Proteomics combined with mass spectrometry (MS) has become a powerful paradigm for the examination of proteins in a global manner in the post-genomics era, and thereby the discovery of cancer biomarkers and drug targets. While transcriptomics provides the tool for unraveling gene-expression networks, proteomics links these networks to protein products and provides further insight into posttranslational modifications that regulate cellular functions,

thereby complementing genomic analyses (reviewed in reference 4). Identification of differentially expressed proteins in HNSCCs using proteomics revealed that expression patterns of proteins may have some predictive power for clinical outcome and personalized risk assessment (4-11). Differential tagging with isotopic reagents, such as isotope-coded
5 affinity tags (ICAT) (12) or the more recent variation that uses isobaric tagging reagents, iTRAQ (Applied Biosystems, Foster City, CA), followed by multidimensional liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis is emerging as one of the more powerful methodologies in the search of disease biomarkers. Recent studies conducted by the inventors using iTRAQ reagents resulted in identification and relative
10 quantification of proteins leading to discovery of potential cancer markers (PCMs) for head-and-neck cancer (13-17).

SUMMARY OF THE INVENTION

Herein the inventors used iTRAQ labeling in combination with multidimensional LC-
15 MS/MS analysis to head-and-neck cancer for comparison of protein profiles of HNSCC and non-cancerous head-and-neck tissues in an attempt to identify potential biomarkers, as well as to identify in a global fashion molecular pathways that are deregulated in head-and-neck cancer, which in turn, should aid in drug-target discovery. The iTRAQ experiments were performed on resected HNSCC and non-cancerous tissue homogenates. The rationale for
20 using whole tissue homogenates as opposed to laser capture microdissection (LCM)-procured tumor cells has been discussed (16). Major advantages in the analysis of tissue homogenates are that the relevant proteins are much more abundant in the tissues of interest than in bodily fluids, and there is an automatic link between a protein that is differentially expressed and the tumor itself. Such a link would need to be demonstrated, if the differentially expressed
25 proteins were to be discovered in a bodily fluid, e.g., blood, as every tissue or organ can potentially discharge into blood. Furthermore, the tumor microenvironment plays an important role in cancer progression (18) and the examination of protein expression in tissues from a homogenate of different cell types, takes into account the contributions of the tumor microenvironment.

30 The protein expression profiles of HNSCCs were compared with non-cancerous head and- neck tissues (controls) using the iTRAQ-labelling technique in combination with multidimensional LC-MS/MS analysis. In the iTRAQ technology, tagging is on primary

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amines, thus potentially allowing the tagging of most tryptic peptides. The multiplexing ability afforded by the iTRAQ reagents, which are available in four different tags, is ideally suited for the present study, as it provides a means to perform a proteomic analysis of both paired and non-paired non-cancerous (histologically normal) head-and-neck tissues, while simultaneously comparing them against the cancer samples. This strategy helps to identify proteins that might be differentially expressed due to manifestation of field cancerization (8,19) in clinically normal mucosa, and may be useful in designing strategies for risk prediction of disease recurrence or second primary tumor development. Some of the overexpressed proteins that the Applicants' identified in the tissues by the iTRAQ technology and LC MS/MS analysis were confirmed by immunohistochemistry and Western blotting. These approaches ensured that the proteins selected demonstrate a consistent pattern of overexpression in HNSCCs and greatly increase the confidence of the observations stemming from iTRAQ analysis. Besides their potential utility as biomarkers for HNSCC, these proteins also provide valuable insight into the previously unknown molecular networks and mechanisms that govern the normal-to-malignant conversion of the epithelium.

Applicants have identified markers associated with the head-and-neck. Thus, the invention relates to novel markers for head-and-neck disease, and compositions comprising same.

In an aspect, the invention provides marker sets that distinguish the head-and-neck diseases, and uses therefor. A marker set may comprise a plurality of polypeptides and/or polynucleotides encoding such polypeptides comprising or consisting of at least one marker listed in Table 5 and optionally 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or up to all of the markers listed therein. In specific aspects, the markers consist of at least 2, 3, 4 or 5 polypeptides listed in Table 5. In an aspect the protein marker sets comprise or consist of protein clusters, or proteins in pathways comprising markers listed in Table 5 and Table 2. In one aspect the invention provides markers in Table 1 that are up-regulated or downregulated or expressed in cancer samples as compared to the non-cancer samples.

The up-regulated markers identified in Table 1 (up-regulated in cancer samples versus non-cancer samples), including but not limited to native-sequence polypeptides, isoforms, chimeric polypeptides, all homologs, fragments, and precursors of the markers, including modified forms of the polypeptides and derivatives are referred to and defined herein as "head-and-neck cancer marker(s)". Polynucleotides encoding head-and-neck cancer markers

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are referred to and defined herein as “head-and-neck cancer polynucleotide marker(s)”, “polynucleotides encoding head-and-neck cancer markers”, or “polynucleotides encoding the cancer marker(s)”. The head-and-neck cancer markers and head-and-neck cancer polynucleotide markers are sometimes collectively referred to herein as “marker(s)”.

5 Up-regulated head-and-neck cancer markers listed in Table 1 (in cancer sample versus non-cancer sample), those listed in Table or 2, and polynucleotides encoding the markers, have application in the determination of the status of the head-and-neck cell or tissue and in the detection of a head-and-neck disease such as head-and-neck cancer. Thus, the markers can be used for diagnosis, monitoring (i.e. monitoring progression or therapeutic treatment),
10 prognosis, treatment, or classification of a head-and-neck disease (e.g. head-and-neck cancer), HNSCC or related conditions or as markers before surgery or after relapse. The invention also contemplates methods for assessing the status of a head-and-neck tissue, and methods for the diagnosis and therapy of a head-and-neck disease.

In accordance with methods of the invention, head-and-neck cancer can be assessed or
15 characterized, for example, by detecting the presence in the sample of (a) a head-and-neck cancer marker or fragment thereof; (b) a metabolite which is produced directly or indirectly by a head-and-neck cancer marker; (c) a transcribed nucleic acid or fragment thereof having at least a portion with which a head-and-neck cancer polynucleotide marker is substantially identical; and/or (c) a transcribed nucleic acid or fragment thereof, wherein the nucleic acid
20 hybridizes with a head-and-neck cancer polynucleotide marker.

The levels of head-and-neck cancer markers or head-and-neck cancer polynucleotide markers in a sample may be determined by methods as described herein and generally known in the art. The expression levels may be determined by isolating and determining the level of nucleic acid transcribed from each head-and-neck cancer polynucleotide marker. Alternatively
25 or additionally, the levels of head-and-neck cancer markers translated from mRNA transcribed from a head-and-neck cancer polynucleotide marker may be determined.

In an aspect, the invention provides a method for characterizing or classifying a head-and-neck sample comprising detecting a difference in the expression of a first plurality of head-and-neck cancer markers or head-and-neck cancer polynucleotide markers relative to a
30 control, the first plurality of markers comprising or consisting of at least 2, 3, 4, or 5 of the markers corresponding to the markers listed in Table 5, and optionally 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or up to all of the markers listed therein or those listed in in Table 2 or

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those that are up-regulated in cancer versus control tissue in Table 1. In specific aspects, the plurality of markers consists of at least 3, 4 or 5 of the markers listed in Table 1.

In an aspect, a method is provided for characterizing a head-and-neck tissue by detecting head-and-neck cancer markers or head-and-neck cancer polynucleotide markers associated with a head-and-neck tissue stage or phase, or head-and-neck disease in a subject comprising:

- (a) obtaining a sample from a subject;
- (b) detecting or identifying in the sample head-and-neck cancer markers or head-and-neck cancer polynucleotide markers; and
- 10 (c) comparing the detected amount with an amount detected for a standard.

In an embodiment of the invention, a method is provided for detecting head-and-neck cancer markers or head-and-neck cancer polynucleotide markers associated with head-and-neck cancer in a patient comprising:

- (a) obtaining a sample from a patient;
- 15 (b) detecting in the sample head-and-neck cancer markers or head-and-neck cancer polynucleotide markers; and
- (c) comparing the detected amount with an amount detected for a standard.

The term “detect” or “detecting” includes assaying, imaging or otherwise establishing the presence or absence of the target head-and-neck cancer markers or polynucleotides encoding the markers, subunits thereof, or combinations of reagent bound targets, and the like, or assaying for, imaging, ascertaining, establishing, or otherwise determining one or more factual characteristics of a head-and-neck tissue phase or head-and-neck disease including cancer, metastasis, stage, or similar conditions. The term encompasses diagnostic, prognostic, and monitoring applications for the head-and-neck cancer markers and polynucleotides encoding the markers.

The invention also provides a method of assessing whether a patient is afflicted with or has a pre-disposition for head-and-neck disease, in particular head-and-neck cancer, the method comprising comparing:

- (a) levels of head-and-neck cancer markers or polynucleotides encoding head-and-neck cancer markers associated with the head-and-neck disease in a sample from the patient; and
- (b) normal levels of head-and-neck cancer markers or polynucleotides encoding

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head-and-neck cancer markers associated with the head-and-neck disease in samples of the same type obtained from control patients not afflicted with the disease, wherein altered levels of the head-and-neck cancer markers or the polynucleotides relative to the corresponding normal levels of head-and-neck cancer markers or polynucleotides is an indication that the patient is afflicted with head-and-neck disease.

In an aspect of a method of the invention for assessing whether a patient is afflicted with or has a pre-disposition for head-and-neck cancer, higher levels of head-and-neck cancer markers (e.g., YWHAZ, stratifin, S100A7) in a sample relative to the corresponding normal levels is an indication that the patient is afflicted with head-and-neck cancer.

In another aspect of a method of the invention for assessing whether a patient is afflicted with or has a pre-disposition for head-and-neck cancer, lower levels of head-and-neck cancer markers (e.g., alpha-1-antitrypsin, KPSG lumican) in a sample relative to the corresponding normal levels is an indication that the patient is afflicted with head-and-neck cancer.

In a further aspect, a method for screening a subject for head-and-neck disease is provided comprising (a) obtaining a biological sample from a subject; (b) detecting the amount of head-and-neck cancer markers associated with the disease in said sample; and (c) comparing said amount of head-and-neck cancer markers detected to a predetermined standard, where detection of a level of head-and-neck cancer markers that differs significantly from the standard indicates head-and-neck disease.

In an embodiment, a significant difference between the levels of head-and-neck cancer marker levels in a patient and normal levels is an indication that the patient is afflicted with or has a predisposition to head-and-neck disease.

In a particular embodiment the amount of head-and-neck cancer marker(s) (e.g., prothymosin alpha, APC-binding protein EB1) detected is greater than that of a standard and is indicative of head-and-neck disease, in particular head-and-neck cancer. In another particular embodiment the amount of head-and-neck cancer marker(s) (e.g., alpha-1-antitrypsin, KPSG lumican) detected is lower than that of a standard and is indicative of head-and-neck disease, in particular head-and-neck cancer.

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In aspects of the methods of the invention, the methods are non-invasive for detecting head-and-neck disease which in turn allow for diagnosis of a variety of conditions or diseases associated with the head-and-neck.

In particular, the invention provides a non-invasive non-surgical method for detection, diagnosis or prediction of head-and-neck disease (e.g., head-and-neck cancer or HNSCC) in a subject comprising: obtaining a sample of blood, plasma, serum, urine or saliva or a tissue sample from the subject; subjecting the sample to a procedure to detect head-and-neck cancer markers or head-and-neck cancer polynucleotide markers in the blood, plasma, serum, urine, saliva or tissue; detecting, diagnosing, and predicting head-and-neck disease by comparing the levels of head-and-neck cancer markers or head-and-neck cancer polynucleotide markers to the levels of marker(s) or polynucleotide(s) obtained from a control subject with no head-and-neck disease.

In an embodiment, head-and-neck disease is detected, diagnosed, or predicted by determination of increased levels of markers (e.g one or more Table 1 up-regulated markers, preferable Table 5 up-regulated markers and more preferably one or more Table 2 up-regulated markers) when compared to such levels obtained from the control.

In another embodiment, head-and-neck disease is detected, diagnosed, or predicted by determination of decreased levels of markers (e.g. one or more Table 1 down-regulated markers) when compared to such levels obtained from the control.

The invention also provides a method for assessing the aggressiveness or indolence of a head-and-neck disease in particular cancer (e.g. staging), the method comprising comparing:

- (a) levels of head-and-neck cancer markers or polynucleotides encoding head-and-neck cancer markers associated with the head-and-neck disease in a patient sample; and
- (b) normal levels of the head-and-neck cancer markers or the polynucleotides in a control sample.

In an embodiment, a significant difference between the levels in the sample and the normal levels is an indication that the head-and-neck disease, in particular cancer, is aggressive or indolent. In a particular embodiment, the levels of head-and-neck cancer markers are higher than normal levels. In another particular embodiment, the levels of head-and-neck cancer markers are lower than normal levels.

In an embodiment, a method is provided for diagnosing and/or monitoring HNSCC comprising comparing:

- (a) levels of YWHAZ or polynucleotides encoding YWHAZ in a sample from the patient; and
- 5 (b) normal levels of YWHAZ or polynucleotides encoding same in samples of the same type obtained from control patients not afflicted with head-and-neck cancer or having a different stage of head-and-neck cancer, wherein altered levels of YWHAZ or polynucleotides encoding same compared with the corresponding normal levels is an indication that the patient is afflicted with HNSCC.

10 In an embodiment, a method is provided for diagnosing and/or monitoring HNSCC comprising comparing:

- (a) levels of S100 A7 or polynucleotides encoding S100 A7 in a sample from the patient; and
- 15 (b) normal levels of S100 A7 or polynucleotides encoding same in samples of the same type obtained from control patients not afflicted with head-and-neck cancer or having a different stage of head-and-neck cancer, wherein altered levels of S100 A7 or polynucleotides encoding same compared with the corresponding normal levels is an indication that the patient is afflicted with HNSCC.

20 In an embodiment, a method is provided for diagnosing and/or monitoring HNSCC comprising comparing

- (a) levels of stratifin or polynucleotides encoding stratifin in a sample from the patient; and
- 25 (b) normal levels of stratifin or polynucleotides encoding same in samples of the same type obtained from control patients not afflicted with head-and-neck cancer or having a different stage of head-and-neck cancer, wherein altered levels of stratifin or polynucleotides encoding same compared with the corresponding normal levels is an indication that the patient is afflicted with HNSCC.

In an aspect, the invention provides a method for determining whether a cancer has metastasized or is likely to metastasize in the future, the method comprising comparing:

- 30 (a) levels of head-and-neck cancer markers or polynucleotides encoding head-and-neck cancer markers in a patient sample; and
- (b) normal levels (or non-metastatic levels) of the head-and-neck cancer markers

or polynucleotides in a control sample.

In an embodiment, a significant difference between the levels in the patient sample and the normal levels is an indication that the cancer has metastasized or is likely to metastasize in the future.

5 In another aspect, the invention provides a method for monitoring the progression of head-and-neck disease, in particular head-and-neck cancer in a patient the method comprising:

- (a) detecting head-and-neck cancer markers or polynucleotides encoding the markers associated with the disease in a sample from the patient at a first time point;
- 10 (b) repeating step (a) at a subsequent point in time; and
- (c) comparing the levels detected in (a) and (b), and therefrom monitoring the progression of the head-and-neck disease.

The invention contemplates a method for determining the effect of an environmental factor on the head-and-neck tissue, or head-and-neck disease comprising comparing head-and-
15 neck cancer polynucleotide markers or head-and-neck cancer markers in the presence and absence of the environmental factor.

The invention also provides a method for assessing the potential efficacy of a test agent for inhibiting head-and-neck disease, and a method of selecting an agent for inhibiting head-and-neck disease.

20 The invention contemplates a method of assessing the potential of a test compound to contribute to a head-and-neck disease comprising:

- (a) maintaining separate aliquots of head-and-neck diseased cells in the presence and absence of the test compound; and
- (b) comparing the levels of head-and-neck cancer markers or polynucleotides
25 encoding the markers associated with the disease in each of the aliquots.

A significant difference between the levels of head-and-neck cancer markers or polynucleotides encoding the markers in an aliquot maintained in the presence of (or exposed to) the test compound relative to the aliquot maintained in the absence of the test compound, indicates that the test compound potentially contributes to head-and-neck disease.

30 The invention further relates to a method of assessing the efficacy of a therapy for inhibiting head-and-neck disease in a patient. A method of the invention comprises comparing: (a) levels of head-and-neck cancer markers or polynucleotides encoding the

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markers associated with disease in a first sample from the patient obtained from the patient prior to providing at least a portion of the therapy to the patient; and (b) levels of head-and-neck cancer markers or polynucleotides encoding the markers associated with disease in a second sample obtained from the patient following therapy.

5 In an embodiment, a significant difference between the levels of head-and-neck cancer markers or polynucleotides encoding the markers in the second sample relative to the first sample is an indication that the therapy is efficacious for inhibiting head-and-neck disease.

In a particular embodiment, the method is used to assess the efficacy of a therapy for inhibiting head-and-neck disease (e.g. head-and-neck cancer), where lower levels of head-
10 and-neck cancer markers or polynucleotides encoding same in the second sample relative to the first sample, is an indication that the therapy is efficacious for inhibiting the disease.

The “therapy” may be any therapy for treating head-and-neck disease, in particular head-and-neck cancer, including but not limited to therapeutics, radiation, immunotherapy, gene therapy, and surgical removal of tissue. Therefore, the method can be used to evaluate a
15 patient before, during, and after therapy.

Certain methods of the invention employ binding agents (e.g. antibodies) that specifically recognize head-and-neck cancer markers.

In an embodiment, the invention provides methods for determining the presence or absence of head-and-neck disease, in particular head-and-neck cancer, in a patient, comprising
20 the steps of (a) contacting a biological sample obtained from a patient with one or more binding agent that specifically binds to one or more head-and-neck cancer markers associated with the disease; and (b) detecting in the sample an amount of marker that binds to the binding agent, relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of head-and-neck disease in the patient.

25 In another embodiment, the invention relates to a method for diagnosing and monitoring a head-and-neck disease, in particular head-and-neck cancer, in a subject by quantitating one or more head-and-neck cancer markers associated with the disease in a biological sample from the subject comprising (a) reacting the biological sample with one or more binding agent specific for the head-and-neck cancer markers (e.g. an antibody) that are
30 directly or indirectly labelled with a detectable substance; and (b) detecting the detectable substance.

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In another aspect the invention provides a method of using an antibody to detect expression of one or more head-and-neck marker in a sample, the method comprising: (a) combining antibodies specific for one or more head-and-neck marker with a sample under conditions which allow the formation of antibody:marker complexes; and (b) detecting
5 complex formation, wherein complex formation indicates expression of the marker in the sample. Expression may be compared with standards and is diagnostic of a head-and-neck disease, in particular HNSCC.

Embodiments of the methods of the invention involve (a) reacting a biological sample from a subject with antibodies specific for one or more head-and-neck cancer markers which
10 are directly or indirectly labelled with an enzyme; (b) adding a substrate for the enzyme wherein the substrate is selected so that the substrate, or a reaction product of the enzyme and substrate forms fluorescent complexes; (c) quantitating one or more head-and-neck cancer markers in the sample by measuring fluorescence of the fluorescent complexes; and (d) comparing the quantitated levels to levels obtained for other samples from the subject patient,
15 or control subjects.

In another embodiment the quantitated levels are compared to levels quantitated for control subjects (e.g. normal or benign) without a head-and-neck disease (e.g. cancer) wherein an increase in head-and-neck marker levels compared with the control subjects is indicative of head-and-neck disease.

20 In a further embodiment the quantitated levels are compared to levels quantitated for control subjects (e.g. normal or benign) without a head-and-neck disease (e.g. cancer) wherein a decrease in head-and-neck marker levels compared with the control subjects is indicative of head-and-neck disease.

A particular embodiment of the invention comprises the following steps

- 25 (a) incubating a biological sample with first antibodies specific for one or more head-and-neck cancer markers which are directly or indirectly labeled with a detectable substance, and second antibodies specific for one or more head-and-neck cancer markers which are immobilized;
- (b) detecting the detectable substance thereby quantitating head-and-neck cancer
30 markers in the biological sample; and
- (c) comparing the quantitated head-and-neck cancer markers with levels for a predetermined standard.

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The standard may correspond to levels quantitated for samples from control subjects without head-and-neck cancer (normal or benign), with a different disease stage, or from other samples of the subject. In an embodiment, increased levels of head-and-neck cancer markers as compared to the standard may be indicative of head-and-neck cancer. In another
5 embodiment, lower levels of head-and-neck cancer markers as compared to a standard may be indicative of head-and-neck cancer.

HNSCC marker levels can be determined by constructing an antibody microarray in which binding sites comprise immobilized, preferably monoclonal, antibodies specific to a substantial fraction of marker-derived HNSCC marker proteins of interest.

10 Other methods of the invention employ one or more polynucleotides capable of hybridizing to one or more polynucleotides encoding HNSCC markers. Thus, methods can be used to monitor a head-and-neck disease (e.g. cancer) by detecting head-and-neck cancer polynucleotide markers associated with the disease.

Thus, the present invention relates to a method for diagnosing and monitoring a head-
15 and-neck disease (e.g. head-and-neck cancer, HNSCC or related condition) in a sample from a subject comprising isolating nucleic acids, preferably mRNA, from the sample; and detecting HNSCC marker polynucleotides associated with the disease in the sample. The presence of different levels of HNSCC marker polynucleotides in the sample compared to a standard or control may be indicative of head-and-neck disease, disease stage, and/or a negative or
20 positive prognosis (e.g., longer progression-free and overall survival).

In embodiments of the invention, head-and-neck cancer marker polynucleotide positive tumors (e.g. higher levels of the polynucleotides compared to a control normal or benign sample) are a negative diagnostic indicator. Positive tumors can be indicative of head-
and-neck cancer, advanced stage disease, lower progression-free survival, and/or overall
25 survival.

In other embodiments of the invention, head-and-neck cancer marker polynucleotide negative tumors (e.g. lower levels of the polynucleotides compared to a control normal or benign tissue) are a negative diagnostic indicator. Negative tumors can be indicative of head-
and-neck cancer, advanced stage disease, lower progression-free survival, and/or overall
30 survival.

The invention provides methods for determining the presence or absence of a head-
and-neck disease in a subject comprising detecting in the sample levels of nucleic acids that

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hybridize to one or more polynucleotides encoding head-and-neck cancer markers associated with the disease, comparing the levels with a predetermined standard or cut-off value, and therefrom determining the presence or absence of head-and-neck disease in the subject. In an embodiment, the invention provides methods for determining the presence or absence of head-and-neck cancer, such as HNSCC in a subject comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to one or more polynucleotides encoding head-and-neck cancer markers; and (b) detecting in the sample a level of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of head-and-neck cancer in the subject.

10 Within certain embodiments, the amount of polynucleotides that are mRNA are detected via polymerase chain reaction using, for example, oligonucleotide primers that hybridize to one or more polynucleotides encoding HNSCC markers, or complements of such polynucleotides. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing oligonucleotide probes that hybridize to one or more
15 polynucleotides encoding HNSCC markers, or complements thereof.

When using mRNA detection, the method may be carried out by combining isolated mRNA with reagents to convert to cDNA according to standard methods; treating the converted cDNA with amplification reaction reagents (such as cDNA PCR reaction reagents) in a container along with an appropriate mixture of nucleic acid primers; reacting the contents
20 of the container to produce amplification products; and analyzing the amplification products to detect the presence of one or more HNSCC polynucleotide markers in the sample. For mRNA the analyzing step may be accomplished using Northern Blot analysis to detect the presence of HNSCC polynucleotide markers. The analysis step may be further accomplished by quantitatively detecting the presence of HNSCC polynucleotide markers in the
25 amplification product, and comparing the quantity of marker detected against a panel of expected values for the known presence or absence of the markers in normal and malignant tissue derived using similar primers.

Therefore, the invention provides a method wherein mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to
30 cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic acid primers that hybridize to one or more HNSCC polynucleotide markers to produce amplification products; (d) analyzing the amplification products to detect an amount of

mRNA encoding the HNSCC markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and diseased tissue (e.g. malignant tissue) derived using similar nucleic acid primers.

In particular embodiments of the invention, the methods described herein utilize the HNSCC polynucleotide markers placed on a microarray so that the expression status of each of the markers is assessed simultaneously.

In a particular aspect, the invention provides a head-and-neck microarray comprising a defined set of genes (i.e., at least 2, 3 4, or 5 genes listed in Table 5 or Table 2) whose expression is significantly altered by head-and-neck phase or head-and-neck disease. The invention further relates to the use of the microarray as a prognostic tool to predict head-and-neck phase or head-and-neck disease. In an embodiment, the head-and-neck microarray discriminates between head-and-neck disease resulting from different etiologies.

In an embodiment, the invention provides for oligonucleotide arrays comprising marker sets described herein. The microarrays provided by the present invention may comprise probes to markers able to distinguish head-and-neck phase or disease. In particular, the invention provides oligonucleotide arrays comprising probes to a subset or subsets of at least 5 to 10 gene markers up to a full set of markers which distinguish head-and-neck disease.

The invention also contemplates a method comprising administering to cells or tissues imaging agents that carry labels for imaging and bind to HNSCC markers and optionally other markers of a head-and-neck disease, and then imaging the cells or tissues.

In an aspect the invention provides an *in vivo* method comprising administering to a subject an agent that has been constructed to target one or more HNSCC markers.

In a particular embodiment, the invention contemplates an *in vivo* method comprising administering to a mammal one or more agent that carries a label for imaging and binds to one or more HNSCC marker, and then imaging the mammal.

According to a particular aspect of the invention, an *in vivo* method for imaging head-and-neck cancer is provided comprising:

- (a) injecting a patient with an agent that binds to one or more HNSCC cancer marker, the agent carrying a label for imaging the head-and-neck cancer;
- (b) allowing the agent to incubate *in vivo* and bind to one or more HNSCC cancer marker associated with the head-and-neck cancer; and
- (c) detecting the presence of the label localized to the HNSCC cancer.

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In an embodiment of the invention the agent is an antibody which recognizes a head-and-neck cancer marker. In another embodiment of the invention the agent is a chemical entity which recognizes a head-and-neck cancer marker.

An agent carries a label to image a head-and-neck marker and optionally other markers. Examples of labels useful for imaging are radiolabels, fluorescent labels (e.g. fluorescein and rhodamine), nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes can also be employed.

The invention also contemplates the localization or imaging methods described herein using multiple markers for a head-and-neck disease (e.g. head-and-neck cancer, HNSCC or related conditions).

The invention also relates to kits for carrying out the methods of the invention. In an embodiment, a kit is for assessing whether a patient is afflicted with a head-and-neck disease (e.g. head-and-neck cancer or HNSCC or related conditions) and it comprises reagents for assessing one or more head-and-neck cancer markers or polynucleotides encoding the markers.

The invention further provides kits comprising marker sets described herein. In an aspect the kit contains a microarray ready for hybridization to target HNSCC oligonucleotide markers, plus software for the data analyses.

The invention also provides a diagnostic composition comprising an HNSCC marker or a polynucleotide encoding the marker. A composition is also provided comprising a probe that specifically hybridizes to HNSCC polynucleotide markers, or a fragment thereof, or an antibody specific for HNSCC markers or a fragment thereof. In another aspect, a composition is provided comprising one or more head-and-neck cancer polynucleotide marker specific primer pairs capable of amplifying the polynucleotides using polymerase chain reaction methodologies. The probes, primers or antibodies can be labeled with a detectable substance.

Still further the invention relates to therapeutic applications for head-and-neck diseases, in particular head-and-neck cancer, employing head-and-neck cancer markers and polynucleotides encoding the markers, and/or binding agents for the markers.

In an aspect, the invention relates to compositions comprising markers or parts thereof

associated with a head-and-neck disease, or antibodies specific for HNSCC markers associated with a head-and-neck disease, and a pharmaceutically acceptable carrier, excipient, or diluent. A method for treating or preventing a head-and-neck disease, in particular head-and-neck cancer (e.g. HNSCC), in a patient is also provided comprising administering to a patient in need thereof, markers or parts thereof associated with a head-and-neck disease, antibodies specific for HNSCC markers associated with a head-and-neck disease, or a composition of the invention. In an aspect the invention provides a method of treating a patient afflicted with or at risk of developing a head-and-neck disease (e.g. head-and-neck cancer) comprising inhibiting expression of head-and-neck cancer markers.

10 In an aspect, the invention provides antibodies specific for HNSCC markers associated with a disease (e.g. HNSCC) that can be used therapeutically to destroy or inhibit the disease (e.g. the growth of HNSCC marker expressing cancer cells), or to block HNSCC marker activity associated with a disease. In an aspect, HNSCC markers may be used in various immunotherapeutic methods to promote immune-mediated destruction or growth inhibition of tumors expressing HNSCC markers.

The invention also contemplates a method of using head-and-neck cancer markers or parts thereof, or antibodies specific for HNSCC markers in the preparation or manufacture of a medicament for the prevention or treatment of a head-and-neck disease (e.g. head-and-neck cancer, HNSCC or related conditions).

20 Another aspect of the invention is the use of HNSCC markers, peptides derived therefrom, or chemically produced (synthetic) peptides, or any combination of these molecules, for use in the preparation of vaccines to prevent a head-and-neck disease and/or to treat a head-and-neck disease.

The invention contemplates vaccines for stimulating or enhancing in a subject to whom the vaccine is administered production of antibodies directed against one or more HNSCC markers.

The invention also provides a method for stimulating or enhancing in a subject production of antibodies directed against one or more HNSCC marker. The method comprises administering to the subject a vaccine of the invention in a dose effective for stimulating or enhancing production of the antibodies.

The invention further provides a method for treating, preventing, or delaying recurrence of a head-and-neck disease (e.g. head-and-neck cancer, HNSCC or related

conditions). The method comprises administering to the subject a vaccine of the invention in a dose effective for treating, preventing, or delaying recurrence of a head-and-neck disease (e.g. head-and-neck cancer, HNSCC or related conditions).

The invention contemplates the methods, compositions, and kits described herein using additional markers associated with a head-and-neck disease (e.g. head-and-neck cancer, HNSCC or related conditions). The methods described herein may be modified by including reagents to detect the additional markers, or polynucleotides for the markers.

In particular, the invention contemplates the methods described herein using multiple markers for HNSCC cancer. Therefore, the invention contemplates a method for analyzing a biological sample for the presence of HNSCC markers and polynucleotides encoding the markers, and other markers that are specific indicators of cancer, in particular head-and-neck cancer. The methods described herein may be modified by including reagents to detect the additional markers, or nucleic acids for the additional markers.

In embodiments of the invention the methods, compositions and kits use one or more of the markers listed in Table 5, in particular those listed in Table 2. In another embodiment, the method uses a panel of markers selected from the markers listed in Table 5, and in one embodiment of those listed in Table 2 in particular a panel comprising two, three or four or more of the markers in Table 5.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE TABLES AND DRAWINGS

The invention will now be described in relation to the Tables and drawings:

Table Legends

Table 1: iTRAQ ratios for HNSCC and non-cancerous head-and-neck tissue samples: HNSCC samples (C1 – C5, cancer of the buccal mucosa; C6 – C10, cancer of the tongue); nonpaired non-cancerous samples (N1, N4, N5); and paired non-cancerous samples (N2, N3) versus the pooled non-paired non-cancerous control. (Grey boxes, not detected; NQ, not quantified; 9999, no expression observed in the pooled sample).

Table 2: Receiver operating characteristics from the iTRAQ ratios of a panel of three bestperforming biomarkers – YWHAZ, stratifin, and S100A7 – individually and as a panel.

Table 3: Receiver operating characteristics from the IHC scores of a panel of three bestperforming biomarkers – YWHAZ, stratifin, and S100A7 – individually and as a panel.

5 Table 4: Comparison of receiver operating characteristics from the iTRAQ ratios of the panel of three best-performing biomarkers: non-paired non-cancerous tissues give better sensitivity and specific as a comparator than pair non-cancerous tissues.

Table 5: Differentially expressed proteins not previously described in head and neck malignancies/cancer.

10 Table 6: Analysis of Stratifin and YWHAZ in HNSCCs: Correlation with clinicopathological parameters.

Figure Legends

Figure 1: Flow diagram for online 2D LC-MS/MS analysis. In position 1, ports 1-2, 3-4, 5-6, 7-8 and 9-10 are connected; in position 2, ports 2-3, 4-5, 6-7, 8-9 and 10-1 are
15 connected. In the diagram, the valves are shown at the initial (Time = 0 min) positions.

Figure 2: Receiver-operating-characteristic curves of a panel of three best-performing biomarkers, YWHAZ, stratifin, and S100 A7: (a) iTRAQ ratios, and (b) IHC scores. See the text for details.

Figure 3: Immunohistochemical verification of iTRAQ-discovered potential cancer
20 markers, YWHAZ, Stratifin and S100 A7 in HNSCCs and non-cancerous head-and-neck tissues. Positive staining is brown and is intense in HNSCCs. The left panel shows the noncancerous (histologically normal) tissues and the right panel depicts the HNSCC tissue sections. Panel A: the HNSCC sample shows intense cytoplasmic and nuclear staining for YWHAZ, while the normal mucosa shows no detectable immunostaining. Panel B:
25 HNSCC tissue section shows cytoplasmic staining for stratifin in tumor cells, while the normal mucosa shows no detectable immunostaining. Panel C: the HNSCC tissue section shows intense cytoplasmic staining for S100A7 in tumor cells, while the normal mucosa shows no detectable immunoreactivity. All panels show x200 magnifications.

Figure 4: Western blot analyses of YWHAZ, Stratifin and S100 A7 in HNSCCs and
30 paired noncancerous head-and-neck tissues. Equal amounts of protein lysates from HNSCCs and paired non-cancerous head-and-neck tissues were employed. See the text for details. The Panels show increased expression of (i) YWHAZ, (ii) stratifin, and (iii) S100A7 in HNSCCs

(C1 – C3) as compared to paired non-cancerous head-and-neck tissues (N1 –N3). Alpha-tubulin (Panel 4) was used as the loading control.

Figure 5: RT-PCR analyses of YWHAZ, stratifin, and S100 A7 in HNSCCs and non-cancerous head-and-neck tissues: Panel (i) shows increased levels of YWHAZ transcripts in HNSCCs (C1 – C3) as compared to the non-cancerous head-and-neck tissues that show basal levels (N2 and N3) and no detectable level (N1) of YWHAZ transcripts. Panel (ii) shows increased levels of stratifin transcripts in HNSCCs (C1 – C3) as compared to the non-cancerous head-and-neck tissues that show basal level (N3) and no detectable level (N1 and N2) of stratifin transcripts. Panel (iii) shows increased levels of S100A7 transcripts in HNSCCs (C1 – C3) as compared to the non-cancerous head-and-neck tissues that show basal level (N3) and no detectable level (N1 and N2) of S100A7 transcripts. β -Actin (Panel iv) was used as a control for normalizing the quantity of RNA used.

Figure 6: Identification of stratifin and YWHAZ in HNSCCs by mass spectrometry. The peptides whose MS/MS spectra are shown are colored red and in a larger font. Those that are common between stratifin and YWHAZ are shown in purple. Other peptides observed are in blue. The matched *b* ions are shown in green, and the matched *y* ions in red.

Figure 7: Immunohistochemical analysis of stratifin in head-and-neck cancer tissues. Paraffin-embedded HNSCC tissue sections and non-malignant mucosa were stained using anti-stratifin antibody (all x100 magnifications): Panel a shows normal oral mucosa with no detectable stratifin immunostaining; Panel b shows HNSCC with strong cytoplasmic and nuclear stratifin immunostaining in the tumor cells; Panel c shows HNSCC negative control with lack of staining in the tumor cells.

Figure 8: Box-Plot analysis: The box plot shows distribution of total scores of stratifin in HNSCCs and non-malignant head-and-neck tissues.

Figure 9: Co-immunoprecipitation assay and Western blot analysis. Immunoprecipitation assays of stratifin, YWHAZ, NF κ B, Bcl-2 and β -catenin proteins were carried out using specific antibodies in head-and-neck cancer cells, HSC2: Figure 9a shows immunoblot analysis for stratifin, demonstrating the binding of stratifin with YWHAZ, NF κ B, Bcl-2, and β -catenin, and the lack of binding in the negative control. Similarly, reverse immunoprecipitation assays were carried out using specific antibodies for YWHAZ, NF κ B, Bcl-2, and β -catenin: Figure 9b shows immunoblot analysis for: (i) YWHAZ, (ii) NF κ B, (iii) Bcl-2, and (iv) β -catenin confirming the binding of these proteins with stratifin.

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Figure 10: Kaplan-Meier estimation of cumulative proportion of disease-free survival: 10a, stratifin protein expression; the median time for disease-free survival (no recurrence/metastasis) in patients with stratifin-positive tumors was 19 months, whereas in those with stratifin-negative tumors it was 38 months ($p = 0.06$). 10b, YWHAZ protein expression; the median time for disease-free survival (no recurrence/metastasis) in patients with YWHAZ-positive tumors was 23 months, whereas in those with YWHAZ-negative tumors it was 35 months ($p = 0.08$). 10c, concomitant stratifin and YWHAZ expressions; the median time for disease-free survival of patients with HNSCCs showing concomitant expressions of stratifin and YWHAZ (Stratifin+/YWHAZ+) was 13 months, as compared to patients with tumors that did not show increased expression of either of these proteins with the median time for disease-free survival being 38 months ($p = 0.019$).

DETAILED DESCRIPTION OF THE INVENTION

Multidimensional liquid chromatography – mass spectrometry (LC-MS/MS) has been used for the analysis of biological samples labeled with isobaric mass tags (iTRAQ) to identify proteins that are differentially expressed in human head-and-neck squamous-cell carcinomas (HNSCCs) in relation to non-cancerous head-and-neck tissues (control) for cancer biomarker discovery. Fifteen individual samples (cancer and non-cancerous tissues) were compared against a pooled non-cancerous control (prepared by pooling equal amounts of proteins from six noncancerous tissues) in five sets by online and offline separation. Eight hundred and eleven (811) non-redundant proteins in HNSCCs were identified, including structural proteins, signaling components, enzymes, receptors, transcription factors and chaperones. A panel of proteins showing consistent differential expression in HNSCC relative to the non-cancerous controls was discovered. Some of the proteins include stratifin (14-3-3 sigma), YWHAZ (14-3-3 zeta), three calcium-binding proteins of the S100 family, S100A 2, S100A 7 (psoriasin) and S100A 11 (calgizarrin), prothymosin alpha (PTHA), L-lactate dehydrogenase A chain (LDH-A), glutathione S transferase-Pi, APCbinding protein EB1, and fascin. Peroxiredoxin2, carbonic anhydrase I, flavin reductase, histone H3 and polybromo-1D (BAF180) were underexpressed in HNSCCs. A panel of the three best performing biomarkers – YWHAZ, stratifin and S100A7 – achieved a sensitivity of 0.92 and a specificity of 0.91 in discriminating cancerous from non-cancerous head-and-neck tissues. Verification of differential expression of YWHAZ, stratifin and S100A7 proteins in clinical samples of

HNSCCs and paired and non-paired non-cancerous tissues by immunohistochemistry, immunoblotting, and RT-PCR confirmed their overexpression in head-and-neck cancer. Verification of YWHAZ, stratifin and S100A7 in an independent set of HNSCCs achieved a sensitivity of 0.92 and a specificity of 0.87 in discriminating cancerous from non-cancerous
 5 head-and-neck tissues, thereby confirming their overexpressions and utility as credible cancer biomarkers.

Methods are provided for detecting the presence of a head-and-neck disease (e.g. head-and-neck cancer) in a sample, the absence of a disease (e.g. head-and-neck cancer) in a sample, the stage or grade of the disease, and other characteristics of head-and-neck diseases
 10 that are relevant to prevention, diagnosis, characterization, and therapy of head-and-neck diseases such as cancer in a patient, for example, the benign or malignant nature of a head-and-neck cancer, the metastatic potential of a head-and-neck cancer, assessing the histological type of neoplasm associated with a head-and-neck cancer, the indolence or aggressiveness of a head-and-neck cancer, and other characteristics of head-and-neck diseases that are relevant to
 15 prevention, diagnosis, characterization, and therapy of head-and-neck diseases such as cancer in a patient. Methods are also provided for assessing the efficacy of one or more test agents for inhibiting a head-and-neck disease, assessing the efficacy of a therapy for a head-and-neck disease, monitoring the progression of a head-and-neck disease, selecting an agent or therapy for inhibiting a head-and-neck disease, treating a patient afflicted with a head-and-neck
 20 disease, inhibiting a head-and-neck disease in a patient, and assessing the disease (e.g. carcinogenic) potential of a test compound.

Abbreviation Index

For convenience, certain abbreviations used in the specification, tables, figures and appended claims are provided for here: iTRAQ, isobaric tags for relative and absolute
 25 quantitation; LC, liquid chromatography; MS/MS, tandem mass spectrometry; PCM, potential cancer marker; HNSCC, head-and-neck squamous cell carcinoma; LCM, laser capture microdissection; PBS, phosphate-buffered saline; SCX, strong cation exchange; ID, internal diameter; RP, reverse phase; IDA, information-dependent acquisition; TBS, tris-buffered saline; SFN, stratifin or 14-3-3 sigma; YWHAZ, 14-3-3 zeta; LDH-A, L-lactate
 30 dehydrogenase A; SD, standard deviation; ROC, receiver operating characteristics; PPIA, peptidyl prolyl isomerase A; PV, predictive values; PPV, positive predictive values; PTHA,

prothymosin alpha; PKM2, pyruvate kinase isozyme M2; AUC, area under the curve; RSD, relative standard deviation; TMA, tissue microarray.

Glossary

For convenience, certain terms employed in the specification, examples, and appended
5 claims are collected here.

The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term “about.” Further, it is to be understood that “a,” “an,” and “the” include plural
10 referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition or method comprising “a head-and-neck marker” includes two or more head-and-neck cancer markers. The term “about” means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made.

15 “Head-and-neck disease” refers to any disorder, disease, condition, syndrome or combination of manifestations or symptoms recognized or diagnosed as a disorder of the head and neck, including but not limited to hyperplasia and cancer precursors, head-and-neck cancer or carcinoma.

“Head-and-neck cancer” or “head-and-neck carcinoma” includes malignant head-and-
20 neck disease including but not limited to squamous cell and adenocarcinomas.

The terms “sample”, “biological sample”, and the like mean a material known or suspected of expressing or containing one or more head-and-neck cancer polynucleotide markers or one or more head-and-neck cancer markers. A test sample can be used directly as obtained from the source or following a pretreatment to modify the character of the sample.
25 The sample can be derived from any biological source, such as tissues, extracts, or cell cultures, including cells (e.g. tumor cells), cell lysates, and physiological fluids, such as, for example, whole blood, plasma, serum, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, synovial fluid, peritoneal fluid, lavage fluid, and the like. The sample can be obtained from animals, preferably mammals, most preferably humans. The
30 sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, the addition of reagents, and the like.

In embodiments of the invention the sample is a mammalian tissue sample. In a particular embodiment, the tissue is head-and-neck tissue.

In another embodiment the sample is a human physiological fluid. In a particular embodiment, the sample is human serum.

5 The samples that may be analyzed in accordance with the invention include polynucleotides from clinically relevant sources, preferably expressed RNA or a nucleic acid derived therefrom (cDNA or amplified RNA derived from cDNA that incorporates an RNA polymerase promoter). The target polynucleotides can comprise RNA, including, without
10 limitation total cellular RNA, poly(A)⁺ messenger RNA (mRNA) or fraction thereof, cytoplasmic mRNA, or RNA transcribed from cDNA (i.e., cRNA; see, for example., Linsley & Schelter, U.S. patent application Ser. No. 09/411,074, or U.S. Pat. Nos. 5,545,522, 5,891,636, or 5,716,785). Methods for preparing total and poly(A)⁺ RNA are well known in the art, and are described generally, for example, in Sambrook et al., (1989, *Molecular Cloning – A Laboratory Manual* (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold
15 Spring Harbor, N.Y.) and Ausubel et al., eds. (1994, *Current Protocols in Molecular Biology*, vol. 2, Current Protocols Publishing, New York). RNA may be isolated from eukaryotic cells by procedures involving lysis of the cells and denaturation of the proteins contained in the cells. Additional steps may be utilized to remove DNA. Cell lysis may be achieved with a nonionic detergent, followed by microcentrifugation to remove the nuclei and hence the bulk
20 of the cellular DNA. (See Chirgwin et al., 1979, *Biochemistry* 18:5294-5299). Poly(A)⁺RNA can be selected using oligo-dT cellulose (see Sambrook et al., 1989, *Molecular Cloning—A Laboratory Manual* (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). In the alternative, RNA can be separated from DNA by organic extraction, for example, with hot phenol or phenol/chloroform/isoamyl alcohol.

25 It may be desirable to enrich mRNA with respect to other cellular RNAs, such as transfer RNA (tRNA) and ribosomal RNA (rRNA). Most mRNAs contain a poly(A) tail at their 3' end allowing them to be enriched by affinity chromatography, for example, using oligo(dT) or poly(U) coupled to a solid support, such as cellulose or SephadexTM (see Ausubel et al., eds., 1994, *Current Protocols in Molecular Biology*, vol. 2, Current Protocols
30 Publishing, New York). Bound poly(A)⁺mRNA is eluted from the affinity column using 2 mM EDTA/0.1% SDS.

A sample of RNA can comprise a plurality of different mRNA molecules each with a

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different nucleotide sequence. In an aspect of the invention, the mRNA molecules in the RNA sample comprise at least 100 different nucleotide sequences.

Target polynucleotides can be detectably labeled at one or more nucleotides using methods known in the art. The label is preferably uniformly incorporated along the length of the RNA, and more preferably, is carried out at a high degree of efficiency. The detectable label can be a luminescent label, fluorescent label, bio-luminescent label, chemi-luminescent label, radiolabel, and colorimetric label. In a particular embodiment, the label is a fluorescent label, such as a fluorescein, a phosphor, a rhodamine, or a polymethine dye derivative. Commercially available fluorescent labels include, for example, fluorescent phosphoramidites such as FluorePrime (Amersham Pharmacia, Piscataway, N.J.), Fluoredite (Millipore, Bedford, Mass.), FAM (ABI, Foster City, Calif.), and Cy3 or Cy5 (Amersham Pharmacia, Piscataway, N.J.).

Target polynucleotides from a patient sample can be labeled differentially from polynucleotides of a standard. The standard can comprise target polynucleotides from normal individuals (i.e., those not afflicted with or pre-disposed to head-and-neck disease), in particular pooled from samples from normal individuals. The target polynucleotides can be derived from the same individual, but taken at different time points, and thus indicate the efficacy of a treatment by a change in expression of the markers, or lack thereof, during and after the course of treatment.

The terms “subject”, “individual” and “patient” refer to a warm-blooded animal such as a mammal. In particular, the terms refer to a human. A subject, individual or patient may be afflicted with or suspected of having or being pre-disposed to head-and-neck disease or a condition as described herein. The terms also includes domestic animals bred for food or as pets, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals.

Methods herein for administering an agent or composition to subjects/individuals/patients contemplate treatment as well as prophylactic use. Typical subjects for treatment include persons susceptible to, suffering from or that have suffered a condition or disease described herein. In particular, suitable subjects for treatment in accordance with the invention are persons that are susceptible to, suffering from or that have suffered head-and-neck cancer.

The term “head-and-neck marker” refers to a marker associated with normal or diseased head-and-neck tissue and comprises or consists of one or more of the polypeptides

that are up-regulated in cancer samples as compared to normal samples in Table 1, those listed in Table 5, in particular The term includes native-sequence polypeptides, isoforms, chimeric polypeptides, complexes, all homologs, fragments, precursors, and modified forms and derivatives of the markers.

5 A head-and-neck marker may be associated with a head-and-neck disease, in particular it may be a head-and-neck cancer marker. The term “head-and-neck cancer marker” includes a marker associated with head-and-neck cancer, in particular a marker listed in Table 5.

The terms “YWHAZ”, “YWHAZ polypeptide” and “YWHAZ protein” include human YWHAZ, in particular the native-sequence polypeptide, isoforms, chimeric
10 polypeptides, all homologs, fragments, precursors, complexes, and modified forms and derivatives of human YWHAZ. The amino acid sequence for native human YWHAZ includes the amino acid sequences referenced in NCBI Gene ID: Q86V33, including GenBank Accession Nos. P29213, P29312, Q32P43, Q5XJ08, Q6GPI2, Q6IN74, Q6NUR9, Q6P3U9, and the exemplary sequences shown in SEQ ID NOs.P63104 (sequence provided in the sequence
15 listing provide herein). Corresponding terms for “S100A7” and “stratifin” have similar meanings. The amino acid sequence for native human S100A7 includes the amino acid sequences referenced in NCBI Gene ID: P31151, including GenBank Accession Nos. Q6FGE3, Q9H1E2, and the exemplary sequences shown in SEQ ID NOs.P31151 (sequence provided in the sequence listing provide herein). The amino acid sequence for native human
20 stratifin includes the amino acid sequences referenced in NCBI Gene ID: P31947, including GenBank Accession Nos. Q6FH30, Q6FH51, Q96DH0, and the exemplary sequences shown in SEQ ID NOs.P31947 (sequence provided in the sequence listing provide herein).

A “native-sequence polypeptide” comprises a polypeptide having the same amino acid sequence of a polypeptide derived from nature. Such native-sequence polypeptides can be
25 isolated from nature or can be produced by recombinant or synthetic means. The term specifically encompasses naturally occurring truncated or secreted forms of a polypeptide, polypeptide variants including naturally occurring variant forms (e.g. alternatively spliced forms or splice variants), and naturally occurring allelic variants.

The term “polypeptide variant” means a polypeptide having at least about 45%, 50%,
30 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% amino acid sequence identity, particularly at least about 70-80%, more particularly at least about 85%, still more particularly at least about 90%, most particularly at least about 95% amino acid sequence

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identity with a native-sequence polypeptide. Particular polypeptide variants have at least 70-80%, 85%, 90%, 95% amino acid sequence identity to the sequences identified in Table 5 or 2. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length or mature sequences of the polypeptide, including variants from other species, but excludes a native-sequence polypeptide. In aspects of the invention variants retain the immunogenic activity of the corresponding native-sequence polypeptide.

Percent identity of two amino acid sequences, or of two nucleic acid sequences is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues in a polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid or nucleic acid sequence identity can be achieved in various conventional ways, for instance, using publicly available computer software including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990). Skilled artisans can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Methods to determine identity and similarity are codified in publicly available computer programs.

An allelic variant may also be created by introducing substitutions, additions, or deletions into a polynucleotide encoding a native polypeptide sequence such that one or more amino acid substitutions, additions, or deletions are introduced into the encoded protein. Mutations may be introduced by standard methods, such as site-directed mutagenesis and PCR-mediated mutagenesis. In an embodiment, conservative substitutions are made at one or more predicted non-essential amino acid residues. A “conservative amino acid substitution” is one in which an amino acid residue is replaced with an amino acid residue with a similar side chain. Amino acids with similar side chains are known in the art and include amino acids with basic side chains (e.g. Lys, Arg, His), acidic side chains (e.g. Asp, Glu), uncharged polar side chains (e.g. Gly, Asp, Glu, Ser, Thr, Tyr and Cys), nonpolar side chains (e.g. Ala, Val, Leu,

Iso, Pro, Trp), beta-branched side chains (e.g. Thr, Val, Iso), and aromatic side chains (e.g. Tyr, Phe, Trp, His). Mutations can also be introduced randomly along part or all of the native sequence, for example, by saturation mutagenesis. Following mutagenesis the variant polypeptide can be recombinantly expressed and the activity of the polypeptide may be
5 determined.

Polypeptide variants include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of a native polypeptide which include fewer amino acids than the full length polypeptides. A portion of a polypeptide can be a polypeptide which is for example, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90,
10 100 or more amino acids in length. Portions in which regions of a polypeptide are deleted can be prepared by recombinant techniques and can be evaluated for one or more functional activities such as the ability to form antibodies specific for a polypeptide.

A naturally occurring allelic variant may contain conservative amino acid substitutions from the native polypeptide sequence or it may contain a substitution of an amino acid from a
15 corresponding position in a polypeptide homolog, for example, a murine polypeptide.

A head-and-neck marker may be part of a chimeric or fusion protein. A “chimeric protein” or “fusion protein” comprises all or part (preferably biologically active) of a head-and-neck marker operably linked to a heterologous polypeptide (i.e., a polypeptide other than a head-and-neck marker). Within the fusion protein, the term “operably linked” is intended to
20 indicate that a head-and-neck marker and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of a head-and-neck marker. A useful fusion protein is a GST fusion protein in which a head-and-neck marker is fused to the C-terminus of GST sequences. Another example of a fusion protein is an immunoglobulin fusion protein in which all or part of a head-and-neck marker is
25 fused to sequences derived from a member of the immunoglobulin protein family. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

A modified form of a polypeptide referenced herein includes modified forms of the polypeptides and derivatives of the polypeptides, including post-translationally modified forms such as glycosylated, phosphorylated, acetylated, methylated or lapidated forms of the
30 polypeptides. For example, an N-terminal methionine may be cleaved from a polypeptide, and a new N-terminal residue may or may not be acetylated. In particular, for chaperonin 10 the first residue, methionine, can be cleaved and the second first residue, alanine can be N-

acetylated.

Head-and-neck cancer markers may be prepared by recombinant or synthetic methods, or isolated from a variety of sources, or by any combination of these and similar techniques.

“Head-and-neck cancer polynucleotide marker(s)”, “polynucleotides encoding the marker(s)”, and “polynucleotides encoding head-and-neck cancer markers” refer to polynucleotides that encode head-and-neck cancer markers including native-sequence polypeptides, polypeptide variants including a portion of a polypeptide, an isoform, precursor, complex, a chimeric polypeptide, or modified forms and derivatives of the polypeptides. A head-and-neck cancer polynucleotide marker comprises or consists of one or more of the polynucleotides encoding the polypeptides listed in Table 5.

In an aspect, a polynucleotide of the invention encodes YWHAZ, for example a polynucleotide sequence referenced in NCBI Gene ID. NM_003406, NP_003397.1, NM_145690.1, or NP_663723.1, more particularly GenBank Accession Nos. gi|21735625; gi|119612231; gi|85567424; gi|4507953; gi|83754473; gi|68085578; gi|119612234; gi|30354619; gi|14278218; gi|119612230; gi|68085278; gi|14278221; gi|6137540; gi|14278219; gi|68085909; gi|6137543; gi|83754467; gi|68085317; gi|83754468; gi|80477445; gi|49119653; gi|119612233; gi|14278220; gi|189953; gi|6137539; gi|68084347; gi|899459; gi|6137544; gi|75517642; gi|71533983; gi|119612232; gi|52000887; gi|119612235; gi|83754474 [and see for example SEQ ID NOs. NM_003406 in the sequence listing provided herein], or a fragment thereof.

In an aspect, a polynucleotide of the invention encodes stratifin for example a polynucleotide sequence referenced in NCBI Gene ID. NM_006142.3 or NP_006133.1, more particularly GenBank Accession Nos. gi|398953; gi|2702355; gi|7981260; gi|49456765; gi|12653125; gi|187302; gi|12654345; gi|62738853; gi|16306737; gi|23940; gi|62738854; gi|5454052; gi|23270783; gi|61680850; gi|61680851; gi|2702353; gi|49456807; gi|119628191; gi|12804273 [and see for example SEQ ID NOs. NM_006142.3 in the sequence listing provided herein], or fragment thereof.

In an aspect, a polynucleotide of the invention encodes S100 A7 for example a polynucleotide sequence referenced in NCBI Gene ID. NM_002963.3 or NP_002954.2, more particularly GenBank Accession Nos. gi|4389153; gi|400892; gi|4389154; gi|49457324; gi|190668; gi|11228051; gi|12053626; gi|5542542; gi|5542543; gi|21961601; gi|119573712;

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gi|157835758,[and see for example SEQ ID NOs. NM_002963.3 in the sequence listing provided herein] or a fragment thereof.

Head-and-neck cancer polynucleotide markers include complementary nucleic acid sequences, and nucleic acids that are substantially identical to these sequences (e.g. having at
5 least about 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% sequence identity).

Head-and-neck cancer polynucleotide markers also include sequences that differ from a native sequence due to degeneracy in the genetic code. As one example, DNA sequence polymorphisms within the nucleotide sequence of a head-and-neck marker may result in silent
10 mutations that do not affect the amino acid sequence. Variations in one or more nucleotides may exist among individuals within a population due to natural allelic variation. DNA sequence polymorphisms may also occur which lead to changes in the amino acid sequence of a polypeptide.

Head-and-neck cancer polynucleotide markers also include nucleic acids that hybridize
15 under stringent conditions, preferably high stringency conditions to a head-and-neck cancer polynucleotide marker, in particular a head-and-neck cancer polynucleotide marker. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, 6.0 x sodium chloride/sodium citrate (SSC) at
20 about 45°C, followed by a wash of 2.0 x SSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

25 Head-and-neck cancer polynucleotide markers also include truncated nucleic acids or nucleic acid fragments and variant forms of the nucleic acids that arise by alternative splicing of an mRNA corresponding to a DNA.

The head-and-neck cancer polynucleotide markers are intended to include DNA and RNA (e.g. mRNA) and can be either double stranded or single stranded. A polynucleotide
30 may, but need not, include additional coding or non-coding sequences, or it may, but need not, be linked to other molecules and/or carrier or support materials. The polynucleotides for use in the methods of the invention may be of any length suitable for a particular method. In

certain applications the term refers to antisense polynucleotides (e.g. mRNA or DNA strand in the reverse orientation to sense cancer polynucleotide markers).

“Statistically different levels”, “significantly altered levels”, or “significant difference” in levels of markers in a patient sample compared to a control or standard (e.g. normal levels or levels in other samples from a patient) may represent levels that are higher or lower than the standard error of the detection assay. In particular embodiments, the levels may be 1.5, 2, 3, 4, 5, or 6 times higher or lower than the control or standard.

“Microarray” and “array,” refer to nucleic acid or nucleotide arrays or protein or peptide arrays that can be used to detect biomolecules associated with head and neck cell or tissue phase and head-and-neck disease, for instance to measure gene expression. A variety of arrays are made in research and manufacturing facilities worldwide, some of which are available commercially. By way of example, spotted arrays and *in situ* synthesized arrays are two kinds of nucleic acid arrays that differ in the manner in which the nucleic acid materials are placed onto the array substrate. A widely used *in situ* synthesized oligonucleotide array is GeneChip™ made by Affymetrix, Inc. Oligonucleotide probes that are 20- or 25-base long can be synthesized *in silico* on the array substrate. These arrays can achieve high densities (e.g., more than 40,000 genes per cm²). Generally spotted arrays have lower densities, but the probes, typically partial cDNA molecules, are much longer than 20- or 25-mers. Examples of spotted cDNA arrays include LifeArray made by Incyte Genomics and DermArray made by IntegriDerm (or Invitrogen). Pre-synthesized and amplified cDNA sequences are attached to the substrate of spotted arrays. Protein and peptide arrays also are known (see for example, Zhu et al., *Science* 293:2101 (2001)).

“Binding agent” refers to a substance such as a polypeptide or antibody that specifically binds to one or more head-and-neck cancer markers. A substance “specifically binds” to one or more head-and-neck cancer markers if it reacts at a detectable level with one or more head-and-neck cancer markers, and does not react detectably with peptides containing an unrelated or different sequence. Binding properties may be assessed using an ELISA, which may be readily performed by those skilled in the art (see for example, Newton et al., *Develop. Dynamics* 197: 1-13, 1993).

A binding agent may be a ribosome, with or without a peptide component, an aptamer, an RNA molecule, or a polypeptide. A binding agent may be a polypeptide that comprises one or more head-and-neck marker sequence, a peptide variant thereof, or a non-peptide mimetic

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of such a sequence. By way of example, a YWHAZ sequence may be a peptide portion of a YWHAZ that is capable of modulating a function mediated by YWHAZ.

An aptamer includes a DNA or RNA molecule that binds to nucleic acids and proteins. An aptamer that binds to a protein (or binding domain) of a head-and-neck marker or a head-and-neck cancer polynucleotide marker can be produced using conventional techniques, without undue experimentation. (For example, see the following publications describing *in vitro* selection of aptamers: Klug et al., Mol. Biol. Reports 20:97-107 (1994); Wallis et al., Chem. Biol. 2:543-552 (1995); Ellington, Curr. Biol. 4:427-429 (1994); Lato et al., Chem. Biol. 2:291-303 (1995); Conrad et al., Mol. Div. 1:69-78 (1995); and Uphoff et al., Curr. Opin. Struct. Biol. 6:281-287 (1996)).

Antibodies for use in the present invention include but are not limited to monoclonal or polyclonal antibodies, immunologically active fragments (e.g. a Fab or (Fab)₂ fragments), antibody heavy chains, humanized antibodies, antibody light chains, genetically engineered single chain F_v molecules (Ladner et al, U.S. Pat. No. 4,946,778), chimeric antibodies, for example, antibodies which contain the binding specificity of murine antibodies, but in which the remaining portions are of human origin, or derivatives, such as enzyme conjugates or labeled derivatives.

Antibodies including monoclonal and polyclonal antibodies, fragments and chimeras, may be prepared using methods known to those skilled in the art. Isolated native or recombinant head-and-neck cancer markers may be utilized to prepare antibodies. (See, for example, Kohler et al. (1975) Nature 256:495-497; Kozbor et al. (1985) J. Immunol Methods 81:31-42; Cote et al. (1983) Proc Natl Acad Sci 80:2026-2030; and Cole et al. (1984) Mol Cell Biol 62:109-120 for the preparation of monoclonal antibodies; Huse et al. (1989) Science 246:1275-1281 for the preparation of monoclonal Fab fragments; and, Pound (1998) Immunochemical Protocols, Humana Press, Totowa, N.J for the preparation of phagemid or B-lymphocyte immunoglobulin libraries to identify antibodies). Antibodies specific for a head-and-neck marker may also be obtained from scientific or commercial sources.

In an embodiment of the invention, antibodies are reactive against a head-and-neck marker if they bind with a K_a of greater than or equal to 10⁻⁷ M.

30 Markers

The invention provides a set of markers correlated with head-and-neck disease. In an aspect, the invention provides a set of markers identified as useful for detection, diagnosis,

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prevention and therapy of head-and-neck disease comprising or consisting of one or more of the markers listed in Table 5. In another aspect, the invention provides the head-and-neck cancer markers in Table 2 for detection, diagnosis and prognosis of a head and neck disease. The invention also provides a method of using head-and-neck cancer markers listed in Table 5
 5 or Table 2, to distinguish head-and-neck disease.

In an embodiment, the markers comprise or consist of 1, 2, 3, 4 or more other markers listed in Table 5 and Table 2.

In embodiments of the invention, a marker is provided which is selected from the group consisting of the polypeptides set forth in Table 5 which polypeptides are up-regulated
 10 biomarkers in head-and-neck cancer and optionally at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 polypeptides set forth in Table 5 and/or 2 or polypeptides in Table 1 that are up-regulated biomarkers in head-and-neck cancer.

In embodiments of the invention, a marker is provided which is selected from the group consisting of at least one marker of Table 2 and at least 2, 3, 4, 5, 6, 7, 8, 9, or 10
 15 polypeptides set forth in Table 5.

The invention provides marker sets that distinguish head-and-neck disease and uses therefor. In an aspect, the invention provides a method for classifying a head-and-neck disease comprising detecting a difference in the expression of a first plurality of head-and-neck cancer markers or head-and-neck cancer polynucleotide markers relative to a control, the first
 20 plurality of head-and-neck cancer markers or head-and-neck cancer polynucleotide markers comprising or consisting of at least 2, 3, 4, or 5 of the markers listed in Table 5. In specific aspects, the plurality of markers consists of the markers listed in Table 2 and optionally at least 5 to 10 of the markers listed in Table 5. In specific aspects, a control comprises markers derived from a pool of samples from individual patients with no head-and-neck disease.

25 Any of the markers provided herein may be used alone or with other markers of head-and-neck disease, or with markers for other phenotypes or conditions.

Also please note that all of the sequences provided herein are representative only and there may be other sequences for particular protein or coding sequences or related sequences. The invention is not intended to be limited to the sequences herein provided.

30 **Detection Methods**

A variety of methods can be employed for the diagnostic and prognostic evaluation of head-and-neck disease or head-and-neck status involving one or more head-and-neck cancer

markers and polynucleotides encoding the markers, and the identification of subjects with a predisposition to head-and-neck diseases or that are receptive to *in vitro* fertilization and embryo transfer procedures. Such methods may, for example, utilize head-and-neck cancer polynucleotide markers, and fragments thereof, and binding agents (e.g. antibodies) against one or more head-and-neck cancer markers, including peptide fragments. In particular, the polynucleotides and antibodies may be used, for example, for (1) the detection of the presence of head-and-neck cancer polynucleotide marker mutations, or the detection of either over- or under-expression of head-and-neck marker mRNA relative to a non-disorder state or different head and neck cell or tissue phase, or the qualitative or quantitative detection of alternatively spliced forms of head-and-neck cancer polynucleotide marker transcripts which may correlate with certain conditions or susceptibility toward such conditions; and (2) the detection of either an over- or an under-abundance of one or more head-and-neck cancer markers relative to a non- disorder state or a different head and neck cell or tissue phase or the presence of a modified (e.g., less than full length) head-and-neck marker which correlates with a disorder state or a progression toward a disorder state.

The invention contemplates a method for detecting the phase of a head-and-neck tissue, in particular a secretory head-and-neck tissue, comprising producing a profile of levels of one or more head-and-neck marker associated with a known head and/or neck cell or tissue phase and/or polynucleotides encoding the markers, and optionally other markers associated with the phase in cells from a patient, and comparing the profile with a reference to identify a profile for the test cells indicative of the phase. In an aspect, the head-and-neck cancer markers comprise those of Table 5, preferably Table 2 or fragments thereof.

The invention also contemplates a method for detecting a head-and-neck disease, in particular a head-and-neck cancer, comprising producing a profile of levels of one or more head-and-neck marker associated with a head-and-neck disease and/or polynucleotides encoding the markers, and other markers associated with head-and-neck disease in cells from a patient, and comparing the profile with a reference to identify a profile for the test cells indicative of disease. In an aspect, the head-and-neck cancer markers are one or more of those listed in Table 5 or Table 2.

The methods described herein may be used to evaluate the probability of the presence of malignant or pre-malignant cells, for example, in a group of cells freshly removed from a host. Such methods can be used to detect tumors, quantitate their growth, and help in the

diagnosis and prognosis of head-and-neck disease. The methods can be used to detect the presence of cancer metastasis, as well as confirm the absence or removal of all tumor tissue following surgery, cancer chemotherapy, and/or radiation therapy. They can further be used to monitor cancer chemotherapy and tumor reappearance.

5 The methods described herein can be adapted for diagnosing and monitoring head-and-neck tissue status or a head-and-neck disease by detecting one or more head-and-neck cancer markers or polynucleotides encoding the markers in biological samples from a subject. These applications require that the amount of markers or polynucleotides quantitated in a sample from a subject being tested be compared to a predetermined standard or cut-off value.
10 The standard may correspond to levels quantitated for another sample or an earlier sample from the subject, or levels quantitated for a control sample. Levels for control samples from healthy subjects, different head-and-neck tissue phases, or subjects with a head-and-neck disease may be established by prospective and/or retrospective statistical studies. Healthy subjects who have no clinically evident disease or abnormalities may be selected for statistical
15 studies. Diagnosis may be made by a finding of statistically different levels of detected head-and-neck cancer markers associated with disease or polynucleotides encoding same, compared to a control sample or previous levels quantitated for the same subject.

 The methods described herein may also use multiple markers for a head-and-neck disease, in particular head-and-neck cancer. Therefore, the invention contemplates a method
20 for analyzing a biological sample for the presence of one or more head-and-neck cancer markers and polynucleotides encoding the markers, and other markers that are specific indicators of a head-and-neck disease. The methods described herein may be modified by including reagents to detect the additional markers, or polynucleotides for the markers.

Nucleic Acid Methods/Assays

25 As noted herein a head-and-neck disease or phase may be detected based on the level of head-and-neck cancer polynucleotide markers in a sample. Techniques for detecting polynucleotides such as polymerase chain reaction (PCR) and hybridization assays are well known in the art.

 Probes may be used in hybridization techniques to detect head-and-neck cancer
30 polynucleotide markers. The technique generally involves contacting and incubating nucleic acids (e.g. recombinant DNA molecules, cloned genes) obtained from a sample from a patient or other cellular source with a probe under conditions favorable for the specific annealing of

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the probes to complementary sequences in the nucleic acids. After incubation, the non-annealed nucleic acids are removed, and the presence of nucleic acids that have hybridized to the probe if any are detected.

Nucleotide probes for use in the detection of nucleic acid sequences in samples may be constructed using conventional methods known in the art. Suitable probes may be based on nucleic acid sequences encoding at least 5 sequential amino acids from regions of a head-and-neck cancer polynucleotide marker, preferably they comprise 10-200, more particularly 10-30, 10-40, 20-50, 40-80, 50-150, 80-120 nucleotides in length.

The probes may comprise DNA or DNA mimics (e.g., derivatives and analogues) corresponding to a portion of an organism's genome, or complementary RNA or RNA mimics. Mimics are polymers comprising subunits capable of specific, Watson-Crick-like hybridization with DNA, or of specific hybridization with RNA. The nucleic acids can be modified at the base moiety, at the sugar moiety, or at the phosphate backbone.

DNA can be obtained using standard methods such as polymerase chain reaction (PCR) amplification of genomic DNA or cloned sequences. (See, for example, in Innis et al., eds., 1990, PCR Protocols: A Guide to Methods and Applications, Academic Press Inc., San Diego, Calif.). Computer programs known in the art can be used to design primers with the required specificity and optimal amplification properties, such as Oligo version 5.0 (National Biosciences). Controlled robotic systems may be useful for isolating and amplifying nucleic acids.

A nucleotide probe may be labeled with a detectable substance such as a radioactive label that provides for an adequate signal and has sufficient half-life such as ^{32}P , ^3H , ^{14}C or the like. Other detectable substances that may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and luminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The nucleic acid probes may be used to detect head-and-neck cancer polynucleotide markers, preferably in human cells. The nucleotide probes may also be useful in the diagnosis of a

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head-and-neck disease involving one or more head-and-neck cancer polynucleotide markers, in monitoring the progression of such disorder, or monitoring a therapeutic treatment.

The detection of head-and-neck cancer polynucleotide markers may involve the amplification of specific gene sequences using an amplification method such as polymerase chain reaction (PCR), followed by the analysis of the amplified molecules using techniques
5 known to those skilled in the art. Suitable primers can be routinely designed by one of skill in the art.

By way of example, at least two oligonucleotide primers may be employed in a PCR based assay to amplify a portion of a polynucleotide encoding one or more head-and-neck
10 marker derived from a sample, wherein at least one of the oligonucleotide primers is specific for (i.e. hybridizes to) a polynucleotide encoding the head-and-neck marker. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis.

In order to maximize hybridization under assay conditions, primers and probes
15 employed in the methods of the invention generally have at least about 60%, preferably at least about 75%, and more preferably at least about 90% identity to a portion of a polynucleotide encoding a head-and-neck marker; that is, they are at least 10 nucleotides, and preferably at least 20 nucleotides in length. In an embodiment the primers and probes are at least about 10-40 nucleotides in length.

20 Hybridization and amplification techniques described herein may be used to assay qualitative and quantitative aspects of head-and-neck cancer polynucleotide marker expression. For example, RNA may be isolated from a cell type or tissue known to express a head-and-neck cancer polynucleotide marker and tested utilizing the hybridization (e.g. standard Northern analyses) or PCR techniques referred to herein.

25 The primers and probes may be used in the above-described methods *in situ* i.e. directly on tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections.

In an aspect of the invention, a method is provided employing reverse transcriptase-polymerase chain reaction (RT-PCR), in which PCR is applied in combination with reverse
30 transcription. Generally, RNA is extracted from a sample tissue using standard techniques (for example, guanidine isothiocyanate extraction as described by Chomcynski and Sacchi, Anal. Biochem. 162:156-159, 1987) and is reverse transcribed to produce cDNA. The cDNA is used

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as a template for a polymerase chain reaction. The cDNA is hybridized to a set of primers, at least one of which is specifically designed against a head-and-neck marker sequence. Once the primer and template have annealed a DNA polymerase is employed to extend from the primer, to synthesize a copy of the template. The DNA strands are denatured, and the procedure is repeated many times until sufficient DNA is generated to allow visualization by ethidium bromide staining and agarose gel electrophoresis.

Amplification may be performed on samples obtained from a subject with a suspected head-and-neck disease and an individual who is not afflicted with a head-and-neck disease. The reaction may be performed on several dilutions of cDNA spanning at least two orders of magnitude. A statistically significant difference in expression in several dilutions of the subject sample as compared to the same dilutions of the non-disease sample may be considered positive for the presence of a head-and-neck disease.

In an embodiment, the invention provides methods for determining the presence or absence of a head-and-neck disease in a subject comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to head-and-neck cancer polynucleotide markers; and (b) detecting in the sample a level of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of a head-and-neck disease in the subject. In an aspect, the head-and-neck disease is cancer and the head-and-neck cancer markers are one or more of those listed in tAble 5, or in another embodiment Table 2.

The invention provides a method wherein a head-and-neck marker mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic acid primers that hybridize to one or more head-and-neck marker polynucleotides, to produce amplification products; (d) analyzing the amplification products to detect amounts of mRNA encoding head-and-neck polynucleotide markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and malignant tissue derived using similar nucleic acid primers.

Head-and-neck cancer marker-positive samples or alternatively higher levels in patients compared to a control (e.g. non-cancerous tissue) may be indicative of late stage disease, and/or that the patient is not responsive to chemotherapy. Alternatively, negative

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samples or lower levels compared to a control (e.g. non-cancerous tissue or negative samples) may be indicative of progressive disease and shorter overall survival.

In another embodiment, the invention provides methods for determining the presence or absence of head-and-neck cancer in a subject comprising (a) contacting a sample obtained
5 from the subject with oligonucleotides that hybridize to one or more head-and-neck cancer polynucleotide markers; and (b) detecting in the sample levels of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of head-and-neck cancer in the subject. In an embodiment, the head-and-neck cancer polynucleotide markers encode one or more polypeptides listed in Table 5.

10 In particular, the invention provides a method wherein YWHAZ, S100 A7, and/or stratifin mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic acid primers that hybridize to a polynucleotide encoding YWHAZ, S100 A7, and/or stratifin, to produce amplification products; (d) analyzing
15 the amplification products to detect an amount of mRNA encoding YWHAZ, S100 A7, and/or stratifin; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and malignant tissue derived using similar nucleic acid primers.

Head-and-neck cancer marker-positive samples or alternatively higher levels, in particular significantly higher levels of YWHAZ, S100 A7, and/or stratifin polynucleotides in
20 patients compared to a control (e.g. normal or benign) are indicative of head-and-neck cancer. Negative samples or lower levels (e.g., of alpha-1-antitrypsin or KPSG lumican polynucleotides) compared to a control (e.g. normal or benign) may also be indicative of progressive disease and poor overall survival.

Oligonucleotides or longer fragments derived from a head-and-neck cancer
25 polynucleotide marker may be used as targets in a microarray. The microarray can be used to simultaneously monitor the expression levels of large numbers of genes and to identify genetic variants, mutations, and polymorphisms. The information from the microarray may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

30 The preparation, use, and analysis of microarrays are well known to a person skilled in the art. (See, for example, Brennan, T. M. et al. (1995) U.S. Pat. No. 5,474,796; Schena, et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995), PCT Application

WO95/251116; Shalon, D. et al. (I 995) PCT application WO95/35505; Heller, R. A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M. J. et al. (1997) U.S. Pat. No. 5,605,662.)

Thus, the invention also includes an array comprising one or more head-and-neck
5 cancer polynucleotide markers (in particular the markers listed in Table 5) and/or markers listed in Table 2 or other markers. The array can be used to assay expression of head-and-neck cancer polynucleotide markers in the array. The invention allows the quantitation of expression of one or more head-and-neck cancer polynucleotide markers.

Microarrays typically contain at separate sites nanomolar quantities of individual
10 genes, cDNAs, or ESTs on a substrate (e.g. nitrocellulose or silicon plate), or photolithographically prepared glass substrate. The arrays are hybridized to cDNA probes using conventional techniques with gene-specific primer mixes. The target polynucleotides to be analyzed are isolated, amplified and labeled, typically with fluorescent labels, radiolabels or phosphorous label probes. After hybridization is completed, the array is inserted into the
15 scanner, where patterns of hybridization are detected. Data are collected as light emitted from the labels incorporated into the target, which becomes bound to the probe array. Probes that completely match the target generally produce stronger signals than those that have mismatches. The sequence and position of each probe on the array are known, and thus by complementarity, the identity of the target nucleic acid applied to the probe array can be
20 determined.

Microarrays are prepared by selecting polynucleotide probes and immobilizing them to a solid support or surface. The probes may comprise DNA sequences, RNA sequences, copolymer sequences of DNA and RNA, DNA and/or RNA analogues, or combinations thereof. The probe sequences may be full or partial fragments of genomic DNA, or they may
25 be synthetic oligonucleotide sequences synthesized either enzymatically *in vivo*, enzymatically *in vitro* (e.g., by PCR), or non-enzymatically *in vitro*.

The probe or probes used in the methods of the invention can be immobilized to a solid support or surface which may be either porous or non-porous. For example, the probes can be attached to a nitrocellulose or nylon membrane or filter covalently at either the 3' or the
30 5' end of the polynucleotide probe. The solid support may be a glass or plastic surface. In an aspect of the invention, hybridization levels are measured to microarrays of probes consisting of a solid support on the surface of which are immobilized a population of polynucleotides,

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such as a population of DNA or DNA mimics, or, alternatively, a population of RNA or RNA mimics. A solid support may be a nonporous or, optionally, a porous material such as a gel.

In accordance with embodiments of the invention, a microarray is provided comprising a support or surface with an ordered array of hybridization sites or “probes” each representing one of the markers described herein. The microarrays can be addressable arrays, and in particular positionally addressable arrays. Each probe of the array is typically located at a known, predetermined position on the solid support such that the identity of each probe can be determined from its position in the array. In preferred embodiments, each probe is covalently attached to the solid support at a single site.

Microarrays used in the present invention are preferably (a) reproducible, allowing multiple copies of a given array to be produced and easily compared with each other; (b) made from materials that are stable under hybridization conditions; (c) small, (e.g., between 1 cm² and 25 cm², between 12 cm² and 13 cm², or 3 cm²; and (d) comprise a unique set of binding sites that will specifically hybridize to the product of a single gene in a cell (e.g., to a specific mRNA, or to a specific cDNA derived therefrom). However, it will be appreciated that larger arrays may be used particularly in screening arrays, and other related or similar sequences will cross hybridize to a given binding site.

In accordance with an aspect of the invention, the microarray is an array in which each position represents one of the markers described herein (e.g. the markers listed in Table 1 and optionally Table 2). Each position of the array can comprise a DNA or DNA analogue based on genomic DNA to which a particular RNA or cDNA transcribed from a genetic marker can specifically hybridize. A DNA or DNA analogue can be a synthetic oligomer or a gene fragment. In an embodiment, probes representing each of the head-and-neck cancer markers and head-and-neck cancer polynucleotide markers is present on the array. In a preferred embodiment, the array comprises at least 5 of the head-and-neck cancer polynucleotide markers.

Probes for the microarray can be synthesized using N-phosphonate or phosphoramidite chemistries (Froehler et al., 1986, Nucleic Acid Res. 14:5399-5407; McBride et al., 1983, Tetrahedron Lett. 24:246-248). Synthetic sequences are typically between about 10 and about 500 bases, 20-100 bases, or 40-70 bases in length. Synthetic nucleic acid probes can include non-natural bases, such as, without limitation, inosine. Nucleic acid analogues such as peptide

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nucleic acid may be used as binding sites for hybridization. (see, e.g., Egholm et al., 1993, Nature 363:566-568; U.S. Pat. No. 5,539,083).

Probes can be selected using an algorithm that takes into account binding energies, base composition, sequence complexity, cross-hybridization binding energies, and secondary structure (see Friend et al., International Patent Publication WO 01/05935, published Jan. 25, 2001).

Positive control probes, (e.g., probes known to be complementary and hybridize to sequences in the target polynucleotides), and negative control probes, (e.g., probes known to not be complementary and hybridize to sequences in the target polynucleotides) are typically included on the array. Positive controls can be synthesized along the perimeter of the array or synthesized in diagonal stripes across the array. A reverse complement for each probe can be next to the position of the probe to serve as a negative control.

The probes can be attached to a solid support or surface, which may be made from glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, gel, or other porous or nonporous material. The probes can be printed on surfaces such as glass plates (see Schena et al., 1995, Science 270:467-470). This method may be particularly useful for preparing microarrays of cDNA (See also, DeRisi et al., 1996, Nature Genetics 14:457-460; Shalon et al., 1996, Genome Res. 6:639-645; and Schena et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 93:10539-11286).

High-density oligonucleotide arrays containing thousands of oligonucleotides complementary to defined sequences, at defined locations on a surface can be produced using photolithographic techniques for synthesis in situ (see, Fodor et al., 1991, Science 251:767-773; Pease et al., 1994, Proc. Natl. Acad. Sci. U.S.A. 91:5022-5026; Lockhart et al., 1996, Nature Biotechnology 14:1675; U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270) or other methods for rapid synthesis and deposition of defined oligonucleotides (Blanchard et al., Biosensors & Bioelectronics 11:687-690). Using these methods oligonucleotides (e.g., 60-mers) of known sequence are synthesized directly on a surface such as a derivatized glass slide. The array produced may be redundant, with several oligonucleotide molecules per RNA.

Microarrays can be made by other methods including masking (Maskos and Southern, 1992, Nuc. Acids. Res. 20:1679-1684). In an embodiment, microarrays of the present invention are produced by synthesizing polynucleotide probes on a support wherein the

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nucleotide probes are attached to the support covalently at either the 3' or the 5' end of the polynucleotide.

The invention provides microarrays comprising a disclosed marker set. In one embodiment, the invention provides a microarray for distinguishing head-and-neck disease samples comprising a positionally-addressable array of polynucleotide probes bound to a support, the polynucleotide probes comprising a plurality of polynucleotide probes of different nucleotide sequences, each of the different nucleotide sequences comprising a sequence complementary and hybridizable to a plurality of genes, the plurality consisting of at least 2, 3, 4, 5, or 6 of the genes corresponding to the markers listed in Table 5 and optionally at least 2 to 18, 5 to 16, or 10 to 15 of the genes corresponding to the markers listed in Table 2. An aspect of the invention provides microarrays comprising at least 4, 5, or 6 of the polynucleotides encoding the markers listed in Table 5.

The invention provides gene marker sets that distinguish head-and-neck disease and uses therefor. In an aspect, the invention provides a method for classifying a head and neck disease comprising detecting a difference in the expression of a first plurality of genes relative to a control, the first plurality of genes consisting of at least 3, 4, 5, or 6 of the genes encoding the markers listed in Table 5. In specific aspects, the plurality of genes consists of at least 4 or 5 of the genes encoding the markers listed in Table 5 and optionally at least 2 to 18, 5 to 16, or 10 to 15 of the genes corresponding to the markers listed in Table 5. In another specific aspect, the control comprises nucleic acids derived from a pool of samples from individual control patients.

The invention provides a method for classifying a head-and-neck disease by calculating the similarity between the expression of at least 3, 4, 5, or 6 polynucleotides encoding markers listed in Table 5 in a sample to the expression of the same markers in a control pool, comprising the steps of:

- (a) labeling nucleic acids derived from a sample, with a first fluorophore to obtain a first pool of fluorophore-labeled nucleic acids;
- (b) labeling with a second fluorophore a first pool of nucleic acids derived from two or more head-and-neck disease samples, and a second pool of nucleic acids derived from two or more control samples;
- (c) contacting the first fluorophore-labeled nucleic acid and the first pool of second fluorophore-labeled nucleic acid with a first microarray under

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- conditions such that hybridization can occur, and contacting the first fluorophore-labeled nucleic acid and the second pool of second fluorophore-labeled nucleic acid with a second microarray under conditions such that hybridization can occur, detecting at each of a plurality of discrete loci on the first microarray a first fluorescent emission signal from the first fluorophore-labeled nucleic acid and a second fluorescent emission signal from the first pool of second fluorophore-labeled genetic matter that is bound to the first microarray and detecting at each of the marker loci on the second microarray the first fluorescent emission signal from the first fluorophore-labeled nucleic acid and a third fluorescent emission signal from the second pool of second fluorophore-labeled nucleic acid;
- (d) determining the similarity of the sample to patient and control pools by comparing the first fluorescence emission signals and the second fluorescence emission signals, and the first emission signals and the third fluorescence emission signals; and
- (e) classifying the sample as head-and-neck disease where the first fluorescence emission signals are more similar to the second fluorescence emission signals than to the third fluorescent emission signals, and classifying the sample as non-head-and-neck disease where the first fluorescence emission signals are more similar to the third fluorescence emission signals than to the second fluorescent emission signals, wherein the first microarray and the second microarray are similar to each other, exact replicas of each other, or are identical, and wherein the similarity is defined by a statistical method such that the cell sample and control are similar where the p value of the similarity is less than 0.01.

In aspects of the invention, the array can be used to monitor the time course of expression of one or more head-and-neck cancer polynucleotide markers in the array. This can occur in various biological contexts such as tumor progression.

The array is also useful for ascertaining differential expression patterns of head-and-neck cancer polynucleotide markers, and optionally other markers, in normal and abnormal cells. This may provide a battery of nucleic acids that could serve as molecular targets for diagnosis or therapeutic intervention.

Protein Methods

Binding agents may be used for a variety of diagnostic and assay applications. There are a variety of assay formats known to the skilled artisan for using a binding agent to detect a target molecule in a sample. (For example, see Harlow and Lane, Antibodies: A Laboratory
5 Manual, Cold Spring Harbor Laboratory, 1988). In general, the presence or absence of a head-and-neck disease (e.g. cancer) in a subject may be determined by (a) contacting a sample from the subject with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined standard or cut-off value.

10 In particular embodiments of the invention, the binding agent is an antibody. Antibodies specifically reactive with one or more head-and-neck marker, or derivatives, such as enzyme conjugates or labeled derivatives, may be used to detect one or more head-and-neck marker in various samples (e.g. biological materials). They may be used as diagnostic or prognostic reagents and they may be used to detect abnormalities in the level of expression of
15 one or more head-and-neck marker, or abnormalities in the structure, and/or temporal, tissue, cellular, or subcellular location of one or more head-and-neck marker. Antibodies may also be used to screen potentially therapeutic compounds *in vitro* to determine their effects on disorders (e.g. head-and-neck cancer) involving one or more head-and-neck cancer markers, and other conditions. *In vitro* immunoassays may also be used to assess or monitor the
20 efficacy of particular therapies.

In an aspect, the invention provides a method for monitoring or diagnosing a head-and-neck disease (e.g. cancer) in a subject by quantitating one or more head-and-neck cancer markers in a biological sample from the subject comprising reacting the sample with antibodies specific for one or more head-and-neck cancer markers, which are directly or
25 indirectly labeled with detectable substances and detecting the detectable substances. In a particular embodiment of the invention, head-and-neck cancer markers are quantitated or measured.

In an aspect of the invention, a method for detecting a head-and-neck disease (e.g. cancer) is provided comprising:

- 30 (a) obtaining a sample suspected of containing one or more head-and-neck cancer markers associated with a head-and-neck disease;
- (b) contacting said sample with antibodies that specifically bind to the head-and-

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neck cancer markers under conditions effective to bind the antibodies and form complexes;

(c) measuring the amount of head-and-neck cancer markers present in the sample by quantitating the amount of the complexes; and

5 (d) comparing the amount of head-and-neck cancer markers present in the samples with the amount of head-and-neck cancer markers in a control, wherein a change or significant difference in the amount of head-and-neck cancer markers in the sample compared with the amount in the control is indicative of a head-and-neck disease.

10 In an embodiment, the invention contemplates a method for monitoring the progression of a head-and-neck disease (e.g. cancer) in an individual, comprising:

(a) contacting antibodies which bind to one or more head-and-neck cancer markers with a sample from the individual so as to form complexes comprising the antibodies and one or more head-and-neck cancer markers in
15 the sample;

(b) determining or detecting the presence or amount of complex formation in the sample;

(c) repeating steps (a) and (b) at a point later in time; and

(d) comparing the result of step (b) with the result of step (c), wherein a difference
20 in the amount of complex formation is indicative of disease, disease stage, and/or progression of the disease in said individual.

The amount of complexes may also be compared to a value representative of the amount of the complexes from an individual not at risk of, or afflicted with, a head-and-neck disease at different stages. A significant difference in complex formation may be indicative of
25 advanced disease e.g. advanced head-and-neck cancer, or an unfavourable prognosis.

In aspects of the invention for diagnosis and monitoring of head-and-neck cancer, the head-and-neck cancer markers are one or more of those upregulated in cancer samples as compared to normal samples in Table 1, those listed in Table 5, and/or YWHAZ, S100 A7, and/or stratifin.

30 In embodiments of the methods of the invention, YWHAZ, S100 A7, and/or stratifin is detected in samples and higher levels, in particular significantly higher levels compared to a

control (normal or benign) is indicative of the prognosis of head-and-neck cancer patient outcome.

In aspects of the invention for characterizing head and neck disease the head-and-neck cancer markers comprise YWHAZ, S100 A7, and/or stratifin and fragments thereof.

5 Antibodies may be used in any known immunoassays that rely on the binding interaction between antigenic determinants of one or more head-and-neck marker and the antibodies. Immunoassay procedures for *in vitro* detection of antigens in fluid samples are also well known in the art. [See for example, Paterson et al., Int. J. Can. 37:659 (1986) and Burchell et al., Int. J. Can. 34:763 (1984) for a general description of immunoassay
10 procedures]. Qualitative and/or quantitative determinations of one or more head-and-neck marker in a sample may be accomplished by competitive or non-competitive immunoassay procedures in either a direct or indirect format. Detection of one or more head-and-neck marker using antibodies can be done utilizing immunoassays which are run in either the forward, reverse or simultaneous modes. Examples of immunoassays are radioimmunoassays
15 (RIA), enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, histochemical tests, and sandwich (immunometric) assays. These terms are well understood by those skilled in the art. A person skilled in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

 According to an embodiment of the invention, an immunoassay for detecting one or
20 more head-and-neck cancer markers in a biological sample comprises contacting binding agents that specifically bind to head-and-neck cancer markers in the sample under conditions that allow the formation of first complexes comprising a binding agent and head-and-neck cancer markers and determining the presence or amount of the complexes as a measure of the amount of head-and-neck cancer markers contained in the sample. In a particular embodiment,
25 the binding agents are labeled differently or are capable of binding to different labels.

 Antibodies may be used to detect and quantify one or more head-and-neck cancer markers in a sample in order to diagnose and treat pathological states. In particular, the antibodies may be used in immunohistochemical analyses, for example, at the cellular and sub-subcellular level, to detect one or more head-and-neck cancer markers, to localize them to
30 particular head-and-neck cells and tissues (e.g. tumor cells and tissues), and to specific subcellular locations, and to quantitate the level of expression.

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Immunohistochemical methods for the detection of antigens in tissue samples are well known in the art. For example, immunohistochemical methods are described in Taylor, Arch. Pathol. Lab. Med. 102:112 (1978). Briefly, in the context of the present invention, a tissue sample obtained from a subject suspected of having a head-and-neck-related problem is contacted with antibodies, preferably monoclonal antibodies recognizing one or more head-and-neck cancer markers. The site at which the antibodies are bound is determined by selective staining of the sample by standard immunohistochemical procedures. The same procedure may be repeated on the same sample using other antibodies that recognize one or more head-and-neck cancer markers. Alternatively, a sample may be contacted with antibodies against one or more head-and-neck cancer markers simultaneously, provided that the antibodies are labeled differently or are able to bind to a different label. The tissue sample may be normal head-and-neck tissue, a cancer tissue or a benign tissue.

An antibody microarray in which binding sites comprise immobilized, preferably monoclonal, antibodies specific to a substantial fraction of marker-derived head-and-neck cancer markers of interest can be utilized in the present invention. Antibody arrays can be prepared using methods known in the art [(see for example, Zhu et al., *Science* 293:2101 (2001) and reference 20].

Antibodies specific for one or more head-and-neck marker may be labelled with a detectable substance and localised in biological samples based upon the presence of the detectable substance. Examples of detectable substances include, but are not limited to, the following: radioisotopes (e.g., ^3H , ^{14}C , ^{35}S , ^{125}I , ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), luminescent labels such as luminol; enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase), biotinyl groups (which can be detected by marked avidin e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods), predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached via spacer arms of various lengths to reduce potential steric hindrance. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualised by electron microscopy.

One of the ways an antibody can be detectably labeled is to link it directly to an enzyme. The enzyme when later exposed to its substrate will produce a product that can be detected. Examples of detectable substances that are enzymes are horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase, malate
5 dehydrogenase, ribonuclease, urease, catalase, glucose-6-phosphate, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, triose phosphate isomerase, asparaginase, glucose oxidase, and acetylcholine esterase.

For increased sensitivity in an immunoassay system a fluorescence-emitting metal atom such as Eu (europium) and other lanthanides can be used. These can be attached to the
10 desired molecule by means of metal-chelating groups such as DTPA or EDTA.

A bioluminescent compound may also be used as a detectable substance. Bioluminescence is a type of chemiluminescence found in biological systems where a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent molecule is determined by detecting the presence of luminescence. Examples
15 of bioluminescent detectable substances are luciferin, luciferase and aequorin.

Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against one or more head-and-neck cancer markers. By way of example, if the antibody having specificity against one or more head-and-neck marker is a rabbit IgG
20 antibody, the second antibody may be goat anti-rabbit gamma-globulin labelled with a detectable substance as described herein.

Methods for conjugating or labelling the antibodies discussed above may be readily accomplished by one of ordinary skill in the art. (See for example Inman, Methods In Enzymology, Vol. 34, Affinity Techniques, Enzyme Purification: Part B, Jakoby and Wichek
25 (eds.), Academic Press, New York, p. 30, 1974; and Wilchek and Bayer, "The Avidin-Biotin Complex in Bioanalytical Applications," Anal. Biochem. 171:1-32, 1988 re methods for conjugating or labelling the antibodies with enzyme or ligand binding partner).

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect one or more head-and-neck cancer markers.
30 Generally, antibodies may be labeled with detectable substances and one or more head-and-neck cancer markers may be localised in tissues and cells based upon the presence of the detectable substances.

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In the context of the methods of the invention, the sample, binding agents (e.g. antibodies specific for one or more head-and-neck cancer markers), or one or more head-and-neck cancer markers may be immobilized on a carrier or support. Examples of suitable carriers or supports are agarose, cellulose, nitrocellulose, dextran, Sephadex, Sepharose, liposomes, carboxymethyl cellulose, polyacrylamides, polystyrene, gabbros, filter paper, magnetite, ion-exchange resin, plastic film, plastic tube, glass, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The support material may have any possible configuration including spherical (e.g. bead), cylindrical (e.g. inside surface of a test tube or well, or the external surface of a rod), or flat (e.g. sheet, test strip). Thus, the carrier may be in the shape of, for example, a tube, test plate, well, beads, disc, sphere, etc. The immobilized antibody may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling. An antibody may be indirectly immobilized using a second antibody specific for the antibody. For example, mouse antibody specific for a head-and-neck marker may be immobilized using sheep anti-mouse IgG Fc fragment specific antibody coated on the carrier or support.

Where a radioactive label is used as a detectable substance, one or more head-and-neck marker may be localized by radioautography. The results of radioautography may be quantitated by determining the density of particles in the radioautographs by various optical methods, or by counting the grains.

Time-resolved fluorometry may be used to detect a signal. For example, the method described in Christopoulos TK and Diamandis EP Anal Chem 1992:64:342-346 may be used with a conventional time-resolved fluorometer.

In accordance with an embodiment of the invention, a method is provided wherein one or more head-and-neck marker antibodies are directly or indirectly labelled with enzymes, substrates for the enzymes are added wherein the substrates are selected so that the substrates, or a reaction product of an enzyme and substrate, form fluorescent complexes with a lanthanide metal (e.g. europium, terbium, samarium, and dysprosium, preferably europium and terbium). A lanthanide metal is added and one or more head-and-neck cancer markers are quantitated in the sample by measuring fluorescence of the fluorescent complexes. Enzymes are selected based on the ability of a substrate of the enzyme, or a reaction product of the enzyme and substrate, to complex with lanthanide metals such as europium and terbium.

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Suitable enzymes and substrates that provide fluorescent complexes are described in U.S. Patent No. 5,3112,922 to Diamandis. Examples of suitable enzymes include alkaline phosphatase and β -galactosidase. Preferably, the enzyme is alkaline phosphatase.

5 Examples of enzymes and substrates for enzymes that provide such fluorescent complexes are described in U.S. Patent No. 5,312,922 to Diamandis. By way of example, when the antibody is directly or indirectly labelled with alkaline phosphatase the substrate employed in the method may be 4-methylumbelliferyl phosphate, 5-fluorosalicyl phosphate, or diflunisal phosphate. The fluorescence intensity of the complexes is typically measured using a time-resolved fluorometer e.g. a CyberFluor 615 Imunoanalyzer (Nordion
10 International, Kanata, Ontario).

One or more head-and-neck marker antibodies may also be indirectly labelled with an enzyme. For example, the antibodies may be conjugated to one partner of a ligand binding pair, and the enzyme may be coupled to the other partner of the ligand binding pair. Representative examples include avidin-biotin, and riboflavin-riboflavin binding protein. In
15 an embodiment, the antibodies are biotinylated, and the enzyme is coupled to streptavidin. In another embodiment, an antibody specific for head-and-neck marker antibody is labeled with an enzyme.

In accordance with an embodiment, the present invention provides means for determining one or more head-and-neck cancer markers in a sample by measuring one or
20 more head-and-neck cancer markers by immunoassay. It will be evident to a skilled artisan that a variety of immunoassay methods can be used to measure one or more head-and-neck cancer markers. In general, an immunoassay method may be competitive or noncompetitive. Competitive methods typically employ an immobilized or immobilizable antibody to one or more head-and-neck marker and a labeled form of one or more head-and-neck marker. Sample
25 head-and-neck cancer markers and labeled head-and-neck cancer markers compete for binding to antibodies to head-and-neck cancer markers. After separation of the resulting labeled head-and-neck cancer markers that have become bound to antibodies (bound fraction) from that which has remained unbound (unbound fraction), the amount of the label in either bound or unbound fraction is measured and may be correlated with the amount of head-and-neck cancer
30 markers in the test sample in any conventional manner, e.g., by comparison to a standard curve.

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In an aspect, a non-competitive method is used for the determination of one or more head-and-neck cancer markers, with the most common method being the "sandwich" method. In this assay, two antibodies to head-and-neck cancer markers are employed. One of the antibodies to head-and-neck cancer markers is directly or indirectly labeled (sometimes referred to as the "detection antibody") and the other is immobilized or immobilizable (sometimes referred to as the "capture antibody"). The capture and detection antibodies can be contacted simultaneously or sequentially with the test sample. Sequential methods can be accomplished by incubating the capture antibody with the sample, and adding the detection antibody at a predetermined time thereafter (sometimes referred to as the "forward" method); or the detection antibody can be incubated with the sample first and then the capture antibody added (sometimes referred to as the "reverse" method). After the necessary incubation(s) have occurred, to complete the assay, the capture antibody is separated from the liquid test mixture, and the label is measured in at least a portion of the separated capture antibody phase or the remainder of the liquid test mixture. Generally it is measured in the capture antibody phase since it comprises head-and-neck cancer markers bound by ("sandwiched" between) the capture and detection antibodies. In an embodiment, the label may be measured without separating the capture antibodies and liquid test mixture.

In a typical two-site immunometric assay for head-and-neck cancer markers, one or both of the capture and detection antibodies are polyclonal antibodies or one or both of the capture and detection antibodies are monoclonal antibodies (i.e. polyclonal/polyclonal, monoclonal/monoclonal, or monoclonal/polyclonal). The label used in the detection antibody can be selected from any of those known conventionally in the art. The label may be an enzyme or a chemiluminescent moiety, but it can also be a radioactive isotope, a fluorophor, a detectable ligand (e.g., detectable by a secondary binding by a labeled binding partner for the ligand), and the like. In a particular aspect, the antibody is labelled with an enzyme which is detected by adding a substrate that is selected so that a reaction product of the enzyme and substrate forms fluorescent complexes. The capture antibody may be selected so that it provides a means for being separated from the remainder of the test mixture. Accordingly, the capture antibody can be introduced to the assay in an already immobilized or insoluble form, or can be in an immobilizable form, that is, a form which enables immobilization to be accomplished subsequent to introduction of the capture antibody to the assay. An immobilized capture antibody may comprise an antibody covalently or noncovalently attached to a solid

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phase such as a magnetic particle, a latex particle, a microtiter plate well, a bead, a cuvette, or other reaction vessel. An example of an immobilizable capture antibody is antibody which has been chemically modified with a ligand moiety, e.g., a hapten, biotin, or the like, and which can be subsequently immobilized by contact with an immobilized form of a binding partner
5 for the ligand, e.g., an antibody, avidin, or the like. In an embodiment, the capture antibody may be immobilized using a species specific antibody for the capture antibody that is bound to the solid phase.

The above-described immunoassay methods and formats are intended to be exemplary and are not limiting.

10 **Computer Systems**

Analytic methods contemplated herein can be implemented by use of computer systems and methods described below and known in the art. Thus, the invention provides computer readable media comprising one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other
15 markers (e.g. markers of head-and-neck cancer). "Computer readable media" refers to any medium that can be read and accessed directly by a computer, including but not limited to magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. Thus, the invention
20 contemplates computer readable medium having recorded thereon markers identified for patients and controls.

"Recorded" refers to a process for storing information on computer readable medium. The skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising information
25 on one or more head-and-neck cancer markers, and optionally other markers.

A variety of data processor programs and formats can be used to store information on one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and other markers on computer readable medium. For example, the information can be represented in a word processing text file, formatted in
30 commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. Any number of dataprocessor structuring formats (e.g., text file or database) may be

adapted in order to obtain computer readable medium having recorded thereon the marker information.

By providing the marker information in computer readable form, one can routinely access the information for a variety of purposes. For example, one skilled in the art can use
5 the information in computer readable form to compare marker information obtained during or following therapy with the information stored within the data storage means.

The invention provides a medium for holding instructions for performing a method for determining or whether a patient has a head-and-neck disease (e.g. head-and-neck cancer) or a pre-disposition to a head-and-neck disease (e.g. cancer), comprising determining the presence
10 or absence of one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers, and based on the presence or absence of the one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers, determining uterine head-and-neck receptivity, head-and-neck disease (e.g. cancer) or a pre-
15 disposition to a head-and-neck disease (e.g. cancer), and optionally recommending a procedure or treatment.

The invention also provides in an electronic system and/or in a network, a method for determining whether a subject has a head-and-neck disease (e.g. cancer) or a pre-disposition to a head-and-neck disease (e.g. cancer), comprising determining the presence or absence of one
20 or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers (e.g. cancer markers), and based on the presence or absence of the one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers, determining whether the subject has a head-and-neck disease (e.g. cancer) or a pre-disposition
25 to a head-and-neck disease (e.g. cancer), and optionally recommending a procedure or treatment.

The invention further provides in a network, a method for determining whether a subject is receptive to *in vitro* fertilization, has a head-and-neck disease (e.g. cancer) or a pre-disposition to a head-and-neck disease (e.g. cancer) comprising: (a) receiving phenotypic
30 information on the subject and information on one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers associated with samples from the subject; (b) acquiring information from the

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network corresponding to the one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers; and (c) based on the phenotypic information and information on the one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers, determining whether the subject is receptive to *in vitro* fertilization, has a head-and-neck disease (e.g. cancer) or a pre-disposition to a head-and-neck disease (e.g. cancer); and (d) optionally recommending a procedure or treatment.

The invention still further provides a system for identifying selected records that identify a diseased head-and-neck cell or tissue (e.g. cancer cell or tissue) or an head and neck cell or tissue phase. A system of the invention generally comprises a digital computer; a database server coupled to the computer; a database coupled to the database server having data stored therein, the data comprising records of data comprising one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other head-and-neck cancer markers, and a code mechanism for applying queries based upon a desired selection criteria to the data file in the database to produce reports of records which match the desired selection criteria.

In an aspect of the invention a method is provided for detecting head-and-neck cancer tissue or cells using a computer having a processor, memory, display, and input/output devices, the method comprising the steps of:

- (a) creating records of one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers of cancer identified in a sample suspected of containing head-and-neck cancer cells or tissue;
- (b) providing a database comprising records of data comprising one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally other markers of cancer; and
- (c) using a code mechanism for applying queries based upon a desired selection criteria to the data file in the database to produce reports of records of step (a) which provide a match of the desired selection criteria of the database of step (b) the presence of a match being a positive indication that the markers of step (a) have been isolated from cells or tissue that are head-and-neck cancer cells or tissue.

The invention contemplates a business method for determining whether a subject is receptive to *in vitro* fertilization, has a head-and-neck disease (e.g. cancer) or a pre-disposition to head-and-neck cancer comprising: (a) receiving phenotypic information on the subject and information on one or more head-and-neck cancer markers, and/or polynucleotides encoding the markers, and optionally other markers, associated with samples from the subject; (b) acquiring information from a network corresponding to one or more head-and-neck cancer markers, and/or polynucleotides encoding the markers, and optionally other markers; and (c) based on the phenotypic information, information on one or more head-and-neck cancer markers, and/or polynucleotides encoding the markers, and optionally other markers, and acquired information, determining whether the subject is receptive to *in vitro* fertilization, has a head-and-neck disease (e.g. cancer) or a pre-disposition to a head-and-neck disease (e.g. cancer); and (d) optionally recommending a procedure or treatment.

In an aspect of the invention, the computer systems, components, and methods described herein are used to monitor disease or determine the stage of disease.

15 **Imaging Methods**

Binding agents, in particular antibodies, specific for one or more head-and-neck cancer markers may also be used in imaging methodologies in the management of a head-and-neck disease.

In an aspect, the invention provides a method for imaging tumors associated with one or more head-and-neck cancer markers.

The invention also contemplates imaging methods described herein using multiple markers for a head-and-neck disease. Preferably each agent is labeled so that it can be distinguished during the imaging.

In an embodiment the method is an *in vivo* method and a subject or patient is administered one or more agents that carry an imaging label and that are capable of targeting or binding to one or more head-and-neck cancer markers. The agent is allowed to incubate *in vivo* and bind to the head-and-neck cancer markers associated with head-and-neck cells or tissues of a particular phase or associated with diseased cells or tissues, (e.g. a head-and-neck tumor). The presence of the label is localized to the head-and-neck cells or tissues, and the localized label is detected using imaging devices known to those skilled in the art.

The agent may be an antibody or chemical entity that recognizes the head-and-neck cancer markers. In an aspect of the invention the agent is a polyclonal antibody or monoclonal

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antibody, or fragments thereof, or constructs thereof including but not limited to, single chain antibodies, bifunctional antibodies, molecular recognition units, and peptides or entities that mimic peptides. The antibodies specific for the head-and-neck cancer markers used in the methods of the invention may be obtained from scientific or commercial sources, or isolated
 5 native head-and-neck cancer markers or recombinant head-and-neck cancer markers may be utilized to prepare antibodies etc. as described herein.

An agent may be a peptide that mimics the epitope for an antibody specific for a head-and-neck marker and binds to the marker. The peptide may be produced on a commercial synthesizer using conventional solid phase chemistry. By way of example, a peptide may be
 10 prepared that includes either tyrosine, lysine, or phenylalanine to which N_2S_2 chelate is complexed (See U.S. Patent No. 4,897,255). An anti-endocrine marker peptide conjugate is then combined with a radiolabel (e.g. sodium ^{99m}Tc pertechnetate or sodium ^{188}Re perrhenate) and it may be used to locate a head-and-neck marker producing cell or tissue (e.g. tumor).

The agent carries a label to image the head-and-neck cancer markers. The agent may
 15 be labelled for use in radionuclide imaging. In particular, the agent may be directly or indirectly labelled with a radioisotope. Examples of radioisotopes that may be used in the present invention are the following: ^{277}Ac , ^{211}At , ^{128}Ba , ^{131}Ba , ^7Be , ^{204}Bi , ^{205}Bi , ^{206}Bi , ^{76}Br , ^{77}Br , ^{82}Br , ^{109}Cd , ^{47}Ca , ^{11}C , ^{14}C , ^{36}Cl , ^{48}Cr , ^{51}Cr , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{165}Dy , ^{155}Eu , ^{18}F , ^{153}Gd , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{72}Ga , ^{198}Au , ^3H , ^{166}Ho , ^{111}In , ^{113m}In , ^{115m}In , ^{123}I , ^{125}I , ^{131}I , ^{189}Ir , ^{191m}Ir , ^{192}Ir ,
 20 ^{194}Ir , ^{52}Fe , ^{55}Fe , ^{59}Fe , ^{177}Lu , ^{15}O , $^{191m-191}\text{Os}$, ^{109}Pd , ^{32}P , ^{33}P , ^{42}K , ^{226}Ra , ^{186}Re , ^{188}Re , ^{82m}Rb , ^{153}Sm , ^{46}Sc , ^{47}Sc , ^{72}Se , ^{75}Se , ^{105}Ag , ^{22}Na , ^{24}Na , ^{89}Sr , ^{35}S , ^{38}S , ^{177}Ta , ^{96}Tc , ^{99m}Tc , ^{201}Tl , ^{202}Tl , ^{113}Sn , ^{117m}Sn , ^{121}Sn , ^{166}Yb , ^{169}Yb , ^{175}Yb , ^{88}Y , ^{90}Y , ^{62}Zn and ^{65}Zn . Preferably the radioisotope is ^{131}I , ^{125}I , ^{123}I , ^{111}I , ^{99m}Tc , ^{90}Y , ^{186}Re , ^{188}Re , ^{32}P , ^{153}Sm , ^{67}Ga , ^{201}Tl , ^{77}Br , or ^{18}F , and is imaged with a photoscanning device.

25 Procedures for labeling biological agents with the radioactive isotopes are generally known in the art. U.S. Pat. No. 4,302,438 describes tritium labeling procedures. Procedures for iodinating, tritium labeling, and ^{35}S labeling especially adapted for murine monoclonal antibodies are described by Goding, J. W. (supra, pp 124-126) and the references cited therein. Other procedures for iodinating biological agents, such as antibodies, binding portions thereof,
 30 probes, or ligands, are described in the scientific literature [see Hunter and Greenwood, Nature 144:945 (1962), David et al., Biochemistry 13:1014-1021 (1974), and U.S. Pat. Nos. 3,867,517 and 4,376,110]. Iodinating procedures for agents are described by Greenwood, F. et

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al., Biochem. J. 89:114-123 (1963); Marchalonis, J., Biochem. J. 113:299-305 (1969); and Morrison, M. et al., Immunochemistry, 289-297 (1971). ^{99m}Tc-labeling procedures are described by Rhodes, B. et al. in Burchiel, S. et al. (eds.), Tumor Imaging: The Radioimmunochemical Detection of Cancer, New York: Masson 111-123 (1982) and the
 5 references cited therein. Labelling of antibodies or fragments with technetium-99m are also described for example in U.S. Pat. No. 5,317,091, U.S. Pat. No. 4,478,815, U.S. Pat. No. 4,478,818, U.S. Pat. No. 4,472,371, U.S. Pat. No. Re 32,417, and U.S. Pat. No. 4,311,688. Procedures suitable for ¹¹¹In-labeling biological agents are described by Hnatowich, D. J. et al., J. Immunol. Methods, 65:147-157 (1983), Hnatowich, D. et al., J. Applied Radiation,
 10 35:554-557 (1984), and Buckley, R. G. et al., F.E.B.S. 166:202-204 (1984).

An agent may also be labeled with a paramagnetic isotope for purposes of an *in vivo* method of the invention. Examples of elements that are useful in magnetic resonance imaging include gadolinium, terbium, tin, iron, or isotopes thereof. (See, for example, Schaefer et al., (1989) JACC 14, 472-480; Shreve et al., (1986) Magn. Reson. Med. 3, 336-340; Wolf, G L.,
 15 (1984) Physiol. Chem. Phys. Med. NMR 16, 93-95; Wesbey et al., (1984) Physiol. Chem. Phys. Med. NMR 16, 145-155; Runge et al., (1984) Invest. Radiol. 19, 408-415 for discussions on *in vivo* nuclear magnetic resonance imaging.)

In the case of a radiolabeled agent, the agent may be administered to the patient, it is localized to the cell or tissue (e.g. tumor) having a head-and-neck marker with which the agent
 20 binds, and is detected or "imaged" *in vivo* using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. [See for example, A. R. Bradwell et al., "Developments in Antibody Imaging", Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp. 65-85 (Academic Press 1985)]. A positron emission transaxial tomography scanner, such as designated Pet VI located at
 25 Brookhaven National Laboratory, can also be used where the radiolabel emits positrons (e.g., ¹¹C, ¹⁸F, ¹⁵O, and ¹³N).

Whole body imaging techniques using radioisotope labeled agents can be used for locating diseased cells and tissues (e.g. primary tumors and tumors which have metastasized). Antibodies specific for head-and-neck cancer markers, or fragments thereof having the same
 30 epitope specificity, are bound to a suitable radioisotope, or a combination thereof, and administered parenterally. For head-and-neck cancer, administration preferably is intravenous. The bio-distribution of the label can be monitored by scintigraphy, and accumulations of the

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label are related to the presence of head-and-neck cancer cells. Whole body imaging techniques are described in U.S. Pat. Nos. 4,036,945 and 4,311,688. Other examples of agents useful for diagnosis and therapeutic use that can be coupled to antibodies and antibody fragments include metallothionein and fragments (see, U.S. Pat. No. 4,732,864). These agents
5 are useful in diagnosis staging and visualization of cancer, in particular head-and-neck cancer, so that surgical and/or radiation treatment protocols can be used more efficiently.

An imaging agent may carry a bioluminescent or chemiluminescent label. Such labels include polypeptides known to be fluorescent, bioluminescent or chemiluminescent, or, that act as enzymes on a specific substrate (reagent), or can generate a fluorescent, bioluminescent
10 or chemiluminescent molecule. Examples of bioluminescent or chemiluminescent labels include luciferases, aequorin, obelin, mnemiopsin, berovin, a phenanthridinium ester, and variations thereof and combinations thereof. A substrate for the bioluminescent or chemiluminescent polypeptide may also be utilized in a method of the invention. For example, the chemiluminescent polypeptide can be luciferase and the reagent luciferin. A substrate for
15 a bioluminescent or chemiluminescent label can be administered before, at the same time (e.g., in the same formulation), or after administration of the agent.

An imaging agent may comprise a paramagnetic compound, such as a polypeptide chelated to a metal, e.g., a metalloporphyrin. The paramagnetic compound may also comprise a monocrystalline nanoparticle, e.g., a nanoparticle comprising a lanthanide (e.g., Gd) or iron
20 oxide; or, a metal ion comprising a lanthanide. "Lanthanides" refers to elements of atomic numbers 58 to 70, a transition metal of atomic numbers 21 to 29, 42 or 44, a Gd(III), a Mn(II), or an element comprising a Fe element. Paramagnetic compounds can also comprise a neodymium iron oxide (NdFeO₃) or a dysprosium iron oxide (DyFeO₃). Examples of elements that are useful in magnetic resonance imaging include gadolinium, terbium, tin, iron, or
25 isotopes thereof. (See, for example, Schaefer et al., (1989) JACC 14, 472-480; Shreve et al., (1986) Magn. Reson. Med. 3, 336-340; Wolf, G L., (1984) Physiol. Chem. Phys. Med. NMR 16, 93-95; Wesbey et al., (1984) Physiol. Chem. Phys. Med. NMR 16, 145-155; Runge et al., (1984) Invest. Radiol. 19, 408-415 for discussions on *in vivo* nuclear magnetic resonance imaging.)

30 An image can be generated in a method of the invention by computer assisted tomography (CAT), magnetic resonance spectroscopy (MRS) image, magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed

tomography (SPECT), or bioluminescence imaging (BLI) or equivalent.

Computer assisted tomography (CAT) and computerized axial tomography (CAT) systems and devices well known in the art can be utilized in the practice of the present invention. (See, for example, U.S. Patent Nos. 6,151,377; 5,946,371; 5,446,799; 5,406,479; 5,208,581; 5,109,397). The invention may also utilize animal imaging modalities, such as MicroCAT.TM. (ImTek, Inc.).

Magnetic resonance imaging (MRI) systems and devices well known in the art can be utilized in the practice of the present invention. For a description of MRI methods and devices see, for example, U.S. Patent Nos. 6,151,377; 6,144,202; 6,128,522; 6,127,825; 6,121,775; 6,119,032; 6,115,446; 6,111,410; 6,02,891; 5,555,251; 5,455,512; 5,450,010; 5,378,987; 5,214,382; 5,031,624; 5,207,222; 4,985,678; 4,906,931; 4,558,279. MRI and supporting devices are commercially available for example, from Bruker Medical GMBH; Caprius; Esaote Biomedica; Fonar; GE Medical Systems (GEMS); Hitachi Medical Systems America; Intermagnetics General Corporation; Lunar Corp.; MagneVu; Marconi Medicals; Philips Medical Systems; Shimadzu; Siemens; Toshiba America Medical Systems; including imaging systems, by, e.g., Silicon Graphics. The invention may also utilize animal imaging modalities such as micro-MRIs.

Positron emission tomography imaging (PET) systems and devices well known in the art can be utilized in the practice of the present invention. For example, a method of the invention may use the system designated Pet VI located at Brookhaven National Laboratory. For descriptions of PET systems and devices see, for example, U.S. Pat. Nos. 6,151,377; 6,072,177; 5,900,636; 5,608,221; 5,532,489; 5,272,343; 5,103,098. Animal imaging modalities such as micro-PETs (Corcorde Microsystems, Inc.) can also be used in the invention.

Single-photon emission computed tomography (SPECT) systems and devices well known in the art can be utilized in the practice of the present invention. (See, for example, U.S. Patents. Nos. 6,115,446; 6,072,177; 5,608,221; 5,600,145; 5,210,421; 5,103,098.) The methods of the invention may also utilize animal imaging modalities, such as micro-SPECTs.

Bioluminescence imaging includes bioluminescence, fluorescence or chemiluminescence or other photon detection systems and devices that are capable of detecting bioluminescence, fluorescence or chemiluminescence. Sensitive photon detection systems can be used to detect bioluminescent and fluorescent proteins externally; see, for

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example, Contag (2000) Neoplasia 2:41-52; Zhang (1994) Clin. Exp. Metastasis 12:87-92. The methods of the invention can be practiced using any such photon detection device, or variation or equivalent thereof, or in conjunction with any known photon detection methodology, including visual imaging. By way of example, an intensified charge-coupled device (ICCD) camera coupled to an image processor may be used in the present invention. (See, e.g., U.S. Pat. No. 5,650,135). Photon detection devices are also commercially available from Xenogen, Hamamatsue.

Screening Methods

The invention also contemplates methods for evaluating test agents or compounds for their ability to inhibit a head-and-neck disease (e.g. cancer), potentially contribute to a head-and-neck disease (e.g. cancer). Test agents and compounds include but are not limited to peptides such as soluble peptides including Ig-tailed fusion peptides, members of random peptide libraries and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries), antibodies [e.g. polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, single chain antibodies, fragments, (e.g. Fab, F(ab)₂, and Fab expression library fragments, and epitope-binding fragments thereof)], and small organic or inorganic molecules. The agents or compounds may be endogenous physiological compounds or natural or synthetic compounds.

The invention provides a method for assessing the potential efficacy of a test agent for inhibiting a head-and-neck disease (e.g. cancer) in a patient, the method comprising comparing:

- (a) levels of one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers in a first sample obtained from a patient and exposed to the test agent; and
- (b) levels of one or more head-and-neck cancer markers and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers, in a second sample obtained from the patient, wherein the sample is not exposed to the test agent, wherein a significant difference in the levels of expression of one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers, and optionally the other markers, in the first sample, relative to the second sample, is an indication that the test

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agent is potentially efficacious for inhibiting a head-and-neck disease (e.g. cancer) in the patient.

The first and second samples may be portions of a single sample obtained from a patient or portions of pooled samples obtained from a patient.

5 In an aspect, the invention provides a method of selecting an agent for inhibiting a head-and-neck disease (e.g. cancer) in a patient comprising:

- (a) obtaining a sample from the patient;
- (b) separately maintaining aliquots of the sample in the presence of a plurality of test agents;
- 10 (c) comparing one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers, in each of the aliquots; and
- (d) selecting one of the test agents which alters the levels of one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck
- 15 cancer markers, and optionally other markers in the aliquot containing that test agent, relative to other test agents.

In a further aspect, the invention provides a method of selecting an agent for inhibiting or enhancing a head and neck cell or tissue phase in a patient comprising:

- (a) obtaining a sample of head and neck cell or tissue in a selected phase;
- 20 (b) separately maintaining aliquots of the sample in the presence of a plurality of test agents;
- (c) comparing one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers, in each of the aliquots; and
- 25 (d) selecting one of the test agents which alters the levels of one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers in the aliquot containing that test agent, relative to other test agents.

30 Still another aspect of the present invention provides a method of conducting a drug discovery business comprising:

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- (a) providing one or more methods or assay systems for identifying agents that inhibit a head-and-neck disease (e.g. head-and-neck cancer) or affect a head and neck cell or tissues phase in a patient;
- (b) conducting therapeutic profiling of agents identified in step (a), or further analogs thereof, for efficacy and toxicity in animals; and
- (c) formulating a pharmaceutical preparation including one or more agents identified in step (b) as having an acceptable therapeutic profile.

In certain embodiments, the subject method can also include a step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and may optionally include establishing a sales group for marketing the pharmaceutical preparation.

The invention also contemplates a method of assessing the potential of a test compound to contribute to a head-and-neck disease (e.g. head-and-neck cancer) comprising:

- (a) maintaining separate aliquots of cells or tissues from a patient with a head-and-neck disease (e.g. cancer) in the presence and absence of the test compound; and
- (b) comparing one or more head-and-neck cancer markers, and/or polynucleotides encoding head-and-neck cancer markers, and optionally other markers in each of the aliquots.

A significant difference between the levels of the markers in the aliquot maintained in the presence of (or exposed to) the test compound relative to the aliquot maintained in the absence of the test compound, indicates that the test compound possesses the potential to contribute to a head-and-neck disease (e.g. head-and-neck cancer).

Kits

The invention also contemplates kits for carrying out the methods of the invention. Kits may typically comprise two or more components required for performing a diagnostic assay. Components include but are not limited to compounds, reagents, containers, and/or equipment.

The methods described herein may be performed by utilizing pre-packaged diagnostic kits comprising one or more specific head-and-neck marker polynucleotide or antibody described herein, which may be conveniently used, e.g., in clinical settings to screen and diagnose patients and to screen and identify those individuals exhibiting a predisposition to developing a head-and-neck disease.

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In an embodiment, a container with a kit comprises a binding agent as described herein. By way of example, the kit may contain antibodies or antibody fragments which bind specifically to epitopes of one or more head-and-neck cancer markers and optionally other markers, antibodies against the antibodies labelled with an enzyme; and a substrate for the enzyme. The kit may also contain microtiter plate wells, standards, assay diluent, wash buffer, adhesive plate covers, and/or instructions for carrying out a method of the invention using the kit.

In an aspect of the invention, the kit includes antibodies or fragments of antibodies which bind specifically to an epitope of one or more polypeptide listed in Table 1 that is upregulated in cancer samples as compared to normal samples, or those listed in Table 5 or Table 2 and means for detecting binding of the antibodies to their epitope associated with tumor cells, either as concentrates (including lyophilized compositions), which may be further diluted prior to use or at the concentration of use, where the vials may include one or more dosages. Where the kits are intended for *in vivo* use, single dosages may be provided in sterilized containers, having the desired amount and concentration of agents. Containers that provide a formulation for direct use, usually do not require other reagents, as for example, where the kit contains a radiolabelled antibody preparation for *in vivo* imaging.

A kit may be designed to detect the level of polynucleotides encoding one or more head-and-neck cancer polynucleotide markers in a sample. In an embodiment, the polynucleotides encode one or more polynucleotides encoding a polypeptide listed in Table 1 that is upregulated in cancer samples as compared to normal samples, or those listed in Table 5 or Table 2. Such kits generally comprise at least one oligonucleotide probe or primer, as described herein, that hybridizes to a polynucleotide encoding one or more head-and-neck cancer markers. Such an oligonucleotide may be used, for example, within a PCR or hybridization procedure. Additional components that may be present within the kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate detection of a polynucleotide encoding one or more head-and-neck cancer markers.

The invention provides a kit containing a microarray described herein ready for hybridization to target head-and-neck cancer polynucleotide markers, plus software for the data analysis of the results. The software to be included with the kit comprises data analysis methods, in particular mathematical routines for marker discovery, including the calculation of correlation coefficients between clinical categories and marker expression. The software

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may also include mathematical routines for calculating the correlation between sample marker expression and control marker expression, using array-generated fluorescence data, to determine the clinical classification of the sample.

5 The reagents suitable for applying the screening methods of the invention to evaluate compounds may be packaged into convenient kits described herein providing the necessary materials packaged into suitable containers.

The invention contemplates a kit for assessing the presence of head-and-neck cells, wherein the kit comprises antibodies specific for one or more head-and-neck cancer markers, or primers or probes for polynucleotides encoding same, and optionally probes, primers or
10 antibodies specific for other markers associated with a head-and-neck disease (e.g. cancer).

The invention relates to a kit for assessing the suitability of each of a plurality of test compounds for inhibiting a head-and-neck disease (e.g. head-and-neck cancer) in a patient. The kit comprises reagents for assessing one or more head-and-neck cancer markers or polynucleotides encoding same, and optionally a plurality of test agents or compounds.

15 Additionally the invention provides a kit for assessing the potential of a test compound to contribute to a head-and-neck disease (e.g. cancer). The kit comprises head-and-neck diseased cells (e.g. cancer cells) and reagents for assessing one or more head-and-neck cancer markers, polynucleotides encoding same, and optionally other markers associated with a head-and-neck disease.

20 **Therapeutic Applications**

One or more head-and-neck cancer markers may be targets for immunotherapy. Immunotherapeutic methods include the use of antibody therapy, *in vivo* vaccines, and *ex vivo* immunotherapy approaches.

In one aspect, the invention provides one or more head-and-neck marker antibodies
25 that may be used systemically to treat a head-and-neck disease associated with the marker. In particular, the head-and-neck disease is head-and-neck cancer and one or more head-and-neck marker antibodies may be used systemically to treat head-and-neck cancer. Preferably antibodies are used that target the tumor cells but not the surrounding non-tumor cells and tissue.

30 Thus, the invention provides a method of treating a patient susceptible to, or having a disease (e.g. cancer) that expresses one or more head-and-neck marker (in particular a marker up-regulated in head-and-neck cancer, for example, an up-regulated marker in Table 1, or that

of Table 5 and/or 2), comprising administering to the patient an effective amount of an antibody that binds specifically to one or more head-and-neck marker.

In another aspect, the invention provides a method of inhibiting the growth of tumor cells expressing one or more head-and-neck cancer markers, comprising administering to a patient an antibody which binds specifically to one or more head-and-neck cancer markers in an amount effective to inhibit growth of the tumor cells.

One or more head-and-neck marker antibodies may also be used in a method for selectively inhibiting the growth of, or killing a cell expressing one or more head-and-neck marker (e.g. tumor cell expressing one or more head-and-neck cancer marker) comprising reacting one or more head-and-neck marker antibody immunoconjugate or immunotoxin with the cell in an amount sufficient to inhibit the growth of, or kill the cell.

By way of example, unconjugated antibodies to head-and-neck cancer markers may be introduced into a patient such that the antibodies bind to head-and-neck cancer marker expressing cancer cells and mediate growth inhibition of such cells (including the destruction thereof), and the tumor, by mechanisms which may include complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, altering the physiologic function of one or more head-and-neck cancer markers, and/or the inhibition of ligand binding or signal transduction pathways. In addition to unconjugated antibodies to head-and-neck cancer markers, one or more head-and-neck cancer marker antibodies conjugated to therapeutic agents (e.g. immunoconjugates) may also be used therapeutically to deliver the agent directly to one or more head-and-neck cancer marker expressing tumor cells and thereby destroy the tumor. Examples of such agents include abrin, ricin A, *Pseudomonas* exotoxin, or diphtheria toxin; proteins such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; and biological response modifiers such as lymphokines, interleukin-1, interleukin-2, interleukin-6, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, or other growth factors.

Cancer immunotherapy using one or more head-and-neck cancer marker antibodies may utilize the various approaches that have been successfully employed for cancers, including but not limited to colon cancer (Arlen et al., 1998, Crit Rev Immunol 18: 133-138), multiple myeloma (Ozaki et al., 1997, Blood 90: 3179-3186; Tsunenati et al., 1997, Blood 90: 2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res 52: 2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J Immunother Emphasis Tumor Immunol 19: 93-101),

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leukemia (Zhong et al., 1996, Leuk Res 20: 581-589), colorectal cancer (Moun et al., 1994, Cancer Res 54: 6160-6166); Velders et al., 1995, Cancer Res 55: 4398-4403), and breast cancer (Shepard et al., 1991, J Clin Immunol 11: 117-127).

5 In the practice of a method of the invention, head-and-neck cancer marker antibodies capable of inhibiting the growth of cancer cells expressing head-and-neck cancer markers are administered in a therapeutically effective amount to cancer patients whose tumors express or overexpress one or more head-and-neck cancer markers. The invention may provide a specific, effective and long-needed treatment for head-and-neck cancer. The antibody therapy methods of the invention may be combined with other therapies including chemotherapy and
10 radiation.

Patients may be evaluated for the presence and level of expression or overexpression of one or more head-and-neck cancer markers in diseased cells and tissues (e.g. tumors), in particular using immunohistochemical assessments of tissue, quantitative imaging as described herein, or other techniques capable of reliably indicating the presence and degree of
15 expression of one or more head-and-neck cancer markers. Immunohistochemical analysis of tumor biopsies or surgical specimens may be employed for this purpose.

Head-and-neck marker antibodies useful in treating disease (e.g. cancer) include those that are capable of initiating a potent immune response against the disease (e.g. tumor) and those that are capable of direct cytotoxicity. In this regard, head-and-neck marker antibodies
20 may elicit cell lysis by either complement-mediated or antibody-dependent cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins.

Head-and-neck marker antibodies that exert a direct biological effect on tumor growth may also be useful in the practice of the invention. Such antibodies may not require the
25 complete immunoglobulin to exert the effect. Potential mechanisms by which such directly cytotoxic antibodies may act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular antibody exerts an anti-tumor effect may be evaluated using any number of *in vitro* assays designed to determine ADCC, antibody-
30 dependent macrophage-mediated cytotoxicity (ADMMC), complement-mediated cell lysis, and others known in the art.

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The anti-tumor activity of a particular head-and-neck cancer marker antibody, or combination of head-and-neck cancer marker antibodies, may be evaluated *in vivo* using a suitable animal model. Xenogenic cancer models, where human cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, may be employed.

The methods of the invention contemplate the administration of single head-and-neck marker antibodies as well as combinations, or "cocktails", of different individual antibodies such as those recognizing different epitopes of other markers. Such cocktails may have certain advantages inasmuch as they contain antibodies that bind to different epitopes of head-and-neck cancer markers and/or exploit different effector mechanisms or combine directly cytotoxic antibodies with antibodies that rely on immune effector functionality. Such antibodies in combination may exhibit synergistic therapeutic effects. In addition, the administration of one or more head-and-neck marker specific antibodies may be combined with other therapeutic agents, including but not limited to chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL2, GM-CSF). The head-and-neck marker specific antibodies may be administered in their "naked" or unconjugated form, or may have therapeutic agents conjugated to them.

The head-and-neck marker specific antibodies used in the methods of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the antibodies retains the function of the antibody and is non-reactive with the subject's immune systems. Examples include any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16^{sup}.th Edition, A. Osal., Ed., 1980).

One or more head-and-neck marker specific antibody formulations may be administered via any route capable of delivering the antibodies to the a disease (e.g. tumor) site. Routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. Preferably, the route of administration is by intravenous injection. Antibody preparations may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

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Treatment will generally involve the repeated administration of the antibody preparation via an acceptable route of administration such as intravenous injection (IV), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including the type of disease and the severity, grade, or stage of the disease, the binding affinity and half life of the antibodies used, the degree of head-and-neck marker expression in the patient, the extent of circulating head-and-neck markers, the desired steady-state antibody concentration level, frequency of treatment, and the influence of any chemotherapeutic agents used in combination with the treatment method of the invention. Daily doses may range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg antibodies per week may be effective and well tolerated, although even higher weekly doses may be appropriate and/or well tolerated. A determining factor in defining the appropriate dose is the amount of a particular antibody necessary to be therapeutically effective in a particular context. Repeated administrations may be required to achieve disease inhibition or regression. Direct administration of one or more head-and-neck marker antibodies is also possible and may have advantages in certain situations.

Patients may be evaluated for serum cancer markers in order to assist in the determination of the most effective dosing regimen and related factors. The head-and-neck cancer assay methods described herein, or similar assays, may be used for quantitating circulating head-and-neck marker levels in patients prior to treatment. Such assays may also be used for monitoring throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters such as serum levels of head-and-neck cancer markers.

The invention further provides vaccines formulated to contain one or more head-and-neck marker or fragment thereof.

In an embodiment, the invention provides a method of vaccinating an individual against one or more head-and-neck marker listed in Table 1 and optionally one or more marker listed in Table 2, comprising the step of inoculating the individual with the marker or fragment thereof that lacks activity, wherein the inoculation elicits an immune response in the individual thereby vaccinating the individual against the marker.

The use in anti-cancer therapy of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity is well known and, for example, has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer

63: 231-237; Fong et al., 1997, J. Immunol. 159: 3113-3117). These and similar methods can be practiced by employing one or more head-and-neck cancer markers, or fragment thereof, or head-and-neck cancer polynucleotide markers and recombinant vectors capable of expressing and appropriately presenting head-and-neck marker immunogens.

5 By way of example, viral gene delivery systems may be used to deliver one or more head-and-neck cancer polynucleotide markers. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems
10 may also be employed by using naked DNA encoding one or more head-and-neck cancer marker or fragment thereof introduced into the patient (e.g., intramuscularly) to induce an anti-tumor response.

Various *ex vivo* strategies may also be employed. One approach involves the use of cells to present one or more head-and-neck marker to a patient's immune system. For example,
15 autologous dendritic cells which express MHC class I and II, may be pulsed with one or more head-and-neck marker or peptides thereof that are capable of binding to MHC molecules, to thereby stimulate the patients' immune systems (See, for example, Tjoa et al., 1996, Prostate 28: 65-69; Murphy et al., 1996, Prostate 29: 371-380).

Anti-idiotypic head-and-neck marker specific antibodies can also be used in therapy as
20 a vaccine for inducing an immune response to cells expressing one or more head-and-neck marker. The generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic head-and-neck cancer marker specific antibodies that mimic an epitope on one or more head-and-neck cancer markers (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J Clin Invest 96: 334-342; Herlyn et al., 1996,
25 Cancer Immunol Immunother 43: 65-76). Such an antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against antigens associated with disease (e.g. tumor antigens).

Genetic immunization methods may be utilized to generate prophylactic or therapeutic humoral and cellular immune responses directed against cells expressing one or more head-
30 and-neck cancer marker. One or more DNA molecules encoding head-and-neck cancer markers, constructs comprising DNA encoding one or more head-and-neck cancer markers/immunogens and appropriate regulatory sequences may be injected directly into

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muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded head-and-neck cancer markers/immunogens. The head-and-neck cancer markers/immunogens may be expressed as cell surface proteins or be secreted. Expression of one or more head-and-neck cancer markers results in the generation of prophylactic or therapeutic humoral and cellular immunity against the disease (e.g. cancer). Various prophylactic and therapeutic genetic immunization techniques known in the art may be used.

The invention further provides methods for inhibiting cellular activity (e.g., cell proliferation, activation, or propagation) of a cell expressing one or more head-and-neck marker. This method comprises reacting immunoconjugates of the invention (e.g., a heterogeneous or homogenous mixture) with the cell so that head-and-neck cancer markers form complexes with the immunoconjugates. A subject with a neoplastic or preneoplastic condition can be treated when the inhibition of cellular activity results in cell death.

In another aspect, the invention provides methods for selectively inhibiting a cell expressing one or more head-and-neck marker by reacting any one or a combination of the immunoconjugates of the invention with the cell in an amount sufficient to inhibit the cell. Amounts include those that are sufficient to kill the cell or sufficient to inhibit cell growth or proliferation.

Vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used to deliver polynucleotides encoding head-and-neck cancer markers to a targeted organ, tissue, or cell population. Methods well known to those skilled in the art may be used to construct recombinant vectors that will express antisense polynucleotides for head-and-neck cancer markers. (See, for example, the techniques described in Sambrook et al (supra) and Ausubel et al (supra)).

Methods for introducing vectors into cells or tissues include those methods discussed herein and which are suitable for *in vivo*, *in vitro* and *ex vivo* therapy. For *ex vivo* therapy, vectors may be introduced into stem cells obtained from a patient and clonally propagated for autologous transplant into the same patient (See U.S. Pat. Nos. 5,399,493 and 5,437,994). Delivery by transfection and by liposome are well known in the art.

Genes encoding head-and-neck cancer markers can be turned off by transfecting a cell or tissue with vectors that express high levels of a desired head-and-neck marker-encoding fragment. Such constructs can inundate cells with untranslatable sense or antisense sequences.

Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies are disabled by endogenous nucleases.

Modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA or PNA, to the regulatory regions of a gene encoding a head-and-neck marker, i.e., the promoters, enhancers, and introns. Preferably, oligonucleotides are derived from the transcription initiation site, e.g. between -10 and +10 regions of the leader sequence. The antisense molecules may also be designed so that they block translation of mRNA by preventing the transcript from binding to ribosomes. Inhibition may also be achieved using "triple helix" base-pairing methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Therapeutic advances using triplex DNA were reviewed by Gee J E et al (In: Huber B E and B I Carr (1994) Molecular and Immunologic Approaches, Futura Publishing Co, Mt Kisco N.Y.).

Ribozymes are enzymatic RNA molecules that catalyze the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. The invention therefore contemplates engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding a head-and-neck marker.

Specific ribozyme cleavage sites within any potential RNA target may initially be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once the sites are identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be determined by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

One or more head-and-neck cancer markers and polynucleotides encoding the markers, and fragments thereof, may be used in the treatment of a head-and-neck disease (e.g. head-and-neck cancer) in a subject. In an aspect the head-and-neck cancer markers and polynucleotides encoding the markers are head-and-neck cancer markers that are down-regulated in head-and-neck cancer, for example, mucin 5B and one or more of the down-regulated markers listed in Table 2. The markers or polynucleotides may be formulated into

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compositions for administration to subjects suffering from a head-and-neck disease. Therefore, the present invention also relates to a composition comprising one or more head-and-neck cancer markers or polynucleotides encoding the markers, or a fragment thereof, and a pharmaceutically acceptable carrier, excipient or diluent. A method for treating or preventing a head-and-neck disease in a subject is also provided comprising administering to a patient in need thereof, one or more head-and-neck cancer markers or polynucleotides encoding the markers, or a composition of the invention.

The invention further provides a method of inhibiting a head-and-neck disease (e.g. head-and-neck cancer) in a patient comprising:

- (a) obtaining a sample comprising diseased cells from the patient;
- (b) separately maintaining aliquots of the sample in the presence of a plurality of test agents;
- (c) comparing levels of one or more head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers in each aliquot;
- (d) administering to the patient at least one of the test agents which alters the levels of the head-and-neck cancer markers, and/or polynucleotides encoding one or more head-and-neck cancer markers in the aliquot containing that test agent, relative to the other test agents.

An active therapeutic substance described herein may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the substance from the action of enzymes, acids and other natural conditions that may inactivate the substance. Solutions of an active compound as a free base or pharmaceutically acceptable salt can be prepared in an appropriate solvent with a suitable surfactant. Dispersions may be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack

Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, the active substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

5 The compositions are indicated as therapeutic agents either alone or in conjunction with other therapeutic agents or other forms of treatment. The compositions of the invention may be administered concurrently, separately, or sequentially with other therapeutic agents or therapies.

10 The therapeutic activity of compositions and agents/compounds identified using a method of the invention and may be evaluated *in vivo* using a suitable animal model.

EXAMPLES

The following non-limiting example is illustrative of the present invention:

Example 1

Experimental Procedures

15 *Samples and Reagents*

Head-and-neck cancer tissues were retrieved from an in-house, dedicated, research head-and-neck tissue bank, with approval from the Human Ethics Committee of All India Institute of Medical Sciences, New Delhi, India. With patient consent, surgically resected specimens of HNSCCs and paired non-cancerous tissues (each taken from a distant site) were
20 collected and banked from patients undergoing curative surgery at the Department of Otorhinolaryngology, All India Institute of Medical Sciences. Normal tissues with no evidence of cancer (non-paired noncancerous controls) were collected from patients attending the Dental Outpatient Department of All India Institute of Medical Sciences for tooth extraction, after consent of the patients. After excision, tissues were flash-frozen in liquid
25 nitrogen within 20 min of devitalization and stored at -80°C until further use; one tissue piece was collected in 10% formalin and embedded in paraffin for histopathological analysis. The clinical and pathological data were recorded in a pre-designed proforma. These included clinical TNM staging [tumor, node, metastasis based on International Union Against Cancer's classification of malignant tumors, 1988 (20)], site of the lesion, histopathological
30 differentiation, age, and gender of the patients.

The histologic diagnosis for each sample was reconfirmed using microscopic examination of a hematoxylin-and-eosin-stained frozen section of each research tissue block.

The tissue from the mirror face of the histologic section was then washed three times in approximately 1 ml of phosphate-buffered saline (PBS) with a cocktail of protease inhibitors as described previously (1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 10 μ M leupeptin, 1 μ g/ml aprotinin, and 1 μ M pepstatin) (13). The washed tissue was then homogenized in 0.5 ml
 5 PBS with protease inhibitors, using a handheld homogenizer. These homogenates were then flash frozen in liquid nitrogen and stored at -80 °C until use. Samples were thawed and clarified by centrifugation and the protein concentration determined by a Bradford-type assay using Bio-Rad protein quantification reagent (Bio-Rad, Mississauga, ON, Canada).

The iTRAQ experiments were performed in five sets of four samples each. A pool of
 10 non-paired non-cancerous head-and-neck tissue homogenates was used as a control in each set of experiments: equal amounts of total protein from the lysates of six non-cancerous samples (nonpaired controls) were pooled to generate a common reference “control sample” against which all the HNSCC samples were compared. Each sample contained 200 μ g of proteins. Trypsin digestion and labeling were performed according to the manufacturer’s (Applied
 15 Biosystems’) protocol; however, as double the manufacturer’s recommended amounts were used, two individual vials of each reagent were used for labeling each sample. iTRAQ labeling was performed as follows: control (non-paired non-cancerous pool), iTRAQ reagent 114; two cancer samples, iTRAQ 115 and 117; individual non-cancerous tissue sample (paired or non-paired sample), iTRAQ 116. A total of five iTRAQ sets were analyzed
 20 resulting in ten cancer (five buccal mucosa and five tongue) samples and two paired non-cancerous plus three non-paired noncancerous samples being compared to the control sample. The paired non-cancerous samples originated from patients with cancer that were resected from sites a minimum of 2 cm away from the advancing edge of the cancer. Each iTRAQ set was analyzed with one run each of online 2D LC-MS/MS and offline 2D LC-MS/MS
 25 analyses.

Strong cation exchange (SCX) separation conditions

For the offline 2D LC-MS/MS analysis, each set of labeled samples was first separated by SCX fractionation using an HP1050 high-performance liquid chromatograph (Agilent, Palo Alto, CA, U.S.) with a 2.1-mm internal diameter (ID) x 100-mm length Polysulfoethyl A
 30 column packed with 5- μ m beads with 300 Å pores (The Nest Group, Southborough, MA) as described previously (17). A 2.1-mm ID x 10-mm length guard column of the same material was fitted immediately upstream of the analytical column. Separation was performed as

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previously described (17). Briefly, each pooled sample set was diluted with the loading buffer (15 mM KH₂PO₄ in 25% acetonitrile, pH 3.0) to a total volume of 2 ml and the pH adjusted to 3.0 with phosphoric acid. Samples were then filtered using a 0.45- μ m syringe filter (Millipore, Cambridge, ON, Canada) before loading onto the column. Separation was performed using a linear binary gradient over one hour. Buffer A was identical in composition to the loading buffer, while Buffer B was Buffer A containing 350 mM KCl. Fractions were collected every two minutes using an SF-2120 Super Fraction Collector (Advantec MFS, Dublin, CA), after an initial wait of 2 minutes to accommodate the void volume. This resulted in a total of 30 SCX fractions per sample set. These fractions were dried by speed vacuuming (Thermo Savant SC110 A, Holbrook, NY) and resuspended in 30 μ l of 0.1% formic acid each.

For the online 2D LC-MS/MS analysis, an SCX cartridge (BioX-SCX, LC Packings, The Netherlands) was plumbed upstream of the reverse phase (RP) desalting cartridge and analytical column. This SCX cartridge was connected through a second valve on the Switchos unit as shown in Figure 1. Samples were separated on this SCX cartridge using 10 μ l step elutions with increasing concentration of ammonium acetate (10 mM, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, 500 mM and 1M). Each step elution was loaded onto the RP desalting column using the switching program as shown in Figure 1, where the eluting peptides were desalted before loading onto the analytical column that was subsequently brought inline with the desalting column. The flow path used for these steps was designed to ensure that there was never any flow reversal through either of the cartridges (SCX or RP). Separation on the RP analytical column was effected as described for the second stage of the offline LC-MS/MS analysis described below.

LC-MS/MS Run conditions

The SCX fractions from 6 to 30 were analyzed by nanoLC-MS/MS using the LC Packings Ultimate instrument (Amsterdam, The Netherlands) fitted with a 10- μ l sample loop. Samples were loaded, using a μ l pick-up mode, onto a 5-mm RP C18 pre-column (LC Packings) at 50 μ l/min and washed for 4 min before switching the precolumn inline with the separation column. The separation column used was either a 75- μ m ID x 150-mm length PepMap RP column from LC Packings packed with 3 μ m C18 beads with 100 Å pores, or an in-house equivalent packed with similar beads (Kromasil; The Nest Group, Southborough, MA). The flow rate used for separation on the RP column was 200 nl/min with the following gradient:

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Time(min)	0	10	15	125	145	150	160	162	188
%B	5	5	15	35	60	80	80	5	Stop

5 Samples were analyzed on a QSTAR Pulsar i mass spectrometer (Applied Biosystems / MDS SCIEX, Foster City, CA) in information-dependent acquisition (IDA) mode with the scan cycles set up to perform a 1-s MS scan followed by five MS/MS scans of the five most abundant peaks for 2 s each. Every fourth scan the peak that was closest in intensity to the threshold of 10 counts was selected for MS/MS. Data acquisition was performed without any
10 repetitions and with a dynamic exclusion of 30 s. Relative protein abundances were determined using the MS/MS scans of iTRAQ-labeled peptides (17). The iTRAQ-labeled peptides fragmented under collision-induced dissociation (CID) conditions to give reporter ions at 114.1, 115.1, 116.1, and 117.1 Th.

The ratios of peak areas of the iTRAQ reporter ions reflect the relative abundances of
15 the peptides and, consequently, the proteins in the samples. Larger, sequence-information-rich fragment ions were also produced under these conditions and gave the identity of the protein from which the peptide was derived.

Data Analysis

The software used for data acquisition was Analyst QS 1.1 (Applied Biosystems /
20 MDS SCIEX). Data were analyzed using ProteinPilot (17, 21) and the database searched was the Celera human database (human KBMS 20041109) with a total of 178,243 entries, both provided by Applied Biosystems Inc. Identified proteins were grouped by the software to minimize redundancy. All peptides used for the calculation of protein ratios were unique to the given protein or proteins within the group; peptides that were common to other isoforms
25 or proteins of the same family that were reported separately were ignored. The ProteinPilot cutoff score used was 1.3, which corresponds to a confidence limit of 95%.

Statistical analysis

The average iTRAQ ratios from different runs were calculated for each protein in the offline and online analyses. Thereafter, the iTRAQ ratios for each protein in the two analyses
30 were averaged. Proteins that were selected for further analysis met the following criteria: (1) detection in ≥ 6 out of the 10 cancer samples, $\geq 50\%$ of which showed differential expression

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≥ 1.5 -fold relative to the control sample, and/or (2) known to be of interest from other studies. These proteins are listed in Table 1 along with two housekeeping proteins (to contrast the performance of the potential biomarkers).

Biomarker Panel analysis

5 To identify a panel of best-performing proteins that can distinguish between HNSCC and non-cancerous tissues, each protein in Table 1 was individually assessed for its ability to discriminate between normal and cancer samples by evaluating its receiver operator characteristic (ROC) curve based on the iTRAQ ratios. Plotting ROC curves and calculating the area-under-the-curve (AUC) and other attributes were performed using the
10 ROCR package within the R statistical computing environment (22). Proteins giving the highest AUC values were selected for biomarker panel analysis and used as input variables into a Naïve Bayes model, implemented in JAVA (23) using the WEKA package (24). Given a sample i that has iTRAQ ratios (or IHC scores, see later) in the vector $x(i)$, the Naïve Bayes model has the form:

$$15 \quad P(i=\text{cancer} \mid x(i)) = P(\text{cancer}) \times P(x(i) \mid i=\text{cancer}) / P(x(i))$$

where $P(i=\text{cancer} \mid x(i))$ is the probability that i is a cancer sample given its $x(i)$ values. This is the posterior probability and is calculated using Bayes theorem. A value ≥ 0.5 is considered a positive hit. $P(x(i) \mid i=\text{cancer})$ is the probability that within the cancer samples, $x(i)$ exists
20 within them. $P(\text{cancer})$ is the probability of i being a cancer sample; this is the prior probability. $P(x(i))$ is the probability of i occurring and is a normalization factor. Nine trials of three-fold cross validation were used for each biomarker panel input into the Naïve Bayes model. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each trial.

25

Verification of candidate potential cancer markers (PCMs) by immunohistochemistry

The three best-performing proteins from the above biomarker panel analysis were selected for immunohistochemical verification using an independent, larger sample set. Antibodies against these three biomarkers were available commercially (Santa Cruz
30 Biotechnology Inc., Santa Cruz, CA). Each antibody was first optimized with respect to dilution and the use of microwave heating in citrate buffer (0.01 M, pH 6.0) to expose the antigen (“antigen retrieval”). Paraffin-embedded sections (5 μ m) of human HNSCCs (25

cases) and paired head-and-neck non-cancerous tissues from these patients (25 samples), as well as nonpaired non-cancerous head-and-neck tissues (10 samples) were collected on gelatin-coated slides. For histopathological analysis, representative sections were stained with hematoxylin and eosin; immunostaining was done on serial sections as previously described (25). Following the application of a protein blocker for 10 min, deparaffinized tissue sections were first incubated with the primary antibodies for 1 h at room temperature or for 16 h at 4°C, followed by the respective secondary antibody conjugated with biotin. The primary antibody was detected using the streptavidin-biotin complex (DAKO LSAB plus kit, DAKO Cytomation, Denmark) and diaminobenzidine as chromogen. Slides were washed with 3x Tris-buffered saline (TBS, 0.1 M, pH=7.4) after every step. Finally, the sections were counterstained with Mayer's hematoxylin and mounted with DPX mountant. In the negative controls, the primary antibody was replaced by non-immune mouse IgG of the same isotype to ensure specificity. HNSCC tissue sections with known immunopositivity for specific proteins were used as positive controls in each batch of sections analyzed (25).

15 *Evaluation of immunohistochemical staining*

The immunopositive staining was evaluated in five areas. Sections were scored as positive if epithelial cells showed immunopositivity in the cytoplasm, plasma membrane and/or nucleus when judged independently by two scorers who were blinded to the clinical outcome, i.e., the slides were coded and the pathologists did not have prior knowledge of the local tumor burden, lymphonodular spread, and grading of the tissue samples, while scoring the immunoreactivity. First, a quantitative score was performed by estimating the percentage of immunopositive-stained cells: 0 < 10% cells, 1 = 10–30% cells, 2 = 30–50% cells, 3 = 50–70% cells, and 4 = >70% cells. Second, the intensity of staining was scored by evaluating the average staining intensity of the positive cells (0, none; 1, weak; 2, intermediate; and 3, strong). Finally, a total score (ranging from 0 to 7) was obtained by adding the quantitative score and the intensity score for each of the 60 sections. The immunohistochemical data were subjected to statistical analysis as described above for the MS results.

Western Blot Analysis of proteins in HNSCCs and normal tissues

Whole-cell lysates were prepared from five HNSCCs and five non-cancerous head-andneck tissues. Frozen tissue samples were homogenized and lysed in a buffer containing 50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 10 mM MgCl₂, 1 mM ethylenediamine tetraacetate (pH 8.0), 1% Nonidet P-40, 100 mM sodium fluoride, 1 mM phenylmethanesulfonyl fluoride,

and 2 µl/ml protease inhibitor cocktail (Sigma). Protein concentrations were determined using the Bradford reagent (Sigma), and equal amounts of proteins (80 µg/lane) from the HNSCCs and non-cancerous tissues were resolved on 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel. The proteins were then electro-transferred onto polyvinylidenedifluoride (PVDF) membranes. After blocking with 5% non-fat powdered milk in TBS (0.1M, pH=7.4), blots were incubated with the respective primary antibodies (1:200 dilution) at 4°C overnight. The protein abundance of alpha-tubulin was used as a control for protein loading, and was determined with mouse monoclonal anti-alpha-tubulin antibody (Clone B7, Santa Cruz Biotechnology Inc.). Membranes were incubated with the respective secondary antibody, HRP-conjugated rabbit/goat/mouse anti-IgG (goat anti-rabbit IgG, 1:5000; rabbit anti-goat IgG, 1:4000; or rabbit anti-mouse IgG, 1:2000, DAKO Cytomation, Denmark), and diluted with 1% bovine serum albumin for 2 h at room temperature. After each step, blots were washed three times with Tween (0.2%)-TBS. Protein bands were detected by the enhanced chemiluminescence method (Santa Cruz Biotechnology Inc.) on XO-MAT film.

Results

The offline and online 2D LC-MS/MS analyses collectively resulted in the identification of a total of 811 non-redundant proteins. Only a few of these proteins displayed consistent differential expression in the HNSCC samples (measured in ≥ 6 out of the 10 samples and with $\geq 50\%$ showing ≥ 1.5 -fold differential expression relative to the control sample) that warranted further analysis. These proteins, all confidently identified with ≥ 2 peptide matches (except APC-binding protein EB1 and superoxide dismutase [Mn]) are given in Table 1 along with two structural proteins, actin and β -2-tubulin, as controls. (See Table S1 for peptide sequences and coverage, and Figures S1 and S2 for the CID spectra of the single-peptide identifications in the Supplemental Data section.) As the nanoLC analyses were performed on 25 SCX fractions, the acquired data files were searched in two groups out of necessity (the version of ProteinPilot software available at that time was incapable of handling a large number of data files each with a large amount of data). Fractions 6–15 were, therefore, searched in one group, while fractions 16–30 were searched in a second group. The ProteinPilot result files from these two halves were then exported into an Excel spreadsheet where the proteins of interest from the two searches were combined by averaging the ratios for the protein in each sample. It is noteworthy that each of the ratios reported by searching either half of the fractions is itself comprised of the ratios from multiple peptides identified in the

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given protein. ProteinPilot automatically only includes unique and high-confidence matches of peptides for any particular protein in the ratios reported (i.e., it excludes those that are shared between different isoforms of any protein or lowconfidence matches to peptides). These averaged ratios from the offline and online analyses were then again averaged and reported in Table I. Of all the individual expression ratios (two offline and two online), 56.4% varied by less than 10% from their respective average shown, and 82.0% varied by less than 20%. It is reassuring that the expression ratios from different analyses and separate handling were comparable.

Nine proteins that did not meet the cutoff criteria stated above – cytokeratin 14, polybromo 1D, PKM2, annexin A1, nucleophosmin 1, Hsp27, cystatin B, GRP 94, and MARCKS – were also included in Table 1 for further analysis, as these had been reported in head-and-neck cancer or are of biological relevance in cancer. The HNSCCs analyzed included five squamous-cell carcinomas (SCCs) of buccal mucosa and five SCCs of the tongue. The rationale for the choice of these two SCC types was to determine if there are site-specific protein expressions or not. The best-performing proteins that can differentiate between HNSCC and non-cancerous tissues were identified by determining the individual receiver-operatorcharacteristic (ROC) curves of the proteins in Table 1 (as described in the Experimental section). The three proteins with the highest AUC values – YWHAZ, Stratifin and S100 A7 – are listed in Table 2 together with their individual and collective figures-of-merit, including sensitivity (cancer samples correctly identified as cancer samples) and specificity (normal samples correctly identified as normal samples). As a panel, the three best-performing biomarkers achieved a sensitivity of 0.92 and a specificity of 0.91 in discriminating HNSCC from non-cancerous headand- neck tissues (Table 2 and Figure 2a). A number of proteins, e.g., prothymosin alpha and APC-binding protein EB1, were predominantly overexpressed in SCCs of buccal mucosa (Table 1) and showed some promise in differentiating between SCCs of buccal mucosa and the tongue; however, as the number of samples are small, this possibility will need to be fully investigated in a future study involving more samples of both types.

Verification of candidate protein biomarkers is a necessary step in moving from the initial discovery to possible application. The panel of three best-performing biomarkers identified by MS analysis – YWHAZ, Stratifin and S100 A7 – were chosen for verification in a different and larger set of HNSCCs and non-cancerous head-and-neck tissues. Verification

exercises included immunohistochemical (Figure 3) and Western blot analyses (Figure 4) at the protein level, as well as RT-PCR analysis (Figure 5) at the mRNA level. All verification results support the above MS findings. In the immunohistochemical analysis, the biomarker panel of YWHAZ, Stratifin and S100A7 achieved a sensitivity of 0.92 and a specificity of 0.87 (Table 3 and Figure 2b) in discriminating HNSCCs from non-cancerous head-and-neck tissues. The paired non-cancerous head-and-neck tissues obtained from HNSCC patients might have altered protein expressions prior to histological changes. To investigate this possibility, the noncancerous tissues were segregated into paired and non-paired groups and evaluated separately with the HNSCCs. Significantly, the panel of the three biomarkers – YWHAZ, Stratifin and S100A7 – appears to perform better in discriminating HNSCC tissues against the non-paired non-cancerous head-and-neck tissues (sensitivity, 0.96; specificity, 0.96) than against the paired non-cancerous tissues (sensitivity, 0.92; specificity, 0.83) (see Table 4). These results appear to support the notion of protein-expression alterations prior to histological changes and caution the use of only paired samples.

15 Discussion

Multidimensional LC- MS/MS has been used for the analysis of clinical samples of HNSCCs labeled with isobaric mass tags (iTRAQ) to identify proteins that are differentially expressed in head-and-neck cancer in relation to non-cancerous head-and-neck tissues. The expression ratios were consistent between the online and offline 2D LC-MS/MS methods used, demonstrating that the methodologies were rugged and reproducible, even though the conditions and details used in peptide elution from the SCX columns in the two methods were different. Expectedly, the numbers of proteins identified by the online analysis (431) was lower than those identified by the offline analysis (580), due to the lower capacity of the SCX cartridge used in the former (50 ng of total peptides versus up to 1 mg in the latter). However, the online analysis was advantageous in terms of shorter data acquisition time and lower amounts of total sample required.

Development of HNSCC is a multistep process that often involves field cancerization, a phenomenon in which not only the site of the primary tumor, but the entire mucosa of the upper aerodigestive tract, is prone to undergoing malignant transformation or progression at multiple sites (19). It is now evident that molecular changes underlying field cancerization are not localized to areas with altered histology, but may persist beyond the histological border of precancerous lesions; a large fraction of the carcinogen-exposed field may harbor molecular

aberrations without presenting clinical or morphological symptoms (8 and references therein). Identification of proteins with altered expression as a manifestation of field cancerization is important in identification of biomarkers for prediction of risk of recurrence, as well as for development of second primary tumors in patients treated for HNSCC. Thus, the selection of normal controls for HNSCCs in a differential expression analysis, including the current study, is non-straightforward and requires careful planning. To address this issue, we have included two types of non-cancerous histologically confirmed normal tissues in our analysis: (1) noncancerous tissues obtained from HNSCC patients from a site distant to the tumor, and (2) normal tissues obtained from individuals with no evidence of cancer or pre-cancerous lesions. In a recent proteomic study, Roesch-Ely et al. (8) investigated changes in protein expressions occurring in different stages of tumorigenesis and field cancerization in HNSCCs. A number of reported differentially expressed proteins, including calgizarrin, stratifin, histone H4, and cystatin A, are also identified in this current study. This study appears to be the first reporting differential expression of calmodulin-like protein 5, polybromo-1D, APC-binding protein EB1, α -1-antitrypsin precursor, carbonic anhydrase I, mast cell tryptase β III, histone H2B.1, L-plastin, and peptidylprolyl isomerase A (PPIA) in HNSCC.

Among the differentially expressed proteins identified, no single protein emerged as a unique marker for HNSCC. However, a panel of three best-performing biomarkers YWHAZ, Stratifin and S100 A7 performed satisfactorily, as determined by both MS and immunohistochemistry on independent sets of samples. Significantly, YWHAZ has previously been identified by us to be overexpressed in oral cancer at the mRNA level and has subsequently been verified using immunohistochemical analysis (26). This serves as an independent validation of and complements the current results. Furthermore, YWHAZ has also been reported to be overexpressed in stomach cancer (27), and in breast and prostate tumor model systems (28, 29). More importantly, YWHAZ is not overexpressed in head-and-neck and lung-cancer tissue samples (18,30), thus illustrating the fact that this protein can provide some selectivity in discriminating among different cancer types.

Stratifin has been reported to be overexpressed in HNSCC. A recent proteomic study reported a 3.6-fold stratifin overexpression (31), thus corroborating the results obtained in this study. A second independent study also showed stratifin overexpression in the range of 2.8 – 9.1-fold in cancer samples (32). In addition, a study of 300 patients with pancreatic ductal adenocarcinoma showed stratifin overexpression in 82% of primary infiltrating

adenocarcinomas, while another 15% showed weak immunopositivity. Overexpression of stratifin correlated with poor prognosis (33). Interestingly and significantly, stratifin was reported to be downregulated in HNSCCs by Roesch-Ely *et al.* (8), whereas we observed consistent overexpression of stratifin in iTRAQ and in IHC verification analysis. The HNSCCs in the study of Roesch-Ely *et al.* (8) are from the German population with tobacco smoking and alcohol consumption being the major risk factors, while the clinical samples in this study, and in Lo *et al.* (12,31) and Chen *et al.* (32) are from Asian populations, where in addition, chewing tobacco and/or betel quid, and bidi smoking are important risk factors. These differences in the risk factors may account for the observed variations in stratifin expression and warrant in-depth investigation in a larger study.

14-3-3 proteins recognize phosphoserine/threonine (pS/T)-containing motifs used by a variety of signal transduction pathways to bind over 200 target proteins that play important roles in the regulation of various cellular processes, including mitogenic and cell-survival signaling, cell-cycle control and apoptotic cell death, epithelial-mesenchymal transition, and cell adhesion, invasion and metastasis (34). The involvement of 14-3-3 proteins in the regulation of oncogenes and tumor suppressor genes points to a potential role in tumorigenesis (35); multiple pathways can be targeted by modulation of these proteins, underscoring their potential as candidate drug targets. Although it might be argued that 14-3-3 proteins are, therefore, too pleiotropic to be targets for therapeutic inhibition, it has been shown that simultaneous inactivation of all 14-3-3 proteins sensitizes cancer cells to DNA-damaging agents. Selective inactivation of stratifin leads to an increased sensitivity towards cancer chemotherapeutic agents. Recent studies have shown that stratifin forms homodimers, while YWHAZ forms homodimers and also heterodimers with other isoforms (36,37). Stratifin has been extensively investigated; by contrast, YWHAZ remains largely unexplored. It is noteworthy that the potential success of strategies aimed at modulating 14-3-3 availability in the cell for cancer therapy is provided in studies showing that reducing cellular 14-3-3 increases chemosensitivity (38,39).

Cytokeratin 14 has also been demonstrated to be overexpressed in many squamous cell carcinomas. A study using immunohistochemical analysis demonstrated that 67 of 74 cases of squamous cell carcinomas showed immunoreactivity regardless of origin, suggesting cytokeratin to be a useful marker for squamous cell carcinoma (40). Another study, comparing

mRNA levels of cytokeratin 14 between oral squamous cell carcinoma and leukoplakia samples reported that the former showed a higher amount of cytokeratin 14 (41).

Prothymosin alpha, found to be overexpressed here in HNSCC, has recently been proposed to be a potential marker of proliferation in patients with thyroid cancer (42). This protein was implicated in various other cancers, including gastric, lung, liver, colon, and breast cancers (43-48). Prothymosin alpha was proposed to be a surrogate marker for the diagnosis of estrogen-negative breast-cancer cases (47), and a urinary marker for the detection and monitoring of bladder cancer (49). Prothymosin alpha expression has been observed in lymph nodes and tonsils (50). This expression in lymph nodes of HNSCC patients would correlate with locoregional spread of the disease and may be a determinant of disease prognosis. Prothymosin alpha is a small, highly acidic, nuclear protein that has been proposed to play a role in cell proliferation and immune regulation (51). Protein changes related to cytoskeletal reorganization, cellular metabolism, and protein-protein interactions have been observed, based on which a model for its immunological mode of action has been proposed (51). Interestingly, some of the proteins identified in that study, such as Lplastin, HSP90, vinculin, aldolaseA, meosin and galectin 3 are found to be overexpressed in the present study as well, although not all of them are included in Table 1. L-plastin is expressed by hematopoietic cells and by most human cancer cell lines, including human submandibular gland cell lines (52, 53). Yet, its functional importance in tumor tissues is controversial: its expression correlates with tumor progression in colon cancer, but not in breast cancer; in melanoma, Lplastin phosphorylation promotes tumor cell invasion (reviewed in 52). Intriguingly, L-plastin has also been proposed to represent a novel target for cancer therapy, and the constitutive activity of its promoter in non-hematopoietic tumors presents novel perspectives for cancer gene therapy using L-plastin promoter-driven viral vectors (52).

S100A7, a small calcium-binding protein of the S100 protein family, originally identified in psoriatic keratinocytes, is up-regulated in abnormally differentiating keratinocytes, squamous carcinomas of different organs, and in a subset of breast tumors (54-58). Incidentally, S100A7 was also identified in oral premalignant epithelia by microarray analysis and proposed to be a marker for invasion (54). It has been hypothesized to play a role in breast-tumor progression by promoting angiogenesis and enhancing the selection of cells that overcome their anti-invasive function (58). This hypothesis has also been suggested to explain why S100A7 expression is high in high-grade or estrogen-receptor negative tumors, as

these are associated with increased hypoxia and reactive oxygen species (ROS), a scenario in which the angiogenic effects of S100A7 are most important. It is noteworthy that increased hypoxia and ROS also occur in head-and-neck tumors and might explain the observed changes in S100A7 expression here. Another study in breast cancer showed that BRCA1 is a transcriptional repressor of S100A7; BRCA1 and c-Myc form a complex on S100A7 promoter, and BRCA1-mediated repression is dependent on a functional c-Myc (59). Further, BRCA1 mutations in tumors abrogate the repression of S100A7. In the absence of BRCA1, S100A7 is induced by topoisomerase II poison and etoposide, as well as increases the cellular sensitivity to etoposide, suggesting a mechanism for BRCA1-mediated resistance to etoposide (59). Incidentally, BRCA1 alterations have been reported in head-and-neck cancer (60,61). However, a correlation, if any, between BRCA1 alterations and S100A7 expression in head-and-neck cancer remains to be demonstrated.

Calgizzarin (S100 A11) has also been previously linked with cancer and was reported as a potential marker for head-and-neck cancer (14). Likewise, S100 A2, which shows overexpression in HNSCC is also known to be overexpressed in other forms of cancer, such as non-small cell lung cancer and uterine leiomyoma (62,63). It has been demonstrated that calgizzarin plays an anti-apoptotic role in uterine leiomyosarcoma cell line (63). Fascin has been discovered to be an early marker for esophageal squamous cell carcinoma (64). We have previously reported pyruvate kinase M2 to be overexpressed in head-and-neck cancers (14, 17). Several studies suggest that PKM2 is present primarily in a dimeric form in tumors, and is useful as a biomarker in their early detection (65-69). PKM2 overexpression in tumor cells is explained on the basis of its key role in the generation of ATP in the glycolytic pathway. Under hypoxic conditions that are typical for tumors, this pathway is a critical route by which tumors satisfy the higher energy requirements needed for proliferation (reviewed in 70,71).

Two of the more interesting proteins discovered in this current HNSCC study are the APC-binding protein EB1 and polybromo-1D. End-binding protein 1 (EB1) was initially discovered as a protein that binds adenomatous polyposis coli protein (APC) at its C-terminal region (72). More recently, however, it has also been shown to bind tubulin and has been detected to associate with the microtubules that form the mitotic spindle during mitosis (72). The EB1 interaction with APC is of particular interest as APC is a tumor suppressor whose inactivation leads to a significantly enhanced level of susceptibility for malignant transformation in colorectal cancer (72). Among others, APC binds to β -catenin and possibly

controls β -catenin's availability in the cytoplasm (72). By virtue of APC's binding to tubulin, EB1 participates in microtubule-dependent processes, including intracellular vesicle trafficking, organization of organelles within the cell, and even cell migration (72). One possible proposed explanation for the mechanism of action of EB1, is that overexpression of EB1, at least in esophageal squamous cell carcinoma, affects the interaction between APC and β -catenin, and that this overexpression correlates with the nuclear accumulation of β -catenin (73). Normally, APC in combination with glycogen synthase kinase 3 β (GSK 3 β) and axin forms a destruction complex that phosphorylates free β -catenin in the cytoplasm, which in turn targets it for ubiquitination and degradation (73). Disruption of APC interaction with β -catenin by EB1 overexpression leads to increased levels of β -catenin in the nucleus, which in turn binds to T-cell factor/lymphoid-enhancing factor (TCF/LEF) and activates transcription of target genes such as c-myc and cyclin D1. Thus, the overexpression of EB1 is thought to play a role in the development of esophageal squamous cell carcinoma by indirectly causing the activation of the β -catenin/TCF pathway (73). It is, therefore, possible that overexpression of EB1 in this study could be the first evidence for the same process occurring in HNSCC.

Polybromo 1D (PB1), also known as BRG1-associated factor 180 (BAF180), is a relatively new member of the SWI/SNF-B (PBAF) chromatin remodeling complex that is a homolog of the yeast rsc protein complex, which is required for progression through mitosis (74). In fact, antibodies against BAF180 localize to the kinetochores during mitosis (74). The fact that both PB1 and EB1 are known to be involved with mitosis is also noteworthy, but requires further investigation to ascertain if a direct relationship between the two exists. Other studies have shown that in yeast, rsc can act as an activator as well as a suppressor of transcription, and that it can be functionally linked with the PKC pathway (75,76). Additionally, it was shown that temperature-sensitive mutants of one of the proteins (nps1) in the rsc complex, when placed at the restrictive temperature, can be rescued by the overexpression of not only the yeast homolog of PKC, PKC1 (as well as other proteins downstream of the PKC1 signal pathway), but also by Bim1p, which is the yeast homolog of EB1 (76). It was further demonstrated that there is no direct interaction between Pkc1p and Bim1p, or any activation of BIM1 transcription or post-transcriptional regulation by Pkc1p, but that suppression of the activity of overexpressed Pkc1p requires a functional Bim1p (76).

In addition to the possibility of this potentially significant link between PB1 and EB1, there is also independent evidence suggesting that PB1 is a tumor suppressor and that this

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activity is found in lung cancer but not in breast cancer (77). This was verified when transfection of BAF180 gene into breast tumor cell lines, possessing a truncated version of the same gene, resulted in growth inhibition (77). Other members of this complex that have been associated with cancer include hSNF5/INI1 and BRG1 itself. HSNF5/INI1 mutations have been found in malignant rhabdoid tumors, while mutations in BRG1 have been noted in various cell lines including carcinomas of the breast, lung, pancreas and prostate. Implication of other members of the PBAF complex in suppression of various cancers, in addition to the above evidence which suggests that PB1 may be a tumor suppressor itself, makes PB1 an exciting discovery in our study. In light of PB1's suggested tumor suppressor role, the presently observed lower expression of this protein in the HNSCC samples is consistent with expectations and warrants in-depth investigation of its role in head-and-neck tumorigenesis.

In conclusion, the use of iTRAQ-labeling of head-and-neck cancers combined with LCMS/MS has led to the discovery of several novel, differentially expressed proteins in these tumors. A panel of the three best-performing biomarkers achieved a sensitivity of 0.92 and a specificity of 0.91. This performance was verified using immunohistochemistry on a larger, independent set of clinical samples of HNSCCs.

EXAMPLE 2

The previous Example identified a consistently increased expression of a panel of proteins, including stratifin (14-3-3 σ) and YWHAZ (14-3-3 ζ) that may serve as cancer biomarkers. In this Example the prognostic utility of these two candidate biomarkers for head-and-neck/oral squamous cell carcinoma (HNSCC) is described. To determine the clinical significance of stratifin and YWHAZ in head-and-neck tumorigenesis, their expressions were analyzed in HNSCCs (51 cases) and non-malignant tissues (39 cases) using immunohistochemistry. Significant increase in stratifin expression was observed in the HNSCCs as compared to the non-malignant mucosa [$p=0.03$, Odd's Ratio (OR) = 3.8, 95% CI = 1.6-9.2]. Kaplan-Meier survival analysis reveals correlation of stratifin overexpression with reduced disease-free survival of HNSCC patients ($p=0.06$). The most intriguing finding is the significant decrease in median disease-free survival (13 months) in HNSCC patients showing overexpression of both stratifin and YWHAZ proteins, as compared to patients that did not show overexpression of these proteins (median disease-free survival = 38 months, $p = 0.019$), underscoring their utility as adverse prognosticators for HNSCCs. Co-immunoprecipitation

assays show the formation of stratifin-YWHAZ heterodimers in HNSCC and interactions with NF κ B, β -catenin, and Bcl-2 proteins. These results indicate the involvement of these proteins in the development of head-and-neck cancer and their association with adverse disease outcome.

5 **Materials and Methods**

Tissues. Following institutional human ethics committee approval, 51 anonymized HNSCCs and 39 non-malignant head-and-neck tissues dating from 2002 and 2006 were retrieved from the Research Tissue Bank at All India Institute of Medical Sciences, New Delhi, India. The tissue specimens, surgically resected human HNSCCs and non-malignant
10 tissues (taken from a distant site) had been collected from patients undergoing curative surgery (with prior written patient consents).⁸¹ After excision, tissues were immediately snap-frozen in liquid nitrogen and stored at -80°C in the Research Tissue Bank. One piece from each patient was collected in 10% formalin and embedded in paraffin for histopathological analysis; the rest was banked. Clinical and pathological data were recorded in a pre-designed
15 performa; these included clinical TNM staging (tumor, node, and metastasis classification of malignant tumors of the International Union Against Cancer (UICC)),⁸² site of the lesion, histopathological differentiation, age, and gender.

Follow-up Study. Fifty-one HNSCC patients who underwent treatment of primary
20 HNSCC between 2002 and 2006 were investigated and evaluated in the head-and-neck cancer follow-up clinic. Survival status of the patients was verified and regularly updated from the records of the Tumor Registry, Institute Rotary Cancer Hospital, as of May, 2007. Patients were monitored for a maximum period of 42 months. As per the hospital protocol, HNSCC patients with T₁ and T₂ tumors were treated with radical radiotherapy or surgery
25 alone, whereas the majority of patients with T₃ and T₄ diseases were treated using a combination of radical surgery followed by postoperative radical radiotherapy. The patients were revisited clinically on a regular basis and the time to recurrence was recorded. If a patient died, the survival time was censored at the time of death; the medical history, clinical examination, and radiological evaluation were used to determine whether the death had
30 resulted from recurrent cancer (relapsing patients) or from any other cause. Disease-free survivors were defined as patients free from clinical and radiological evidence of local, regional, or distant relapse at the time of the last follow-up. Loco-regional relapse/death was

observed in 17 of 51 (30%) patients monitored in this study. Thirty-four patients who did not show recurrence were alive until the end of the follow-up period. Only disease-free survival was evaluated in the present study, as the number of deaths due to disease progression did not allow a reliable statistical analysis. Disease-free survival was expressed as the number of
 5 months from the date of surgery to the loco-regional relapse.

Immunohistochemistry. Paraffin-embedded sections (5 μ m thick) of human oral normal tissues (n = 39) and HNSCCs (n = 51) were collected on gelatin-coated slides. For histopathological analysis, representative sections were stained with hematoxylin and eosin, whereas immunostaining was performed on serial sections as described previously⁸¹. Briefly,
 10 the sections were deparaffinized in xylene, hydrated, and pretreated in a microwave oven in citrate buffer [0.01M (pH = 6.0)] for antigen retrieval. The sections were incubated with hydrogen peroxide (0.3% v/v) in methanol for 20 min to quench the endogenous peroxidase activity. Non-specific binding was blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS, 0.01M, pH = 7.2) for 1 h. Thereafter, slides were incubated
 15 with the primary antibody, (1 μ g/ml) (anti-14-3-3 σ , goat polyclonal antibody, Santa Cruz Biotechnology Inc., Santa Cruz, CA) for 16 h at 4 °C and washed with PBS. The primary antibody was detected using the streptavidin-biotin complex (Dako LSAB plus kit, Dako, Copenhagen, Denmark) and diaminobenzidine as the chromogen. All incubations were performed at room temperature in a moist chamber. Slides were washed with 3x Tris-buffered
 20 saline (TBS, 0.1M, pH = 7.4) after every step. Finally, the sections were counterstained with Mayer's hematoxylin and mounted with DPX mountant. In negative controls, the primary antibody was replaced by non-immune mouse IgG of the same isotype to ensure specificity.

Positive criteria for immunohistochemical staining. Immunopositive staining was evaluated in five areas of the tissue section. For stratifin expression, sections were scored as
 25 positive if epithelial cells showed immunopositivity in the cytoplasm, plasma membrane, and /or nucleus as evaluated by two independent scorers blinded to the clinical outcome (the slides were coded and the scorers did not have prior knowledge of the local tumor burden, lymphonodular spread, and grading of the tissue samples). These sections were rated based on the percentage of cells showing immunopositivity as follows: 0, < 10%; 1, 10-30%; 2, 30-
 30 50%; 3, 50-70%; and 4, >70%. Sections were also rated on the basis of stain intensity as follows: 0, none; 1, mild; 2, moderate; 3, intense, as described by Perathoner *et al.*⁸³ Finally, a total score (ranging from 0 to 7) was obtained by adding the scores of percentage positivity

and intensity. The sections were considered positive if the total score was ≥ 5 .⁸¹

Cell Culture. Human oral squamous carcinoma cell line, HSC2, was used in this study.⁸¹ Cells were grown in monolayer cultures in Dulbecco's modified eagle medium (DMEM-F12) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, MO), 100
5 $\mu\text{g/ml}$ streptomycin and 100 U/ml Penicillin in a humidified incubator (5% carbon-dioxide, 95% air) at 37 °C as described.⁸¹

Co-immunoprecipitation. Co-immunoprecipitation (Co-IP) assays were carried out as described earlier.⁸¹ Briefly, oral cancer cells, HSC2, were rinsed in ice-cold PBS and lysed in lysis buffer. Lysates were incubated on ice for 30 min and cell debris was removed by
10 centrifugation. Lysates were pre-cleared by adding 20 μl of Protein A-Sepharose (GE Healthcare Biosciences, Sweden), followed by overnight incubation with polyclonal stratifin, YWHAZ, and NF κ B antibodies, or monoclonal β -catenin and Bcl-2 antibodies (1:200 dilution) (Santa Cruz Biotechnology, CA) on a rocker at 4 °C. Immunocomplexes were pulled down by incubating with Protein A-Sepharose for 2 h at 4 °C, followed by washing with 4x
15 ice-cold lysis buffer to eliminate non-specific interactions. In negative controls, the primary antibody was replaced by non-immune mouse IgG of the same isotype to ensure specificity. Protein A-Sepharose-bound immunocomplexes were then resuspended in Laemmli sample buffer (10 mM Tris, 10% v/v glycerol, 2% w/v SDS, 5 mM EDTA, 0.02% bromophenol blue, and 6% β -mercaptoethanol, pH = 7.4), boiled for 5 min, and analyzed by Western blotting
20 using specific antibodies.¹² The proteins were electro-transferred onto polyvinylidenedifluoride (PVDF) membrane. After blocking with 5% non-fat powdered milk in TBS (0.1 M, pH = 7.4), blots were incubated with anti-14-3-3 σ antibody (1:200 dilution) at 4°C overnight. α -Tubulin served as a control for protein loading and was determined with mouse monoclonal anti- α -tubulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).
25 Membranes were incubated with secondary antibody, HRP-conjugated goat/mouse anti-IgG (Dako CYTOMATION, Denmark), diluted at an appropriate dilution in 1% BSA, for 2 h at room temperature. After each step, blots were washed with 3x Tween (0.2%)-TBS (TTBS). Protein bands were detected by the enhanced chemiluminescence method (Santa Cruz Biotechnology, CA) on XO-MAT film.

30 **Statistical Analysis.** The immunohistochemical data were subjected to statistical analysis using SPSS 10.0 software. The relationship between the protein expression and

clinicopathological parameters were tested by Chi-Square and Fischer's exact test. Two sided p-values were calculated and $p \leq 0.05$ was considered to be significant. Box plots were prepared to determine the distribution of total score of stratifin expression in HNSCCs and non-malignant tissues. The correlation of stratifin and or YWHAZ staining with patient survival was evaluated using life tables constructed from survival data with Kaplan-Meier plots.

Results

The amino acid sequences of stratifin and YWHAZ with peptides identified by MS and MS/MS are given in Figure 6A and 6B, respectively.

10 Immunohistochemical analysis of stratifin in HNSCCs and non-malignant tissues.

Results of the immunohistochemical analysis of stratifin expression in HNSCCs and non-malignant mucosa, and the relationship with clinicopathological parameters are summarized in Table 1. Chi-Square analysis shows significant increase in stratifin expression in HNSCCs as compared to non-malignant mucosa ($p = 0.03$, OR= 3.8, 95% CI=1.6-9.2). In histologically normal oral tissues, 31% of the cases show weak immunostaining of stratifin (Figure 7a). Increased stratifin expression was observed in 63% of HNSCCs. Intense nuclear / membranous staining, in addition to cytoplasmic staining, was observed in the epithelial cells of HNSCCs (Figure 7b). No immunostaining was observed in tissue sections used as negative controls where the primary antibody was replaced by isotype specific IgG (Figure 7c). No significant correlation was observed between stratifin overexpression and clinicopathological parameters including age, gender, histological differentiation, tumor stage, and nodal status of HNSCCs (Table 1). Increased expressions of stratifin were observed in HNSCCs with a median score of 6 (range 4-7) as compared to non-malignant (histologically normal) oral tissues median score of 5 (range 4-6) shown in the box plot analysis in Figure 8.

25 **Co-immunoprecipitation.** To determine the functional significance of stratifin in head-and-neck carcinogenesis, we identified its binding partners in oral cancer cells, HSC2, using co-IP assays followed by Western blotting. IP of stratifin reveals its binding to YWHAZ, NF κ B, β -catenin, and Bcl-2 proteins as shown in Figure 9a. Reverse IP assay using specific antibodies for these proteins followed by Western blotting confirmed their binding to stratifin (Figure 9b). No band was observed in the immunoblot analysis of the negative controls. It is noteworthy that all these proteins--stratifin, YWHAZ, NF κ B, β -catenin and Bcl-2 -- have 14-3-3 binding motif, Mode 1, as reported earlier by us.⁸¹

Association of Stratifin and YWHAZ Expression with Disease Outcome. Kaplan-Meier Survival analysis reveals reduced disease-free survival for HNSCC patients overexpressing stratifin ($p=0.06$, Figure 10a). The median disease-free survival was 19 months in HNSCC patients showing stratifin overexpression as compared to 38 months in HNSCC patients who did not. Patients with YWHAZ-positive tumors had a shorter disease-free survival (median = 23 months) than YWHAZ-negative tumors (median = 35 months; $p = 0.08$, Figure 10b). Most remarkably, HNSCC patients showing overexpressions of both stratifin and YWHAZ have a significantly decreased median disease-free survival of 13 months ($p = 0.019$, Figure 10c) in HNSCCs, as compared to patients showing no overexpression of these two proteins (median = 38 months), underscoring the utility of these proteins as adverse prognosticators for HNSCCs.

Discussion

This investigation is one of the very few studies that demonstrate the prognostic utility of candidate biomarkers identified using MS-based proteomics. Recently, a comparison of protein profiles in tumor-distant head-and-neck tissues with clinical outcomes was reported to reveal a significant association between aberrant profiles and tumor-relapse events, suggesting that proteomic profiling in conjunction with protein identification may have significant predictive power for clinical outcome.⁸ The concept of using a panel of biomarkers for the purpose of improved diagnostics has taken hold in recent years. For example, DeSouza *et al.*¹⁷ and Dubé *et al.*¹⁸ demonstrated that the use of a panel of three biomarkers – chaperonin 10, pyruvate kinase M2, and α -1-antitrypsin – markedly increases the sensitivity and specificity for differentiating between head-and-neck carcinoma and non-malignant head-and-neck tissues. For head-and-neck cancers, Example 1 determined that a panel of biomarkers – stratifin, YWHAZ, and S100 A7 – performs better than any of the individual biomarkers for the detection of HNSCC.⁸⁰ In this current study, it is found that a panel of two biomarkers – stratifin and YWHAZ – shows promise as prognostic markers for HNSCCs. Although not wishing to be bound by any particular theory, the enhanced performance of the combination of stratifin and YWHAZ versus either protein individually, in prognosing the clinical outcome of HNSCCs, can be understood on the basis of their biological functions detailed below.

Results of *in vitro* studies demonstrate the formation of stratifin-YWHAZ heterodimers and binding of stratifin to NF κ B, β -catenin, and Bcl-2, implicating stratifin's

involvement in many cellular processes associated with tumorigenesis. Similar to the present invention, Bhawal *et al.*,⁸⁴ have very recently reported increased expression of the stratifin transcript and protein in OSCCs. These results are in accordance with findings of Chen *et al.*³² and Lo *et al.*^{31,11} who reported overexpression of stratifin in OSCCs. In addition, Lo *et al.*¹¹ demonstrated ten-fold increases in stratifin expression in HPV18-positive OSCCs in comparison with HPV18-negative OSCCs. It is of note that all these studies are on Asian populations. By contrast, studies on European populations reported decreased expression of stratifin in HNSCCs.⁸ If this geographical difference stands up to further scrutiny, it points to the importance of genetic and/or risk factors in developing HNSCCs. Furthermore, epigenetic inactivation of stratifin has been shown to be associated with *p16* gene silencing and HPV negativity in OSCCs.⁸⁵ Thus these results suggest different mechanisms at work in HNSCC tumorigenesis exhibiting overexpression of stratifin and tumorigenesis that does not. Whether these different mechanisms can be attributed to the presence or absence of HPV infection and/or differences in risk factors, such as smoking and drinking in the European and Western populations, and chewing of betel quid and/or tobacco in the Asian population, remains to be determined.

Overexpression of stratifin has been observed in other human cancers. Perathoner *et al.*⁸³ suggested that stratifin overexpression promotes tumor proliferation and/or prevents apoptotic signal transduction in colorectal carcinoma. Samuel *et al.*⁸⁶ demonstrated the role of stratifin in prevention of apoptosis by influencing the sub-cellular distribution of the pro-apoptotic protein, Bax, in colorectal cancer cells. Deletion of stratifin has been correlated with increased sensitivity of colorectal cells to doxorubicin. Similarly, Guweidhi *et al.*⁸⁷ proposed an anti-apoptotic role for stratifin in pancreatic cancer cells by inhibiting bad-mediated apoptosis. Liu *et al.*⁸⁸ showed that elevated stratifin expression contributes considerably to the observed drug resistance in MCF7/AdVp3000 cells. Stratifin has been shown to be a pivotal MDM2 regulator, involved in blocking a variety of MDM2 activities, including MDM2-mediated cytoplasmic localization of p53. Stratifin overexpression leads to destabilization of MDM2 by enhancing its self-ubiquitination and, thereby, stabilizing the cellular p53⁸⁹. In previous studies, the inventors reported that *p53* mutations are infrequent in OSCCs in the Indian population, despite stratifin overexpression; a currently unknown mechanism must be involved in stabilizing p53 in these OSCC patients.^{90,91} The present inventors have also reported overexpression of MDM2 and cyclinD1 in OSCCs⁹². Investigation of the relationship

between stratifin overexpression, p53 stabilization, MDM2 and cyclinD1 expressions in HNSCCs is currently underway.

The inventors, in a recent study aimed at delineation of early changes in expression of proteins in hyperplasia, demonstrated increased expressions of NF κ B and COX-2 in early pre-malignant stages of the development of oral cancer and sustained elevation along the tumorigenic pathway⁹³. Further, increased expression of YWHAZ in different stages of the development of OSCC and YWHAZ's involvement in cell signaling pathways involved in inflammation, cell proliferation and abrogation of apoptosis during oral carcinogenesis⁸¹ was shown. Herein the inventors extend these findings by demonstrating the binding of stratifin with YWHAZ, thus suggesting the formation of stratifin-YWHAZ heterodimers and binding to NF κ B in oral cancer. These findings are supported by the study of Aguilera *et al.*⁹⁴ which showed the requirement of 14-3-3 proteins for efficient export of the p65 sub-unit of NF κ B. Taken together with our earlier findings of YWHAZ, it is hypothesized that 14-3-3 proteins may be an important link between chronic inflammation and cancer that warrants further investigation.

The Co-IP results show stratifin binding also with β -catenin and Bcl-2 proteins. Earlier, the inventors showed that these proteins interact with YWHAZ, thereby supporting the hypothesis that these complexes may be responsible for altered functions of stratifin. Fang *et al.*⁹⁵ recently showed that AKT, which is activated downstream from epidermal growth factor receptor signaling, phosphorylates β -catenin at Ser-552 *in vitro* and *in vivo*, causing its dissociation from cell-cell contacts and accumulation in both the cytosol and nucleus, and enhancing its interaction with YWHAZ via a binding motif containing Ser-552. This phosphorylation of β -catenin by AKT increases β -catenin's transcriptional activity and promotes tumor cell invasion, indicating that AKT-dependent regulation of β -catenin plays a critical role in tumor invasion and development. The oncogenic role of YWHAZ has been proposed in a recent study using *siRNA* for knocking down its expression in cancer cells⁹⁶. Downregulation of YWHAZ sensitizes cells to stress-induced apoptosis and JNK/p38 signaling; in addition, it enforces cell-cell contacts and expression of adhesion proteins. YWHAZ's oncogenic properties is also supported by a Web-based meta-analysis (Onco-mine) that reveals its overexpression in various types of carcinomas.^{34,97} To unravel the functional significance of 14-3-3 proteins in tumor development, the present inventors have investigated the functional significance of the interactions between stratifin and YWHAZ, and their

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respective roles in the development and progression of HNSCC. All indications are that targeting specific 14-3-3 isoforms may serve as a plausible strategy for cancer therapy.

In conclusion, stratifin is overexpressed in HNSCCs relative to normal tissues. Increased concomitant expression of stratifin and YWHAZ serves as adverse prognosticator in HNSCCs and underscores the importance of these proteins in head-and-neck tumorigenesis. Increased expression of stratifin forming stratifin-YWHAZ heterodimers and binding to NF κ B, β -catenin, and Bcl-2 proteins suggest the implication of these complexes in diverse cellular processes in head-and-neck carcinogenesis. It is submitted that targeting the stratifin-YWHAZ heterodimer, using small molecule modulator/peptide inhibitor that intervenes with 14-3-3 client protein interactions, would serve as a plausible therapeutic strategy for head-and-neck cancer.

Table 1

Table 1

Accession #	Protein name	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	N2	N3	N1	N4	N6
np1P51947	Stratificin			1.73	1.71	1.69				1.86	1.84	0.84	0.77	0.82	0.88	1.08
nm1Q8V723	YYHA2			1.46	0.84	1.23	1.88		1.56	1.68	1.82	0.85	0.81	0.88	0.83	0.83
np1P28234	3106A 2			1.86	1.8		1.88			1.88	1.88	1.87	1.28	0.86	1.84	1.88
np1P21131	3106A 7								1.21	1.44		0.88	1.01	0.81	1.08	1.28
np1P04434	Prothymosin Alpha					1.43	1.06		1.28	1.78	1.28	0.88	1.63	1.04	1.18	1.88
nm1Q8V741	Fascin			0.86		1.88				1.43	1.78	1.18		1.14	1.02	1.01
np1P51948	Calgizzarin												1	1.88	1.3	1.18
np1P04032	Macpin Precursor			1.78	1.78	1.02					0.84		1.88	0.88	1.08	0.88
np1P13238	Annexin A8	1.3			1.88	1.8	1.88			1.71	1.68	0.83		1.88	1.18	1.12
np1Q3W271	Calmodulin-like protein 6	1.81	1.18		1.78	0.78	1.84	1.77		1.17	0.88	0.82		0.88	0.82	1.08
gslAAC1388.1	Glutathione S Transferase-P	1.88	1.88	1.28	1.1	1.78	1.71	0.82	1.88	1.78	1.18	1.88	0.87	0.88	1.01	0.82
np1P04738	LDM A		1.74	1.87	1.82	1.88	1.77		1.48	1.84	1.28	1.03	1.88	0.88	0.84	0.83
nm1Q8V7E9	PPIA	1.88	1.88	1.18	0.88	1.88	1.84		1.48	1.17	1.88	0.88	0.88	0.88	0.82	0.87
np1Q19881	APC-binding protein EB1						1.88			1.88		0.83	0.87	1.88	1.48	
nm1Q8V7M7	Superoxide dismutase [Mn]		1.81		1	0.81					0.81				0.81	1.18
nm1Q8V7E8	L Pistatin	1.78	1		0.88	1.88	1.88			1.88	1.62			0.88		1.08
np1P04738	Beta Actin	1.11	1.84	1.88	0.88	1.14	0.88	0.82	1.3	1.08	0.87	0.83	1.88	1.07	0.81	1.1
nm1Q8V7E9	Tubulin beta2	1.2	0.81	1.82	0.88	1.18	1.18		1.05	1.2	1.82	0.88	0.88	1.18	1	
nm1Q8V7U39	PACAP	0.8		0.88		0.81	0.48	0.73	0.82		0.82	0.88	0.81	1.88	1.44	1.02
nm1Q71D3	Histone H8	0.88		0.67	0.68		0.88		0.8	0.82			1.88	1.28	1.24	1.84
np1P04282	Histone H4	0.88		0.62				0.87	0.84	1.24		1.2		1.88		
np1P01028	Alpha 1 Anti-Trypsin Precursor	0.87	0.78	0.36	0.88	0.6	0.82	0.22	0.36		0.78	1	0.81		0.88	0.82
np1P51384	K4PO Lumican	0.81	0.88			0.81	0.88	0.86	0.86	0.8	0.83				1.02	0.8
nm1Q8V7E7	Mac cell fryplace beta 3	0.81				0.84	0.84	0.82	0.88		0.83	1.88	1.88	0.83	0.82	1.78
nm1Q8V7E8	Histone H3B.1	0.88	0.8	0.81	0.87	0.82	0.88		0.48	0.43	1.88			1.2	1.48	1.78
np1P04738	Vimentin	0.78	0.4	0.82	0.81	0.88	0.38	0.38	0.46	0.43		0.81		1.03	0.88	0.78
np1P22378	Peroxiredoxin 2	0.43	0.13	0.88	0.88		0.48	0.88	1.11	0.88	0.81	0.84	0.43	0.84	0.88	0.78
np1P04738	Carbonic Anhydrase I	0.34		0.48	0.57	0.37	0.37	0.37	0.87	0.88	0.88	0.88	0.88	0.81	0.87	0.83
np1P04042	Flavin reductase	0.64		0.64		0.6	0.68	0.1	0.88				0.87	0.6	0.88	0.81
gslKRM7E	Cytokeratin 14	1.27	1.21	1.84		1.48	1.28	0.24		1.01	1.71	0.81	0.88	0.81	1.04	1.18
nm1Q8V7E8	Polydromio-10			0.84		0.88					0.88	1.88			1.07	
np1P14878	PK M2	1.61	1.48	1.18	1.04	1.18	1.84		1.81	1.4	1.21	0.8	0.84	0.88	0.84	0.87
np1P04282	Annexin A1	0.88	1.88	1.2	1.88	1.4	1.88	0.83	1.24	0.81	1.88	0.88	0.84	1.08	1.27	1.4
gslAAC16788.1	Nucleophosmin 1	1.87		1.88	1.48	1.4	1.8		1.18	1.18	1.88		0.84	1.18	1.88	0.88
np1P04738	Hsp27	1.2	1.8	1.28	1.88	1.28	1.48	1.88	1.88	1.88	1.42	1.01	1.01	0.8	1.88	1.12
np1P04282	Cytalin B	1.84	1.88	0.88	0.88	0.88	1.21	0.87	0.88	0.88	0.82	0.88	0.87	0.88	0.88	0.88
np1P14878	GRP 94	0.8	0.88		0.8		0.88	0.88		0.8				1.17	0.81	0.78
np1P07882	MARCKS	0.88	1.88	1.28		0.84	1.88		1.8	0.88	0.84	1.18	1.1	0.88	0.88	0.88

<0.4 <0.5 <0.67 <0.8 >1.25 >1.8 >2.0 >2.8

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Table 2.

Biomarkers	Sensitivity	Specificity	PPV	NPV	AUC
YWHAZ	0.30	1.00	1.00	0.42	1.00
Stratfin	0.80	1.00	1.00	0.71	0.98
S100A7	0.70	1.00	1.00	0.63	0.90
YWHAZ, stratfin, S100A7	0.92	0.91	0.95	0.85	0.96

Table 3.

Biomarkers	Sensitivity	Specificity	PPV	NPV	AUC
YWHAZ	1.00	0.71	0.71	1.00	0.90
Stratfin	0.92	0.60	0.62	0.91	0.85
S100A7	0.96	0.71	0.71	0.96	0.90
YWHAZ, stratfin, S100A7	0.92	0.87	0.83	0.94	0.91

Table 4.

Comparison	Sensitivity	Specificity	PPV	NPV	AUC
Cancer versus Paired Normal	0.92	0.83	0.85	0.92	0.89
Cancer versus Non-Paired normal	0.96	0.96	0.98	0.90	0.97

Table 5
Differentially Expressed Proteins in Head and Neck Malignancies/Cancer

Protein name	Gene Name	Accession #	Expression in Head and Neck Cancer
Calmodulin-like protein 5	CALML5	Peptide: spt Q9NZT1 mRNA/DNA coding: NM_017422	Up regulated
Polybromo-1D	PBRM1	Peptide: trm Q86U86 mRNA/DNA coding: NM_181042	Down regulated
APC-binding protein EB1	MAPRE1	Peptide: spt Q15691 mRNA/DNA coding: NM_012325	Up regulated
Alpha 1 Anti-Trypsin Precursor	SERPINA1 or AAT	Peptide: spt P01009 mRNA/DNA coding: NM_001002236	Down regulated
Carbonic anhydrase I	CA1	Peptide:	Down

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		spt P00915 mRNA/DNA coding: NM_001738	regulated
Mast cell tryptase beta III	trypt aseB	Peptide: trm Q96RZ7 mRNA/DNA coding: NM_024164	Down regulated
Histone H2B.1	HIS T2H 2BE	Peptide: spt Q16778 mRNA/ DNA coding: NM_003528	Down regulated
Plastin 3	PLS 3	Peptide: spt P13797 mRNA/DNA coding: NM_005032	Up regulated
Histone H4	HIS T1H 4A	Peptide: spt P62805 mRNA/DNA coding: NM_003495	Down regulated
Cyclophilin A	PPI A	Peptide: trm Q6NTE9 mRNA/DNA coding: NM_021130	Up regulated
S100 CBP A7	S100 A7	Peptide: spt P31151	Up regulated

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		mRNA/DNA coding: NM_002963.3	
Prothymosin Alpha	PTM A	Peptide: spt P06454 mRNA/DNA coding: NM_00109928 5	Up regulated
PACAP (proapoptotic caspase adaptor protein)	MG C295 06	Peptide: trm Q8WU39 mRNA/DNA coding: NM_016459	Down regulated
LDH A	LDH A	Peptide: spt P00338 mRNA/DNA coding: NM_005566	Up regulated
KSPG Luminican	LU M	Peptide: spt P51884 mRNA/DNA coding: NM_002345	Down regulated
Peroxiredoxin 2	PRD X2	Peptide: spt P32119 mRNA/DNA coding: NM_181738	Down regulated
Superoxide dismutase 2 (SOD2 protein)	SOD 2	Peptide: trm AAH16934	Up regulated

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		mRNA/DNA coding: BC016934	
Histone H3	HIS T2H 3A	Peptide: trm Q71DI3 mRNA/DNA coding: NM_021059	Down regulated
MARCKS	MA CS	Peptide: gb AAA59555. 1 mRNA/DNA coding: M68956	Up regulated
GRP-94	HSP 90B1	Peptide: spt P14625 mRNA/DNA coding: NM_003299	Down regulated
Nucleophosmin 1	NP M1	Peptide: gb AAH16768. 1 mRNA/DNA coding: BC016768	Up regulated
PK M2	PK M2	Peptide: spt P14618 MRNA/DNA coding: NM_182471	Up regulated

Table 6 : Analysis of Stratifin and YWHAZ in HNSCCs: Correlation with clinicopathological parameters

Clinicopathological Features	Total Cases	STRATIFIN ⁺		STRATIFIN ⁺ -YWHAZ ⁺		STRATIFIN ⁺ /YWHAZ ⁺	
	N	n	(%)	n	(%)	n	(%)
Non-malignant tissue	39	12	(31)	8	(20)	25	(64)
HNSCC#	51	32	(63)	28	(55)	43	(84)
Differentiation							
WDSCC	29	18	(62)	16	(55)	26	(90)
MDSCC	19	12	(63)	10	(53)	15	(79)
PDSCC	3	2	(67)	2	(67)	2	(67)
Tumor Stage							
T ₁	6	5	(83)	5	(83)	6	(100)
T ₂	15	6	(40)	4	(27)	11	(73)
T ₃	13	9	(69)	8	(61)	12	(92)
T ₄	17	12	(71)	11	(65)	14	(82)
Nodal Status							
N ⁻	28	17	(61)	15	(54)	23	(82)
N ⁺	23	15	(65)	13	(56)	23	(87)

WDSCC, well differentiated squamous cell carcinoma; MDSCC, moderately differentiated squamous cell carcinoma; PDSCC, poorly differentiated squamous cell carcinoma.

For HNSCCs vs Non-malignant tissues: a) Stratifin⁺, (p=0.030,OR= 3.8 , 95% CI= 1.6-9.2) ; b) YWHAZ⁺, (p=0.024,OR=2.8, 95% CI=1.2-6.8) ; c) Stratifin⁺-YWHAZ⁺, (p=0.001,OR= 4.7, 95% CI=1.8-12.2) ; d) SFN⁺/YWHAZ⁺, (p=0.027,OR= 3.1, 95% CI=1.1-8.2) .

5 The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing
10 description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

 All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its
15 entirety. All publications, patents and patent applications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the antibodies, methodologies etc. which are reported therein which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

20 Below full citations are set out for references.

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SEQUENCE LISTING

SEQ ID NO: 1

Stratifin: Accn #P31947 (Up regulated in HNCa)
 gi|398953|sp|P31947.1|1433S_HUMAN 14-3-3 protein sigma (Stratifin)
 (Epithelial cell marker protein 1)
 MERASLIQKAKLAEQAERYEDMAAFMKGAVEKGEELSCEERNLLSVAYKNVVGQRAAWRVLSSIEQKSNEEGSEE
 KGPEVREYREKVETELQGVCDTVLGLLDShLIKEAGDAESRVFYLMKMGDYRYLAEVATGDDKKRIIDSARSAYQ
 EAMDISKKEMPPTNPIRLGLALNFSVFHYEIANSPEEAISLAKTTFDEAMADLHTLSEDSYKDSTLIMQLLRDNLTL
 LWTADNAGEEGGEAPQEPQS

SEQ ID NO: 2

YWHAZ: Accn # P63104 (Up regulated in HNCa)
 gi|52000887|sp|P63104.1|1433Z_HUMAN 14-3-3 protein zeta/delta (Protein
 kinase C inhibitor protein 1) (KCIP-1)
 MDKNELVQKAKLAEQAERYDDMAACMKSVTEQGAELSNEERNLLSVAYKNVVGARRSSWRVSVSSIEQKTEGAEEKQ
 QMAREYREKIETELRDICNDVLSLLEKFLIPNASQAESKVFYLMKMGDYRYLAEVAAGDDKKGIVDQSQAYQEA
 FEISKKEMQPTHPIRLGLALNFSVFYIEILNSPEKACSLAKTAFDEAIAELDTLSEESYKDSTLIMQLLRDNLTLW
 TSDTQGDAAEAGEGGEN

SEQ ID NO: 3

S100 A2: Accn # P29034 (Up regulated in HNCa)
 gi|114152869|sp|P29034.3|S10A2_HUMAN Protein S100-A2 (S100 calcium-binding
 protein A2) (Protein S-100L) (CAN19)
 MMCSSLEQALAVLVTTTFHKYSCQEGDKFKLSKGEMKELLHKELPSFVGEKVDDEGLKKLMGSLDENSQQQVDFQY
 AVFLALITVMCNDFQGCPRP

SEQ ID NO: 4

S100 A7: Accn # P31151 (Up regulated in HNCa)
 gi|400892|sp|P31151.3|S10A7_HUMAN Protein S100-A7 (S100 calcium-binding
 protein A7) (Psoriasin)
 MSNTQAERSIIGMIDMFHKYTRRDDKIDKPSLLTMMKENFPNFLSACDKKGTNYLADVFEKKDKNEDKKIDFSEFL
 SLLGDIATDYHKQSHGAAPCSGGSQ

SEQ ID NO: 5

Prothymosin alpha: Accn # P06454 (Up regulated in HNCa)
 gi|135834|sp|P06454.2|PTMA_HUMAN Prothymosin alpha [Contains: Thymosin
 alpha-1]
 MSDAAVDTSSSEITTKDLKEKKEVVVEEAENGRDAPANGNAENEENGEQADNEVDDEEEEGGEEEEEEEEEGDGEEED
 GDEDEEAESATGKRAAEDDEDDVDTKKQKTDEDD

SEQ ID NO: 6

Fascin: Accn # Q96IH1 (Up regulated in HNCa)
 gi|74732058|sp|Q96IH1|Q96IH1_HUMAN FSCN1 protein
 SASSTATMTANGTAEAVQIQFGLINCGNKYLTAFAFGFKVNASASSLKKKQIWTLEQPPDEAGSAAVCLRSHLGRY
 LAADKDGNTVCEREVPGPCRFLIVAHDDGRWSLQSEAHRRYFGGTEDRLSCFAQTVSPAEEKWSVHIAMHPQVNIY
 SVTRKRYAHLARPADIAVDRDVPWGVDSLITLAFQDQRYSVQTADHRFLRHDGRLVARPEPATGYTLEFRSGKV
 AFRDCEGRYLAPSGPSGTLKAGKATKVGKDELFALEQSCAQVVLQAANERNVSTRQGMDSLANSQDEETDQETFQLE
 IDRDTKKCAFRTHGTGKYWTLTATGGVQSTASSKNASCYFDIEWRDRRITLRASNGKFVTSKKNQQLAASVETAGDS
 ELFLMKLINRPIIVFRGEHGFICRKVTGTLNANRSSYDVFQLEFNDGAYNIKDSTGKYWTVGSDSAVTSSGDTPV
 DFFFEFCDYNKVAIKVGGRYLKGDHAGVLKASAETVDPASLWEY

SEQ ID NO: 7

Calgizzarin: Accn # P31949 (Up regulated in HNCa)
 gi|1710818|sp|P31949.2|S10AB_HUMAN Protein S100-A11 (S100 calcium-binding
 protein A11) (Protein S100C) (Calgizzarin) (MLN 70)
 MAKISSPTETERCIESLIAVFQKYAGKDGNYNTLSKTEFLSFMNTELAFAFTKNQKDPGVLDMMKKLDTNSDGQLD
 FSEFLNLIGGLAMACHDSFLKAVPSQKRT

SEQ ID NO: 8

Maspin: Accn # P36952 (Up regulated in HNCa)
 gi|547892|sp|P36952.1|SPB5_HUMAN Serpin B5 precursor (Maspin) (Protease
 inhibitor 5)

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MDALQLANSFAVDLFLKQLCEKEPLGNVLFSPICLSTSLSLAQVGAKGDTANEIGQVLHFENVKDIPFGFQTVTSD
 VNKLSSFYSLKLIKRLYVDKSLNLSTEFISSTKRPYAKELETVDKDKLEETKGQINNSIKDLTDGHFENILADNS
 VNDQTKILVVNAAYFVGKWMKKFPESETKECPRLNKTDTKPVQMMNMEATFCMGNIDSINCKIIELPFQNKHLSM
 FILLPKDVEDESTGLEKIEKQLNSESLSQWTNPSTMANAKVKLSIPKFKVEKMIDPKACLENLGLKHIFSEDTSDF
 SGMSETKGVALSNVIHKVCLEITEDGGDSIEVPGARILQHKDELNADHPFIYIIRHNKTRNIIFFGKFCSP

SEQ ID NO: 9

Annexin A8: Accn # P13928 (Up regulated in HNCa)
 gi|126302519|sp|P13928.2|ANXA8_HUMAN Annexin A8 (Annexin-8) (Annexin VIII)
 (Vascular anticoagulant-beta) (VAC-beta)
 MAWWKAWIEQEGVTVKSSSHFNPDPAETLYKAMKGIGTNEQAIIDVLTKRSNTQRQQIAKSFKAQFGKDLTETLK
 SELSGKFERLIVALMYPYRYEAKELHDAMKGLGTKEGVIEILASRTKNQLREIMKAYEEDYGSSLEEDIQADTS
 GYLERILVCLLQGSRDDVSSFVDPGLALQDAQDLYAAGEKIRGTDEMKFITILCTRSATHLLRVFEEYEKIANKSI
 EDSIKSETHGSLEEAMLTVMKCTQNLHSYFAERLYYAMKGAGTRDGLTIRNIVSRSEIDLNLIKCHFKKMYGKTLS
 SMIMEDTSGDYKNALLSLVGSDP

SEQ ID NO: 10

Calmodulin like protein 5: Accn # Q9NZT1 (Up regulated in HNCa)
 gi|14285407|sp|Q9NZT1.1|CALL5_HUMAN Calmodulin-like protein 5 (Calmodulin-
 like skin protein)
 MAGELTPEEEAQYKKAFAVDTDGNGTINAQELGAALKATGKNLSEAQLRKLISEVDSGDGEISFQEFLTAARKA
 RAGLEDLQVAFRAFDQDGDGHITVDELRRAMAGLGQPLPQEELDAMIREADVDDQGRVNYEEFARMLAQE

SEQ ID NO: 11

GST P: Accn # AAC13869.1 (Up regulated in HNCa)
 gi|726098|gb|AAC13869.1| glutathione S-transferase-P1c [Homo sapiens]
 MPPYTVVYFPVRGRCAALRMLLADQGSWKEEVTVETWQEGSLKASCLYGQLPKFQDGDLTLYQSNTILRHLGRT
 LGLYGKDQQEAALVDMVNDGVEDLRCKYVSLIYTNYEVGKDDYVKALPGQLKPFETLLSQNQGGKTFIVGDQISFA
 DYNLLDLLLIHEVLAPGCLDAFPLLSAYVGRLSARPKLKAFLASPEYVNLPIGNGKQ

SEQ ID NO: 12

LDH A: Accn # P00338 (Up regulated in HNCa)
 gi|126047|sp|P00338.2|LDHA_HUMAN L-lactate dehydrogenase A chain (LDH-A)
 (LDH muscle subunit) (LDH-M) (Renal carcinoma antigen NY-REN-59) (Cell
 proliferation-inducing gene 19 protein)
 MATLKDQLIYNLLKEEQTPQNKITVVGVGAVGMACAISILMKDLADELALVDVIEDKCLKGEMMDLQHGSFLRTPK
 IVSGKDYNVTANSKLVIIITAGARQQEGESRLNLVQRNVNIFKFIIPNVVKYSPNCKLLIVSNPVDILTYVAWKISG
 FPKNRVIGSGCNLDSARFRYLMGERLGVHPLSCHGWVLGEHGDSSVPVWSGMNVAGVSLKTLHPDLGTDKDKEQWK
 EVHKQVVESAYEVIKLGKGYTSAIGLSVADLAESIMKNLRRVHPVSTMIKGLYGIKDDVFLSVPCILGQNGISDLV
 KVTLTSEEEARLKKSADTLWGIQKELQF

SEQ ID NO: 13

PPIA: Accn # P62937 (Up regulated in HNCa)
 gi|51702775|sp|P62937.2|PPIA_HUMAN Peptidyl-prolyl cis-trans isomerase A
 (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein)
 MVNPTVFFDIAVDGEPLGRVSFELFADKVPKTAENFRALSTGEKGFYKSGCFHRIIPGFMCQGGDFTRHNGTGK
 SIYGEKFEDENFILKHTGPGILSMANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNIVEAMERFGSRNGKT
 SKKITIADCGQLE

SEQ ID NO: 14

APC binding protein EB1: Accn # Q15691 (Up regulated in HNCa)
 gi|20138589|sp|Q15691.3|MARE1_HUMAN Microtubule-associated protein RP/EB
 family member 1 (APC-binding protein EB1) (End-binding protein 1) (EB1)
 MAVNVYSTSVTSDNLSRHDMWLAWINESLQLNLTKIEQLCSGAAYCQFMDMLFPGSIALKKVKFQAKLEHEYIQNFK
 ILQAGFKRMGVDKIIPVDKLVKGFQDNFEFVQWFKKFFDANYDGKDYDPVAARQGOETAVAPSLVAPALNPKPKP
 LTSSSAAPQRPISTQRTAAAPKAGPGVVRKNPGVGNDDAAELMQQVNVKLTVEDLEKERDFYFGKLRNIELIC
 QENEGENDPVLQRIVDILYATDEGFVIPDEGGPQEEQEEY

SEQ ID NO: 15

SOD (Mn): Accn # AAH16934 (Up regulated in HNCa)
 gi|16877367|gb|AAH16934.1| SOD2 protein [Homo sapiens]
 MLGRAVCGTSRQLAPALGYLGSRQKHSPLDLPYDYGALPHINAQIMQLHHSKHHAAYVNNLNVTEEKYQEALAKG
 RFQAERREAVPGRGDPREPGPIRTGLSVEENSLRICTGSEFSRHSLSFKHVMVYLIVEGVPRWV

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SEQ ID NO: 16

L-Plastin: Accn # P13797 (Up regulated in HNCa)
 gi|2506254|sp|P13797.3|PLST_HUMAN Plastin-3 (T-plastin)
 MATTQISKDELDELKEAFKVDLNSNGFICDYELHELFKEANMPLPGYKVREIIQKLMLDGDNRKDGKISFDEFVY
 IFQEVKSSDIKTFRKAINRKEGICALGGTSELSSEGTQHSYSEEEKYAFVNWINKALENDPDCRHVIPMNPNTDD
 LFKAVGDGIVLCKMINLSVPDTIDERAINKKKLTPIIQENLNLALNSASAIGCHVVNIGAEDLRAGKPHLVGLL
 WQIIKIGLGFADIELSRNEALALLRDGETLEELMKLSPEELLRWANFHLENSGWQKINNFSADIKDSKAYFHLLN
 QIAPKGQKEGEPRIDINMSGFNETDDLKRAESMLQQADKLGCRQFVTPADVVSNGNPKLNLAFFVANLNFNKPALTKP
 ENQDIDWTLLEGETREERTFRNWMNSLGVNPHVNHLYADLQDALVILQLYERIKVPVDWSKVNKPPYPKLGANMKK
 LENCNYA VELGKHPAKFSLVGIGGQDLNDGNQTL
 TLALVWQLMRRYTLNVLEDLGDGQKANDDIIVNWVNRTLSEAGKSTSIQSFKDKTISSSLAVVDLIDAIQPGCINY
 DLVKSGNLTEDDKHNNAKYAVSMARRIGARVYALPEDLVEVKPKMVMVTFACLMGRGMKRV

SEQ ID NO: 17

PACAP: Accn # Q8WU39 (Down regulated in HNCa)
 gi|74730663|sp|Q8WU39|Q8WU39_HUMAN PACAP protein
 MRSLPLLLLLLLGAWAIPGGLGDRAPLTATAPQLDDEEMYSAHMPAHLRCDACRAVAYQMWQNLAKAETKLHTSNS
 GGRRELSELVYTDVLDLRSCSRNWQDYGVREVDQVKRLTGPGGLSEGPEPSISVMVTGGPWPTRLSRTCLHYLGEFGE
 DQIYEAHQQGRGALEALLCGGPQGACSEKVSATREEL

SEQ ID NO: 18

Histone H3: Accn # Q71DI3 (Down regulated in HNCa)
 gi|74758899|sp|Q71DI3.3|H32_HUMAN Histone H3.2 (H3/m) (H3/o)
 MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIA
 QDFKTDLRFQSSAVMALQEASEAYLVGLFEDTNLCIAHAKRVTIMPKDIQLARRIGERA

SEQ ID NO: 19

Histone H4: Accn # P62805 (Down regulated in HNCa)
 gi|51317339|sp|P62805.2|H4_HUMAN Histone H4
 MSGRGKGGKGLGKGGAKRHRKVLDRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFLENVIRDAVITYTEH
 AKRKTVTAMDVVYALKRQGRITLYGFGG

SEQ ID NO: 20

Alpha-1-antitrypsin: Accn # P01009 (Down regulated in HNCa)
 gi|1703025|sp|P01009.3|A1AT_HUMAN Alpha-1-antitrypsin precursor (Alpha-1
 protease inhibitor) (Alpha-1-antiproteinase)
 MPSSVSWGILLLAGLCLVPVSLAEDPQGDAQAQKTDTSHHDDHPTFNKITPNLAFAFSLYRQLAHQSNSTNIFF
 SPVSIATAFAMLSLGTKADTHDEILEGLNFNLTETPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVD
 KFLEDVKKLYHSEAFTVNFGDTEEAQKQINDYVEKGTQGKIVDLVKELDRDRTVFALVNYIFFKGKWERPFVVKDTE
 EEDFHVDQVTTVKVPMKRLGMFNIQHCKKLSSWVLLMKYLGNAIAIFFLPDEGKLQHLENELTHDIITKFLNED
 RRSASLHLPKLSITGTDLKSVLGQLGITKVFNSGADLSGVTEEAPLKLKSAVHKAVLTIDEKGTEAAGAMFLEAI
 PMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

SEQ ID NO: 21

KPSG lumican: Accn # P51884 (Down regulated in HNCa)
 gi|20141464|sp|P51884.2|LUM_HUMAN Lumican precursor (Keratan sulfate
 proteoglycan lumican) (KSPG lumican)
 MSLSAFTLFLALIGGTSGQYYDYDFPLSIYQSSPNCAPCNCPEYPSAMYCDELKLKSVPMVPPGIKYLYLRNN
 QIDHIDEKAFENVTDLQWLILDHNLLENSKIKGRVFSKLKQLKKLHINHNNLTESVGPLPKSLEDLQLTHNKITKL
 GSFEGLVNLTFIHLQHNRLKEDAVSAAFKGLKSLEYLDLSFNQIARLPSGLPVSLTLYLDNNKISNIPDEYFKRF
 NALQYLRLSHNELADSGIPGNSFNVSLLVELDLSYNKLKNIPTVNNENLENYYLEVNQLEKFDIKSFCKILGPLSYS
 KIKHLRLDGNRISETSLPPDMYECLRVANEVTLN

SEQ ID NO: 22

Mast cell tryptase beta III: Accn # Q96RZ7 (Down regulated in HNCa)
 gi|74761085|sp|Q96RZ7|Q96RZ7_HUMAN Mast cell tryptase beta III
 MLNLLLLALPVLASRAYAAPAGQALQRVGIVGGQAPRSKWPVQVSLRVRDRYWMHFCGGS LIHPQWVLTAAHCV
 GPDVKDLAALRVQLREQHLYYQDQLLPVSRIIVHPQFYTAQIGADIALELEPVNVSSHVHTVTLPPASETFPPG
 MPCWVTGWGDVDNDERLPPPFPLKQVKVPIMENHICDAKYHLGAYTGDDVRIVRDDMLCAGNTRRDSCQVATAPHT
 FPAPS

SEQ ID NO: 23

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Histone H2B.1: Accn # Q16778 (Down regulated in HNCa)
 gi|7387736|sp|Q16778.3|H2B2E_HUMAN Histone H2B type 2-E (H2B.q) (H2B/q)
 (H2B-GL105)
 MPEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSIYVYKVLKQVHPDTGISSKAMGIMNSFVNDIFERIAG
 EASRLAHYNKRSTITSREIQTAVRLLLPGELAKHAVSEGTKAVTKYTSSK

SEQ ID NO: 24

Vimentin: Accn # P08670 (Down regulated in HNCa)
 gi|55977767|sp|P08670.4|VIME_HUMAN Vimentin
 MSTRSVSSSSYRRMFGGPGTASRPSSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGGVYATRSSAVRLRSSVPG
 VRLQLQDSVDFSLADAINTEFKNTRTNEKVELQELNDRFANYIDKVRFLQQNKILLAELEQLKGQKSRLGDL YEE
 EMRELRRQVDQLTNDKARVEVERDNLAEDIMRLREKLQEEMLQREEAENTLQSFQDQVDNASLARLDLERKVESLQ
 EEIAFLKKLHEEEIQELQAQIQEQHVQIDVDVSKPDLTAALRDVRRQYESVAAKNLQEAEEWYKSKFADLSEAANR
 NNDALRQAKQESTEYRRQVQSLTCEVDALKGTNESLERQMRMEENFAVEAANYQDTIGRLQDEIQNMKEEMARHL
 REYQDLLNVKMALEDIEIATYRKLLGEEESRISLPLPNFSSNLNRETNLDSLPLVDTHSKRTLLIKTVETRDGQVIN
 ETSQHDDLE

SEQ ID NO: 25

Peroxiredoxin 2: Accn # P32119 (Down regulated in HNCa)
 gi|2507169|sp|P32119.5|PRDX2_HUMAN Peroxiredoxin-2 (Thioredoxin peroxidase
 1) (Thioredoxin-dependent peroxide reductase 1) (Thiol-specific antioxidant
 protein) (TSA) (PRP) (Natural killer cell-enhancing factor B) (NKEF-B)
 MASGNARIGKPAPDFKATAVVDGAFKEVKLSYKGYVVLFFYPLDFTFVCPTEIIAFSNRAEDFRKLGCEVLGVS
 VDSQFTHLAWINTPRKEGGLGPLNIPLLADVTRRLSEYGVLTDEGIAYRGLFIIDGKGVLRQITVNDLPVGRSV
 DEALRLVQAFQYTDEHGEVCPAGWKPGSDTIKPNVDDSKYFSKH

SEQ ID NO: 26

Carbonic anhydrase I: Accn # P00915 (Down regulated in HNCa)
 gi|115449|sp|P00915.2|CAH1_HUMAN Carbonic anhydrase 1 (Carbonic anhydrase I)
 (Carbonate dehydratase I) (CA-I)
 MASPDWGYDDKNGPEQWSKLYPIANGNNQSPVDIKTSETKHDTSLKPISVSYNPATAKEIINVGHSHFVNFEDNDN
 RSVLKGGPFSDSYRLFQFHFHWGSTNEHGSEHTVDGVKYSaelhVAHWNSAKYSSSLAEAAASKADGLAVIGVLMKVG
 EANPKLQKVLDALQAIKTKGKRAPFTNFDPTLLPSSLDFTWYTPGSLTHPPLYESVTWIICKESISVSSEQLAQFR
 SLLSNVEGDNAVPMQHNNRPTQPLKGRTVRAS

SEQ ID NO: 27

Flavin reductase: Accn # P30043 (Down regulated in HNCa)
 gi|1706870|sp|P30043.3|BLVRB_HUMAN Flavin reductase (FR) (NADPH-dependent
 diaphorase) (NADPH-flavin reductase) (FLR) (Biliverdin reductase B) (BVR-B)
 (Biliverdin-IX beta-reductase) (Green heme-binding protein) (GHBP)
 MAVKKIAIFGATGQTGLTTLAQAVQAGYEVTVLVRDSSRLPSEGPRPAHVVGVDVLQAADV DKT VAGQDAVIVLLG
 TRNDLSPTTVMSEGARNIVAAMKAHGVDKVVACTSAFLLDWPTKVP PRLQAVTDDHIRMHKVLRRESGLKYVAVMPP
 HIGDQPLTGAYTVTL DGRGPSRVISKHDLGHFMLRCLTTDEYDGHSTYPSHQYQ

SEQ ID NO: 28

Cytokeratin 14: Accn # NP_000517 (Up regulated in HNCa)
 gi|15431310|ref|NP_000517.2| keratin 14 [Homo sapiens]
 MTTCSRQFTSSSSMKGSCGIGGGIGGGSSRISSVLAGGSCRAPSTYGGGLSVSSSRFSSGGAYGLGGGYGGGFSSS
 SSSFGSGFGGGYGGGLGAGLGGGFAGGDGLLVGSEKVTMQLNDRLASYLKVRAL EEAANADLEV KIRDWYQ
 RQRP AEIKDYSPYFKTIEDLRNKILTATVDNANVLLQIDNARLAADDFRTKYETELNLRMSVEADINGLRRLVDEL
 TLARADLEMQIESLKEELAYLKKNHEEEMNALRGQVG DVNVEMDAAPGVDLSRILNEMRDQYEKMAEKNRKDAEE
 WFFTKEELNREVATNSELVQSGKSEISELRRTMQLNLEIQLQSLMKASLENSLEETKGRYCMQLAQIQEMIGSV
 EEQLAQLRCMEQQNQEYKILLDVKTRLEQEIATYRRLLEGEDAHLSSSQFSSGSQSSRDVTSSSRQIRTKVMDVH
 DGKVVSTHEQVLRKN

SEQ ID NO: 29

Polybromo-1D: Accn # Q86U86 (Down regulated in HNCa)
 gi|73921624|sp|Q86U86.1|PB1_HUMAN Protein polybromo-1 (hPB1) (Polybromo-1D)
 (BRG1-associated factor 180) (BAF180)
 MGSKRRRATSPSSSVSGDFDDGHHSVSTPGPSRKRRRLSNLPTVDPIAVCHELYNTIRDYKDEQGRLLCEL FIRAP
 KRRNQPDYEVVSQPIDLMKIQQKLKMEYDDVNLLTADFQLLFNNAKSYYKPDSP EYKAACKLWDLYLRTRNEFV
 QKGEADDEDDDDGQDNQGTVTEGSSPAYLKEILEQLLEAIVVATNP SGRLISELFQKLPSKVQYPDYYAIIKEPI
 DLKTIAQRIQNGSYKSIHAMAKDIDLLAKNAKTYNEPGSQVFKDANSIKKIFYMKKA EIEHHEMAKSSLRMRTPSN

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LAAARLTGPSHSKGS LG EERNPTSKYYRNKRAVQGGRLSAITMALQYGESESEEDAALAAARYEEGESEAESITSFM
 DVS NPFYQLYDTVRSCRNNQGQLIAEPFYHLPSKKKYPDYYQQIKMPISLQQIRTKLKNQEYETLDHLECDLNLMF
 ENAKRYNVPNSAIYKRVLKLQQVMQAKKKELARR
 DDIEDGDSMISSATS DTGS AKRKS KKNIRKQRMKILFNVVLEAREPGSGRRLCDLFMVKPSKKDYPDYYKIILEPM
 DLKII EHNIRNDKYAGEEGMIEDMKLMFRNARHYNEEGSQVYNDAHILEKLLKEKRKELGPLPDDDDMASPKLKLS
 RKSGISPKKSKYMT PMQQKLNEVYEAVKNYTDKRGRRLSAIFLRLPSRSELPDYYLTIKKPMDEKIRSHMMANKY
 QDIDSMVEDFVMMFNACTYNEPESLIYKDALVLHKLVLLETRDLEGDEDSHVPNVTLTIQELIHNLFVSVMSHQD
 DEGRCYSDSLAEI PAVDPNFPNKPPLTFDIIRKNVENNRYRRLDLFQEHMFVLERARRMNRTDSEIYEDAVELQQ
 FFIKIRDELCKNGEILLSPALSYTTKHLHNDVEKERKEKLPKEIEEDKLRREEKREAEKSEDSSGAAGLSGLHRT
 YSQDCSFKN SMYHVGDYVYVEPAEANLQPHIVCI
 ERLWEDSAGEKWLYGCWFYRPNETFHLATRKFEKEVFKSDYYNKVPVSKILGKCVVMFVKEYFKLCPENFRDEDV
 FVCESRYSAKTSFKKIKLWTMPISSVRFVPRDVPLPVVRVASVFANADKGDDEKNTDNSEDSRAEDNFNLEKEKE
 DVPVEMSNGE PGCHYFEQLHYNDMWLVGDCVFIKSHGLVRPRVGRIEKVWVRDGAAYFYGPIFIHPEETEHEPTK
 MFYKKEVFLSNLEETCPMTCILGKCAVLSFKDFLSCRPT EIPENDILLCESRYNESDKQMKKFKGLKRFSLSAKVV
 DDEIYYFRKPIVPQKEPSLLEKKIQLEAKFAELEGGDDDIEMGEEDSEVIEPPSLPQLQTPPLASELDLMPYTP
 PQSTPKSAKGS AKKEGSKRKINMSGYILFSSEMR AVIKAQHPDYSFGELSRLVGTEWRNLETAKKAEYEERA AKVA
 EQQERERAAQQQQPSASPRAGTPVGALMGVPPPP
 TPMGMLNQQLTPVAGMMGGYPPGLPPLQGPVDGLVSMGSMQPLHPGGPPPHLPPGVPGLPGI PPPGVMNQGVAPM
 VGTPAPGGS PYGQVGLGPPGQAPPPYPGHPAGPPV IQQPTT PMFVAPPPKTQRLHSEAYLK YIEGLSAESN
 SISKWDQTLAARRRDVHLSKEQESRLPSHWLKS KGAHTT MADALWRLRDLMLRDTLNRQAYNLENV

SEQ ID NO: 30

PK M2: Accn # P14618 (Upregulated in many cancers and possibly HNCa)
 gi|20178296|sp|P14618.4|KPYM_HUMAN Pyruvate kinase isozymes M1/M2 (Pyruvate
 kinase muscle isozyme) (Pyruvate kinase 2/3) (Cytosolic thyroid hormone-
 binding protein) (CTHBP) (THBP1)
 MSKPHSEAGTAFIQ TQQLHAAMADTFLEHMCRLDIDSPITARN TGIICTIGPASRSVETLKEMIKSGMNVARLNF
 SHGTHEYHAETIKNVRTATESFASDPILYRPVAVALDTKGPEIRTGLIKSGTAEVELKKGATLKITLDNAYMEKC
 DENILWLDYKNICKVVEVGSKIYVDDGLISLQVKQKGAFLVTEVENGGSLGSKKGVNLPGA AVDLPAVSEKDIQD
 LKFGVEQD VDMVFASFIRKASDVHEVRKVLGEKGKNIKIISKIENHEGVRRFDEILEASDGIMVARGDLGIEIPAE
 KVFLAQKMMIGRCNRAGKPVICATQMLESMIKKPRPTRAEGSDVANAVLDGADCIMLSGETAKGDYPLEAVRMQHL
 IAREAEAAIYHLQLFEELRRLAPITSDPTEATAVGAVEASFKCCSGAIIVLTKSGRSAHQVARYRPRAPIIAVTRN
 PQTARQAHL YRGIFPVLC KDPVQEA WAEDVDLRVNFAMNVGKARGFFKKGDVVIVLTGWRPGSGFTNTMRVVPVP

SEQ ID NO: 31

Annexin A1: Accn # P04083 (Upregulated in HNCa)
 gi|113944|sp|P04083.2|ANXA1_HUMAN Annexin A1 (Annexin-1) (Annexin I)
 (Lipocortin I) (Calpactin II) (Chromobindin-9) (p35) (Phospholipase A2
 inhibitory protein)
 MAMVSEFLKQAWFIENEEQEYVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEATI IDILTKRNN AQ
 RQQIKAAYLQETGKPLDETLKKALTGHLEEVVLALLKTPAQF DADELRAAMKGLGTDEDTLIEILASRTNKEIRDI
 NRVYREELKRD LAKDITS DTSGDFRNALLSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGT DVNVNTILTTR
 SYPQLRRVFQKYTKYSKHD MNKVL DLELKGDI EKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSR
 EIDMNDIKAFYQKMYGISLCQA ILDETKGDY EKILVALCGGN

SEQ ID NO: 32

Nucleophosmin I: Accn # AAH16768.1 (Upregulated in HNCa)
 gi|16876992|gb|AAH16768.1| Nucleophosmin (nucleolar phosphoprotein B23,
 numatrin) [Homo sapiens]
 MEDSMDMDMSPLRPQNYLFGCELKADKDYHFKVDNDENEHQLSLRTVSLGAGAKDELHIVEAEAMNYEGSPIKVTL
 ATLKMSVQPTVSLGGFEITPPVVLRLKCGSGPVHISGQHLVAVEEDA ESEDEEEEDVKLLSISGKRSAPGGGSKVP
 QKKVKLA ADEDDDDDEEDDDDDGDDDFDDEEAEEKAPVKKSIRDTPAKNAQKSNQNGKDSKPSSTPRSKGQESF
 KKQEKTPKTPKGPSSVEDIKAKMQASIEKGGSLPKVEAKFINCVKNCFRMTDQEA IQDLWQWRKSL

SEQ ID NO: 33

Hsp 27: Accn # P04792 (Upregulated in HNCa)
 gi|19855073|sp|P04792.2|HSPB1_HUMAN Heat shock protein beta-1 (HspB1) (Heat
 shock 27 kDa protein) (HSP 27) (Stress-responsive protein 27) (SRP27)
 (Estrogen-regulated 24 kDa protein) (28 kDa heat shock protein)
 MTERRVPFSLLRGPSWDPF RDWYPHSRLFDQAFGLPRLPEEWSQWLGGSSWPGYVRPLPPAAIESPAVAAPAYSRA
 LSRQLSSGVSEIRHTADRWRVSLDVNHFAPELTVKTKDGVVEITGKHEERQDEHGYISRCFTRKYTLPPGVDPTQ
 VSSSLSP EGTLTVEAPMPKLATQSN EITIPVTFESRAQLGGPEAAKSDETA AK

Cystatin B: Accn # P04080 (Downregulated in HNCa)
gi|1706278|sp|P04080.2|CYTB_HUMAN Cystatin-B (Stefin-B) (Liver thiol
proteinase inhibitor) (CPI-B)
MMCGAPSATQPATAETQHIADQVRSQLEEKENKKFPVFKAVSFKSQVVAGTNYFIKVHVGDEDFVHLRVFQSLPHE
NKPLTLSNYOTNKAHKDELTYF

GRP 94: Accn # P14625 (Downregulated in HNCa)
gi|119360|sp|P14625.1|ENPL_HUMAN Endoplasmic precursor (Heat shock protein
90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP94) (gp96
homolog) (Tumor rejection antigen 1)
MRALWVLGLCCVLLTFGSVRADDEVVDGTVEEDLGKSREGSRTDDEVVQREEEAIQLDGLNASQIRELREKSEKF
AFQAEVNRMMKLIINSLYKNKEIFLRELISNASDALDKIRLISLTDENALSGNEELTVKIKCDKEKNLLHVTDTGV
GMTREELVKNLGTIAKSGTSEFLNKMTEAQEDGQSTSELIGQFGVGFYSAFLVADKVIIVTSKHNNDTQHIWESDSN
EFSVIADPRGNTLGRGTTITLVLKEEASDYLELDTIKNLVKKYSQFINFPIYVWSSKTETVEEPMEEEEAAKEEKE
ESDDEAAVEEEEEEEKPKTKKVEKTVWDWELMNDIKPIWQRPSKEVEEDEYKAFYKSFSKESDDPMAYIHFTAEGE
VTFKSILFVPTSAPRGLFDEYGSKKS DYIKLYVRRVFITDDFHDMMPKYLN FVKGVVDSDDLPLNVSRET LQQHKL
LKVIRKKLVKRKTLDMIKKIADDKYNDTFWKEFGTNIKLG VIEDHSNRTRLAKLLRFQSSHHPTDITSLDQYVERMK
EKQDKIYFMAGSSRKEAESSPFVERLLKKGYEVIYLT E PVDEYCIQALPEFDGKRFQNVAKEGVKFD ESEKTKESR
EAVEKEFEPELLNWMKDKALKDKIEKAVVSQRLTESPCALVASQYGSNMERIMKAQAYQTGKDISTNY YASQKKT
FEINPRHPLIRDMLRRIKED EDDKTVLDLAVVLFETATLRSGYLLPDTKAYGDRIERMLRLSLNIDPDAKVEEEPE
EEPEETAEDTTEDTEODEDEEMDVGTDEEEETAKESTAEKDEL

MARCKS: Accn # gb|AAA59555.1 (Protein of interest, possibly upregulated in HNCa)
gi|187387|gb|AAA59555.1| myristoylated alanine-rich C-kinase substrate
MGAQFSKTAAKGEAAERPGEA AVASSPSKANGQHGVKVN GDAS PAAAESGAKEELQANGSAPAADKEEPAAAG
SGAASPSAAEKGE PAAAAPEAGAS PVEKEAPAEGEAAEPGSPTAAEGEAASAASSTSSPKAEDGATPSPSNETPK
KKKKRFSFKKSFKL SGFSFKKNKKEAGEGGEAEAPAAEGGKDEAAGGAAAAAAEAGAASGEQAAAPGEEAAAGEEG
AAGGDSQEAKPQEAAVAPEKPPASDETKAAE EPSKVEEKKAEEAGASAAACEAPSAAGLVCPRRGGSPRGGARGRR
SLNQACAAPSOEAOPECSPEAPPAEAAE

[illegible]

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RefSeq Accession Number: NM_003406

gi|21735623|ref|NM_003406.2| Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ), transcript variant 1, mRNA

GCCCACTCCCACCGCCAGCTGGAACCCTGGGGACTACGACGTCCCTCAAACCTTGCTTCTAGGAGATAAA
AAGAACATCCAGTCATGGATAAAAATGAGCTGGTTCAGAAGGCCAAACTGGCCGAGCAGGCTGAGCGATA
TGATGACATGGCAGCCTGCATGAAGTCTGTAAGTGAAGGAGCTGAATTATCCAATGAGGAGAGGAAT
CTTCTCTCAGTTGCTTATAAAAATGTTGTAGGAGCCCGTAGGTTCATCTTGGAGGGTCGTCTCAAGTATTG
AACAAAAGACGGAAGGTGCTGAGAAAAAACAGCAGATGGCTCGAGAATACAGAGAGAAAATTGAGACGGA
GCTAAGAGATATCTGCAATGATGTACTGTCTCTTTTGGAAAAGTTCTTGATCCCCAATGCTTCACAAGCA
GAGAGCAAAGTCTTCTATTTGAAAATGAAAGGAGATTACTACCGTTACTTGGCTGAGGTTGCCGCTGGTG
ATGACAAGAAAGGGATTGTCGATCAGTCACAACAAGCATACCAAGAAGCTTTTGAAATCAGCAAAAAGGA
AATGCAACCAACACATCCTATCAGACTGGGTCTGGCCCTTAACCTCTCTGTGTTCTATTATGAGATTCTG
AACTCCCCAGAGAAAGCCTGCTCTCTTGCAAAGACAGCTTTTGATGAAGCCATTGCTGAACTTGATACAT
TAAGTGAAGAGTCATACAAAGACAGCACGCTAATAATGCAATTACTGAGAGACAACTTGACATTGTGGAC
ATCGGATACCCAAGGAGACGAAGCTGAAGCAGGAGAAGGAGGGGAAAATTAACCGGCCTTCCAACCTTTTG
TCTGCCTCATTCTAAAATTTACACAGTAGACCATTTGTCATCCATGCTGTCCACAAATAGTTTTTTGTT
TACGATTTATGACAGGTTTATGTTACTTCTATTTGAATTTCTATATTTCCCATGTGGTTTTTATGTTTAA
TATTAGGGGAGTAGAGCCAGTTAACATTTAGGGAGTTATCTGTTTTTCATCTTGAGGTGGCCAATATGGGG
ATGTGGAATTTTTATACAAGTTATAAGTGTGTTGGCATAGTACTTTTGGTACATTGTGGCTTCAAAGGGC
CAGTGTAAGTCTGCTTCCATGTCTAAGCAAAGAAACTGCCTACATACTGGTTTGTCTGGCGGGGAATA
AAAGGGATCATTTGGTCCAGTCACAGGTGTAGTAATTGTGGGTACTTTAAGGTTTGGAGCACTTACAAGG
CTGTGGTAGAATCATACCCCATGGATACCACATATTAACCATGTATATCTGTGGAATACTCAATGTGTA
CACCTTTGACTACAGCTGCAGAAGTGTTCTTTAGACAAAGTTGTGACCCATTTTACTCTGGATAAGGGC
AGAAACGGTTCACATTCCATTATTTGTAAAGTTACCTGCTGTAGCTTTCATTATTTTTGCTACACTCAT
TTTATTTGTATTTAAATGTTTTAGGCAACCTAAGAACAATGTAAAAGTAAAGATGCAGGAAAAATGAAT
TGCTTGGTATTCATTACTTCATGTATATCAAGCACAGCAGTAAACAAAAACCCATGTATTTAACTTTTT
TTTAGGATTTTTGCTTTTGTGATTTTTTTTTTTTTTTTTTTTGATACTGCCTAACATGCATGTGCTGTAAA
AATAGTTAACAGGGAAATAACTTGAGATGATGGCTAGCTTTGTTAATGTCTTATGAAATTTTCATGAAC
AATCCAAGCATAATTGTTAAGAACACGTGTATTAAATTCATGTAAGTGAATAAAAGTTTTATGAATGGA
CTTTTCACTACTTTCTCTACAGCTTTTCATGTAAATTAGTCTTGGTCTGAAACTTCTCTAAAGGAAAT
TGTAATTTTTTGAATTTATTCCTTATTCCTCTTGGCAGCTAATGGGCTCTTACCAAGTTTAAACACA
AAATTTATCATAACAAAAATACTACTAATAATACTACTGTTTCCATGTCCCATGATCCCCTCTCTTCCTC
CCCACCCTGAAAAAATGAGTTCCTATTTTTTCTGGGAGAGGGGGGATTGATTAGAAAAAATGTAGTG
TGTTCCATTTAAATTTTGGCATATGGCATTTTCTAAGTGAAGCCACAATGTTCTTGGCCCATCATG
ACATTGGGTAGCATTAAGTGTGCTTCCAAATCACTTTTTTGGTTTTTAAGAATTTCTTGATA
CTCTTATAGCCTGCCTTCAATTTTGATCCTTTATTCTTTCTATTTGTGAGGTGCACAAGATTACCTTCCT
GTTTTAGCCTTCTGTCTTGTACCAACCATTCTTACTTGGTGGCCATGTACTTGGAAAAAGGCCGCATGA
TCTTTCTGGCTCCACTCAGTGTCTAAGGCACCCTGCTTCCTTTGCTTGCATCCCACAGACTATTTCCCTC
ATCCTATTTACTGCAGCAAATCTCTCCTTAGTTGATGAGACTGTGTTTATCTCCCTTTAAACCCCTACCT
ATCCTGAATGGTCTGTCTGCTGCTGCTTTTAAATCCTTCCTCTTTCTTCTCCTCTATTCTCTAAATAA
TGATGGGGCTAAGTTATACCCAAAGCTCACTTTACAAAATATTTCTCAGTACTTTGCAGAAAACACCAA
ACAAAAATGCCATTTTAAAAAAGGTGTATTTTTTCTTTTAGAATGTAAGCTCCTCAAGAGCAGGGACAAT
GTTTTCTGTATGTTCTATTGTGCCTAGTACACTGTAAATGCTCAATAAATATTGATGATGGGAGGCAGTG
AGTCTTGATGATAAGGGTGAGAACTGAAATCCC

SEQ ID NO: 39

Gene name: S100A2

RefSeq Accession Number: NM_005978

gi|45269153|ref|NM_005978.3| Homo sapiens S100 calcium binding protein A2 (S100A2), mRNA

CTCCCCTCACCCCGGTCCAGGATGCCCAGTCCCCACGACACCTCCCCTTCCCCTGTGGCCTGGGTGGG
CTCAGGGGGCTGCCCTTGACCTGGCCTAGAGCCCTCCCCAGCTGGTGGTGGAGCTGGCACTCTCTGGGAG
GGAGGGGGCTGGGAGGGAATGAGTGGGAATGGCAAGAGGCCAGGGTTTGGTGGGATCAGGTTGAGGCAGG
TTTGGTTTTCCTTAAATGCCAAGTTGGGGGCCAGTGGGGCCACATATAAATCCTCACCCCTGGGAGCCTG
GCTGCCTTGCTCTCCTTCCTGGGTCTGTCTCTGCCACCTGGTCTGCCACAGATCCATGATGTGCAGTTCT
CTGGAGCAGGCGCTGGCTGTGCTGGTCACTACCTTCCACAAGTACTCCTGCCAAGAGGGGCGACAAGTTCA
AGCTGAGTAAGGGGGAAATGAAGGAACTTCTGCACAAGGAGCTGCCCAGCTTTGTGGGGGAGAAAGTGGA
TGAGGAGGGGGCTGAAGAAGCTGATGGGCAGCCTGGATGAGAACAGTGACCAGCAGGTGGACTTCCAGGAG
TATGCTGTTTTCTGGCACTCATCACTGTCTGCAATGACTTCTTCCAGGGCTGCCAGACCGACCCT
GAAGCAGAATCTTGACTTCTGCCATGGATCTCTTGGGCCAGGACTGTTGATGCCTTTGAGTTTTGTA
TTCAATAAACTTTTTTTGTCTGTTGATAATATTTAATTGCTCAGTGATGTTCCATAACCCGGCTGGCTC
AGCTGGAGTGCTGGGAGATGAGGGCCTCCTGGATCCTGCTCCCTTCTGGGCTCTGACTCTCCTGGAAATC

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TCTCCAAGGCCAGAGCTATGCTTTAGGTCTCAATTTTGAATTTCAAACACCAGCAAAAAATTGGAAATC
GAGATAGGTTGCTGACTTTTATTTTGTCAAATAAAGATATTAAAAAAGGCCAAAAA

SEQ ID NO: 40

Gene Name: S100A7

RefSeq Accession Number: NM_002963.3

gi|115298656|ref|NM_002963.3| Homo sapiens S100 calcium binding protein A7 (S100A7), mRNA

GTCCAAACACACACATCTCACTCATCCTTCTACTCGTGACGCTTCCCAGCTCTGGCTTTTGGAAAGCAAA
GATGAGCAACACTCAAGCTGAGAGGTCCATAATAGGCATGATCGACATGTTTCACAAATACACCAGACGT
GATGACAAGATTGAGAAGCCAAGCCTGCTGACGATGATGAAGGAGAACTTCCCCAACTTCCTTAGTGCCT
GTGACAAAAAGGGCACAAATTACCTCGCCGATGTCTTTGAGAAAAAGGACAAGAATGAGGATAAGAAGAT
TGATTTTTCTGAGTTTCTGTCCTTGCTGGGAGACATAGCCACAGACTACCACAAGCAGAGCCATGGAGCA
GCGCCCTGTTCCGGGGGCGAGCCAGTGACCCAGCCCCACCAATGGGCCTCCAGAGACCCCAGGAACAATAA
AATGTCTTCTCCACAGAAAAA

SEQ ID NO: 41

Gene Name: PTMA

RefSeq Accession Number: NM_001099285

gi|151101406|ref|NM_001099285.1| Homo sapiens prothymosin, alpha (gene sequence 28) (PTMA), transcript variant 1, mRNA

CCCCACTGGCTGCTCTGAAAAGCCATCTTGCATTGTTCCCTCATCCGCCTCCTTGCTCGCCGCGAGCCGC
CTCCGCGCGCGCCTCCTCCGCGCGCGGACTCCGGCAGCTTTATCGCCAGAGTCCCTGAACTCTCGCT
TTCTTTTTTAATCCCCTGCATCGGATCACCGGCGTGCCCCACCATGTCAGACGCAGCCGTAGACACCAGCT
CCGAAATCACCAAGGACTTAAAGGAGAAGAAGGAAGTTGTGGAAGAGGCAGAAATGGAAGAGACGC
CCCTGCTAACGGGAATGCTGAGAATGAGGAAAATGGGGAGCAGGAGGCTGACAATGAGGTAGACGAAGAA
GAGGAAGAAGGTGGGGAGGAAGAGGAGGAGGAAGAAGAAGGTGATGGTGAGGAAGAGGATGGAGATGAAG
ATGAGGAAGCTGAGTCAGCTACGGGCAAGCGGGCAGCTGAAGATGATGAGGATGACGATGTCGATACCAA
GAAGCAGAAGACCGACGAGGATGACTAGACAGCAAAAAGGAAAAGTTAACTAAAAAAGGCCGC
CGTGACCTATTACCCCTCCACTTCCCGTCTCAGAATCTAAACGTGGTCACTTTCGAGTAGAGAGGCCCGC
CCGCCCACCGTGGGCAGTGCCACCCGCAGATGACACGCGCTCTCCACCACCAACCCAAACCATGAGAAT
TTGCAACAGGGGAGGAAAAAGAACCAAACTTCCAAGGCCCTGCTTTTTTTCTTAAAGTACTTTAAAA
AGGAAATTTGTTGTATTTTTTATTTACATTTTATTTTTGTACATATTGTTAGGGTCAGCCATTTTAA
ATGATCTCGGATGACCAACAGCCTTCGGAGCGTTCTCTGTCTACTTCTGACTTTACTTGTGGTGTGA
CCATGTTTATTATAATCTCAAAGGAGAAAAAACCTTGTAAGGAGCAAAATGACAACAGAAAAACA
ATCTTATTCGAGCATTCCAGTAACTTTTTGTGTATGTACTTAGCTGTACTATAAGTAGTTGGTTTGT
TGAGATGGTTAAAAAGGCCAAAGATAAAAGTTTCTTTTTTTTCTTTTTTGTCTATGAAGTTGCTGTT
TATTTTTTTTGGCCTGTTTGATGTATGTGTGAACAATGTTGTCCAACAATAAACAGGAATTTTATTTTG
CTGAGTTGTTCTAACAAAAA

SEQ ID NO: 42

Gene Name: FSCN1

RefSeq Accession Number: NM_003088

gi|49472815|ref|NM_003088.2| Homo sapiens fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus) (FSCN1), mRNA

GCTGCGGAGGGTGCGTGCGGGCCGCGGCAGCCGAACAAAGGAGCAGGGGCGCCGCGCAGGGACCCGCCA
CCCACCTCCCGGGGCGCGCAGCGGCCTCTCGTCTACTGCCACCATGACCGCCAACGGCACAGCCGAGGC
GGTGACAGATCCAGTTCGGCCTCATCACTGCGGCAACAAGTACCTGACGGCCGAGGCGTTCGGGTTCAG
GTGAACGCGTCCGCCAGCAGCCTGAAGAAGAAGCAGATCTGGACGCTGGAGCAGCCCCCTGACGAGGCGG
GCAGCGCGGCGGTGTGCCTGCGCAGCCACCTGGGCGGCTACCTGGCGGCGGACAAGGACGGCAACGTGAC
CTGCGAGCGGAGGTGCCCCGGTCCCGACTGCCGTTTCTCATCGTGGCGCACGACGACGGTCGCTGGTCG
CTGCAGTCCGAGGCGCACCGGCGCTACTTCGGCGGCACCGAGGACCGCCTGTCTGCTTCGCGCAGACGG
TGTCCCCCGCCGAGAAGTGAGCGTGCACATCGCCATGCACCCTCAGGTCAACATCTACAGCGTCACCCG
TAAGCGCTACGCGCACCTGAGCGCGCGGCGGCGGACGAGATCGCCGTGGACCGCGACGTGCCCTGGGGC
GTCGACTCGCTCATCACCTCGCCTTCCAGGACCAGCGCTACAGCGTGCAGACCGCCGACCACCGCTTCC
TGCGCCACGACGGGCGCCTGGTGGCGCGCCCCGAGCCGGCCACTGGCTACACGCTGGAGTTCCGCTCCGG
CAAGGTGGCCTTCCGCGACTGCGAGGGCCGTTACCTGGCGCCGTGGGGGCCAGCGGCACGCTCAAGGCG
GGCAAGGCCACCAAGGTGGGCAAGGACGAGCTCTTTGCTCTGGAGCAGAGCTGCGCCCAGGTCTGTGCTGC
AGGCGGCCAACGAGAGGAACGTGTCCACGCGCCAGGGTATGGACCTGTCTGCCAATCAGGACGAGGAGAC
CGACCAGGAGACCTTCCAGCTGGAGATCGACCGCGACACCAAAAAGTGTGCCTTCCGTACCCACACGGGC
AAGTACTGGACGCTGACGGCCACCGGGGGCGTGACGTCCACCGCCTCCAGCAAGAATGCCAGCTGCTACT
TTGACATCGAGTGGCGTGACCGGCGCATCACACTGAGGGCGTCCAATGGCAAGTTTGTGACCTCCAAGAA
GAATGGGCAGCTGGCCGCTCGGTGGAGACAGCAGGGGACTCAGAGCTCTTCCTCATGAAGCTCATCAAC

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CGCCCCATCATCGTGTTCGCGGGGAGCATGGCTTCATCGGCTGCCGCAAGGTCACGGGCACCCTGGACG
CCAACCGCTCCAGCTATGACGTCTTCCAGCTGGAGTTCAACGATGGCGCCTACAACATCAAAGACTCCAC
AGGCAAATACTGGACGGTGGGCAGTGACTCCGCGGTACACAGCAGCGGCGACACTCCTGTGGACTTCTTC
TTCGAGTTCTGCGACTATAACAAGGTGGCCATCAAGGTGGGCGGGCGCTACCTGAAGGGCGACCACGCAG
GCGTCTGAAGGCCTCGGCGGAAACCGTGGACCCCGCCTCGCTCTGGGAGTACTAGGGCCGGCCCGTCCT
TCCCCGCCCCCTGCCACATGGCGGCTCCTGCCAACCCCTCCCTGCTAACCCCTTCTCCGCCAGGTGGGCTC
CAGGGCGGGAGGCAAGCCCCCTTGCCTTTCAAACCTGGAAACCCAGAGAAAACGGTGCCCCACCTGTGCG
CCCCTATGGACTCCCCACTCTCCCTCCGCCCCGGGTTCCTACTCCCTCGGGTCAGCGGCTGCGGCCTG
GCCCTGGGAGGGATTTAGATGCCCTGCCCTCTTGTCTGCCACGGGGCGAGTCTGGCACCTCTTCTTC
TGACCTCAGACGGCTCTGAGCCTTATTTCTCTGGAAGCGGCTAAGGGACGGTTGGGGGCTGGGAGCCCTG
GGCGTGTAGTGTAACCTGGAATCTTTTGCCTCTCCAGCCACCTCCTCCAGCCCCCAGGAGAGCTGGGC
ACATGTCCCAAGCCTGTGAGTGGCCCTCCCTGGTGCAGTGTCCCCGAAACCCCTGCTTGGAAGGGAAGC
TGTGCGGTGGGCTAGGACTGACCCTTGTGGTGTTTTTTTGGGTGGTGGCTGGAAACAGCCCCCTCTCCAC
GTGGCAGAGGCTCAGCCTGGCTCCCTTCCCTGGAGCGGCAGGGCGTGACGGCCACAGGGTCTGCCCCGCTG
CACGTTCTGCCAAGGTGGTGGTGGCGGGCGGGTAGGGGTGTGGGGGCGCTTTCCTCCTGTCTCTTTCCT
TTCACCCTAGCCTGACTGGAAGCAGAAAATGACCAAATCAGTATTTTTTTTAAATGAAATATTATTGCTGG
AGGCGTCCCAGGCAAGCCTGGCTGTAGTAGCGAGTGATCTGGCGGGGGCGTCTCAGCACCCCTCCCCAGG
GGGTGCATCTCAGCCCCCTCTTCCGTCCTTCCCGTCCAGCCCCAGCCCTGGGCCTGGGCTGCCGACACC
TGGGCCAGAGCCCCCTGCTGTGATTGGTGTCTCCCTGGGCCTCCCGGGTGGATGAAGCCAGGCGTCGCCCCC
TCCGGGAGCCCTGGGGTGAAGCCCGGGGGCCCCCTGCTGCCAGCCTCCCCCGTCCCCAACATGCATCTC
ACTCTGGGTGTCTTGGTCTTTTATTTTTTTGTAAGTGTCAATTTGTATAACTCTAAACGCCCATGATAGTAG
CTTCAAACCTGGAAATAGCGAAATAAAATAACTCAGTCTGCAGCCCCAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 43

Gene Name: S100A11

RefSeq Accession Number: NM_005620

gi|5032056|ref|NM_005620.1| Homo sapiens S100 calcium binding protein A11 (S100A11), mRNA

GGGCAAGGCTGGGCGGGAAGGGCGTGGGTTGAGGAGAGGCTCCAGACCCGACGCGCGCGCACAGAGC
TCTCAGCGCCGCTCCCAGCCACAGCCTCCCGCGCCTCGCTCAGCTCCAACATGGCAAAAATCTCCAGCCC
TACAGAGACTGAGCGGTGCATCGAGTCCCTGATTGCTGTCTTCCAGAAGTATGCTGGAAAGGATGGTTAT
AACTACACTCTCTCCAAGACAGAGTTCTTAAGCTTCATGAATACAGAACTAGCTGCCTTCACAAAGAACC
AGAAGGACCCTGGTGTCTTGACCGCATGATGAAGAACTGGACACCAACAGTGATGGTCAGCTAGATTT
CTCAGAATTTCTTAATCTGATTGGTGGCCTAGCTATGGCTTGCCATGACTCCTTCCTCAAGGCTGTCCCT
TCCCAGAAGCGGACCTGAGGACCCCTTGCCCTGGCCTTCAAACCCACCCCTTTCTTCCAGCCTTTCT
GTCATCATCTCCACAGCCCACCCATCCCTGAGCACACTAACCACCTCATGCAGGCCCCACCTGCCAATA
GTAATAAAGCAATGTCACCTTTTTTAAACATGAAA

SEQ ID NO: 44

Gene Name: SERPINB5

RefSeq Accession Number: NM_002639

gi|142377273|ref|NM_002639.3| Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA

TTGTGCTCCTCGCTTGCCTGTTCTTTTCCACGCATTTTCCAGGATAACTGTGACTCCAGGCCCCGAATG
GATGCCCTGCAACTAGCAAATTCGGCTTTTGCCGTTGATCTGTTCAAACAACATATGTGAAAAGGAGCCAC
TGGGCAATGTCTCTTCTCTCAATCTGTCTCTCCACCTCTCTGTCACTTGCTCAAGTGGGTGCTAAAGG
TGACACTGCAAATGAAATTGGACAGGTTCTTCATTTTGAAAATGTCAAAGATATACCCTTTGGATTTCAA
ACAGTAACATCGGATGTAAACAACTTAGTTCCTTTTACTCACTGAACTAATCAAGCGGCTCTACGTAG
ACAAATCTCTGAATCTTTCTACAGAGTTCATCAGCTCTACGAAGAGACCCTATGCAAAGGAATTGGAAAC
TGTTGACTTCAAAGATAAATTGGAAGAAACGAAAGGTCAGATCAACAACCTCAATTAAGGATCTCACAGAT
GGCCACTTTGAGAACATTTTAGCTGACAACAGTGTGAACGACCAGACCAAATCCTTGTGGTTAATGCTG
CCTACTTTGTTGGCAAGTGGATGAAGAAATTTCTGAATCAGAAACAAAAGAATGTCCTTTCAGACTCAA
CAAGACAGACACCAAACAGTGCAGATGATGAACATGGAGGCCACGTTCTGTATGGGAAACATTGACAGT
ATCAATTGTAAGATCATAGAGCTTCCTTTTCAAATAAGCATCTCAGCATGTTTCATCCTACTACCCAAGG
ATGTGGAGGATGAGTCCACAGGCTTGAGAAAGATTGAAAACAACTCAACTCAGAGTCACTGTACAGTG
GACTAATCCCAGCACCATGGCCAATGCCAAGGTCAAACCTCTCCATTCCAAAATTTAAGGTGGAAAAGATG
ATTGATCCCAAGGCTTGTCTGGAAAATCTAGGGCTGAAACATATCTTCAGTGAAGACACATCTGATTTCT
CTGGAATGTGAGAGACCAAGGGAGTGGCCCTATCAAATGTTATCCACAAAGTGTGCTTAGAAATAACTGA
AGATGGTGGGGATTCCATAGAGGTGCCAGGAGCACGGATCCTGCAGCACAAGGATGAATTGAATGCTGAC
CATCCCTTTATTTACATCATCAGGCACAACAAAACCTCGAAACATCATTTTCTTTGGCAAATTTCTGTTCTC
CTTAAGTGGCATAGCCCATGTTAAGTCCTCCCTGACTTTTCTGTGGATGCCGATTTCTGTAAACTCTGCA
TCCAGAGATTCATTTTCTAGATACAATAAATTGCTAATGTTGCTGGATCAGGAAGCCGCCAGTACTTGTC

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ATATGTAGCCTTCACACAGATAGACCTTTTTTTTTTTTCCAATTCTATCTTTTGTTCCTTTTTTCCCATA
AGACAATGACATACGCTTTTAATGAAAAGGAATCACGTTAGAGGAAAAATATTTATTCATTATTTGTCAA
ATTGTCCGGGGTAGTTGGCAGAAATACAGTCTTCCACAAAGAAAATTCTATAAGGAAGATTTGGAAGCT
CTTCTTCCCAGCACTATGCTTTTCTTCTTTGGGATAGAGAATGTTCCAGACATTCTCGCTTCCCTGAAAG
ACTGAAGAAAGTGTAGTGCATGGGACCCACGAAACTGCCCTGGCTCCAGTGAAACTTGGGCACATGCTCA
GGCTACTATAGGTCCAGAAGTCCTTATGTTAAGCCCTGGCAGGCAGGTGTTTATTAAATTTCTGAATTTT
GGGGATTTTCAAAAGATAATATTTTACATACACTGTATGTTATAGAACTTCATGGATCAGATCTGGGGCA
GCAACCTATAAATCAACACCTTAATATGCTGCAACAAAATGTAGAATATTCAGACAAAATGGATACATAA
AGACTAAGTAGCCCATAGGGGTCAAAATTTGCTGCCAAATGCGTATGCCACCAACTTACAAAAACACTT
CGTTCGCAGAGCTTTTCAGATTGTGGAATGTTGGATAAGGAATTATAGACCTCTAGTAGCTGAAATGCAA
GACCCCAAGAGGAAGTTCAGATCTTAATATAAATTCACCTTCATTTTTGATAGCTGTCCCATCTGGTCAT
GTGGTTGGCACTAGACTGGTGGCAGGGGCTTCTAGCTGACTCGCACAGGGATTCTCACAATAGCCGATAT
CAGAATTTGTGTTGAAGGAACCTTGTCTCTTCATCTAATATGATAGCGGGAAAAGGAGAGGAACTACTGC
CTTTAGAAAATATAAGTAAAGTGATTAAAGTGCTCACGTTACCTTGACACATAGTTTTTTCAGTCTATGGG
TTTAGTTACTTTAGATGGCAAGCATGTAACCTTATATTAATAGTAATTTGTAAAGTTGGGTGGATAAGCTA
TCCCTGTTGCCGGTTCATGGATTACTTCTCTATAAAAAATATATATTTACCAAAAAATTTTGTGACATTC
CTTCTCCCATCTCTTCTTGACATGCATTGTAAATAGGTTCTTCTTGTCTGAGATTCAATATTGAATTT
CTCCTATGCTATTGACAATAAAATATTATTGAACCTAC

SEQ ID NO: 45

Gene Name: ANXA8L2

RefSeq Accession Number: NM_001630

gi|91807121|ref|NM_001630.2| Homo sapiens annexin A8-like 2 (ANXA8L2), mRNA

AAAGCCAGGCAGAGCCCGGGGGCGAGGGGGCGGCAGGCAGGTGTAGCGCTGCCCTGGGCGGGCTTGCACC
CCCACACCCAAGTGAGCGGCCTGCTCACTCCTCAGCTGCAGGAGCCAGACGTGTGGAGTCCCAGCAGAGG
CCAACCTGTGTCTTTCATCTCCGTGAGAAAGGTGCCCCGAAGTGAAAGAGATGGCCTGGTGGAAAGCC
TGGATTGAACAGGAGGGTGTACAGTGAAGAGCAGCTCCCACTTCAACCCAGACCCTGATGCAGAGACCC
TCTACAAAGCCATGAAGGGGATCGGGACCAACGAGCAGGCTATCATCGATGTGCTCACCAAGAGAAGCAA
CACGCAGCGGCAGCAGATCGCCAAGTCCTTCAAGGCTCAGTTCGGCAAGGACCTCACTGAGACCTTGAAG
TCTGAGCTCAGTGGCAAGTTTGAGAGGCTCATTGTGGCCCTTATGTACCCGCCATACAGATACGAAGCCA
AGGAGCTGCATGACGCCATGAAGGGCTTAGGAACCAAGGAGGGTGTATCATTGAGATCCTGGCCTCTCG
GACCAAGAACCAGCTGCGGGAGATAATGAAGGCGTATGAGGAAGACTATGGGTCCAGCCTGGAGGAGGAC
ATCCAAGCAGACACAAGTGGCTACCTGGAGAGGATCCTGGTGTGCCTCCTGCAGGGCAGCAGGGATGATG
TGAGCAGCTTTGTGGACCCGGCACTGGCCCTCCAAGACGCACAGGATCTGTATGCGGCAGGCGAGAATAT
TCGTGGGACTGATGAGATGAAATTCATCACCATCCTGTGCACGCGCAGTGCCACTCACCTGCTGAGAGTG
TTTGAAGAGTATGAGAAAATTGCCAACAAGAGCATTGAGGACAGCATCAAGAGTGAGACCCATGGCTCAC
TGGAGGAGGCCATGCTCACTGTGGTGAAATGCACCCAAAACCTCCACAGCTACTTTGCAGAGAGACTCTA
CTATGCCATGAAGGGAGCAGGGACGCGTGATGGGACCCTGATAAGAAACATCGTTTCAAGGAGCGAGATT
GACTTAAATCTTATCAAATGTCACTTCAAGAAGATGTACGGCAAGACCCTCAGCAGCATGATCATGGAAG
ACACCAGCGGTGACTACAAGAACGCCCTGCTGAGCCTGGTGGGCAGCGACCCCTGAGGCACAGAAGAACA
AGAGCAAAGACCATGAAGCCAGAGTCTCCAGGACTCCTCACTCAACCTCGGCCATGGACGCAGGTTGGGT
GTGAGGGGGGTCCCAGCCTTTCGGTCTTCTATTTCCCTATTTCCAGTGCTTTCCAGCCGGGTTTCTGACC
CAGAGGGTGGAAACCGGCCTGGACTCCTCTTCCCAACTTCTCCAGGTCATTTCCCAGTGTGAGCACAATG
CCAACCTTAGTGTTTCTCCAGCCAGACAGATGCCTCAGCATGAAGGGCTTGGGGACTTGTGGATCATTC
TTCCTCCCTGCAGGAGCTTCCCAAGCTGGTCACAGAGTCTCCTGGGCACAGGTTATACAGACCCAGCCC
CATTCCCATCTACTGAAACAGGGTCTCCACAAGAGGGGGCCAGGGAATATGGGTTTTTAACAAGCGTCTTA
CAAAACACTTCTCTATCATGCAGCCGGAGAGCTGGCTGGGAGCCCTTTTGTTTTAGAACACACATCCTTC
AGCAGCTGAGAAACGAACACGAATCCATCCCAACCGAGATGCCATTAACATTCTATAAAATGTTAGGC
TCTAAATGGACGAAAAATTTCTCTCGCCATCTTAATAACAAAATAAACTACAAATTCCTGACCCAAGGACA
CTGTGTTATAAGAGGCGTGGGCTCCCCTGGTGGCTGACCAGGTCAGCTGCCCTGGCCTTGACCCCTCTG
CATGCAGCACAGAAGGGTGTGACCATGCCCTCAGCACCCTCTTGTCCCCACTGAACGGCAACTGAGACT
GGGTACCTGGAGATTCTGAAGTGCCTTTGCTGTGGTTTTCAAAAATAATAAGATTTGTATTCAACTCAAA
AAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 46

Gene Name: CALML5

RefSeq Accession Number: NM_017422

gi|38327636|ref|NM_017422.3| Homo sapiens calmodulin-like 5 (CALML5), mRNA

CCCGATCCCTGCGGCTGCCTGCACTCTGGACCACGAGCTCTGAGAGCAGCAGGTTGAGGGCCGGTGGGC
AGCAGCTCGGAGGCTCCGCGAGGTGCAGGAGACGCAGGCATGGCCGGTGAGCTGACTCCTGAGGAGGAGG
CCCAGTACAAAAAGGCTTTCTCCGCGGTTGACACGGATGGAAACGGCACCATCAATGCCAGGAGCTGGG
CGCGGCGCTGAAGGCCACGGGCAAGAACCTCTCGGAGGCCAGCTAAGGAAACTCATCTCCGAGGTTGAC
AGCGACGGCGACGGCGAAATCAGCTTCCAGGAGTTCCTGACGGCGGCAAGGAAGGCCAGGGCCGGCCTGG

AGGACCTGCAGGTTCGCCTTCCGCGCCTTCGACCAGGATGGCGACGGCCACATCACCGTGGACGAGCTCAG
GCGGGCCATGGCGGGGCTGGGGCAGCCGCTGCCGCAGGAGGAGCTGGACGCCATGATCCGCGAGGCCGAC
GTGGACCAGGACGGGCGGGTGAACACGAGGAGTTCGCGAGGATGCTCGCCCAGGAGTGAGGCTCCCCGC
CTGTGTCCCCCTGGCTGCGCTCTGAGCCTTCAGGGCCACCGCCCGCTGCTGCTTTTGTGCTGGGACTCTC
CGGGGAAACCTGGTTCGGTGGATGGGAAACTGCCTCCCCCTGGGAGGAAGGCTTTGCGCTCCGGGGCCTGG
ATGCGGCGCCCTCGGGCCGCCTGCGAGCCCCCTCTCTGCCTCCAGACCTTGGGCAGAAGGAGGCCTCCTTG
GGCCTGGTCCCCCTTTGCCCTGCAGTGGAATGAGGGCCCCCTCAGCCCCGCATTGATCTAAATAAAGGACT
GCCGAGTTCCAAAA

SEQ ID NO: 47

Gene Name: GST-P1c

Accession Number: U21689

gi|726097|gb|U21689.1|HSU21689 Human glutathione S-transferase-Plc gene,
complete cds

[illegible]

SEQ ID NO: 48

Gene Name: LDHA

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RefSeq Accession Number: NM_005566

gi|5031856|ref|NM_005566.1| Homo sapiens lactate dehydrogenase A (LDHA), mRNA

TGCTGCAGCCGCTGCCGCCGATTCCGGATCTCATTGCCACGCGCCCCGACGACCGCCCGACGTGCATTC
CCGATTCCTTTTGGTTCCAAGTCCAATATGGCAACTCTAAAGGATCAGCTGATTTATAATCTTCTAAAGG
AAGAACAGACCCCCCAGAATAAGATTACAGTTGTTGGGGTTGGTGCTGTTGGCATGGCCTGTGCCATCAG
TATCTTAATGAAGGACTTGGCAGATGAACTTGCTCTTGTTGATGTCATCGAAGACAAATTGAAGGGAGAG
ATGATGGATCTCCAACATGGCAGCCTTTTCCTTAGAACACCAAAGATTGTCTCTGGCAAAGACTATAATG
TAACTGCAAACCTCCAAGCTGGTCATTATCACGGCTGGGGCACGTCAGCAAGAGGGAGAAAGCCGTCTTAA
TTTGGTCCAGCGTAACGTGAACATATTTAAATTCATCATTCCCTAATGTTGTAAAATACAGCCCGAACTGC
AAGTTGCTTATTGTTTCAAATCCAGTGGATATCTTGACCTACGTGGCTTGGAAGATAAGTGGTTTCCCA
AAAACCGTGTTATTGGAAGTGGTTGCAATCTGGATTACGCCCGATTCCGTACCTGATGGGGGAAAGGCT
GGGAGTTACCCCATTAAGCTGTCATGGGTGGGTCTTGGGGAACATGGAGATTCCAGTGTGCCTGTATGG
AGTGGAATGAATGTTGCTGGTGTCTCTCTGAAGACTCTGCACCCAGATTTAGGGACTGATAAAGATAAGG
AACAGTGGAAAGAGGTTTACAAGCAGGTGGTTGAGAGTGCTTATGAGGTGATCAAACCTCAAAGGCTACAC
ATCCTGGGCTATTGGACTCTCTGTAGCAGATTTGGCAGAGAGTATAATGAAGAATCTTAGGCGGGTGCAC
CCAGTTTCCACCATGATTAAGGGTCTTTACGGAATAAAGGATGATGTCTTCCTTAGTGTTCTTGCATTT
TGGGACAGAATGGAATCTCAGACCTTGTGAAGGTGACTCTGACTTCTGAGGAAGAGGCCCGTTTGAAGAA
GAGTGCAGATACACTTTGGGGGATCCAAAAGGAGCTGCAATTTTAAAGTCTTCTGATGTCATATCATTTT
ACTGTCTAGGCTACAACAGGATTCTAGGTGGAGGTTGTGCATGTTGTCTTTTTTATCTGATCTGTGATTA
AAGCAGTAATATTTTAAGATGGACTGGGAAAAACATCAACTCCTGAAGTTAGAAATAAGAATGGTTTGTA
AAATCCACAGCTATATCCTGATGCTGGATGGTATTAATCTTGTGTAGTCTTCAACTGGTTAGTGTGAAAT
AGTTCTGCCACCTCTGACGCACCACTGCCAATGCTGTACGTACTGCATTTGCCCTTGAGCCAGGTGGAT
GTTTACCGTGTGTTATATAACTTCCTGGCTCCTTCACTGAACATGCCTAGTCCAACATTTTTTCCCAGTG
AGTCACATCCTGGGATCCAGTGTATAAATCCAATATCATGTCTTGTGCATAATTCTTCCAAAGGATCTTA
TTTTGTGAACTATATCAGTAGTGTACATTACCATATAATGTAAAAAGATCTACATACAAACAATGCAACC
AACTATCCAAGTGTATACCAACTAAAACCCCAATAAACCTTGAACAGTG

SEQ ID NO: 49

Gene Name: PPIA

RefSeq Accession Number: NM_021130

gi|114520617|ref|NM_021130.3| Homo sapiens peptidylprolyl isomerase A (cyclophilin A) (PPIA), mRNA

GAACGTGGTATAAAAGGGGCGGGAGGCCAGGCTCGTGCCGTTTTGCAGACGCCACCGCCGAGGAAAACCG
TGTAATATTAGCCATGGTCAACCCACCGTGTTCTTCGACATTGCCGTCGACGGCGAGCCCTTGGGCCGC
GTCTCCTTTGAGCTGTTTGCAGACAAGGTCCCAAAGACAGCAGAAAATTTTCGTGCTCTGAGCACTGGAG
AGAAAGGATTTGGTTATAAGGGTTCCTGCTTTACAGAATTATTCCAGGGTTTATGTGTCAGGGTGGTGA
CTTCACACGCCATAATGGCACTGGTGGCAAGTCCATCTATGGGGAGAAATTTGAAGATGAGAACTTCATC
CTAAAGCATACGGGTCTGGCATCTTGTCCATGGCAAATGCTGGACCCAACACAAATGGTTCCCAGTTTTT
TCATCTGCACTGCCAAGACTGAGTGGTTGGATGGCAAGCATGTGGTGTGTTGGCAAAGTGAAAGAAGGCAT
GAATATTGTGGAGGCCATGGAGCGCTTTGGGTCCAGGAATGGCAAGACCAGCAAGAAGATCACCATTGCT
GACTGTGGACAACCTCGAATAAGTTGACTTGTGTTTTATCTTAACCACCAGATCATTCCTTCTGTAGCTC
AGGAGAGCACCCCTCCACCCCATTTGCTCGCAGTATCCTAGAATCTTTGTGCTCTCGCTGCAGTTCCTT
TGGGTTCCATGTTTTCTTGTTCCTCCCATGCCTAGCTGGATTGCAGAGTTAAGTTTATGATTATGAAA
TAAAAACTAAATAACAATTGTCCTCGTTTTGAGTTAAGAGTGTTGATGTAGGCTTTATTTTAAGCAGTAAT
GGGTACTTCTGAAACATCACTTGTTTGCTTAATTCTACACAGTACTTAGATTTTTTTTACTTTCCAGTC
CCAGGAAGTGTCAATGTTTGTGAGTGGAATATTGAAATGTAGGCAGCAACTGGGCATGGTGGCTCACT
GTCTGTAATGTATTACCTGAGGCAGAAGACCACCTGAGGGTAGGAGTCAAGATCAGCCTGGGCAACATAG
TGAGACGCTGTCTTACAAAAAATAATTAGCCTGGCCTGGTGGTGCATGCCTAGTCCCTAGCTGATCTGGA
GGCTGACGTGGGAGGATTGCTTGAGCCTAGAGTGAGCTATTATCATGCCACTGTACAGCCTGGGTGTTCA
CAGATCTTGTGTCTCAAAGGTAGGCAGAGGCAGGAAAAGCAAGGAGCCAGAATTAAGAGGTTGGGTGAGT
CTGCAGTGAGTTCATGCATTTAGAGGTGTTCTTCAAGATGACTAATGTCAAAAATTGAGACATCTGTTGC
GGTTTTTTTTTTTTTTTTTCCCTGGAATGCAGTGGCGTGATCTCAGCTCACTGCAGCCTCCGCCTCCT
GGGTCAAGTGATTCTAGTGCCTCAGCCTCCTGAGTAGCTGGGATAATGGGCGTGTGCCACCATGCCAG
CTAATTTTTGTATTTTTTAGTATAGATGGGGTTTCATCATTTTGACCAGGCTGGTCTCAAACCTCTGACCT
CAGCTGATGCGCCTGCCTTGGCCTCCCAAACCTGCTGAGATTACAGATGTGAGCCACCGCACCTACCTCA
TTTTCTGTAACAAAGCTAAGCTTGAACACTGTTGATGTTCTTGAGGGAAGCATATTGGGCTTTAGGCTGT
AGGTCAAGTTTATACATCTTAATTATGGTGGAATTCCTATGTAGAGTCTAAAAAGCCAGGTAAGTGGTGC
TACAGTCAGTCTCCCTGCAGAGGGTTAAGGCGCAGACTACCTGCAGTGAGGAGGTAAGTGGTGTAGCATA
TAGAGCCTCTCCCTAGCTTTGGTTATGGAGGCTTTGAGGTTTTGCAAACCTGACCAATTTAAGCCATAAG
ATCTGGTCAAAGGGATACCCTTCCCACTAAGGACTTGGTTTTCTCAGGAAATTATATGTACAGTGCTTGCT
GGCAGTTAGATGTGAGGACAATCTAAGCTGAGAAAACCCCTTCTCTGCCACCTTAACAGACCTCTAGGG
TTCTTAACCCAGCAATCAAGTTTGCCTATCCTAGAGGTGGCGGATTTGATCATTTGGTGTGTTGGGCAAT

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TTTTGTTTTACTGTCTGGTTCCTTCTGCGTGAATTACCACCACCACCCTTGTGCATCTCAGTCTTGTGT
GTTGTCTGGTTACGTATTCCCTGGGTGATACCATTCAATGTCTTAATGTACTTGTGGCTCAGACCTGAGT
GCAAGGTGGAAATAAACATCAAACATCTTTTCATTA

SEQ ID NO: 50

Gene Name: MAPRE1

RefSeq Accession Number: NM_012325gi|6912493|ref|NM_012325.1| Homo sapiens microtubule-associated protein,
RP/EB family, member 1 (MAPRE1), mRNA

ACGAGACGAAGACGGAACCGGAGCCGGTTGCGGGCAGTGGACGCGGTTCTGCCGAGAGCCGAAGATGGCA
GTGAACGTATACTCAACGTCAGTGACCAGTGATAACCTAAGTCGACATGACATGCTGGCCTGGATCAATG
AGTCTCTGCAGTTGAATCTGACAAAGATCGAACAGTTGTGCTCAGGGGCTGCGTATTGTCAGTTTATGGA
CATGCTGTTCCCTGGCTCCATTGCCTTGAAGAAAGTGAAATTCGAAGCTAAGCTAGAACACGAGTACATC
CAGAACTTCAAATACTACAAGCAGGTTTTTAAGAGAATGGGTGTTGACAAAATAATTCCTGTGGACAAAT
TAGTAAAAGGAAAGTTTCAGGACAATTTTGAATTCGTTTCAGTGGTTCAAGAAGTTTTTCGATGCAAACTA
TGATGGAAAAGACTATGACCCTGTGGCTGCCAGACAAGGTCAAGAACTGCAGTGGCTCCTTCCCTTGTT
GCTCCAGCTCTGAATAAACCGAAGAAACCTCTCACTTCTAGCAGTGCAGCTCCCCAGAGGCCCATCTCAA
CACAGAGAACCGCTGCGGCTCCTAAGGCTGGCCCTGGTGTGGTGCAGAAAGAACCTGGTGTGGGCAACGG
AGACGACGAGGCAGCTGAGTTGATGCAGCAGGTCAACGTATTGAACTTACTGTTGAAGACTTGGAGAAA
GAGAGGGATTTCTACTTCGGAAAGCTACGGAACATTGAATTGATTTGCCAGGAGAACGAGGGGGAAAACG
ACCCTGTATTGCAGAGGATTGTAGACATTCTGTATGCCACAGATGAAGGCTTTGTGATACCTGATGAAGG
GGGCCCACAGGAGGAGCAAGAAGAGTATTAACAGCCTGGACCAGCAGAGCAACATCGGAATTCCTCACTC
CAAATCATGTGCTTAACTGTAAAATACTCCCTTTTGTTATCCTTAGAGGACTCACTGGTTTCTTTTCATA
AGCAAAAAGTACCTCTTCTTAAAGTGCACTTTGCAGACGTTTCACTCCTTTTCCAATAAGTTTGAGTTAG
GAGCTTTTACCTTGTAGCAGAGCAGTATTAACATCTAGTTGGTTTACCTGGAAACAGAGAGGCTGACCG
TGGGGCTCACCATGCGGATGCGGGTCACACTGAATGCTGGAGAGATGTATGTAATATGCTGAGGTGGCGA
CCTCAGTGGAGAAATGTAAAGACTGAATTGAATTTTAAGCTAATGTGAAATCAGAGAATGTTGTAATAAG
TAAATGCCTTAAGAGTATTTAAATATGCTTCCACATTTCAAATATAAAATGTAACATGACAAGAGATT
TTGCGTTTGACATTGTGTCTGGGAAGGAAGGGCCAGACCTTGGAACCTTTGGAACCTGCTGTCAACAGGT
CTTACAGGGCTGCTTGAACCTCATAGGCCTAGGCTTTGGTCTAAAAGGAACATTTAAAAAGTTGCCCTG
TAAAGTTATTTGGTGTCAATTGACCAATTGCATCCCAGCTAAAAAGCAAGAGGCATCGTTGCCTGGATAAT
AGAGGATGTGTTTCAGCCCTGAGATGTTACAGTTGAAGAGCTTGGTTTCATTGAGCATTTCTCTATTTTT
CCAGTTATCCCGAAATTTCTATGTATTATTTTTTGGGGAAGTGAGGTGTGCCAGTTTTTTAATCTAACA
ACTACTTTTGGGGACTTGCCACATCTCTGGGATTTGAATGGGGATTGTATCCCATTTTACTGTCTTTTA
GGTTTACATTTACCACGTTTCTCTTCTCTGCTCCCCTTGCCACTGGGACTCCTCTTTGGCTCCTTGAAG
TTTGCTGCTTAGAGTTGGAAGTGCAGCAGGCAGGTGATCATGCTGCAAGTTCTTTCTGGACCTCTGGCAA
AGGGAGTGGTCAGTGAAGGCCATCGTTACCTTGGGATCTGCCAGGCTGGGGTGTTCGGTATCTGCTGT
TCACAGCTCTCCACTGTAATCCGAATACTTTGCCAGTGCACATAATCTCTTTGGAGATAAAATTCATTAGT
GTGTTACTAAATGTTAATTTTCTTTTGCAGAAAATACAGTACCGTGTCTGAATTAATTATTAATATTTAA
AATACTTCATTCTTAACTCTCCCTCATTTGCTTTGCCACAGCCTATTCAGTTCCTTTGTTTGGCAGGA
TTCTGCAAAATGTGTCTCACCCACTACTGAGATTGTTTCAGCCCCTGATGTATTTGTATTGATTTGTTTCT
GGTGGTAGCTTGTCTGAAATGTGTGTAGAAAGCAAGTATTTTATGATAAAAATGTTGTGTAGTGCATGC
TCTGTGTGGAATTCAGAGGAAAACCCAGATTGAGTATTAACAATGCCAAAAAATGCAAGTAACTAGCCA
TTGTTCAAATGACAGTGGTGCTATTTCTCTTTTGTGGCCTTTTAGACTTTTGTGCCCCTAAAATTCATT
TTATTGGGAACCCATTTTCCACCTGGTCTTTCTTGACAGGGTTTTTTTCTACTTTAAACAGTTTCTAAAT
AAAATTCTGTATTTCAAAA

SEQ ID NO: 51

Gene Name: SOD2

Accession Number: BC016934gi|16877366:65-487 Homo sapiens superoxide dismutase 2, mitochondrial, mRNA
(cDNA clone MGC:21350 IMAGE:4184203), complete cds

ATGTTGAGCCGGGCAGTGTGCGGCACCAGCAGGCAGCTGGCTCCGGCTTTGGGGTATCTGGGCTCCAGGC
AGAAGCACAGCCTCCCCGACCTGCCCTACGACTACGGCGCCCTGGAACCTCACATCAACGCGCAGATCAT
GCAGCTGCACCACAGCAAGCACCACGCGGCCCTACGTGAACAACCTGAACGTCACCGAGGAGAAGTACCAG
GAGGCGTTGGCCAAGGGTAGGTTCCAGGCTGAGCGGCGGGAGGCAGTCCCCGGCAGAGGCGACCCAGGG
AGCCAGGCCCCATACGGACGGGCCTCTCCGTGGAGGAGAACTCGCTTCGTATTTGTACCGGTTCCGAGTT
TTCCAGGCACGATAGTCTCTCTTTTAAACACATGGTCTACCTCATTGTAGAAGGAGTGCCTCGATGGGTT
TGA

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SEQ ID NO: 52

Gene name: PLS3

RefSeq Accession Number: NM_005032

gi|28416938|ref|NM_005032.3| Homo sapiens plastin 3 (T isoform) (PLS3), mRNA

AAAGATTCCGAGGTGCAGAAAGTTGTCTGAGTGCCTTGGTCGGCGGCAGTCGGGGCCAGACCCAGGACTCTG
 CGACTTTACATCTTTAAATGGATGAGATGGCTACCACTCAGATTTCCAAAGATGAGCTTGATGAACTCAA
 AGAGGCCTTTGCAAAAGTTGATCTCAACAGCAACGGATTCATTTGTGACTATGAACTTCATGAGCTCTTC
 AAGGAAGCTAATATGCCATTACCAGGATATAAAGTGAGAGAAATTATTCAGAAACTCATGCTGGATGGTG
 ACAGGAATAAAGATGGGAAAATAAGTTTTGACGAATTTGTTTATATTTTTCAAGAGGTAAAAAGTAGTGA
 TATTGCCAAGACCTTCCGCAAAGCAATCAACAGGAAAGAAGGTATTTGTGCTCTGGGTGGAACCTCAGAG
 TTGTCCAGCGAAGGAACACAGCATTCTTACTCAGAGGAAGAAAAATATGCTTTTGTAACTGGATAAACA
 AAGCTTTGGAAAATGATCCTGATTGTAGACATGTTATACCAATGAACCCTAACACCGATGACCTGTTCAA
 AGCTGTTGGTGATGGAATTGTGCTTTGTAAAATGATTAACCTTTTCAGTTCTTGATACCATTGATGAAAGA
 GCAATCAACAAGAAGAACTTACACCCTTCATCATTAGGAAAACCTTGAACCTGGCACTGAACTCTGCTT
 CTGCCATTGGGTGTCATGTTGTGAACATTGGTGCAGAAGATTTGAGGGCTGGGAAACCTCATCTGGTTTTT
 GGGACTGCTTTGGCAGATCATTAAGATCGGTTTGTTCGCTGACATTGAATTAAGCAGGAATGAAGCCTTG
 GCTGCTTTACTCCGAGATGGTGAGACTTTGGAGGAACCTTATGAAATTGTCTCCAGAAGAGCTTCTGCTTA
 GATGGGCAAACCTTTCATTTGGAAAACCTCGGGCTGGCAAAAAATTAACAACCTTTAGTGCTGACATCAAGGA
 TTCCAAAGCCTATTTCCATCTTCTCAATCAAATCGCACCAAAAAGGACAAAAGGAAGGTGAACCACGGATA
 GATATTAACATGTCAGGTTTCAATGAAACAGATGATTTGAAGAGAGCTGAGAGTATGCTTCAACAAGCAG
 ATAAATTAGGTTGCAGACAGTTTGTACCCCTGCTGATGTTGTGTCAGTGGAAACCCCAAACCTCAACTTAGC
 TTTCGTGGCTAACCTGTTTAATAAATACCCAGCACTAACTAAGCCAGAGAACCAGGATATTGACTGGACT
 CTATTAGAAGGAGAACTCGTGAAGAAAGAACCTTCCGTAACTGGATGAACTCTCTTGGTGTCATCCTC
 ACGTAAACCATCTCTATGCTGACCTGCAAGATGCCCTGGTAATCTTACAGTTATATGAACGAATTAAAGT
 TCCTGTTGACTGGAGTAAGGTTAATAAACCTCCATACCCGAAACTGGGAGCCAACATGAAAAAGCTAGAA
 AACTGCAACTATGCTGTTGAATTAGGGAAGCATCCTGCTAAATTTCTCCCTGGTTGGCATTGGAGGGCAAG
 ACCTGAATGATGGGAACCAACCCCTGACTTTAGCTTTAGTCTGGCAGCTGATGAGAAGATATACCCCTCAA
 TGTCTGGAAGATCTTGGAGATGGTCAGAAAGCCAATGACGACATCATTGTGAACCTGGGTGAACAGAACG
 TTGAGTGAAGCTGGAAAATCAACTTCCATTAGAGTTTTAAGGACAAGACGATCAGCTCCAGTTTGGCAG
 TTGTGGATTTAATTGATGCCATCCAGCCAGGCTGTATAAACTATGACCTTGTGAAGAGTGGCAATCTAAC
 AGAAGATGACAAGCACAATAATGCCAAGTATGCAGTGTCAATGGCTAGAAGAATCGGAGCCAGAGTGTAT
 GCTCTCCCTGAAGACCTTGTGGAAGTAAAGCCCAAGATGGTCATGACTGTGTTTGCATGTTTGATGGGCA
 GGGGAATGAAGAGAGTGTAAAATAACCAATCTGAATAAAACAGCCATGCTCCAGGTGCATGATTCGCAG
 GTCAGCTATTTCCAGGTGAAGTGCTTATGGCTTAAGGAACTCTTGGCCATTCAAAGGACTTTTCATTTTG
 ATTAACAGGACTAGCTTATCATGAGAGCCCTCAGGGGAAAGGGTTTAAGAAAAACAACCTCCTCTTCCCA
 TAGTCAGAGTTGAATTTGTCAGGCACGCCTGAAATGTGCTCATAGCCAAAACATTTTACTCTCTCCTCCT
 AGAATGCTGCCCTTGACATTTCCCATTTGCTGTATGTTATTTCTTGCTCTGTTATCTTTTGCCCTCTTAGA
 ATGTCCCTCTCTTGGGACTTGCTTAGATGATGGGATATGAATATTATTAGACAGTAATTTTGCTTTCCAT
 CCAGTATGCTAGTTCTTATTCGAGAACTATGGTCAGAGCGTATTTGGATATGAGTATCCTTTGCTTATCT
 TTGTAGTACTGAAAATTTGCCGAAGTAACTGGCTGTGCAGAATGTAATAGAAGCTTTTCTTATTCTTTTA
 TTCTTAAGATCAGTATCTTTTTACAGTATCTTTCTACATGATCCTTTTTTGTACATTTAAGAATATTTT
 GATTATATTAAACAAGACTGCTGATTTTGTACTTTTTTTAAGGGGTCTTCAAGTAAGTAAAACATACAT
 CGTAGCTAGAAGAAAAATGTACCTTAAATTTGCATCTTCCCTCTCATACCCAAGCTGTAAACAATTGAAA
 TATTTTGTCTTAAATCACTTGGTTCAATACATGCTTATTTGTTTTAAACCTGTATCATCAAACCTCTCTC
 TCTAAATTTAAATGCTGTTGAATATGATACTTTTGGAGAGAGAGTGTGCTCAGAACTTAGACGGGATTT
 GGTAGGCCAAGTATGCTAAGTGTACAATATATTTTTTAATTTTACACCTGAAACAAAGAAATGTGGTCAC
 TAAAAATAAAAGTATATATGTAGGAATTAATGTACTCTTGCTTTGTCAAGCTGTTTGCTATAGTTTCCAA
 GGTATTATGTTACTCTAACTCTGAAAAGTGATGTAATCTGGTAGCAATGTAGTAGTTCAAATAAAGGCAT
 TTACATAAT

SEQ ID NO: 53

Gene Name: MGC29506

RefSeq Accession Number: NM_016459

gi|117938313|ref|NM_016459.3| Homo sapiens hypothetical protein MGC29506 (MGC29506), mRNA

AGCACACACACATCTGCACCTCAACCACAGACTACACTTGCTGAACTGGCTCCTGGGGCCATGAGGCTGT
 CACTGCCACTGCTGCTGCTGCTGCTGGGAGCCTGGGCCATCCCAGGGGGCCTCGGGGACAGGGCGCCACT
 CACAGCCACAGCCCCACAACCTGGATGATGAGGAGATGTACTCAGCCCACATGCCCGCTCACCTGCGCTGT
 GATGCCTGCAGAGCTGTGGCTTACCAGATGTGGCAAATCTGGCAAAGGCAGAGACCAAACCTTCATACCT
 CAAACTCTGGGGGGCGGGCGGGAGCTGAGCGAGTTGGTCTACACGGATGTCCTGGACCGGAGCTGCTCCCCG
 GAACTGGCAGGACTACGGAGTTCGAGAAGTGGACCAAGTGAACGTCTCACAGGCCAGGACTTAGCGAG
 GGGCCAGAGCCAAGCATCAGCGTGATGGTCACAGGGGGCCCCTGGCCTACCAGGCTCTCCAGGACATGTT
 TGCATACTTGGGGGAGTTTGGAGAAGACCAGATCTATGAAGCCCACCAACAAGGCCGAGGGGCTCTGGA

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GGCATTGCTATGTGGGGGACCCCAGGGGGCCTGCTCAGAGAAGGTGTCAGCCACAAGAGAAGAGCTCTAG
 TCCTGGACTCTACCCTCCTCTGAAAGAAGCTGGGGCTTGCTCTGACGGTCTCCACTCCCGTCTGCAGGCA
 GCCAGGAGGGCAGGAAGCCCTTGCTCTGTGCTGCCATCCTGCCTCCCTCCTCCAGCCTCAGGGCACTCGG
 GCCTGGGTGGGAGTCAACGCCTTCCCCTCTGGACTCAAATAAAACCCAGTGACCTCAAAAAAAAAAAAAA
 AAAAA

SEQ ID NO: 54

Gene Name: HIST2H3A

RefSeq Accession Number: NM_021059

gi|31742502|ref|NM_021059.2| Homo sapiens histone cluster 2, H3c (HIST2H3C), mRNA

CGCCGCTGCGCTGGTAAGCCTGTGTTTTGGTTCGCTATGGCCCGTACTAAGCAGACTGCTCGCAAGTCGA
 CCGGCGGCAAGGCCCGAGGAAGCAGCTGGCCACCAAGGCGGCCCGCAAGAGCGCGCCGGCCACGGGCGG
 GGTGAAGAAGCCGCACCGCTACCGGCCCGGCACCGTAGCCCTGCGGGAGATCCGGCGCTACCAGAAGTCC
 ACGGAGCTGCTGATCCGCAAGCTGCCCTTCCAGCGGCTGGTACGCGAGATCGCGCAGGACTTTAAGACGG
 ACCTGCGCTTCCAGAGCTCGGCCGTGATGGCGCTGCAGGAGGCCAGCGAGGCCTACCTGGTGGGGCTGTT
 CGAAGACACGAACCTGTGCGCCATCCACGCCAAGCGCGTGACCATTATGCCCAAGGACATCCAGCTGGCC
 CGCCGCATCCGTGGAGAGCGGGCTTAAGAAGTGGCGGTTGCGCCGGAGGTTCCATCGTATCCAAAAGGCT
 CTTTTCAGAGCCACCCA

SEQ ID NO: 55

Gene Name: HIST1H4A

RefSeq Accession Number: NM_003495

gi|18105065|ref|NM_003495.2| Homo sapiens histone cluster 1, H4i (HIST1H4I), mRNA

ATGTCAGGACGCGGCAAAGGAGGTAAGGGCCTGGGGAAAGGGGGTGCCAAGCGCCACCGCAAGGTGCTGC
 GCGACAACATCCAGGGTATCACCAAGCCAGCCATTCGGCGCCTTGCTCGCCGCGGCGGCGTGAAGCGCAT
 TTCTGGCCTCATCTATGAGGAGACCCGCGGAGTGTGAAGGTGTTCTGAGAAACGTGATCCGGGACGCC
 GTGACCTACACGGAGCACGCCAAGCGCAAGACGGTCACCGCCATGGACGTGGTCTACGCGCTCAAGCGCC
 AGGGCCGCACCCTCTATGGCTTCGGCGGCTAAATGGCATTTTGAAGCCCAGTCATTCTCTAAAAGGCC
 TTTTATAGGGCCCCTAAGCTT

SEQ ID NO: 56

Gene Name: SERPINA1; Synonym: AAT

RefSeq Accession Number: NM_001002236

gi|50363218|ref|NM_001002236.1| Homo sapiens serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1), transcript variant 2, mRNA

TGGGCAGGAAGTGGGCACTGTGCCCAGGGCATGCACTGCCTCCACGCAGCAACCCTCAGAGTCCTGAGCT
 GAACCAAGAAGGAGGAGGGGGTCCGGGCTCCGAGGAAGGCCCTAGCCGCTGCTGCTGCCAGGAATTCCAGG
 TTGGAGGGGGCGGCAACCTCCTGCCAGCCTTCAGGCCACTCTCCTGTGCCTGCCAGAAGAGACAGAGCTTG
 AGGAGAGCTTGAGGAGAGCAGGAAAGGGCGGCAGTAAGTCTTCAGCATCAGGCATTTTGGGGTGACTCAG
 TAAATGGTAGATCTTGCTACCAAGTGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGAGGGCCAGCTAA
 GTGGTACTCTCCAGAGACTGTCTGACTCACGCCACCCCTCCACCTTGGACACAGGACGCTGTGGTTTC
 TGAGCCAGGTACAATGACTCCTTTTCGCAGCCTCCCCCGTTGCCCTCTGGATCCACTGCTTAAATACGGA
 CGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAATCGACAATGCCG
 TCTTCTGTCTCGTGGGGCATCCTCCTGCTGGCAGGCCTGTGCTGCCTGGTCCCTGTCTCCCTGGCTGAGG
 ATCCCCAGGGAGATGCTGCCCAGAAGACAGATACATCCCACCATGATCAGGATCACCCAACCTTCAACAA
 GATCACCCCCAACCTGGCTGAGTTCGCCTTCAGCCTATACCGCCAGCTGGCACACCAGTCCAACAGCACC
 AATATCTTCTTCTCCCCAGTGAGCATCGCTACAGCCTTTGCAATGCTCTCCCTGGGGACCAAGGCTGACA
 CTCACGATGAAATCCTGGAGGGCCTGAATTTCAACCTCACGGAGATTCCGGAGGCTCAGATCCATGAAGG
 CTTCAGGAACCTCCTCCGTACCCTCAACCAGCCAGACAGCCAGCTCCAGCTGACCACCGGCAATGGCCTG
 TTCCTCAGCGAGGGCCTGAAGCTAGTGGATAAGTTTTTGGAGGATGTTAAAAAGTTGTACCACTCAGAAG
 CCTTCACTGTCAACTTCGGGGACACCGAAGAGGCCAAGAAACAGATCAACGATTACGTGGAGAAGGGTAC
 TCAAGGGAAAATTGTGGATTTGGTCAAGGAGCTTGACAGAGACACAGTTTTTGCTCTGGTGAATTACATC
 TTCTTTAAAGGCAAATGGGAGAGACCCTTTGAAGTCAAGGACACCGAGGAAGAGGACTTCCACGTGGACC
 AGGTGACCACCGTGAAGGTGCCTATGATGAAGCGTTTAGGCATGTTTAAACATCCAGCACTGTAAGAAGCT
 GTCCAGCTGGGTGCTGCTGATGAAATACCTGGGCAATGCCACCGCCATCTTCTTCTGCTGATGAGGGG
 AAACCTACAGCACCTGGAAAATGAACTACCCACGATATCATCACCAGTTCTGGAAAATGAAGACAGAA
 GGTCTGCCAGCTTACATTTACCCAACTGTCCATTACTGGAACCTATGATCTGAAGAGCGTCTGGGTCA
 ACTGGGCATCACTAAGGTCTTCAGCAATGGGGCTGACCTCTCCGGGGTCAAGAGGAGGCACCCCTGAAG
 CTCTCCAAGGCCGTGCATAAGGCTGTGCTGACCATCGACGAGAAAGGGACTGAAGCTGCTGGGGCCATGT
 TTTTAGAGGCCATACCATGTCTATCCCCCGGAGGTCAAGTTCAACAAACCCCTTGTCTTCTTAATGAT

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TGAACAAAATACCAAGTCTCCCCTCTTCATGGGAAAAGTGGTGAATCCCACCCAAAAATAACTGCCTCTC
GCTCCTCAACCCCTCCCCTCCATCCCTGGCCCCCTCCCTGGATGACATTAAAGAAGGGTTGAGCTGGTCC
CTGCCTGCAAAA

SEQ ID NO: 57

Gene Name: LUM

RefSeq Accession Number: NM_002345

gi|61742794|ref|NM_002345.3| Homo sapiens lumican (LUM), mRNA

ACAGTGAGCTTCCTTATTTGAAGCAGGACTCAATTCTTGGTTAAAAGCTATGGTATTTGAGCTAGTTAAA
CACATATCTCTCTCCCATTCATAGGGAATGAGCTGGGCTGTCCCTTCTCCCCACGTTACCTGCACTTC
GTTAGAGAGCAGTGTTCACATGCCACACCACAAGATCCCCACAATGACATAACTCCATTCAGAGACTGGC
GTGACTGGGCTGGGTCTCCCCACCCCCCTTCAGCTCTTGTATCACTCAGAATCTGGCAGCCAGTTCCGTC
CTGACAGAGTTCACAGCATATATTGGTGGATTCTTGTCCATAGTGCATCTGCTTTAAGAATTAACGAAAG
CAGTGTCAAGACAGTAAGGATTCAAACCATTTGCCAAAAATGAGTCTAAGTGCATTTACTCTCTTCCTGG
CATTGATTGGTGGTACCAGTGGCCAGTACTATGATTATGATTTTCCCCTATCAATTTATGGGCAATCATC
ACCAAAGTGTGCACCAGAATGTAAGTGGCCTGAAAGCTACCCAAGTGGCATGTACTGTGATGAGCTGAAA
TTGAAAAGTGTACCAATGGTGCCTCCTGGAATCAAGTATCTTTACCTTAGGAATAACCAGATTGACCATA
TTGATGAAAAGGCCTTTGAGAATGTAAGTGTGATCTGCAGTGGCTCATTCTAGATCACAACCTTCTAGAAAA
CTCCAAGATAAAAGGGAGAGTTTTCTCTAAATTGAAACAACTGAAGAAGCTGCATATAAACCACAACAAC
CTGACAGAGTCTGTGGGCCCCACTTCCCAAATCTCTGGAGGATCTGCAGCTTACTCATAACAAGATCACAA
AGCTGGGCTCTTTTGAAGGATTGGTAAACCTGACCTTCATCCATCTCCAGCACAATCGGCTGAAAGAGGA
TGCTGTTTCAGCTGCTTTTAAAGGTCTTAAATCACTCGAATACCTTGACTTGAGCTTCAATCAGATAGCC
AGACTGCCTTCTGGTCTCCCTGTCTCTTCTAACTCTCTACTTAGACAACAATAAGATCAGCAACATCC
CTGATGAGTATTTCAAGCGTTTTAATGCATTGCAGTATCTGCGTTTATCTCACAACGAACTGGCTGATAG
TGGAATACCTGGAAATTCTTCAATGTGTATCCCTGGTTGAGCTGGATCTGTCTATAACAAGCTTAAA
AACATACCAACTGTCAATGAAAACCTTGAAAACCTATTACCTGGAGGTCAATCAACTTGAGAAGTTTGACA
TAAAGAGCTTCTGCAAGATCCTGGGGCCATTATCCTACTCCAAGATCAAGCATTTGCGTTTGGATGGCAA
TCGCATCTCAGAAACCAGTCTTCCACCGGATATGTATGAATGTCTACGTGTTGCTAACGAAGTCACTCTT
AATTAATATCTGTATCCTGGAACAATATTTTATGGTTATGTTTTTCTGTGTGTGAGTTTTTCATAGTATCC
ATATTTTATTACTGTTTATTACTTCCATGAATTTTAAATCTGAGGGAAATGTTTTGTAAACATTTATTT
TTTTTAAAGAAAAGATGAAAGGCAGGCCTATTTATCACAAGAACACACACATATACACGAATAGACATC
AAACTCAATGCTTTATTTGTAAATTTAGTGTTTTTTTATTTCTACTGTCAAATGATGTGCAAAACCTTTT
ACTGGTTGCATGGAAATCAGCCAAGTTTTATAATCCTTAAATCTTAATGTTCCCTCAAAGCTTGGATTAAA
TACATATGGATGTTACTCTCTTGCACCAAATTATCTTGATACATTCAAATTTGTCTGGTTAAAAAATAGG
TGGTAGATATTGAGGCCAAGAATATTGCAAAATACATGAAGCTTCATGCACTTAAAGAAGTATTTTTAGA
ATAAGAATTTGCATACTTACCTAGTGAACTTTTCTAGAATTATTTTTCTACTCTAAGTCATGTATGTTTC
TCTTTGATTATTTGCATGTTATGTTTAATAAGCTACTAGCAAAATAAAACATAGCAAATGGCATCACTGT
GTTTGACTTCTTGTGAAATTTCTGTACTTTGTATATAAAATACATAAAACAATAGATTAGAAATCAAAG
ATATCTCTGGCCTGCA

SEQ ID NO: 58

Gene Name: tryptaseB

RefSeq Accession Number: NM_024164

gi|68508969|ref|NM_024164.5| Homo sapiens tryptase beta 2 (TPSB2), mRNA

AGAAGGAACAGGGAGCGGCCAGGATGCTGAATCTGCTGCTGCTGGCGCTGCCCCGTCCTGGCGAGCCGCGC
CTACGCGGCCCTGCCCCAGGCCAGGCCCTGCAGCGAGTGGGCATCGTTGGGGGTGAGGAGGCCCCCAGG
AGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCGCGACCGATACTGGATGCACTTCTGCGGGGGCTCCC
TCATCCACCCCCAGTGGGTGCTGACCGCAGCGCACTGCGTGGGACCGGACGTCAAGGATCTGGCCGCCCT
CAGGGTGCAACTGCGGGAGCAGCACCTCTACTACCAGGACCAGCTGCTGCCGGTCAGCAGGATCATCGTG
CACCCACAGTTCTACACCGCCCAGATCGGAGCGGACATCGCCCTGCTGGAGCTGGAGGAGCCGGTGAACG
TCTCCAGCCACGTCCACACGGTCACCCTGCCCCCTGCCTCAGAGACCTTCCCCCGGGGATGCCGTGCTG
GGTCACTGGCTGGGGCGATGTGGACAATGATGAGCGCCTCCCACCGCCATTTCTCTGAAGCAGGTGAAG
GTCCCCATAATGGAAAACCACATTTGTGACGCAAAATACCACCTTGGCGCCTACACGGGAGACGACGTCC
GCATCGTCCGTGACGACATGCTGTGTGCCGGGAACACCCGGAGGGACTCATGCCAGGGCGACTCCGGAGG
GCCCCCTGGTGTGCAAGGTGAATGGCACCTGGCTGCAGGCGGGCGTGGTCAGCTGGGGCGAGGGCTGTGCC
CAGCCCAACCGGCCTGGCATCTACACCCGTGTACCTACTACTTGGACTGGATCCACCACTATGTCCCCA
AAAAGCCGTGAGTCAGGCCTGGGGTGTCCACCTGGGTCACTGGAGAGCCAGCCCCCTCCTGTCCAAAACAC
CACTGCTTCCCTACCCAGGTGGCGACTGCCCCCACACCTTCCCTGCCCCGTCTGAGTGCCCCCTTCTGT
CCTAAGCCCCCTGCTCTCTTCTGAGCCCCCTTCCCCTGTCTGAGGACCTTCCCCATCCTGAGCCCCCTT
CCCTGTCTTAAGCCTGACGCCTGCACCGGGGCCCTCCGGCCCTCCCCTGCCCAGGCAGCTGGTGGTGGGCG
CTAATCCTCCTGAGTGTGGACCTCATTAAGTGCATGGAAATCA

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SEQ ID NO: 59

Gene Name: HIST2H2BE

RefSeq Accession Number: NM_003528

gi|22027639|ref|NM_003528.2| Homo sapiens histone cluster 2, H2be (HIST2H2BE), mRNA

ACTTCTTTTCTTGGCTAAGCCGCGTTTGTACTGTGTCTTACCATGCCTGAACCGGCAAAATCCGCTCCGG
CCCCTAAAAGGGCTCCAAGAAAGCCGTCACCAAAGCCCAGAAGAAAGACGGCAAGAAGCGCAAGCGCAG
CCGCAAAGAGAGCTACTCCATCTACGTGTACAAGGTGCTGAAGCAGGTCCACCCCGACACCGGCATCTCG
TCCAAGGCCATGGGCATCATGAACCTCTCGTCAACGACATCTTCGAGCGCATCGCGGGAGAGGCTTCCC
GCCTGGCGCACTACAACAAGCGCTCCACCATCACATCCCGCGAGATCCAGACGGCCGTGCGCCTGCTGCT
GCCCCGGCGAGCTGGCCAAGCACGCCGTGTCCGAGGGGCACCAAGGCGGTACCAAGTACACCAGCTCCAAG
TGAGTCCCTGCCGGGACCTGGCGCTCGCTCGCTCGAGTCGCCGGCTGCTTGACTCCAAAGGCTCTTTTCA
GAGCCACCCACCTAATCACTAGAAAAGAGCTTGTTCACTTATTCCCTTAGTTTCTTTTCATAAAGTAAGT
TATTTTAGTGTGAAGGTCATGGGAAATGGCATACTAGCTTTTAACTATTTGGAAGTCGAGGTCCCCAG
TGCGTCATTGGATTTGCTTTTGAATCTAGAGCGTGTCTTTACTCATTGTGCTGCTTAGCCTTCCCAGGAG
TCGGTTCTCAATTAGGCTGTTGGGAATCCGCCTCTTTACCCGCCCCACTCCCGCCCCACACGCGCCCTG
GTGGCTCCTTGGGTCTGTTTCATTCTAAAACGAAGTGGCTGAGTTCGGCTGTCATTTAAGAGAACTCCAG
GACACAATTCAGCCCGGTTCCGCAAACACTGCGTGACAGCTCTGTATGACTGACGCTTGGCAGCAGCTT
TTGTGTCCGGTCACCAAGTTCTGCCGTGCGATGGGGCCTCCTGTGGATAACAGCCGTTCTGTGTATTTTGG
ACGAAGGCCGCGCAGCCGGGTCCCAGCCTTGTCCTGATTGGGCGACAAGAATATTTCAAATTTCTGCGCCT
TTTTCTAATTTGTAGATTTTCAAGTTTCCGTCGTTCACTTTGAGACTTTGAAATTCCTATTTCTCATTTTGT
TGATAATTTCTGCATTTAATGGTCTGTGCTTTAAATGGTAACGCTACGGCCCCAGGTCACTGCGAGGCAC
TTACCATGTAGATACGGGCTCAAAAGTCACCTCTCAGAGACCTACGTCATCCACTCAGGAATTCGCGCCT
CTCATACTTGCTGTCTCATTTTATCTTCTTCTAGCAGCTGTCTGAAATTGGTTCGTCTGTTTTCTTGT
TTATGGTATTCTCAAGCCCTTGACAGACCGGCTAGTGTGGTTTTCCCGTGTCATCTTCAGCCTGGCACATT
ATGGACACTTAAATACTACGTATTGATCTAATATTGTTGGGTAAATTTTCCATCCCACCCTTTTCTTAA
TCGCTTCCGTGGATGGATGAAGGTTGCTGTTTCCATTAGATGTATGTGAAGGCACAGTGAAAATGG
AAATGTTCTTGGAGCTACTTCTCAAATGTATCCTTAGTCACCTCAGTGCAACAGCTGGGAGGGGGCCG
TGTTAAGATTTTTTTTGTCTACAAAGAGGAGGTGGCAATGGTAGATCCACCCTTATGCTTCTCAGTTTAGC
ATAACCTCTTATGGATTTTTCATCAAATTCAGCGTGTGGTCACTGGAAAGAGCCTTTTCTTCTCCTTTT
CTTACTCTCCCCTCATGGTGTTCCTCTTAAAGGAGAGGAGCTTTTAAATTTACACTTACCACCTCATTT
GCTTTTCTGGAGGCCATGCAATATAGGCGGGACTACAGAGTTAATCTCCTTTTACAAATGAGGCCAAGA
GAAGCCTCATTGGTTTACAGTCATGCAGCTCATACTGTCCACCCTTGTATTCTCAGATGCAGGACAATTG
CATTTTAGTTTTATTTTGTGGAGGTGCAGAATATTTACTCTTCTGTCCAACCCTTGATTCTGCCGAGGA
AGACACTGATGGTTTGTGAGTGATTCAGCTGTTTTTGGCTAAGGGCTTTTGGAGCTGATGGCAGGGGTT
TGATGAATCCAAATGAGCTCTAGACATTATCACAGACTGAATAGATCTTAACTGTCTCCTACATGTGTGT
TTTCAAATGTGTATAGATGCTATTGTTATTAATAAAGTTACCAATTAATTTAA

SEQ ID NO: 60

Gene Name: VIM

RefSeq Accession Number: NM_003380

gi|62414288|ref|NM_003380.2| Homo sapiens vimentin (VIM), mRNA

GTCCCCGCGCCAGAGACGACGCCGCGCTCCCACCACCCACACCCACCGCGCCCTCGTTCGCCTCTTCTCC
GGGAGCCAGTCCGCGCCACCGCCGCGCCAGGCCATCGCCACCCTCCGCAGCCATGTCCACCAGGTCCG
TGTCTCGTCTCTCTACCGCAGGATGTTCCGGCGGCGCGGCCACCGCGAGCCGGCCGAGCTCCAGCCGGAG
CTACGTGACTACGTCCACCCGCACCTACAGCCTGGGCAGCGCGCTGCGCCCCAGCACCAGCCGCAGCCTC
TACGCTCGTCCCCGGGCGGCGTGTATGCCACGCGCTCCTCTGCCGTGCGCCTGCGGAGCAGCGTGCCCG
GGGTGCGGCTCCTGCAGGACTCGGTGGACTTCTCGCTGGCCGACGCCATCAACACCGAGTTCAAGAACAC
CCGCACCAACGAGAAGGTGGAGCTGCAGGAGCTGAATGACCGCTTCGCCAACTACATCGACAAGGTGCGC
TTCCTGGAGCAGCAGAATAAGATCCTGCTGGCCGAGCTCGAGCAGCTCAAGGGCCAAGGCAAGTCGCGCC
TGGGGGACCTCTACGAGGAGGAGATGCGGGAGCTGCGCCGGCAGGTGGACCAGCTAACCAACGACAAAGC
CCGCGTCGAGGTGGAGCGCGACAACCTGGCCGAGGACATCATGCGCCTCCGGGAGAAATTGCAGGAGGAG
ATGCTTCAGAGAGAGGAAGCCGAAAACACCCTGCAATCTTTCAGACAGGATGTTGACAATGCGTCTCTGG
CACGTCTTGACCTTGAACGCAAAGTGGAATCTTTGCAAGAAGAGATTGCCTTTTTGAAGAACTCCACGA
AGAGGAAATCCAGGAGCTGCAGGCTCAGATTCAGGAACAGCATGTCCAAATCGATGTGGATGTTTCCAAG
CCTGACCTCACGGCTGCCCTGCGTGACGTACGTACGCAATATGAAAGTGTGGCTGCCAAGAACCTGCAGG
AGGCAGAAGAATGGTACAAATCCAAGTTTGCTGACCTCTCTGAGGCTGCCAACCAGGAACAATGACGCCCT
GCGCCAGGCAAAGCAGGAGTCCACTGAGTACCGGAGACAGGTGCAGTCCCTCACCTGTGAAGTGGATGCC
CTTAAAGGAACCAATGAGTCCCTGGAACGCCAGATGCGTGAAATGGAAGAGAACTTTGCCGTTGAAGCTG
CTAACTACCAAGACACTATTGGCCGCCTGCAGGATGAGATTCAGAATATGAAGGAGGAAATGGCTCGTCA
CCTTCGTGAATACCAAGACCTGCTCAATGTTAAGATGGCCCTTGACATTGAGATTGCCACCTACAGGAAG
CTGCTGGAAGGCGAGGAGAGCAGGATTTCTCTGCCTCTTCCAAACTTTTCTCCTGAACCTGAGGGAAA
CTAATCTGGATTCACCTCCTCTGGTTGATACCCACTCAAAAAGGACACTTCTGATTAAAGACGTTGAAAC

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TAGAGATGGACAGGTTATCAACGAACTTCTCAGCATCACGATGACCTTGAATAAAAATTGCACACACTC
AGTGCAGCAATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAAAGAAACAGCTTTCAAGTGCC
TTTCTGCAGTTTTTCAGGAGCGCAAGATAGATTTGGAATAGGAATAAGCTCTAGTTCTTAACAACCGACA
CTCCTACAAGATTTAGAAAAAGTTTACAACATAATCTAGTTTACAGAAAAATCTTGTGCTAGAATACTT
TTTAAAAGGTATTTTGAATACCATTAAACTGCTTTTTTTTTTCCAGCAAGTATCCAACCAACTTGGTTC
TGCTTCAATAAATCTTTGGAAAACTC

SEQ ID NO: 61

Gene name: PRDX2

RefSeq Accession Number: NM_181738

gi|33188453|ref|NM_181738.1| Homo sapiens peroxiredoxin 2 (PRDX2), nuclear
gene encoding mitochondrial protein, transcript variant 3, mRNA

GCTCGTCCGCTCCCTCCCCCGCGCGGTGCACGTCTTGGTTCCGGGCCGGGCATAAAAGGCTTCGCGGCCCA
GGGCTCACTTGGCGCTGAGAACGCGGGTCCACGCGTGTGATCGTCCGTGCGTCTAGCCTTTGCCACGCA
GCTTTCAGTCATGGCCTCCGGTAACGCGCGCATCGGAAAGCCAGCCCCTGACTTCAAGGCCACAGCGGTG
GTTGATGGCGCCTTCAAAGAGGTGAAGCTGTGCGACTACAAAGGGAAGTACGTGGTCCTCTTTTTCTACC
CTCTGGACTTCACTTTTGTGTGCCCCACCGAGATCATCGGTTTCAAGCAACCGTGCAGAGGACTTCCGCAA
GCTGGGCTGTGAAGTGCTGGGCGTCTCGGTGGACTCTCAGTTCACCCACCTGGCTTGGTATGAGCAGGGG
CCAAAGAGGGAGGTTGCAGCTAAGCTCACACCCTCAGGTCTAGCAGTGTGGCTTCGTGGCCATTGCTCA
ACCTCTGGAACCTGCGTTTCCCCATCGTGAAAATAATGGAAACATTGCCGCCCAAGTCTTTAAGGATGAT
GACAGTAATTAGCATTTGACAACCTAGTTGCCTGGTATATAGAGTTGCAGATGCAACTCAGATGCAACTCT
ATCTACTCTATGTACTTAGTTCCCAGGAGGGAGGCTGTGCTGCCCTATTTTCATGAAGATGGAACTCCAG
TTCACCGAAG

SEQ ID NO: 62

Gene name: CA1

RefSeq Accession Number: NM_001738

gi|4502516|ref|NM_001738.1| Homo sapiens carbonic anhydrase I (CA1), mRNA

GTGGTACCCAGTCCTCAGGTGCAACCCCTGCGTGGTCCTCTGTGGCAGCCTTCTCTCATTCAGAGCTGT
TTTCCACAGAGGTAGTGAAAAGAACTGGATTTTCAAGTTCACTTTGCAAGAGAAAAAGAAAACTCAGTAG
AAGATAATGGCAAGTCCAGACTGGGGATATGATGACAAAAATGGTCCTGAACAATGGAGCAAGCTGTATC
CCATTGCCAATGGAAATAACCAATCCCCTGTTGATATTTAAACCAGTGAAACCAAACATGACACCTCTCT
GAAACCTATTAGTGTCTCCTACAACCCAGCCACAGCCAAAGAAATTATCAATGTGGGGCATTCTTTCCAT
GTAAATTTTGGAGACAACGATAACCGATCAGTGCTGAAAGGTGGTCCTTTCTCTGACAGCTACAGGCTCT
TTCAGTTTCATTTTCACTGGGGCAGTACAAATGAGCATGGTTCAGAACATACAGTGGATGGAGTCAAATA
TTCTGCCGAGCTTCACGTAGCTCACTGGAATTCTGCAAAGTACTCCAGCCTTGCTGAAGCTGCCTCAAAG
GCTGATGGTTTGGCAGTTATTGGTGTTTTGTGATGAAGGTTGGTGAGGCCAACCCAAAGCTGCAGAAAGTAC
TTGATGCCCTCCAAGCAATTAACCAAGGGCAAACGAGCCCCATTACAAATTTTGACCCCTCTACTCT
CCTTCCTTCATCCCTGGATTTCTGGACCTACCCTGGCTCTCTGACTCATCCTCCTCTTTATGAGAGTGTA
ACTTGATCATCTGTAAGGAGAGCATCAGTGTCAGCTCAGAGCAGCTGGCACAATTCCGCAGCCTTCTAT
CAAATGTTGAAGGTGATAACGCTGTCCCCATGCAGCACAAACCGCCCAACCCAACTCTGAAGGGCAG
AACAGTGAGAGCTTCATTTTGTGATGATTCTGAGAAGAACTTGTCTTCTCAAGAACACAGCCCTGCTTC
TGACATAATCCAGTTAAATAATAATTTTAAAGAAATAAATTTATTTCATATTAGCAAGACAGCATGCC
TTCAAATCAATCTGTAAACTAAGAACTTAAATTTTAGTTCTTACTGCTTAATTCAAATAATAATTAGT
AAGCTAGCAAATAGTAATCTGTAAGCATAAGCTTATCTTAAATTCAAGTTTAGTTTGAGGAATTCTTTAA
AATTACAATAAGTGATTTGTATGTCTATTTTTTTTTCAGTTTATTTGAACCAATAAAATAATTTTATCTCT
TTCT

SEQ ID NO: 63

Gene name: BLVRB

RefSeq Accession Number: NM_000713

gi|4502418|ref|NM_000713.1| Homo sapiens biliverdin reductase B (flavin
reductase (NADPH)) (BLVRB), mRNA

GAATTCGAATGCCACCCTCCCAGATGGGGGCAGAGAGCACCGCCCAGCAGCCAGTGGGTTCCCGCGCGTG
CCGAGACTCTGAGGCCTTGCACCCCCACGATCCCGTACGATGGCCGTCAAGAAGATCGCGATCTTCGGCG
CCACTGGCCAGACCGGGCTCACCAACCTGGCGCAGGCGGTGCAAGCAGGTTACGAAGTGACAGTGCTGGT
GCGGGACTCCTCCAGGCTGCCATCAGAGGGGGCCCCGGCGGCCACGTGGTAGTGGGAGATGTTCTGCAG
GCAGCCGATGTGGACAAGACCGTGGCTGGCCAGGACGCTGTCATCGTGCTGCTGGGCACCCGCAATGACC
TCAGTCCCACGACAGTTATGTCCGAGGGCGCCCGGAACATTGTGGCAGCCATGAAGGCTCATGGTGTGGA
CAAGGTCGTGGCCTGCACCTCGGCTTTCCTGCTCTGGGACCCTACCAAGGTGCCCCACGACTGCAGGCT
GTGACTGATGACCACATCCGGATGCACAAGGTGCTGCGGGAATCAGGCCTGAAGTACGTGGCTGTGATGC
CGCCACACATAGGAGACCAGCCACTAACTGGGGCGTACACAGTGACCCTGGATGGACGAGGGCCCTCAAG
GGTCATCTCCAACATGACCTGGGCCATTTTCATGCTGCGCTGCCTCACCACCGATGAGTACGACGGACAC

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AGCACCTACCCCTCCCACCAGTACCAGTAGCACTCTGTCCCCATCTGGGAGGGTGGCATTCTGGACATGA
GGAGCAAAGGAAGGGGGCAATAAATGTTGAGCCAAGAGCTTCAAATTACTCTAG

SEQ ID NO: 64

Gene name: KRT14

RefSeq Accession Number: NM_000526

gi|15431309|ref|NM_000526.3| Homo sapiens keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner) (KRT14), mRNA

ACCCGAGCACCTTCTCTTCACTCAGCCAAGTCTCGCTCGCTCACCTCCCTCCTCTGCACCATGACTACC
TGCAGCCGCCAGTTCACCTCCTCCAGCTCCATGAAGGGCTCCTGCGGCATCGGGGGCGGCATCGGGGGCG
GCTCCAGCCGCATCTCCTCCGTCCTGGCCGGAGGGTCTGCGCGCCCCCAGCACCTACGGGGGCGGCCT
GTCTGTCTCATCCTCCCGCTTCTCCTCTGGGGGAGCCTACGGGCTGGGGGGCGGCTATGGCGGTGGCTTC
AGCAGCAGCAGCAGCAGCTTTGGTAGTGGCTTTGGGGGAGGATATGGTGGTGGCCTTGGTGGTGGCTTGG
GTGGTGGCTTTGGTGGTGGCTTTGCTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
GAACCTCAATGACCGCCTGGCCTCCTACCTGGACAAGGTGCGTGCTCTGGAGGAGGCCAACGCCGACCTG
GAAGTGAAGATCCGTGACTGGTACCAGAGGCAGCGGCCTGCTGAGATCAAAGACTACAGTCCCTACTTCA
AGACCATTGAGGACCTGAGGAACAAGATTCTCACAGCCACAGTGGACAATGCCAATGTCCTTCTGCAGAT
TGACAATGCCCCGTCTGGCCGCGGATGACTTCCGCACCAAGTATGAGACAGAGTTGAACCTGCGCATGAGT
GTGGAAGCCGACATCAATGGCCTGCGCAGGGTGTGGACGAAGTACCCTGGCCAGAGCTGACCTGGAGA
TGCAGATTGAGAGCCTGAAGGAGGAGCTGGCCTACCTGAAGAAGAACCACGAGGAGGAGATGAATGCCCT
GAGAGGCCAGGTGGGTGGAGATGTCAATGTGGAGATGGACGCTGCACCTGGCGTGGACCTGAGCCGCATT
CTGAACGAGATGCGTGACCAGTATGAGAAGATGGCAGAGAAGAACCGCAAGGATGCCGAGGAATGGTTCT
TCACCAAGACAGAGGAGCTGAACCGCGAGGTGGCCACCAACAGCGAGCTGGTGCAGAGCGGCAAGAGCGA
GATCTCGGAGCTCCGGCGCACCATGCAGAACCTGGAGATTGAGCTGCAGTCCCAGCTCAGCATGAAAGCA
TCCCTGGAGAACAGCCTGGAGGAGACCAAAGGTGCTACTGCATGCAGCTGGCCCAGATCCAGGAGATGA
TTGGCAGCGTGGAGGAGCAGCTGGCCCAGCTCCGCTGCGAGATGGAGCAGCAGAACCAGGAGTACAAGAT
CCTGCTGGACGTGAAGACGCGGCTGGAGCAGGAGATCGCCACCTACCGCCGCTGCTGGAGGGCGAGGAC
GCCACCTCTCCTCCTCCCAGTTCTCCTCTGGATCGCAGTCATCCAGAGATGTGACCTCCTCCAGCCGCC
AAATCCGCACCAAGGTCATGGATGTGCACGATGGCAAGGTGGTGTCCACCCACGAGCAGGTCCTTCGCAC
CAAGAACTGAGGCTGCCCAGCCCCGCTCAGGCCTAGGAGGCCCCCGTGTGGACACAGATCCCCTGAA
GATCCCCTCTCCTGCCAAGCACTTCACAGCTGGACCCTGCTTCACCCTCACCCCCTCCTGGCAATCAAT
ACAGCTTCATTATCTGAGTTGCAT

SEQ ID NO: 65

Gene name: PBRM1

RefSeq Accession Number: NM_181042

gi|93102368|ref|NM_181042.2| Homo sapiens polybromo 1 (PBRM1), transcript variant 4, mRNA

TCCATGGGTTCCAAGAGAAGAAGAGCTACCTCCCCTTCCAGCAGTGTGAGCGGGGACTTTGATGATGGGC
ACCATTCTGTGTCAACACCAGGCCCAAGCAGGAAAAGGAGGAGACTTTCCAATCTTCCAAGTGTAGATCC
TATTGCCGTGTGCCATGAACCTCTATAATACCATCCGAGACTATAAGGATGAACAGGGCAGACTTCTCTGT
GAGCTCTTCATTAGGGCACCAAAGCGAAGAAATCAACCAGACTATTATGAAGTGGTTTCTCAGCCCATTG
ACTTGATGAAAATCCAACAGAACTAAAAATGGAAGAGTATGATGATGTTAATTTGCTGACTGCTGACTT
CCAGCTTCTTTTTTAACAATGCAAAGTCCTATTATAAGCCAGATTCTCCTGAATATAAAGCCGCTTGCAAA
CTCTGGGATTTGTACCTTCGAACAAGAAATGAGTTTGTTCAGAAAGGAGAAGCAGATGACGAAGATGATG
ATGAAGATGGGCAAGACAATCAGGGCACAGTGAAGGATCTTCTCCAGCTTACTTGAAGGAGATCCT
GGAGCAGCTTCTTGAAGCCATAGTTGTAGCTACAAATCCATCAGGACGTCTCATTAGCGAACTTTTTTCAG
AACTGCCTTCTAAAGTGCAATATCCAGATTATTATGCAATAATTAAGGAGCCTATAGATCTCAAGACCA
TTGCCCAGAGGATACAGAATGGAAGCTACAAAAGTATTATGCAATGGCCAAAGATATAGATCTCCTCGC
AAAAAATGCCAAACTTATAATGAGCCTGGCTCTCAAGTATTCAAGGATGCAAATTCAAATAAAAAATA
TTTTATATGAAAAGGCTGAAATTGAACATCATGAAATGGCTAAGTCAAGTCTTCGAATGAGGACTCCAT
CCAATTGGCTGCAGCCAGACTGACAGGTCTTACACAGTAAAGGCAGCCTTGGTGAAGAGAGAAATCC
CACTAGCAAGTATTACCGTAATAAAAGAGCAGTACAAGGAGGTGTTTTATCAGCAATTACAATGGCACTT
CAATATGGCTCAGAAAGTGAAGAAGATGCTGCTTTAGCTGCTGCACGCTATGAAGAGGGAGAGTCAGAAG
CAGAAAGCATCACTTCCTTTATGGATGTTTCAAATCCTTTTTATCAGCTTTATGACACAGTTAGGAGTTG
TCGGAATAACCAAGGGCAGCTAATAGCTGAACCTTTTTTACCATTTGCCTTCAAAGAAAAAATACCCTGAT
TATTACCAGCAAATTAATAATGCCCATATCACTACAACAGATCCGAACAAAAGTGAAGAATCAAGAATATG
AACTTTAGATCATTTGGAGTGTGATCTGAATTTAATGTTTGAAAATGCCAAACGCTATAATGTGCCCAA
TTCAGCCATCTACAAGCGAGTTCTAAAATTGCAGCAAGTTATGCAGGCAAAGAAGAAAGAGCTTGCCAGG
AGAGACGATATCGAGGACGGAGACAGCATGATCTTTCAGCCACCTCTGATACTGGTAGTGCCAAAAGAA
AAAGTAAAAAGAACATAAGAAAGCAGCGAATGAAAATCTTATTCAATGTTGTTCTTGAAGCTCGAGAGCC
AGGTTTCAGGCAGAAGACTTTGTGACCTATTTATGGTTAAACCATCCAAAAAGGACTATCCTGATTATTAT
AAAATCATCTTGGAGCCAATGGACTTGAAAATAATTGAGCATAACATCCGCAATGACAAATATGCTGGTG

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AAGAGGGAATGATAGAAGACATGAAGCTGATGTTCCGGAATGCCAGGCACTATAATGAGGAGGGCTCCCA
GGTTTATAATGATGCACATATCCTGGAGAAGTTACTCAAGGAGAAAAGGAAAGAGCTGGGCCCCTGCCT
GATGATGATGACATGGCTTCTCCCAAACCTCAAGCTGAGTAGGAAGAGTGGCATTCTCCTAAAAAATCAA
AATACATGACTCCAATGCAGCAGAACTAAATGAGGTCTATGAAGCTGTAAAGAACTATACTGATAAGAG
GGGTCGCCGCCTCAGTGCCATATTTCTGAGGCTTCCCTCTAGATCTGAGTTGCCTGACTACTATCTGACT
ATTAAAAAGCCCATGGACATGGAAAAAATTCGAAGTCACATGATGGCCAACAAGTACCAAGATATTGACT
CTATGGTTGAGGACTTTGTCATGATGTTTAATAATGCCTGTACATACAATGAGCCGGAGTCTTTGATCTA
CAAAGATGCTCTTGTTCTACACAAAGTCTGCTTGAAACACGCAGAGACCTGGAGGGAGATGAGGACTCT
CATGTCCCAAATGTGACTTTGCTGATTCAAGAGCTTATCCACAATCTTTTTGTGTGTCAGTCATGAGTCATC
AGGATGATGAGGGAAGATGCTACAGCGATTCTTTAGCAGAAATTCCTGCTGTGGATCCCAACTTTCTTAA
CAAACCACCCCTTACATTTGACATAATTAGGAAGAATGTTGAAAATAATCGCTACCGTCGGCTTGATTTA
TTTCAAGAGCATATGTTTGAAGTATTGGAACGAGCAAGAAGGATGAATCGGACAGATTGAGAAATATATG
AAGATGCAGTAGAACTTCAGCAGTTTTTTTATTAATAATTCGTGATGAACTCTGCAAAAATGGAGAGATTCT
TCTTTCACCGGCACTCAGCTATACCACAAAACATTTGCATAATGATGTGGAGAAAGAGAGAAAGGAAAAA
TTGCCAAAAGAAATAGAGGAAGATAAACTAAAACGAGAAGAAGAAAAAGAGAAGCTGAAAAGAGTGAAG
ATTCTCTGGTGCTGCAGGCCTCTCAGGCTTACATCGCACATACAGCCAGGACTGTAGCTTTAAAAACAG
CATGTACCATGTTGGAGATTACGTCTATGTGGAACCTGCAGAGGCCAACCTACAACCACATATCGTCTGT
ATTGAAAGACTGTGGGAGGATTGAGCTGGTGAAAAATGGTTGTATGGCTGTTGGTTTTACCGACCAAATG
AAACATTCCACCTGGCTACACGAAAATTTCTAGAAAAAGAGTTTTTAAGAGTGACTATTACAACAAAGT
TCCAGTTAGTAAATTTCTAGGCAAGTGTGTGGTCATGTTTGTCAAGGAATACTTTAAGTTATGCCCAGAA
AACTTCCGAGATGAGGATGTTTTTGTCTGTGAATCACGGTATTCTGCCAAAACCAAATCTTTTAAGAAAA
TTAAACTGTGGACCATGCCATCAGCTCAGTCAGGTTTTGTCCCTCGGGATGTGCCTCTGCCTGTGGTTTCG
CGTGGCCTCTGTATTTGCAAATGCAGATAAAGGTGATGATGAGAAGAATACAGACAACTCAGAGGACAGT
CGAGCTGAAGACAATTTTAACCTTGAAAAGGAAAAAGAGATGTCCCTGTGGAAATGTCCAATGGTGAAC
CAGGTTGCCACTACTTTGAGCAGCTCCATTACAATGACATGTGGCTGAAGGTTGGCGACTGTGTCTTCAT
CAAGTCCCATGGCCTGGTGCGTCCCTCGTGTGGGCAGAATTGAAAAAGTATGGGTTTCGAGATGGAGCTGCA
TATTTTTATGGCCCCATCTTCATTCACCCAGAAGAAACAGAGCATGAGCCCACAAAAATGTTCTACAAAA
AAGAAGTATTTCTGAGTAATCTGGAAGAAACCTGCCCCATGACATGTATTCTCGGAAAGTGTGCTGTGTT
GTCATTCAAGGACTTCCTCTCCTGCAGGCCAACTGAAATACCAGAAAATGACATTCTGCTTTGTGAGAGC
CGCTACAATGAGAGCGACAAGCAGATGAAGAAATTCAAAGGATTGAAGAGGTTTTCACTCTCTGCTAAAG
TGGTAGATGATGAAATTTACTACTTCAGAAAACCAATTGTTCTCAGAAGGAGCCATCACCTTTGCTGGA
AAAGAAGATCCAGTTGCTAGAAGCTAAATTTGCCGAGTTAGAAGGTGGAGATGATGATATTGAAGAGATG
GGAGAAGAAGATAGTGAGGTCATTGAACCTCCTTCTCTACCTCAGCTTCAGACCCCCCTGGCCAGTGAGC
TGGACCTCATGCCCTACACACCCCCACAGTCTACCCCAAAGTCTGCCAAAGGCAGTGCAAAGAAGGAAGG
CTCCAAACGGAAAATCAACATGAGTGGCTACATCCTGTTGAGCAGTGAGATGAGGGCTGTGATTAAGGCC
CAACACCCAGACTACTCTTTCGGGGAGCTCAGCCGCCTGGTGGGGACAGAATGGAGAAATCTTGAGACAG
CCAAGAAAGCAGAATATGAAGGTGTGATGAACCAAGGAGTGGCCCTATGGTAGGGACTCCAGCACCAGG
TGGAAGTCCATATGGACAACAGGTGGGAGTTTTGGGGCCTCCAGGGCAGCAGGCACCACCTCCATATCCC
GGCCACATCCAGCTGGACCCCTGTCTACAGCAGCCAACAACACCCATGTTTGTAGCTCCCCACCAA
AGACCCAGCGGCTTCTTCACTCAGAGGCCTACCTGAAATACATTGAAGGACTCAGTGCGGAGTCCAACAG
CATTAGCAAGTGGGATCAGACACTGGCAGCTCGAAGACGCGACGTCCATTTGTGCGAAAGAACAGGAGAGC
CGCCTACCTCTCACTGGCTGAAAAGCAAAGGGGCCACACCACCATGGCAGATGCCCTCTGGCGCCTTC
GAGATTTGATGCTCCGGGACACCTCAACATTCGCCAAGCATAACCTAGAAAATGTTTAATCACATCA
TTACGTTTCTTTTATATAGAAGCATAAAGAGTTGTGGATCAGTAGCCATTTTAGTTACTGGGGGTGGGGG
GAAGGAACAAAGGAGGATAATTTTTATTGCATTTTACTGTACATCACAAGGCCATTTTTATATACGGACA
CTTTTAATAAGCTATTTCAATTTGTTTGTATATTAAGTTGACTTTATCAAATACACAAAGATTTTTTTG
CATATGTTTTCCTTCGTTTAAACCAGTTTCATAATTGGTTGTATATGTAGACTTGGAGTTTTATCTTTTT
ACTTGTGTCATGGAAGTGAACCATTAGAGGTTTTTGTCTTGGCTTGGGGTTTTTGTCTTCTTGGTTTT
GGGTTTTTTTTATATATATATATAAAGAACAAAATGAAAAAACACACACACAAGAGTTTACAGATT
AGTTTAAATTGATAATGAAATGTGAAGTTTGTCTAGTTTACATCTTAGAGAGGGGAGTATACTTGTGTT
TGTTTCATGTGCCTGAATATCTTAAGCCACTTTCTGCAAAAGCTGTTTCTTACAGATGAAGTGCTTTCTT
TGAAAGGTGGTTATTTAGGTTTTAGATGTTTAATAGACACAGCAGATTTGCTCTATTAACCTCAGAGGCTC
ACTACAGAAATATGTAATCAGTGCTGTGCATCTGTCTGCAGCTAATGTACCTCCTGGACACCAGGAGGGG
AAAAAGCACTTTTTCAATTGTGCTGAGTTAGACATCTGTGAGTTAGACTATGGTGTGAGTATTTTGCA
GAACACGTGCACAACCCTGAGGTATGTTTAATCTAGGCAGGTACGTTTAAGGATATTTTGATCTATTTAT
AATGAATTCACAATTTATGCCTATAAATTCAGATGATTTAAATTTTAAACCTGTTACATTGAAAAACA
TTGAAGTTCGTCTTGAAGAAAGCATTAAGGTATGCATGGAGGTGATTTATTTTTAAACATAACACCTAAC
CTAACATGGGTAAAGAGAGTATGGAAGTATGAGCTGTATAAGAAGCATAATTGTGAACAAGTAGATT
GATTGCCTTCATATACAAGTATGTTTTAGTATTCCTTATTTCTTATTATCAGATGTATTTTTCTTTTA
AGTTTCAATGTTGTTATAATTCTCAACCAGAAATTTAATACTTTCTAAAATATTTTTTAAATTTAGCTTG
TGCTTTTGAATTACAGGAGAAGGGAATCATAATTTAATAAACGCTTACTAGAAAGACCATTACAGATCC
CAAACACTTGGGTTTGGTGACCCTGTCTTCTTATATGACCCTACAATAAACATTTGAAGGCAGCATAGG
ATGGCAGACAGTAGGAACATTGTTTCACTTGGCGGCATGTTTTTGAAACCTGCTTTATAGTAACTGGGTG

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ATTGCCATTGTGGTAGAGCTTCCACTGCTGTTTATAATCTGAGAGAGTTAATCTCAGAGGATGCTTTTTT
CCTTTTAATCTGCTATGAATCAGTACCCAGATGTTTAATTACTGTACTTATTAAATCATGAGGGCAAAG
AGTGTAGAATGGAAAAAGTCTCTTGTATCTAGATACTTTAAATATGGGAGGCCCTTTAACTTAATTGCC
TTTAGTCAACCACTGGATTTGAATTTGCATCAAGTATTTTAAATAATATTGAATTTAAAAAATGTATTG
CAGTAGTGTGTCAGTACCTTATTGTTAAAGTGAGTCAGATAAATCTTCAATTCCTGGCTATTTGGGCAAT
TGAATCATCATGGACTGTATAATGCAATCAGATTATTTTGTCTTAGACATCCTTGAATTACACCAAAGA
ACATGAAATTTAGTTGTGGTTAAATTATTTATTTATTTTATTCATGCATTCATTTTATTTCCCTTAAGGTCTGG
ATGAGACTTCTTTGGGGAGCCTCTAAAAAATTTTCACTGGGGGCCACGTGGGTCAATTAGAAGCCAGAG
CTCTCCTCCAGGCTCCTTCCCAGTGCCTAGAGGTGCTATAGGAAACATAGATCCAGCCAGGGGCTTCCCT
AAAGCAGTGCAGCACCGGCCAGGGCATCACTAGACAGGCCCTAATTAAGTTTTTTTTTAAAAAGCCTGTG
TATTTATTTTAGAATCATGTTTTTCTGTATATTAACCTGGGGGATATCGTTAATATTTAGGATATAAGAT
TTGAGGTGAGCCATCTTCAAAAAAGAAAAAATTTGACTCAAGAAAGTACAAGTAACTATACACCTTT
TTTTCATAGTTTTTAGGAACTGTAGTAATGTGGCTTAGAAAGTATAATGGCCTAAATGTTTTCAAAATGT
AAGTTCCTGTGGAGAAGAATTGTTTATATTGCAAACGGGGGGACTGAGGGGAACCTGTAGGTTTAAACA
GTATGTTTGTGAGCCAACTGATTTAAAGGCCTTTAACTGTTTTGGTTGTTGTTTTTTTTTTAAGCCACT
CTCCCCCTTCCCTATGAGGAAGAATTGAGAGGGGCACCTATTTCTGTAAAATCCCCAAATTGGTGTTGATGA
TTTTGAGCTTGAATGTTTTCATACCTGATTAAACTTGGTTTATTCTAATTTCTGTATCATATCATCTGA
GGTTTACGTGGTAACTAGTCTTATAACATGTATGTATCTTTTTTTTGTGTTTCATCTAAAGCTTTTTAAT
CCAAATAAATACAGAGTTTGCAAAGTGA

SEQ ID NO: 66

Gene name: PKM2

RefSeq Accession Number: NM_182471gi|33286421|ref|NM_182471.1| Homo sapiens pyruvate kinase, muscle (PKM2),
transcript variant 3, mRNA

CGCCGCGCTTCCTCCTGAAGGTGACTGCGCCCGCGGGGACGCAGGGGGCGGGGCCCGGGTCCGCCGGAGC
CGGGATTGGGCAGAGGGCGGGGCGGCGGAGGATTGCGGCGGCCCGCAGCGGGATAACCTTGAGGCTGAG
GCAGTGGCTCCTTGACAGCAGCTGCACGCGCCGTGGCTCCGGATCTCTTCGTCTTTGCAGCGTAGCCCG
AGTTCGGTCAGCGCCGGAGGACCTCAGCAGCCATGTCGAAGCCCCATAGTGAAGCCGGGACTGCCTTCATT
CAGACCCAGCAGCTGCACGCAGCCATGGCTGACACATTCTGGAGCACATGTGCCGCCTGGACATTGATT
CACCACCCATCACAGCCCGGAACACTGGCATCATCTGTACCATTTGGCCCAGCTTCCCGATCAGTGGAGAC
GTTGAAGGAGATGATTAAGTCTGGAATGAATGTGGCTCGTCTGAACCTTCTCTCATGGAACCTCATGAGTAC
CATGCGGAGACCATCAAGAATGTGCGCACAGCCACGGAAAGCTTTGCTTCTGACCCCATCCTCTACCGGC
CCGTTGCTGTGGCTCTAGACACTAAAGGACCTGAGATCCGAACCTGGGCTCATCAAGGGCAGCGGCACTGC
AGAGGTGGAGCTGAAGAAGGGAGCCACTCTCAAATCACGCTGGATAACGCCTACATGGAAAAGTGTGAC
GAGAACATCCTGTGGCTGGACTACAAGAACATCTGCAAGGTGGTGGAAAGTGGGCAGCAAGATCTACGTGG
ATGATGGGCTTATTTCTCTCCAGGTGAAGCAGAAAGGTGCCGACTTCCTGGTGACGGAGGTGGAAAATGG
TGGCTCCTTGGGCAGCAAGAAGGGTGTGAACCTTCCTGGGGCTGCTGTGGACTTGCCTGCTGTGTGCGAG
AAGGACATCCAGGATCTGAAGTTTGGGGTTCGAGCAGGATGTTGATATGGTGTGTTGCGTCATTCATCCGCA
AGGCATCTGATGTCCATGAAGTTAGGAAGGTCTGGGAGAGAAGGGAAAGAACATCAAGATTATCAGCAA
AATCGAGAATCATGAGGGGGTTCGGAGGTTTGATGAAATCCTGGAGGCCAGTGATGGGATCATGGTGGCT
CGTGGTGATCTAGGCATTGAGATTCCTGCAGAGAAGGTCTTCCTTGCTCAGAAGATGATGATTGGACGGT
GCAACCGAGCTGGGAAGCCTGTCATCTGTGCTACTCAGATGCTGGAGAGCATGATCAAGAAGCCCCGCC
CACTCGGGCTGAAGGCAGTGATGTGGCCAATGCAGTCCTGGATGGAGCCGACTGCATCATGCTGTCTGGA
GAAACAGCCAAAGGGGACTATCCTCTGGAGGCTGTGCGCATGCAGCACCTGATAGCTCGTGAGGCTGAGG
CAGCCATGTTCCACCGCAAGCTGTTTGAAGAACTTGTGCGAGCCTCAAGTCACTCCACAGACCTCATGGA
AGCCATGGCCATGGGCAGCGTGGAGGCTTCTTATAAGTGTTTAGCAGCAGCTTTGATAGTTCTGACGGAG
TCTGGCAGGTCTGCTCACCAGGTGGCCAGATACCGCCACCTGCCCCCATCATTTGCTGTGACCCGGAATC
CCCAGACAGCTCGTCAGGCCACCTGTACCGTGGCATCTTCCCTGTGCTGTGCAAGGACCCAGTCCAGGA
GGCCTGGGCTGAGGACGTGGACCTCCGGGTGAACTTTGCCATGAATGTTGGCAAGGCCCGAGGCTTCTTC
AAGAAGGGAGATGTGGTCATTGTGCTGACCGGATGGCGCCCTGGCTCCGGCTTCACCAACACCATGCGTG
TTGTTCCCTGTGCCGTGATGGACCCAGAGCCCCTCCTCCAGCCCCTGTCCCACCCCTTCCCCAGCCCA
TCCATTAGGCCAGCAACGCTTGTAGAACTCACTCTGGGCTGTAACGTGGCACTGGTAGGTTGGGACACCA
GGGAAGAAGATCAACGCCTCACTGAAACATGGCTGTGTTTGCAGCCTGCTCTAGTGGGACAGCCCAGAGC
CTGGCTGCCCATCATGTGGCCCCACCCAATCAAGGGAAGAAGGAGGAATGCTGGACTGGAGGCCCTGGA
GCCAGATGGCAAGAGGGTGACAGCTTCCTTTCTGTGTGTAATCTGTCCAGTTCCCTTTAGAAAAAATGGA
TGCCAGAGGACTCCCAACCTGGCTTGGGGTCAAGAAACAGCCAGCAAGAGTTAGGGGCCCTTAGGGCAC
TGGGCTGTTGTTCCATTGAAGCCGACTCTGGCCCTGGCCCTTACTTGCTTCTCTAGCTCTCTAGGCCTCT
CCAGTTTGCACCTGTCCCCACCCTCCACTCAGCTGTCTTGCAGCAAACACTCCACCCTCCACCTTCCATT
TTCCCCACTACTGCAGCACCTCCAGGCCTGTTGCTATAGAGCCTACCTGTATGTCAATAAACACAGCT
GAAGCACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 67

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Gene name: ANXA1

RefSeq Accession Number: NM_000700

gi|4502100|ref|NM_000700.1| Homo sapiens annexin A1 (ANXA1), mRNA
AGTGTGAAATCTTCAGAGAAGAATTTCTCTTTAGTTCTTTGCAAGAAGGTAGAGATAAAGACACTTTTTTC
AAAAATGGCAATGGTATCAGAATTCCTCAAGCAGGCCTGGTTTATTGAAAATGAAGAGCAGGAATATGTT
CAAACGTGTGAAGTCATCCAAAGGTGGTCCCGGATCAGCGGTGAGCCCCTATCCTACCTTCAATCCATCCT
CGGATGTCGCTGCCTTGCATAAGGCCATAATGGTTAAAGGTGTGGATGAAGCAACCATCATTGACATTCT
AACTAAGCGAAACAATGCACAGCGTCAACAGATCAAAGCAGCATATCTCCAGGAAACAGGAAAGCCCCTG
GATGAAACACTTAAGAAAGCCCTTACAGGTCACCTTGAGGAGGTTGTTTTAGCTCTGCTAAAACTCCAG
CGCAATTTGATGCTGATGAACCTTCGTGCTGCCATGAAGGGCCTTGGAAGTGAAGATACTCTAATTGA
GATTTTGGCATCAAGAACTAACAAAGAAATCAGAGACATTAACAGGGTCTACAGAGAGGAACTGAAGAGA
GATCTGGCCAAAGACATAACCTCAGACACATCTGGAGATTTTCGGAACGCTTTGCTTTCTCTTGCTAAGG
GTGACCGATCTGAGGACTTTGGTGTGAATGAAGACTTGGCTGATTGAGATGCCAGGGCCTTGTATGAAGC
AGGAGAAAGGAGAAAGGGGACAGACGTAAACGTGTTCAATACCATCCTTACCACCAGAAGCTATCCACAA
CTTCGAGAGTGTTCAGAAATACACCAAGTACAGTAAGCATGACATGAACAAAGTTCTGGACCTGGAGT
TGAAAGGTGACATTGAGAAATGCCTCAGAGCTATCGTGAAGTGCGCCACAAGCAAACCAGCTTTCTTTGC
AGAGAAGCTTCATCAAGCCATGAAAGGTGTTGGAAGTCGCCATAAGGCATTGATCAGGATTATGGTTTCC
CGTTCTGAAATTGACATGAATGATATCAAAGCATTCTATCAGAAGATGTATGGTATCTCCCTTTGCCAAG
CCATCCTGGATGAAACCAAAGGAGATTATGAGAAAATCCTGGTGGCTCTTTGTGGAGGAAACTAAACATT
CCCTTGATGGTCTCAAGCTATGATCAGAAGACTTTAATTATATATTTTCATCCTATAAGCTTAAATAGGA
AAGTTTCTTCAACAGGATTACAGTGTAGCTACCTACATGCTGAAAAATATAGCCTTTAAATCATTTTTAT
ATTATAACTCTGTATAATAGAGATAAGTCCATTTTTTAAAAATGTTTTCCCAAACCATAAAACCTATA
CAAGTTGTTCTAGTAACAATACATGAGAAAGATGTCTATGTAGCTGAAAATAAAATGACGTCACAAGAC

SEQ ID NO: 68

Gene name: NPM1

Accession Number: BC016768

gi|16876991|gb|BC016768.1| Homo sapiens nucleophosmin (nucleolar
phosphoprotein B23, numatrin), mRNA (cDNA clone MGC:22724 IMAGE:4081364),
complete cds

GGGGCCTGGTGTGATTCCGTCCTGCGCGGTTGTTCTCTGGAGCAGCGTTCTTTTATCTCCGTCCGCCTTC
TCTCCTACCTAAGTGCGTGCCGCCACCCGATGGAAGATTGATGGACATGGACATGAGCCCCCTGAGGCC
CCAGAACTATCTTTTCGGTTGTGAACATAAGGCCGACAAAGATTATCACTTTAAGGTGGATAATGATGAA
AATGAGCACCAGTTATCTTTAAGAACGGTCAGTTTAGGGGCTGGTGCAAAGGATGAGTTGCACATTGTTG
AAGCAGAGGCAATGAATTACGAAGGCAGTCCAATTAAAGTAACACTGGCAACTTTGAAAATGTCTGTACA
GCCAACGGTTTCCCTTGGGGGCTTTGAAATAACACCACCAGTGGTCTTAAGGTTGAAGTGTGGTTCAGGG
CCAGTGCATATTAGTGGACAGCACTTAGTAGCTGTGGAGGAAGATGCAGAGTCAGAAGATGAAGAGGAGG
AGGATGTGAAACTCTTAAGTATATCTGGAAAGCGGTCTGCCCCTGGAGGTGGTAGCAAGGTTCCACAGAA
AAAAGTAAACTTGCTGCTGATGAAGATGATGACGATGATGATGAAGAGGATGATGATGAAGATGATGAT
GGTGTGATGATTTTGATGATGAGGAAGCTGAAGAAAAAGCGCCAGTGAAGAAATCTATACGAGATACTCCAG
CCAAAAATGCACAAAAGTCAAATCAGAATGGAAAAGACTCAAACCATCATCAACACCAAGATCAAAGG
ACAAGAATCCTTCAAGAAACAGGAAAAAACTCCTAAAACACCAAAAGGACCTAGTTCTGTAGAAGACATT
AAAGCAAAAATGCAAGCAAGTATAGAAAAAGGTGGTTCTCTTCCCAAAGTGGAAGCCAAATTCATCAATT
GTGTGAAGAATTGCTTCCGGATGACTGACCAAGAGGCTATTCAAGATCTCTGGCAGTGGAGGAAGTCTCT
TTAAGAAAATAGTTTAAACAATTTGTTAAAAAATTTTCCGTCTTATTTTCTGTAACAGTTGATATC
TGGCTGTCTTTTATAATGCAGAGTGAGAACTTTCCCTACCGTGTTTGATAAATGTTGTCCAGGTTCTA
TTGCCAAGAATGTGTTGTCCAAAATGCCGTTTAGTTTTTAAAGATGGAAGTCCACCCTTTGCTTGGTTTT
AAGTATGTATGGAATGTTATGATAGGACATAGTAGTAGCGGTGGTCAGACATGGAAATGGTGGGGAGACA
AAAATATACATGTGAAATAAACTCAGTATTTTAAATAAAGTGAAAAAATAAATAAATAAATAAATAAATAA
A

SEQ ID NO: 69

Gene name: HSPB1

RefSeq accession number: NM_001540

gi|4996892|ref|NM_001540.2| Homo sapiens heat shock 27kDa protein 1 (HSPB1),
mRNA

CTCAAACACCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGCCGAGCCCAGCGCCCCGCA
CTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAGCATGACCGAGCGCCGCGTCCCCTTCTCGCTCCTG
CGGGGCCCCAGCTGGGACCCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGGCCTTCGGGC
TGCCCCGGCTGCCGGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGCTGGCCAGGCTACGTGCGCCCCCT
GCCCCCGCGCCCATCGAGAGCCCCGAGTGGCCGCGCCCGCCTACAGCCGCGCGCTCAGCCGGCAACTC
AGCAGCGGGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGATGTCAACCACTTCG
CCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGTGGTGGAGATCACCGGCAAGCACGAGGAGCGGCA

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GGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAATACACGCTGCCCCCGGTGTGGACCCACC
CAAGTTTCCTCCTCCCTGTCCCCTGAGGGCACACTGACCGTGGAGGCCCCCATGCCAAGCTAGCCACGC
AGTCCAACGAGATCACCATCCCAGTCACCTTCGAGTCGCGGGGCCAGCTTGGGGGGCCAGAAGCTGCAAA
ATCCGATGAGACTGCCGCCAAGTAAAGCCTTAGCCTGGATGCCACCCCTGCTGCCGCCACTGGCTGTGC
CTCCCCCGCCACCTGTGTGTTCTTTTGATACATTTATCTTCTGTTTTTCTCAAATAAAGTTCAAAGCAAC
CACCTGTAAAAA

SEQ ID NO: 70

Gene name: CSTB

RefSeq accession number: NM_000100

gi|20357564|ref|NM_000100.2| Homo sapiens cystatin B (stefin B) (CSTB), mRNA
CCCCTTGTTCCGCCCCGCGTCACGTGACCCAGCGCCTACTTGGGCTGAGGAGCCGCGCTCCCCTC
GCCGAGTCCCCTCGCCAGATTCCCTCCGTGCGGCCAAGATGATGTGCGGGCGCCCTCCGCCACGCAGC
CGGCCACCGCCGAGACCCAGCACATCGCCGACCAGGTGAGGTCCCAGCTTGAAGAGAAAGAAAACAAGAA
GTTCCCTGTGTTTAAGGCCGTGTCAATCAAGAGCCAGGTGGTCGCGGGGACAACTACTTCATCAAGGTG
CACGTGCGCGACGAGGACTTCGTACACCTGCGAGTGTTCCAATCTCTCCCTCATGAAAACAAGCCCTTGA
CCTTATCTAACTACCAGACCAACAAAGCCAAGCATGATGAGCTGACCTATTTCTGATCCTGACTTTGGAC
AAGGCCCTTCAGCCAGAAGACTGACAAAGTCATCCTCCGTCTACCAGAGCGTGCACTTGTGATCCTAAAA
TAAGCTTCATCTCCGGGCTGTGCCCCCTGGGGTGAAGGGGCAGGATTCTGCAGCTGCTTTTGCATTTCT
CTTCCTAAATTTCAATTGTGTTGATTTCTTTCCTTCCCAATAGGTGATCTTAATTACTTTCAGAATATTTT
CAAAATAGATATATTTTTAAATCCTTAAAAA

SEQ ID NO: 71

Gene name: HSP90B1

RefSeq accession number: NM_003299

gi|4507676|ref|NM_003299.1| Homo sapiens heat shock protein 90kDa beta
(Grp94), member 1 (HSP90B1), mRNA
GTGGGCGGACCGCGCGGCTGGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGGTGGAGGCGGCTCCTGCG
ATCGAAGGGGACTTGAGACTCACCGGCCGACGCCATGAGGGCCCTGTGGGTGCTGGGCCTCTGCTGCGT
CCTGCTGACCTTCGGGTGCGTCAGAGCTGACGATGAAGTTGATGTGGATGGTACAGTAGAAGAGGATCTG
GGTAAAAGTAGAGAAGGATCAAGGACGGATGATGAAGTAGTACAGAGAGAGGAAGAAGCTATTCAGTTGG
ATGGATTAAATGCATCACAAATAAGAGAACTTAGAGAGAAGTCGGAAAAGTTTGCCTTCCAAGCCGAAGT
TAACAGAATGATGAACTTATCATCAATTCATTGTATAAAAATAAAGAGATTTTCTGAGAGAACTGATT
TCAAATGCTTCTGATGCTTTAGATAAGATAAGGCTAATATCACTGACTGATGAAAATGCTCTTTCTGGAA
ATGAGGAATAACAGTCAAAATTAAGTGTGATAAGGAGAAGAACCTGCTGCATGTCACAGACACCGGTGT
AGGAATGACCAGAGAAGAGTTGGTTAAAACCTTGGTACCATAGCCAAATCTGGGACAAGCGAGTTTTTA
AACAAAATGACTGAAGCACAGGAAGATGGCCAGTCAACTTCTGAATTGATTGGCCAGTTTGGTGTGCGTT
TCTATTCGCTTCTTGTAGCAGATAAGGTTATTGTCACTTCAAAACACAACAACGATACCCAGCACAT
CTGGGAGTCTGACTCCAATGAATTTTCTGTAATTGCTGACCCAAGAGGAAACACTCTAGGACGGGGAACG
ACAATTACCCTTGTCTTAAAAGAAGAAGCATCTGATTACCTTGAATTGGATACAATTAAAATCTCGTCA
AAAATATTCACAGTTCATAAATTTCTTATTTATGTATGGAGCAGCAAGACTGAACTGTTGAGGAGCC
CATGGAGGAAGAAGAAGCAGCCAAAGAAGAGAAAGAAGATCTGATGATGAAGCTGCAGTAGAGGAAGAA
GAAGAAGAAAAGAAACCAAAGACTAAAAAGTTGAAAAAAGTGTCTGGGACTGGGAACCTTATGAATGATA
TCAAACCAATATGGCAGAGACCATCAAAAGAAGTAGAAGAAGATGAATACAAAGCTTTCTACAAATCAT
TTCAAAGGAAAGTGATGACCCCATGGCTTATATTCATTTACTGCTGAAGGGGAAGTTACCTTCAAATCA
ATTTTATTTGTACCCACATCTGCTCCACGTGGTCTGTTTGACGAATATGGATCTAAAAGAGCGATTACA
TTAAGCTCTATGTGCGCCGTGTATTCATCACAGACGACTTCCATGATATGATGCCTAAATACCTCAATTT
TGTCAGGGTGTGGTGGACTCAGATGATCTCCCTTGAATGTTTCCGCGAGACTCTTCAGCAACATAAA
CTGCTTAAGGTGATTAGGAAGAAGCTTGTTCGTAAAACGCTGGACATGATCAAGAAGATTGCTGATGATA
AATACAATGATACTTTTTGGAAAGAATTTGGTACCAACATCAAGCTTGGTGTGATTGAAGACCACTCGAA
TCGAACACGTCTTGCTAACTTCTTAGGTTCCAGTCTTCTCATCATCAACTGACATTACTAGCCTAGAC
CAGTATGTGGAAGAATGAAGGAAAAACAAGACAAAATCTACTTCATGGCTGGGTCCAGCAGAAAAGAGG
CTGAATCTTCTCCATTTGTTGAGCGACTTCTGAAAAGGGCTATGAAGTTATTTACCTCACAGAACCTGT
GGATGAATACTGTATTACGGCCCTTCCGAATTTGATGGGAAGAGGTTCCAGAATGTTGCCAAGGAAGGA
GTGAAGTTCGATGAAAGTGAGAAAATAAGGAGAGTCGTGAAGCAGTTGAGAAAGAATTTGAGCCTCTGC
TGAATTGGATGAAAGATAAAGCCCTTAAGGACAAGATTGAAAAGGCTGTGGTGTCTCAGCGCCTGACAGA
ATCTCCGTGTGCTTTGGTGGCCAGCCAGTACGGATGGTCTGGCAACATGGAGAGAATCATGAAAGCACAA
GCGTACCAAACGGGCAAGGACATCTCTACAAATTACTATGCGAGTCAGAAGAAAACATTTGAAATTAATC
CCAGACACCCGCTGATCAGAGACATGCTTCGACGAATTAAGGAAGATGAAGATGATAAAACAGTTTTGGA
TCTTGCTGTGGTTTTGTTTGAACAGCAACGCTTCGGTCAAGGTATCTTTTACCAGACACTAAAGCATAT
GGAGATAGAATAGAAAGAATGCTTCGCCTCAGTTTGAACATTGACCCTGATGCAAAGGTGGAAGAAGAGC
CCGAAGAAGAACCTGAAGAGACAGCAGAAGACACAACAGAAGACACAGAGCAAGACGAAGATGAAGAAAT
GGATGTGGGAACAGATGAAGAAGAAGAAACAGCAAAGGAATCTACAGCTGAAAAGATGAATTGTAAATT

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ATACTCTCACCATTGATCCTGTGTGGAGAGGGAATGTGAAATTTACATCATTCTTTTGGGAGAGAC
TTGTTTTGGATGCCCCCTAATCCCCTTCTCCCCTGCACTGTAAATGTGGGATTATGGGTCACAGGAAAA
AGTGGGTTTTTTAGTTGAATTTTTTTTAAATTCTCATGAATGTAAATTTGTACTATTTAACTGACTAT
TCTTGATGTAAATCTTGTCTGTGTATAAAAATAAAAAAGATCCCAAAT

SEQ ID NO: 72

Gene name: MACS

Accession Number: M68956

gi|187386|gb|M68956.1|HUMMARKSG Human myristoylated alanine-rich C-kinase
substrate mRNA, complete cds

TTTATTACTTCTTTTTTTTCGAACTACACTTGGGCTCCTTTTTTTGTGCTCGACTTTTCCACCCTTTTT
CCCTCCCTCCTGTGCTGCTGCTTTTTGATCTCTTCGACTAAAATTTTTTTATCCGGAGTGTATTTAATCG
GTTCTGTTCTGTCTCTCCACCACCCCCACCCCCCTCCCTCCGGTGTGTGTGCCGCTGCCGCTGTTGCCG
CCGCCGCTGCTGCTGCTGCTCGCCCCGTCTGTTACACCAACCCGAGGCTCTTTGTTTCCCCTCTTGGATCT
GTTGAGTTTCTTTGTTGAAGAAGCCAGCATGGGTGCCAGTTCTCCAAGACCGCAGCGAAGGGAGAAGCC
GCCGCGGAGAGGCCTGGGGAGGCGGCTGTGGCCTCGTCGCCTTCCAAAGCGAACGGACAGGAGAATGGCC
ACGTGAAGGTAAACGGCGACGCTTCGCCCCGCGCCGCGGAGTCGGGCGCCAAGGAGGAGCTGCAGGCCAA
CGGCAGCGCCCCGCGCCGCGACAAGGAGGAGCCCCGCGCGCCGCGGGAGCGGGGCGGCGTCGCCCTCCGCG
GCCGAGAAAGGTGAGCCGGCCGCGCCGCTGCCCCGAGGCCGGGGCCAGCCCGGTAGAGAAGGAGGCC
CCGCGGAAGGCGAGGCTGCCGAGCCCGCTCGCCACGGCCGCGGAGGGAGAGGCCGCGTCGGCCGCCTC
CTCGACTTCTTCGCCCAAGGCCGAGGACGGGGCCACGCCCTCGCCCAGCAACGAGACCCCGAAAAAAA
AAGAAGCGCTTTTCCTTCAAGAAGTCTTTCAAGCTGAGCGGCTTCTCCTTCAAGAAGAACAAGAAGGAGG
CTGGAGAAGGCGGTGAGGCTGAGGCGCCCGCTGCCGAAGGCGGCAAGGACGAGGCCCGCGGGGCGCAGC
TGCGGCCGCGCGGAGGCGGGCGCGGCCTCCGGGGAGCAGGCAGCGGCGCGGGCGAGGAGGCAGCAGCG
GGCGAGGAGGGGGCGGCGGGTGGCGACTCGCAGGAGGCCAAGCCCCAGGAGGCCGCTGTGCGGCCAGAGA
AGCCGCCCCGCCAGCGACGAGACCAAGGCCGCCGAGGAGCCCAGCAAGGTGGAGGAGAAAAAGGCCGAGGA
GGCCGGGGCCAGCGCCGCGCCTGCGAGGCCCCCTCCGCCGCGGGCTGGTGTGCCCCCGGAGAGGAGGC
AGCCCCCGCGGAGGAGCCCGCGGCCGCGCAGCCTCAATCAAGCCTGCGCAGCCCCCTCACAGGAGGCC
AGCCCGAGTGCAGTCCAGAAGCCCCCCCCAGCGGAGGCGGCAGAGTAAAGAGCAAGCTTTTGTGAGATAA
TCGAAGAACATTTTCTCCCCGTTTGTGTTGGTGGAGTGGTGCCAGGTACTGGATTTTGGAGAACTTGTC
TACAACCAGGGATTGATTTTAAAGATGTCTTTTTTTATTTTACTTTTTTTTAAGCACCAAATTTTGTGTT
TTTTTTTTTCTCCCCTCCCCACAGATCCCATCTCAAATCATTCTGTAAACCACCATTCACACAGGTCGAG
GAGAGCTTAAACACCTTCTTCCTCTGGCCTTGTTTCTCTTTTATTTTTTATTTTTTCGCATCAGTATTAA
TGTTTTTGCATACTTTGCATCTTTATTCAAAAGTGTAACCTTTCTTTGTCAATCTATGGACATGCCCATA
TATGAAGGAGATGGGTGGGTCAAAAAGGGATATCAAATGAAGTGATAGGGGTACAATGGGGAAATTGAA
GTGGTGCATAACATTGCCAAAATAGTGTGCCACTAGAAATGGTGTAAGGCTGTCTTTTTTTTTTTTTTT
AAAGAAAAGTTATTACCATGTATTTTGTGAGGCAGGTTTACAACACTACAACCTCGTGCCGAATTC

We Claim:

1. A method for detecting one or more head-and-neck cancer markers or polynucleotides encoding the markers associated with a head-and-neck disease or a head-and-neck tissue phase in a subject comprising:
 - 5 (a) obtaining a sample from a subject;
 - (b) detecting in proteins extracted from the sample one or more head-and-neck cancer markers listed in Table 5 or polynucleotides encoding the markers that are associated with the disease or phase; and
 - (c) comparing the detected amount with an amount detected for a standard.
- 10 2. A method for detecting a head-and-neck disease in a subject, the method comprising comparing:
 - (a) levels of one or more head-and-neck cancer markers listed in Table 5 that are extracted from a sample from the subject; and
 - 15 (b) normal levels of expression of the head-and-neck cancer markers in a control sample, wherein a significant difference in levels of head-and-neck cancer markers, relative to the corresponding normal levels, is indicative of head-and-neck disease.
3. A method according to claim 1 or 2 comprising:
 - 20 (a) contacting a biological sample obtained from a subject with one or more binding agent that specifically binds to the head-and-neck cancer markers or parts thereof; and
 - (b) detecting in the sample amounts of head-and-neck cancer markers that bind to the binding agents, relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of the head-and-neck disease in

25 the subject.
4. A method according to claim 3 wherein the binding agent is an antibody.
5. A method for screening a subject for head-and-neck cancer comprising (a) obtaining a biological sample from a subject; (b) detecting in proteins extracted from the sample the amount of one or more head-and-neck cancer markers listed in Table 5; and (c)

30 comparing the amount of head-and-neck cancer markers detected to a predetermined standard, where detection of a level of head-and-neck cancer markers different than that of a standard is indicative of head-and-neck cancer.

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6. A method according to claim 5 wherein the level of head-and-neck cancer markers are significantly higher compared to the standard and are indicative of head-and-neck cancer.
7. A method according to claim 5 wherein the level of head-and-neck cancer markers are significantly lower compared to the standard and are indicative of head-and-neck cancer.
8. A method according to any one of claims 1 to 7 wherein the sample is obtained from tissues, extracts, cell cultures, cell lysates, lavage fluid, or physiological fluids.
9. A method according to claim 8 wherein the sample is obtained from a tumor tissue.
10. A method for determining the presence or absence of head-and-neck cancer markers associated with a head-and-neck disease in a subject comprising detecting one or more polynucleotide encoding a head-and-neck marker listed in Table 5 in a sample from the subject and relating the detected amount to the presence of a head-and-neck disease.
11. A method according to claim 10 wherein the polynucleotide detected is mRNA.
12. A method according to claim 11 wherein the polynucleotide is detected by
 - (a) contacting the sample with oligonucleotides that hybridize to the polynucleotides; and
 - (b) detecting in the sample levels of nucleic acids that hybridize to the polynucleotides relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of a head-and-neck disease in the subject.
13. A method according to claim 11 wherein the mRNA is detected using an amplification reaction.
14. A method according to claim 13 wherein the amplification reaction is a polymerase chain reaction employing oligonucleotide primers that hybridize to the polynucleotides, or complements of such polynucleotides.
15. A method according to claim 11 wherein the mRNA is detected using a hybridization technique employing oligonucleotide probes that hybridize to the polynucleotides or complements of such polynucleotides.
16. A method according to claim 13 wherein the mRNA is detected by (a) isolating mRNA from the sample and combining the mRNA with reagents to convert it to

- cDNA; (b) treating the converted cDNA with amplification reaction reagents and primers that hybridize to the polynucleotides, to produce amplification products; (d) analyzing the amplification products to detect an amount of mRNA encoding one or more head-and-neck cancer markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal tissue derived using similar primers.
17. A method for diagnosing and monitoring head-and-neck cancer in a subject comprising isolating nucleic acids in a sample from the subject; and detecting one or more polynucleotides encoding head-and-neck cancer markers listed in Table 1 in the sample wherein the presence of higher or lower levels of polynucleotides encoding head-and-neck cancer markers in the sample compared to a standard or control is indicative of disease or prognosis.
 18. A method for monitoring the progression of head-and-neck cancer in a subject, the method comprising: (a) detecting in a sample from the subject at a first time point, one or more head-and-neck cancer markers or polynucleotides encoding the markers listed in Table 1; (b) repeating step (a) at a subsequent point in time; and (c) comparing levels detected in steps (a) and (b), and thereby monitoring the progression of head-and-neck cancer.
 19. A method for determining in a subject whether head-and-neck cancer has metastasized or is likely to metastasize in the future, the method comprising comparing (a) levels of one or more head-and-neck cancer markers or polynucleotides encoding the markers listed in Table 5, in a subject sample; and (b) normal levels or non-metastatic levels of the head-and-neck cancer markers or polynucleotides encoding the markers, in a control sample wherein a significant difference between the levels of expression in the subject sample and the normal levels or non-metastatic levels is an indication that the head-and-neck cancer has metastasized.
 20. A method for assessing the aggressiveness or indolence of head-and-neck cancer comprising comparing: (a) levels of expression of one or more head-and-neck cancer markers or polynucleotides encoding the markers listed in Table 1, in a subject sample; and (b) normal levels of expression of the head-and-neck cancer markers or polynucleotides encoding the markers, in a control sample, wherein a significant difference between the levels in the subject sample and normal levels is an indication

that the cancer is aggressive or indolent.

21. A diagnostic composition comprising an agent that binds to a head-and-neck cancer marker listed in Table 5 or hybridizes to a polynucleotide encoding such marker.
22. A method for assessing the potential efficacy of a test agent for inhibiting head-and-neck cancer in a subject, the method comprising comparing: (a) levels of one or more head-and-neck cancer markers listed in Table 5, in a first sample obtained from a subject and exposed to the test agent, wherein the head-and-neck cancer markers, and (b) levels of the head-and-neck cancer markers in a second sample obtained from the subject, wherein the sample is not exposed to the test agent, wherein a significant difference in the levels of expression of the head-and-neck cancer markers in the first sample, relative to the second sample, is an indication that the test agent is potentially efficacious for inhibiting head-and-neck cancer in the subject.
23. A method of assessing the efficacy of a therapy for inhibiting head-and-neck cancer in a subject, the method comprising comparing: (a) levels of one or more head-and-neck cancer markers listed in Table 5 in a first sample obtained from the subject; and (b) levels of the head-and-neck cancer markers in a second sample obtained from the subject following therapy, wherein a significant difference in the levels of expression of the head-and-neck cancer markers in the second sample, relative to the first sample, is an indication that the therapy is efficacious for inhibiting head-and-neck cancer in the subject.
24. A method of selecting an agent for inhibiting head-and-neck cancer in a subject the method comprising (a) obtaining a sample comprising cancer cells from the subject; (b) separately exposing aliquots of the sample in the presence of a plurality of test agents; (c) comparing levels of one or more head-and-neck cancer markers listed in Table 5 in each of the aliquots; and (d) selecting one of the test agents which alters the levels of head-and-neck cancer markers in the aliquot containing that test agent, relative to other test agents.
25. A method of inhibiting head-and-neck cancer in a subject, the method comprising (a) obtaining a sample comprising cancer cells from the subject; (b) separately maintaining aliquots of the sample in the presence of a plurality of test agents; (c) comparing levels of one or more head-and-neck cancer markers listed in Table 5 in each of the aliquots; and (d) administering to the subject at least one of the test agents

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which alters the levels of head-and-neck cancer markers in the aliquot containing that test agent, relative to other test agents.

26. A method of assessing the head-and-neck cancer cell carcinogenic potential of a test compound, the method comprising: (a) maintaining separate aliquots of head-and-neck cancer cells in the presence and absence of the test compound; and (b) comparing expression of one or more head-and-neck cancer markers listed in Table 1, in each of the aliquots, and wherein a significant difference in levels of head-and-neck cancer markers in the aliquot maintained in the presence of the test compound, relative to the aliquot maintained in the absence of the test compound, is an indication that the test compound possesses head-and-neck cancer cell carcinogenic potential.
27. An *in vivo* method for imaging a head-and-neck disease comprising:
 - (a) injecting a subject with one or more agent that binds to a head-and-neck marker listed in Table 5, the agent carrying a label for imaging the head-and-neck marker;
 - (b) allowing the agent to incubate *in vivo* and bind to a head-and-neck marker; and
 - (c) detecting the presence of the label localized to diseased head-and-neck tissue.
28. A method according to claim 27 wherein the agent is an antibody that specifically reacts with a head-and-neck marker.
29. A method according to any preceding claim wherein the head-and-neck cancer markers comprise or consist of those listed in Table 2.
30. A set of head-and-neck cancer markers comprising a plurality of polypeptides comprising or consisting of at least 2, 3, 4, 5, or 6 of the markers listed in Table 5 or Table 2.
31. A set of markers according to claim 30 wherein the polypeptides comprise or are selected from the group consisting of YWHAZ, S100A7 and stratifin.
32. A kit for carrying out a method as claimed in any one of claims 1 to 27.
33. A kit for determining the presence of a head and neck cancer in a subject, comprising a known amount of one or more binding agent that specifically binds to one or more head and neck cancer markers listed in Table 5 wherein the binding agent comprises a detectable substance, or it binds directly or indirectly to a detectable substance.
34. A kit for determining the presence of head and neck cancer in a subject, comprising a known amount of an oligonucleotide that hybridizes to a polynucleotide encoding a

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head and neck cancer marker listed in Table 5 wherein the oligonucleotide is directly or indirectly labeled with a detectable substance.

Application number/numéro de demande: 2618163

Figures: 3a, b, c, 7

Pages: —

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th Floor)

Documents reçus avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10ième étage)

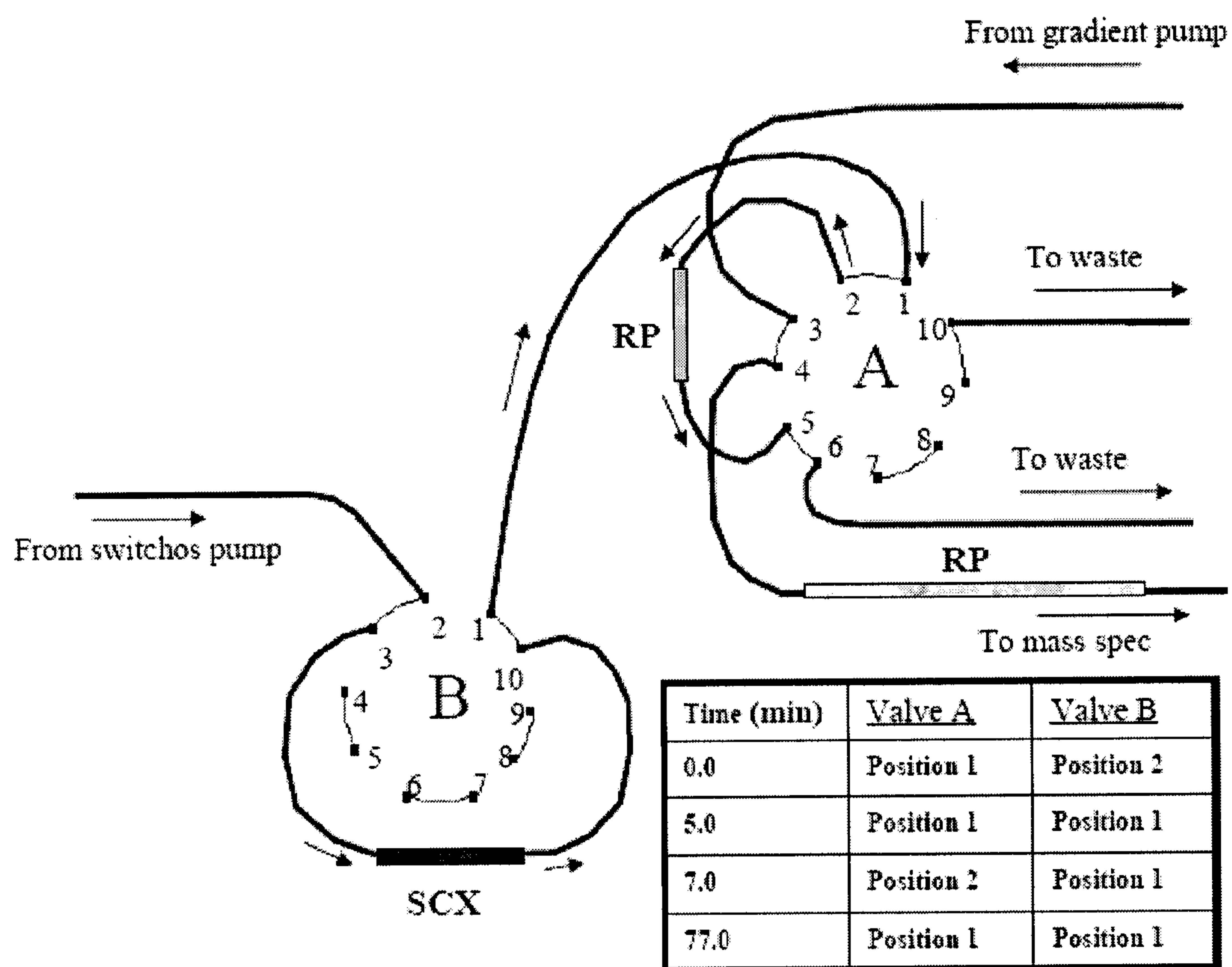
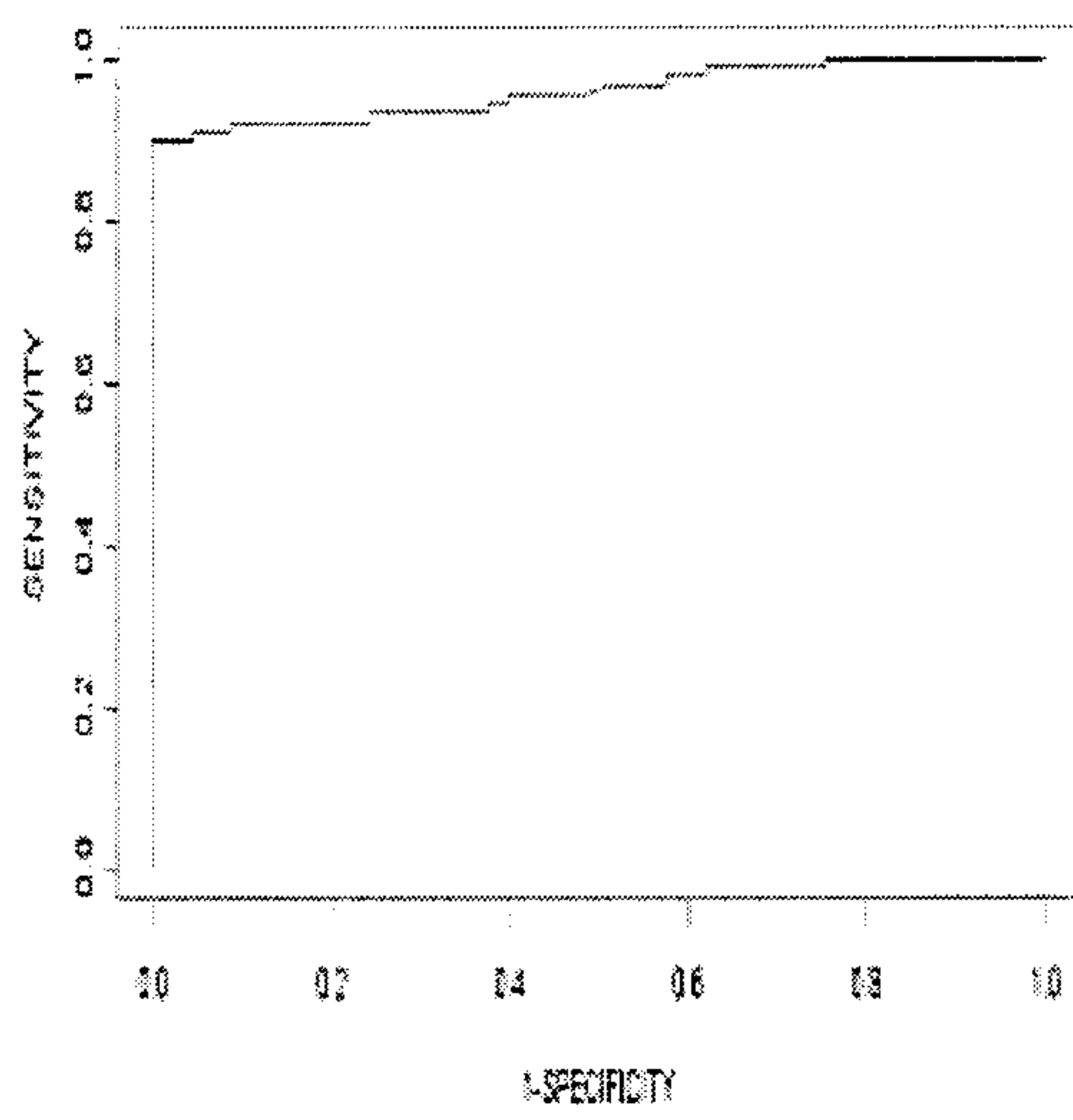


Figure 1

Figure 2:

a) iTRAQ ratios



b) IHC scores

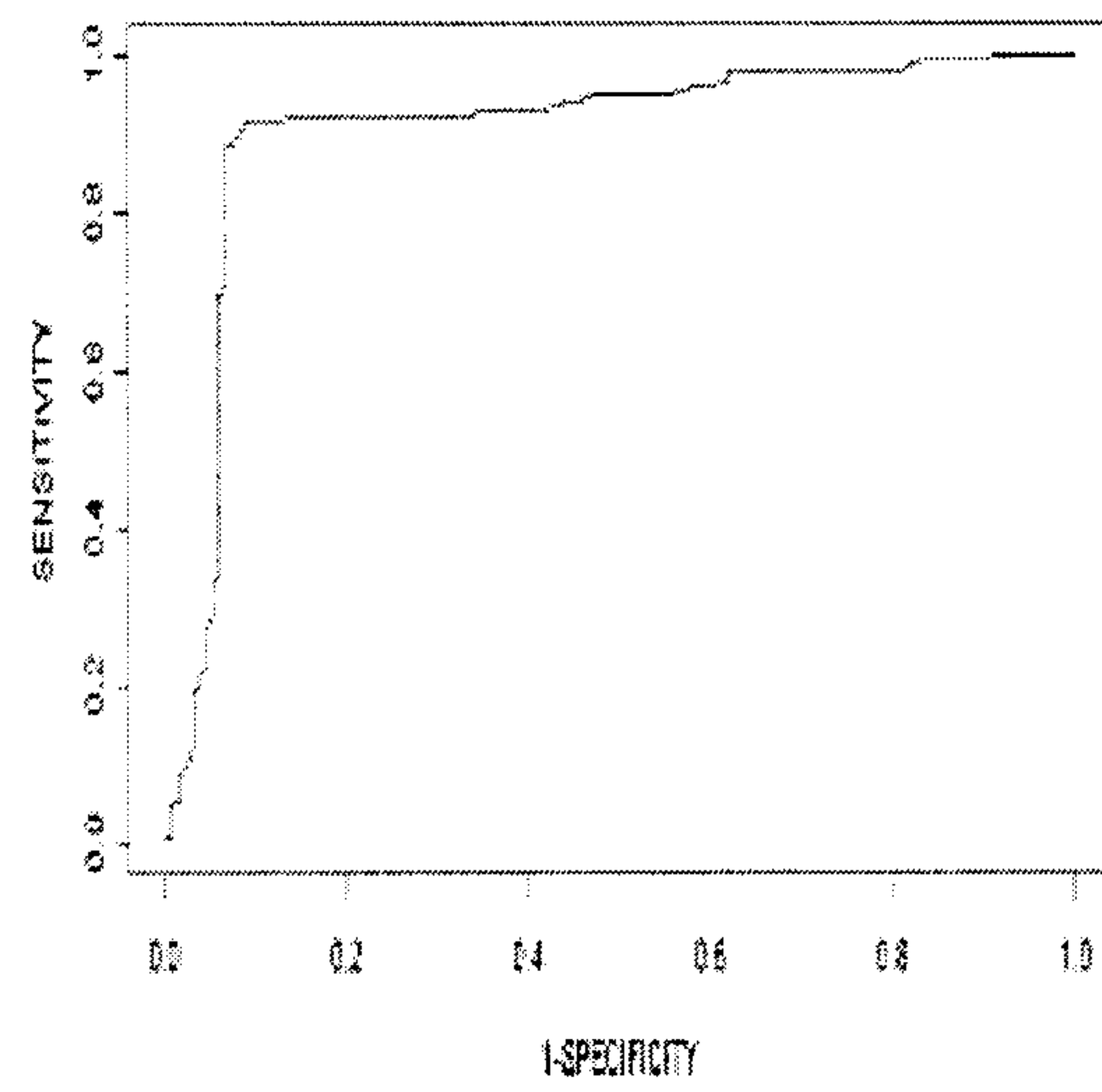
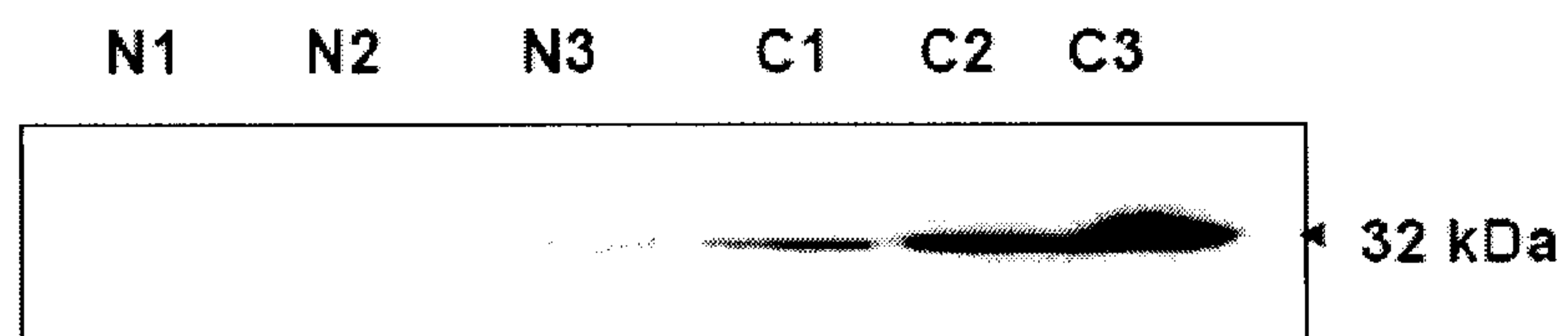
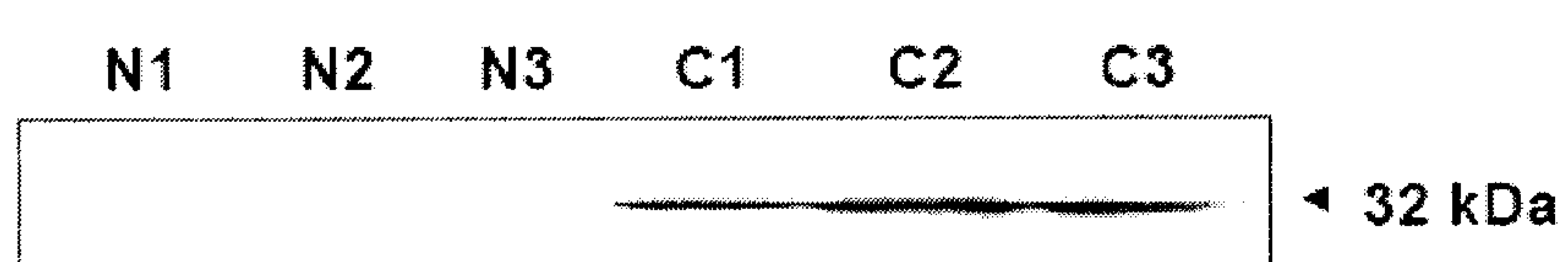


Figure 4

i) YWHAZ



ii) Stratifin



iii) S100 A7

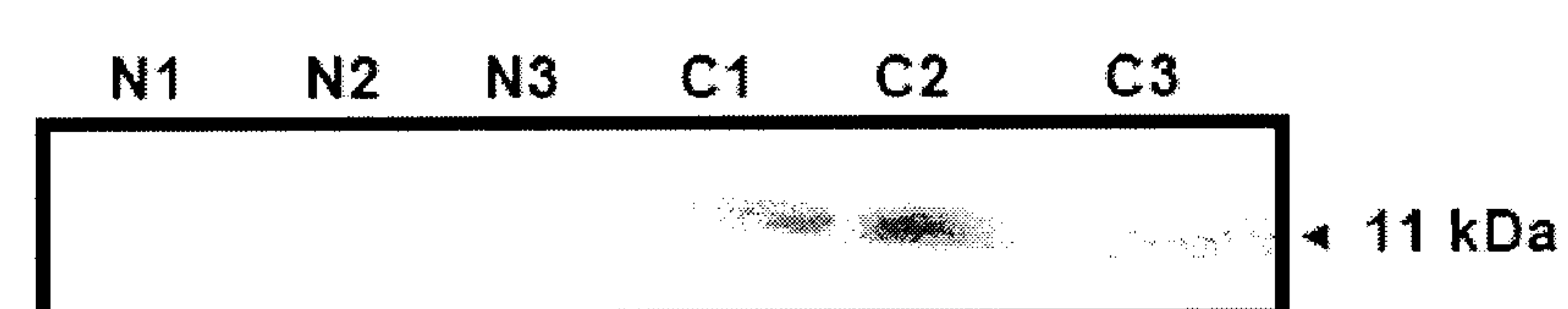
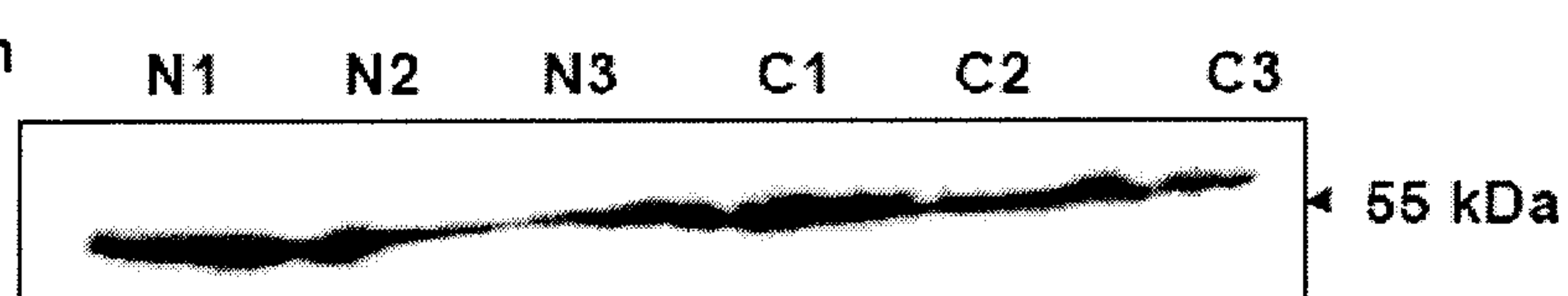
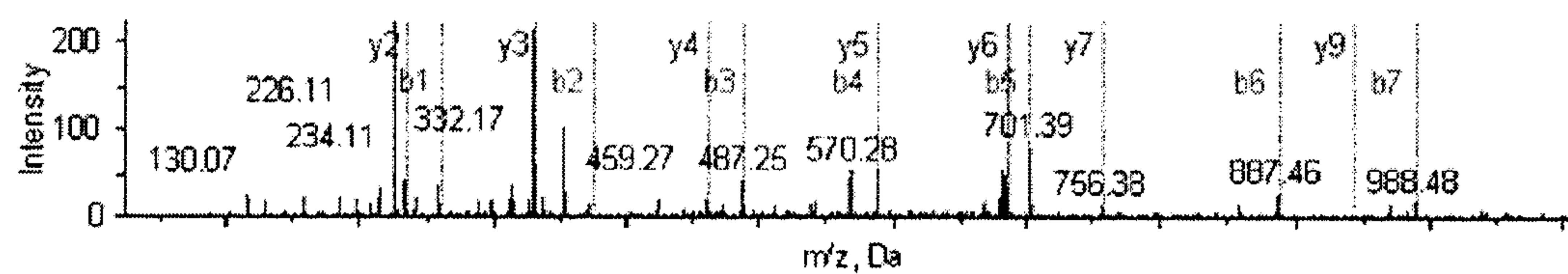
iv) α -tubulin

FIGURE 6a

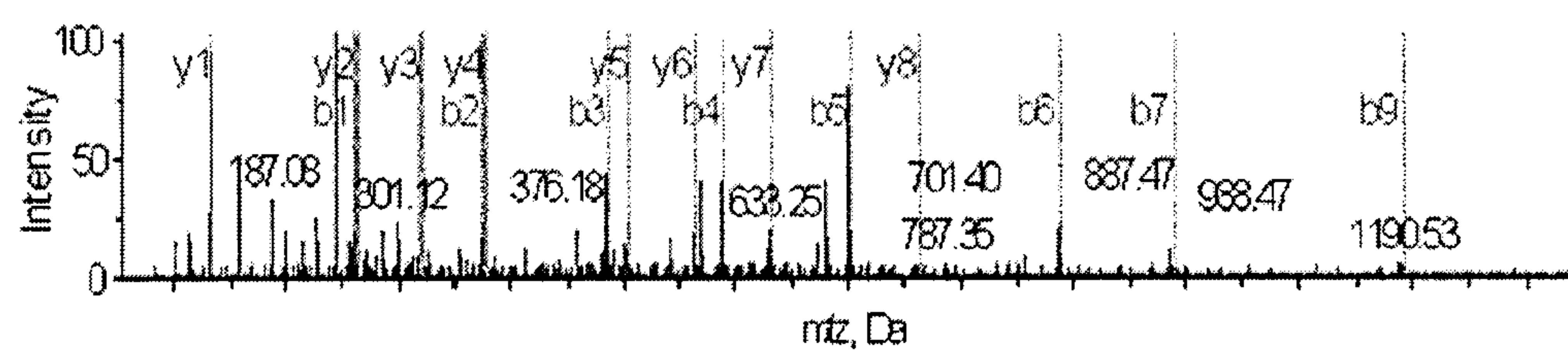
Contrib	Conf	Sequence	Modifications	Δ Mass	Prec MW	z	Sc
2.00	99	DNLTLTADNAGEEGGEAPQEPQS	ITRAQ4plex@N-term	0.1502	2672.3462	3	15



MERASLIQKAKLAEOAERYEDMAAFMKGAVEKGEELSCEERNLLSVAYKNVVGQRAAWRYLSSIEQK
SNEEGSEEKGPEVREYRVFYLMKMGDYYRYLAEVATGDDKKRIIDSARSAYQEAMDISKKEMPPTNPIRL
GLALNFSV FHYEIANSPEEAISLAKTTFDEAMADLHTLSEDSYKDSTLIMQLLRDNLTLTADNAGEEG
GEAPQEPQS

FIGURE 6b

Contrib	Conf	Sequence	Modifications	Δ Mass	Prec MW	z	Sc
2.00	99	DNLTWLWTSQTGGDEAEAGEGGEN	iTRAQ4plex@N-term	0.0967	2552.1875	3	15



RPLPSPSAAPSFPSPSRSLRAGRCGGGTRPSPTPGGGGAAGRGRGPRGPKREGKAWPRAGRSPGL
ARRRPPEHPVMDKNELVQAKLAEEQAERYDDMAACMKSVTEQGAELSNEERNLLSVAYKNVVGAR
RSSWRVVSIEQKTEGAEEKQQMAREYREKIETELRDICNDVLSLLEKFLIPNASQAESKVFYLYKMKGD
YYRYLAEEVAAGDDKKGIVDQSQQAYQEAFEISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAK
TAFDEAIAELDTLSEESYKDBTLIMQLLRDNLTWLWTSQTGGDEAEAGEGGEN

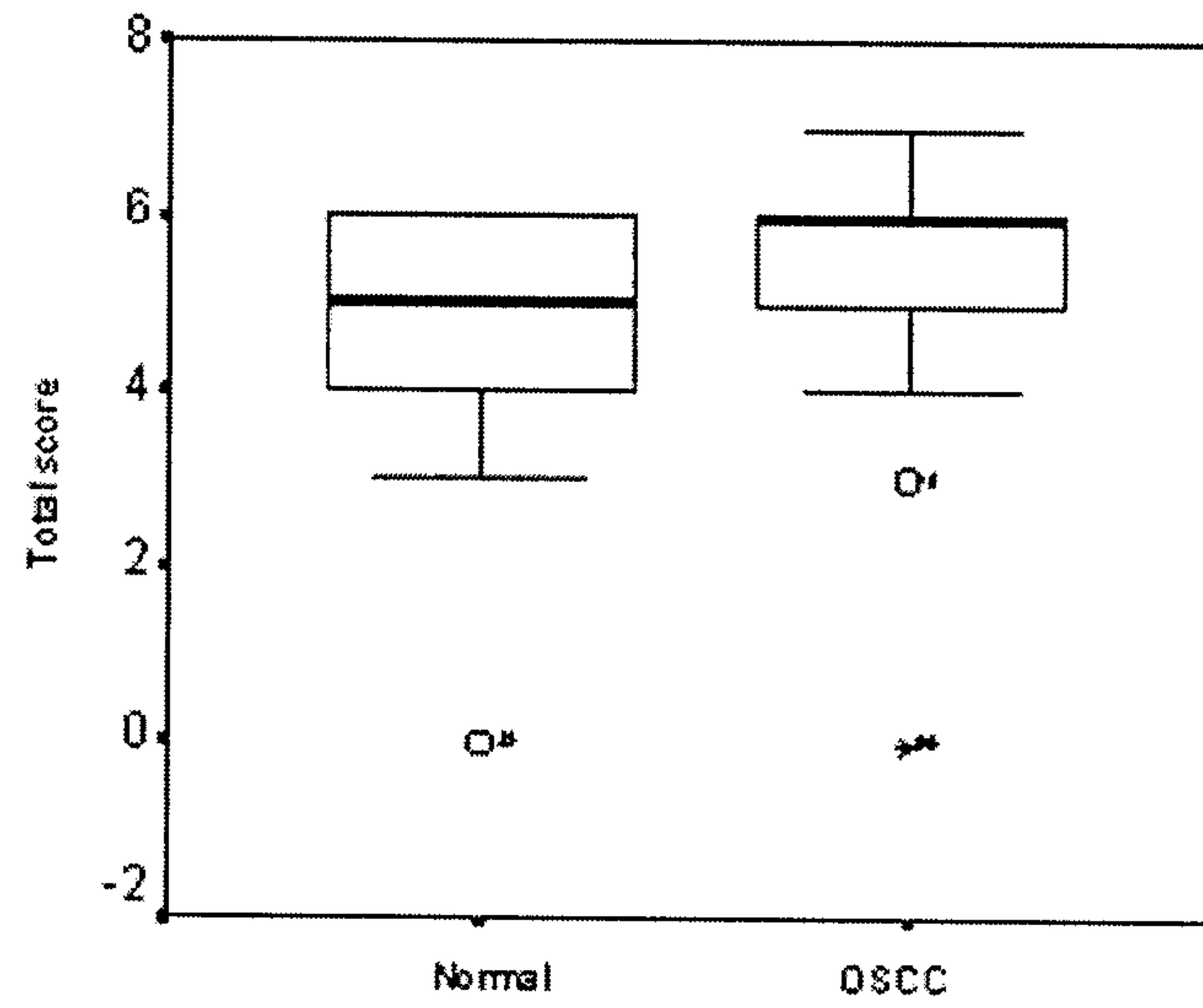
FIGURE 8

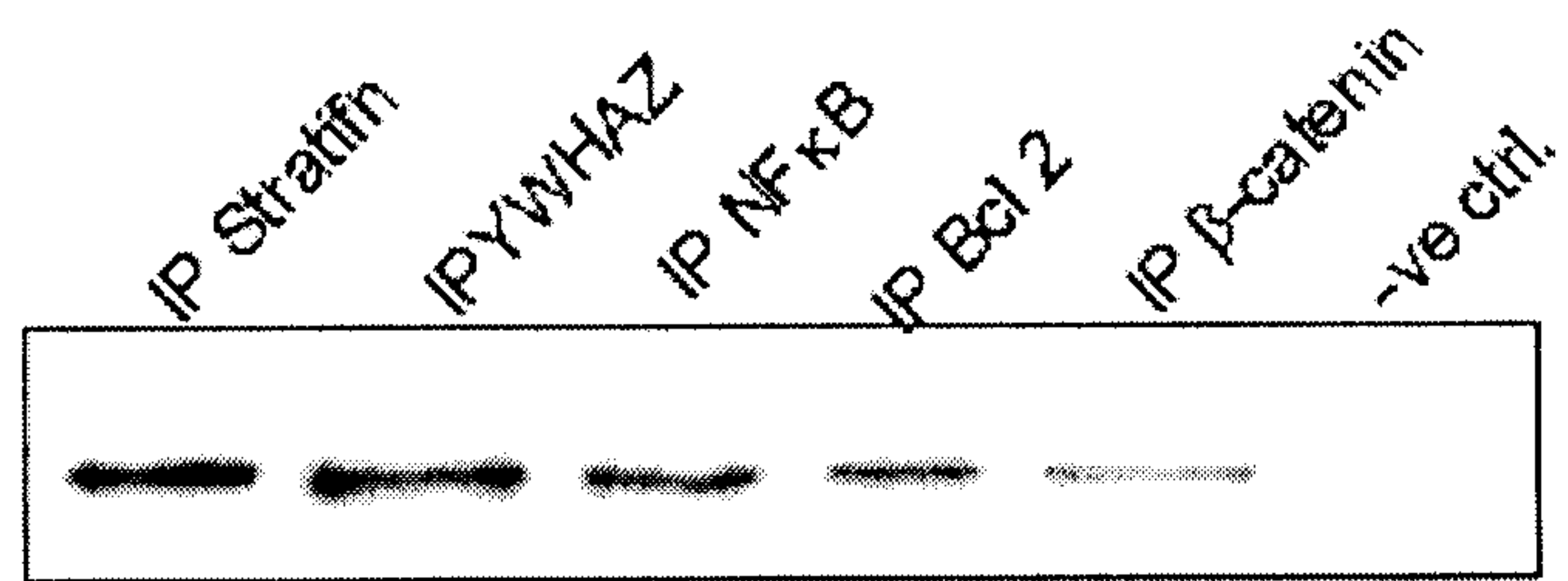
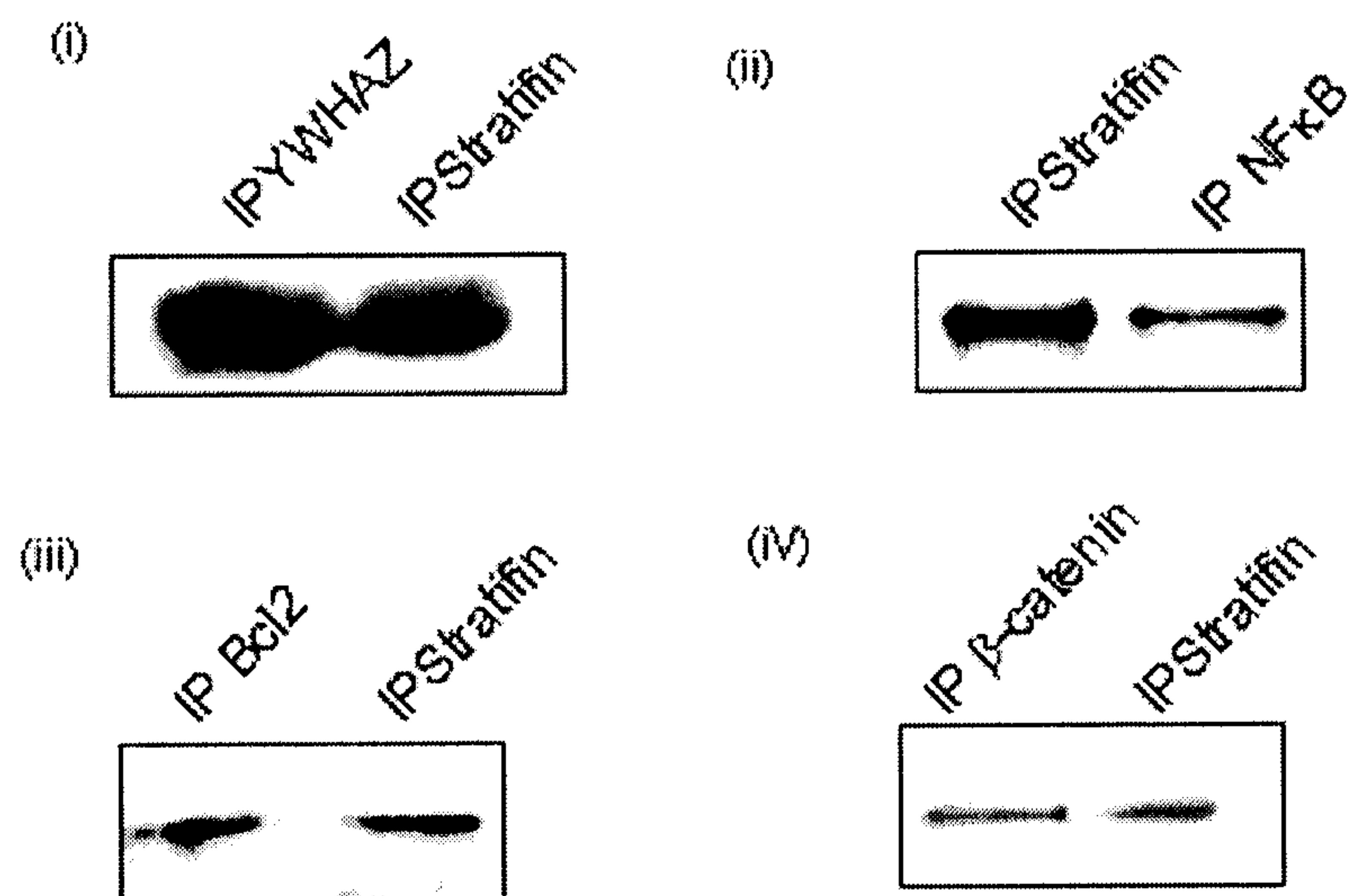
FIGURE 9a**FIGURE 9b**

FIGURE 10