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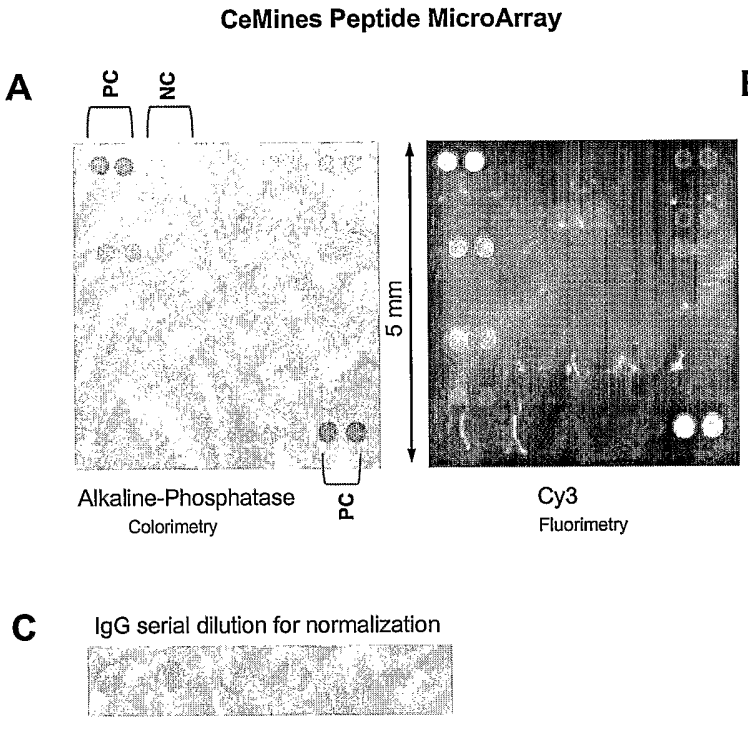

(57) Abstract: The present invention relates to autoantibodies and the detection thereof with peptide epitopes. The invention also relates to autoantibody patterns and their correlation with biological class distinctions.

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COMPOSITIONS AND METHODS FOR CLASSIFYING BIOLOGICAL SAMPLES

BACKGROUND

[001] Cancer is the second leading cause of death in the United States. Despite focused research in conventional diagnostics and therapies, the five-year survival rate has improved only minimally in the past 25 years. Better understanding of the complexity of tumorigenesis is required for the development and commercialization of much-needed, efficacious diagnostic and therapeutic products.

[002] Based on observed immune responses to human tumors, it has been suggested that serum autoantibodies ("aABs") could be used in cancer diagnostics (Fernandez-Madrid et al., Clin Cancer Res. 5:1393-400 (1999)). For example, the presence of certain serum aABs can reportedly predict the manifestation of lung cancer among at-risk patients (Lubin et al., Nat Med. 1995; 1:701-2), as well as the prognosis for non-small cell lung cancer (NSCLC) patients (Blaes et al., Ann Thorac Surg. 2000; 69:254-8). Notably however, such cancer studies have only reported on a small number of markers that are not determinative of the presence or absence of cancer and have invariably focused on the appearance of cancer-related serum aABs and their tumor-associated antigens in cancer patients (Vernino et al., Clin. Cancer Res. 10:7270-5(2004); Metcalfe et al., Breast Cancer Res. 2:438-43 (2000); Tan, J. Clin. Invest. 108:1411-5 (2001); Lubin et al., Nat Med. 1:701-2 (1995); Torchilin et al., Trends Immunol. 22:424-7 (2001); Koziol et al., Clin. Cancer Res. 9:5120-5126, (2003); Zhang et al., Clin. Exp. Immunol. 125:3-9, (2001)). Further, the low frequency with which an autoantibody specific for any individual tumor-associated antigen is detected has precluded the use of autoantibodies as useful diagnostic markers.

[003] Few studies concerning the multiplex analysis of aABs in a disease condition have been reported. The pioneering study by Robinson et al. in this specific area was published in 2002 and described multiple aABs that recognized a variety of biomolecules and were present in eight distinct human autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (Robinson et al., Nat Med. 8:295-301 (2002)). No similar studies concerning cancer have been reported.

[004] All currently used aAB detection strategies have their intrinsic strengths and weaknesses. For example, detection of an individual aAB by ELISA offers simplicity. The major weakness of this approach, however, is that it is silent with respect to other potentially informative aABs and therefore limited in its predictive value. The SEREX analysis (serological analysis of expression cDNA libraries) enables simultaneous identification of different aABs with known specificity (Gure et al., Cancer Res. 58:1034-41 (1998)). This technique, however, is time and labor consuming, and, thus, unsuitable for clinical use. Western blotting with patient sera quickly identifies the size of potential autoantigens in a

protein sample but is restricted in its informative capacity by the protein samples used and the limited resolution of autoantibody:antigen complexes, and provides no further information regarding the identity of autoantigens (Fernandez-Madrid et al., Clin Cancer Res. 5:1393-400 (1999)).

[005] In conclusion, autoantibody patterns determinative for cancer, cancer subtypes, and other aspects of the disease have not been described. Further, high-throughput analytical tools for detecting autoantibodies and autoantibody patterns in biological samples that are relevant to the diagnosis and characterization of cancer would be of great benefit.

SUMMARY OF INVENTION

[006] The present invention concerns the detection of autoantibodies (aABs) in biological samples, and exploits differences in immune status, as determined by autoantibody profiling, to distinguish physiological states or phenotypes (referred to herein as classes) and yield diagnostic and prognostic information. The present invention uses peptide epitopes to mimic antigen-antibody binding and determine autoantibody binding activities (autoantibody profiling) in biological samples as a semi-quantifiable measure of immune status. Methods for selecting sets of informative epitopes useful for autoantibody profiling and class prediction, including diagnostic and prognostic determinations, as well as sets of informative epitopes useful for particular disease class distinctions are provided. In one example, as disclosed herein, patients with different tumor status have detectable differences in their serum aAB profiles, which has diagnostic relevance. A set of synthetic peptides is used to measure autoantibody binding activities in cancer and non-cancer samples, and a subset of informative epitopes is identified and used to characterize the immune status associated with the cancer and provide a highly accurate cancer diagnostic. In another example disclosed herein, a set of informative epitopes useful for distinguishing lung cancer subclasses is provided. Advantageously, the invention uses autoantibody binding activity pattern recognition and sets of informative epitopes because combinations of multiple autoantibody binding activities as composites possess a greater potential to characterize cancer accurately compared with traditional single-entity biomarkers, including single aABs.

[007] In addition to sets of informative epitopes that may be used to detect autoantibody binding activity patterns that are diagnostic for a variety of cancers, the present invention provides sets of informative epitopes that may be used to determine a specific disease stage or the histopathological phenotype of a tumor based on the autoantibody binding activity patterns detected therewith. Additionally provided herein are sets of informative epitopes that may be used to classify a sample as being from an individual at high risk for manifestation of a disease based on the autoantibody binding activity patterns detected therewith. Notably, unlike gene-arrays, the biological samples used for the aAB-tests disclosed herein do not require a biopsy or time-consuming sample purification.

[008] Importantly, the present invention makes use of epitopes, rather than whole proteins or fragments thereof, to probe samples for autoantibodies. As demonstrated herein, epitopes corresponding to different segments of a single protein can exhibit discordant differences in their binding activities between samples from different classes. As a consequence, autoantibody detection with whole proteins or fragments thereof (i.e., composites of multiple epitopes) can be uninformative with respect to class distinction, while the use of individual epitopes within a single protein may be highly informative. For example, a first epitope may have an epitope binding activity present at a certain frequency in non-cancer samples, and lack detectable epitope binding activity in samples from small cell lung cancer patients. A second epitope, corresponding to the same protein and not overlapping with the first epitope, may have an abundant epitope binding activity present at a similar frequency in both normal samples and cancer samples. In this instance, the first epitope would be informative, as discussed herein, while the second epitope and the whole protein would not be informative to class distinction based on these results.

[009] Another important aspect of the diagnostic and prognostic methods disclosed herein is that they take into consideration autoantibodies of varied distribution, notably including epitope binding activities that are present in normal samples and decreased in disease samples. That is, the present methods do not focus solely on autoantibodies that appear in disease conditions in response to the appearance of disease-associated autoantigens. Rather, the present invention utilizes a variety of epitopes, many of which detect high levels of epitope binding activities in normal samples at a certain frequency and reveal low or undetectable levels of epitope binding activities in samples corresponding to a disease condition. Despite the fact that autoantibodies capable of binding such epitopes are frequently not detectable in disease samples, these epitopes are, nonetheless, informative with respect to class distinction, and are useful in the diagnostic and prognostic methods disclosed herein.

[0010] Accordingly, in one aspect, the present invention provides methods of identifying a set of informative epitopes, the autoantibody binding activities of which correlate with a class distinction between samples. The methods comprise sorting epitopes by the degree to which their autoantibody binding activity in samples correlates with a class distinction, and determining whether the correlation is stronger than expected by chance. An epitope for which autoantibody binding activity correlates with a class distinction more strongly than expected by chance is an informative epitope. A set of informative epitopes is identified. In one embodiment, the class distinction is determined between known classes. Preferably, the class distinction is between a disease class and a non-disease class, more preferably a cancer class and a normal class. In another preferred embodiment, the class distinction is between a high risk class and a non-disease class, more preferably a high risk cancer class and a non-cancer class. A known class can also be a class of individuals who respond well to chemotherapy or a class of individuals who do not respond well to chemotherapy.

[0011] In another embodiment, the known class distinction is a disease class distinction, preferably a cancer class distinction, still more preferably a lung cancer class distinction, a breast cancer class distinction, a gastrointestinal cancer class distinction, or a prostate cancer class distinction. In one embodiment, the known class distinction is a lung cancer class distinction between an SCLC class and an NSCLC class.

[0012] Sorting epitopes by the degree to which their autoantibody binding activity in samples correlates with a class distinction and determining the significance of the correlation can be carried out by neighborhood analysis (e.g., employing a signal to noise routine, a Pearson correlation routine, or a Euclidean distance routine) that comprises defining an idealized autoantibody binding activity pattern, wherein the idealized pattern is autoantibody binding activity that is uniformly high in a first class and uniformly low in a second class; and determining whether there is a high density of epitopes for which autoantibody binding activity is similar to the idealized pattern, as compared to an equivalent random pattern. The signal to noise routine is:

[0013] $P(g,c)=(\mu_1(g)- \mu_2(g))/(\sigma_1(g)+\sigma_2(g))$,

[0014] wherein g is the autoantibody binding activity value for an epitope; c is the class distinction, $\mu_1(g)$ is the mean of the autoantibody binding activity values for g for the first class; $\mu_2(g)$ is the mean of the autoantibody binding activity values for g for the second class; $\sigma_1(g)$ is the standard deviation for the first class; and $\sigma_2(g)$ is the standard deviation for the second class.

[0015] In one embodiment, a signal to noise routine is used to determine a weighted vote for an informative epitope for the classification of cancer without neighborhood analysis.

[0016] Another aspect of the present invention is a method of assigning a sample to a known or putative class, comprising determining a weighted vote of one or more informative epitopes (e.g., greater than 20, 50, 100, 150) for one of the classes in accordance with a model built with a weighted voting scheme, wherein the magnitude of each vote depends on the autoantibody binding activity of the sample for the given epitope and on the degree of correlation of the autoantibody binding activity for the given epitope with class distinction; and summing the votes to determine the winning class. The weighted voting scheme is:

[0017] $V_g = a_g (x_g - b_g)$,

[0018] wherein V_g is the weighted vote of the epitope, g; a_g is the correlation between autoantibody binding activity for the epitope and class distinction, P(g,c), as defined herein; $b_g = (\mu_1(g)+\mu_2(g))/2$ which is the average of the mean $\log_{10}$ autoantibody binding activity value for the epitope in a first class and a second class; x_g is the $\log_{10}$ autoantibody binding activity value for the epitope in the

sample to be tested; and wherein a positive V value indicates a vote for the first class, and a negative V value indicates a negative vote for the first class (a vote for the second class). A prediction strength can also be determined, wherein the sample is assigned to the winning class if the prediction strength is greater than a particular threshold, e.g., 0.3. The prediction strength is determined by:

[0019] $(V_{win} - V_{lose})/(V_{win} + V_{lose})$,

[0020] wherein V_{win} and V_{lose} are the vote totals for the winning and losing classes, respectively.

[0021] The invention also encompasses a method of determining a weighted vote for an informative epitope to be used in classifying a sample, comprising determining a weighted vote for one of the classes for one or more informative epitopes, wherein the magnitude of each vote depends on the autoantibody binding activity of the sample for the epitope and on the degree of correlation of the autoantibody binding activity for the epitope with class distinction. The votes may be summed to determine the winning class.

[0022] Yet another embodiment of the present invention is a method for ascertaining a plurality of classifications from two or more samples, comprising clustering samples by autoantibody binding activities to produce putative classes; and determining whether the putative classes are valid by carrying out class prediction based on putative classes and assessing whether the class predictions have a high prediction strength. The clustering of the samples can be performed, for example, according to a self organizing map. The self organizing map is formed of a plurality of Nodes, N, and the map clusters the vectors according to a competitive learning routine. The competitive learning routine is:

[0023] $f_{i+1}(N) = f_i(N) + \tau(d(N,N_p),i)(P - f_i(N))$

[0024] wherein i=number of iterations, N=the node of the self organizing map, τ=learning rate, P=the subject working vector, d=distance, N_p =node that is mapped nearest to P, and f_i (N) is the position of N at i. To determine whether the putative classes are valid the steps for building the weighted voting scheme can be carried out as described herein and class prediction may be performed on the samples.

[0025] The invention also pertains to a method for classifying a sample obtained from an individual into a class, comprising assessing the sample for autoantibody binding activity for at least one epitope; and, using a model built with a weighted voting scheme, classifying the sample as a function of autoantibody binding activity of the sample with respect to that of the model.

[0026] The present invention also pertains to a method, e.g., for use in a computer system, for classifying a sample obtained from an individual. The method comprises providing a model built by a weighted voting scheme; assessing the sample for autoantibody binding activity for at least one epitope, to thereby obtain an autoantibody binding activity value for each epitope; using the model built with a weighted voting scheme, classifying the sample comprising comparing the autoantibody binding activity of the sample to the model, to thereby obtain a classification; and providing an output indication of the classification. The routines for the weighted voting scheme and neighborhood analysis are described herein. The method can be carried out using a vector that represents a series of autoantibody binding activity values for the samples. The vectors are received by the computer system, and then subjected to the above steps. The methods further comprise performing cross-validation of the model. The cross-validation of the model involves eliminating or withholding a sample used to build the model; using a weighted voting routine, building a cross-validation model for classifying without the eliminated sample; and using the cross-validation model, classifying the eliminated sample into a winning class by comparing the autoantibody binding activity values of the eliminated sample to autoantibody binding activity values of the cross-validation model; and determining a prediction strength of the winning class for the eliminated sample based on the cross-validation model classification of the eliminated sample. The methods can further comprise filtering out any autoantibody binding activity values in the sample that exhibit an insignificant change, normalizing the autoantibody binding activity values of the vectors, and/or rescaling the values. The method further comprises providing an output indicating the clusters (e.g., formed working clusters).

[0027] The invention also encompasses a method for ascertaining at least one previously unknown class (e.g., a cancer class) into which at least one sample to be tested is classified, wherein the sample is obtained from an individual. The method comprises obtaining autoantibody binding activity values for a plurality of epitopes from two or more samples; forming respective vectors of the samples, each vector being a series of autoantibody binding activity values indicative of autoantibody binding activities in a corresponding sample; and using a clustering routine, grouping vectors of the samples such that vectors indicative of similar autoantibody binding activities are clustered together (e.g., using a self organizing map) to form working clusters, the working clusters defining at least one previously unknown class. The previously unknown class is validated by using the methods for the weighted voting scheme described herein. The self organizing map is formed of a plurality of Nodes, N, and clusters the vectors according to a competitive learning routine. The competitive learning routine is:

[0028] $f_i+1\ (N)=f_i\ (N)+\tau(d(N,N_p),i)(P-f=_i\ (N))$

[0029] wherein i=number of iterations, N=the node of the self organizing map, τ=learning rate, P=the subject working vector, d=distance, N_p =node that is mapped nearest to P, and f_i (N) is the position of N at i.

[0030] The invention also provides a method for increasing the number of informative epitopes useful for a particular class prediction. The method involves determining the correlation of autoantibody binding activity for an epitope with a class distinction, and determining if the epitope is an informative epitope. In one embodiment, the method involves use of a signal to noise routine. If the epitope is determined to be informative, i.e. as having significant predictive value, it may be combined with other informative epitopes and used in accordance with a weighted voting scheme model as described herein for class prediction.

[0031] In one embodiment, the mean average antibody binding activity (±SEM) for two or more epitopes across samples of a first class is compared to the mean average antibody binding activity (±SEM) for the two or more epitopes across samples of a second class, and a neighborhood analysis using a two-sided Student t-test is done to identify informative epitopes.

[0032] In one embodiment, the invention provides a method for identifying a set of informative epitopes having autoantibody binding activities that correlate with a class distinction between samples, comprising the steps of: (a) determining autoantibody binding activities for a plurality of epitopes in a plurality of samples for each of two or more classes; (b) identifying clusters of epitopes from the plurality of epitopes which have autoantibody binding activities in samples of the same class from the plurality of samples, wherein the clusters of epitopes have autoantibody binding activities that correlate with a class distinction between samples of different classes from the plurality of samples; and (c) determining whether the correlation is stronger than expected by chance; wherein a cluster of epitopes having autoantibody binding activities that correlate with a class distinction more strongly than expected by chance are a set of informative epitopes.

[0033] In a preferred embodiment, a pattern recognition algorithm is used to identify a set of informative epitopes using autoantibody binding activities for a plurality of epitopes in a plurality of samples for each of two or more classes. The pattern recognition algorithm recognizes clusters of autoantibody binding activities that can be used to distinguish classes among the samples. In a preferred embodiment, the pattern recognition algorithm is used to validate the resulting patterns. In a preferred embodiment, a neural network pattern recognition algorithm is used. In another preferred embodiment, a support vector machine algorithm is used for pattern recognition. When a small number of samples are used, a support vector machine algorithm is preferably used. Training may be done using samples from any class that is to be distinguished, e.g., cancer samples or control samples.

[0034] The invention also pertains to a computer apparatus for classifying a sample into a class, wherein the sample is obtained from an individual, wherein the apparatus comprises: a source of autoantibody binding activity values of the sample; a processor routine executed by a digital processor, coupled to receive the autoantibody binding activity values from the source, the processor routine determining classification of the sample by comparing the autoantibody binding activity values of the sample to a model built with a weighted voting scheme or a pattern recognition algorithm and training samples; and an output assembly, coupled to the digital processor, for providing an indication of the classification of the sample. The model is built with a weighted voting scheme, as described herein, or a pattern recognition algorithm and training samples, as described herein. The output assembly comprises a display of the classification.

[0035] Yet another embodiment is a computer apparatus for constructing a model for classifying at least one sample to be tested, wherein the apparatus comprises a source of vectors for autoantibody binding activity values from two or more samples belonging to two or more classes, the vectors being a series of autoantibody binding activity values for the samples; a processor routine executed by a digital processor, coupled to receive the autoantibody binding activity values of the vectors from the source, the processor routine determining relevant epitopes for classifying the sample based on the autoantibody binding activity values, and constructing the model with a portion of the relevant epitopes by utilizing a weighted voting scheme. The apparatus can further include a filter, coupled between the source and the processor routine, for filtering out any of the autoantibody binding activity values in a sample that exhibit an insignificant change; or a normalizer, coupled to the filter, for normalizing the autoantibody binding activity values. The output assembly can be a graphical representation.

[0036] The invention also includes a computer apparatus for constructing a model for classifying at least one sample to be tested, wherein the model is based on autoantibody binding activity patterns established through the use of a pattern recognition algorithm and training samples.

[0037] The invention also involves a machine readable computer assembly for classifying a sample into a class, wherein the sample is obtained from an individual, wherein the computer assembly comprises a source of autoantibody binding activity values of the sample; a processor routine executed by a digital processor, coupled to receive the autoantibody binding activity values from the source, the processor routine determining classification of the sample by comparing the autoantibody binding activity values of the sample to a model built with a weighted voting scheme; and an output assembly, coupled to the digital processor, for providing an indication of the classification of the sample. The invention also includes a machine readable computer assembly for constructing a model for classifying at least one sample to be tested, wherein the computer assembly comprises a source of vectors for autoantibody binding activity values from two or more samples belonging to two or more

classes, the vector being a series of autoantibody binding activity values for the samples; a processor routine executed by a digital processor, coupled to receive the autoantibody binding activity values of the vectors from the source, the processor routine determining relevant epitopes for classifying the sample, and constructing the model with a portion of the relevant epitopes by utilizing a weighted voting scheme.

[0038] The invention also includes a machine readable computer assembly for classifying a sample into a class, comprising a processor routine executed by a digital processor, wherein the processor routine determines classification of the sample by comparing autoantibody binding activities of the sample to a model based on autoantibody binding activity patterns established through the use of a pattern recognition algorithm and training samples.

[0039] In one embodiment, the invention includes a method of determining a treatment plan for an individual having a disease, comprising obtaining a sample from the individual; assessing autoantibody binding activity of the sample for at least one epitope; using a computer model built with a weighted voting scheme, classifying the sample into a disease class as a function of the autoantibody binding activity of the sample with respect to that of the model; and using the disease class, determining a treatment plan. Another application is a method of diagnosing or aiding in the diagnosis of an individual wherein a sample from the individual is obtained, comprising assessing the sample for autoantibody binding activity for at least one epitope; and using a computer model built with a weighted voting scheme, classifying the sample into a class of the disease including evaluating the autoantibody binding activity of the sample with respect to that of the model; and diagnosing or aiding in the diagnosis of the individual. The invention also includes a method for determining the efficacy of a drug designed to treat a disease class, wherein an individual has been subjected to the drug, which method comprises obtaining a sample from the individual subjected to the drug; assessing the sample for autoantibody binding activity for at least one epitope; and using a model built with a weighted voting scheme, classifying the sample into a class of the disease including evaluating the autoantibody binding activity of the sample as compared to that of the model. Yet another application is a method of determining whether an individual belongs to a phenotypic class that comprises obtaining a sample from the individual; assessing the sample for the autoantibody binding activity for at least one epitope; and using a model built with a weighted voting scheme, classifying the sample into a class including evaluating the autoantibody binding activity of the sample as compared to that of the model.

[0040] In another embodiment, the method of determining a treatment plan involves assessing the autoantibody binding activity of a patient sample for two or more epitopes using a computer model based on autoantibody binding activity patterns established through the use of a pattern recognition algorithm and training samples.

[0041] In one aspect, the invention provides a set of epitopes informative for breast cancer diagnosis. In a preferred embodiment, the invention provides a set of informative epitopes, which epitopes are informative for the diagnosis of breast cancer, comprising from 1-27, more preferably from 2-27, more preferably from 5-27, more preferably from 10-27, more preferably from 15-27, more preferably from 20-27, more preferably from 25-27 informative epitopes selected from the group consisting of those disclosed in Figure 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Figure 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Figure 2.

[0042] In another preferred embodiment, the invention provides a set of informative epitopes, which epitopes are informative for the diagnosis of lung cancer, particularly NSCLC, comprising from 1-51, more preferably from 2-51, more preferably from 5-51, more preferably from 10-51, more preferably from 15-51, more preferably from 20-51, more preferably from 25-51, more preferably from 30-51, more preferably from 35-51, more preferably from 40-51, more preferably from 45-51 informative epitopes selected from the group consisting of those disclosed in Table 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 2.

[0043] In one aspect, the invention provides a set of epitopes informative for distinguishing NSCLC and SCLC. In a preferred embodiment, the invention provides a set of informative epitopes, which epitopes are informative for the distinguishing NSCLC and SCLC, comprising from 1-28, more preferably from 2-28, more preferably from 5-28, more preferably from 10-28, more preferably from 15-28, more preferably from 20-28, more preferably from 25-28 informative epitopes selected from the group consisting of those disclosed in Figure 3. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Figure 3. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Figure 3.

[0044] In one aspect, the invention provides a set of epitopes informative for distinguishing NSCLC and SCLC. In a preferred embodiment, the invention provides a set of informative epitopes, which epitopes are informative for the distinguishing NSCLC and SCLC, comprising from 1-51, more preferably from 2-51, more preferably from 5-51, more preferably from 10-51, more preferably from 15-51, more preferably from 20-51, more preferably from 25-51, more preferably from 30-51, more preferably from 35-51, more preferably from 40-51, more preferably from 45-51 informative epitopes selected from the group consisting of those disclosed in Table 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 2.

[0045] In another preferred embodiment, the invention provides a set of informative epitopes, which epitopes are informative for the diagnosis of lung cancer, particularly NSCLC, comprising from 1-25, more preferably from 2-25, more preferably from 5-25, more preferably from 10-25, more preferably from 15-25, more preferably from 20-25 informative epitopes selected from the group consisting of those disclosed in Table 11. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 11. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 11.

[0046] In one aspect, the invention provides sets of peptides useful for identifying a set of informative epitopes for a particular class distinction. In one embodiment, the set of peptides comprises from 1-1448, more preferably from 2-1448, more preferably from 5-1448, more preferably from 10-1448, more preferably from 25-1448, more preferably from 50-1448, more preferably from 100-1448, more preferably from 250-1448, more preferably from 500-1448, more preferably from 750-1448, more preferably from 1000-1448, more preferably from 1250-1448 peptides selected from the group of peptides disclosed in Table 1, and/or from 1-31, more preferably from 2-31, more preferably from 5-31, more preferably from 10-31, more preferably from 15-31, more preferably from 20-31, more preferably from 25-31 peptides selected from the group of peptides disclosed in Table 10, and/or from 1-83, more preferably 2-83, more preferably 5-83, more preferably 10-83, more preferably 15-83, more preferably 20-83, more preferably 25-83, more preferably 50-83, more preferably 75-83 peptides selected from the group of peptides disclosed in Table 9, and/or from 1-42, more preferably 2-42, more preferably 5-42, more preferably 10-42, more preferably 15-42, more preferably 20-42, more preferably 25-42, more preferably 30-42, more preferably 35-42 peptides selected from the group of peptides disclosed in Table 8, and/or from 1-52, more preferably from 2-52, more preferably from 5-52, more preferably from 10-52, more preferably from 15-52, more preferably from 20-52, more preferably from 25-52, more preferably from 30-52, more preferably from 35-52, more preferably from 40-52, more preferably from 45-52 peptides selected from the group of peptides disclosed in Table 7.

[0047] In one aspect, the invention provides epitope microarrays for distinguishing between a plurality of classes for a biological sample, wherein the microarray comprises a plurality of peptides, each peptide independently having a corresponding epitope binding activity in a sample characteristic of a particular class selected from the plurality of particular classes, wherein taken together, the plurality of peptides have corresponding epitope binding activities in a plurality of samples collectively characteristic of all of the plurality of particular classes, wherein the autoantibody binding activity of each peptide is independently higher in a sample characteristic of one of the plurality of particular classes than in a sample characteristic of another one of the plurality of particular classes.

[0048] In a preferred embodiment, the invention provides epitope microarrays for distinguishing between a first class and a second class for a biological sample. The epitope microarrays comprise

a plurality of peptides, each peptide independently having a corresponding epitope binding activity in a sample characteristic of the first class or in a sample characteristic of the second class, wherein taken together, the plurality of peptides have corresponding epitope binding activities in samples collectively characteristic of the first and second classes, wherein the autoantibody binding activity of each peptide is independently higher in a sample characteristic of either the first class or the second class as compared to its autoantibody binding activity in a sample characteristic of the other class.

[0049] Preferred distinct classes include a non-disease class and a disease class, more preferably a non-cancer class and a cancer class, the latter preferably being lung cancer, breast cancer, gastrointestinal cancer, or prostate cancer. Other preferred distinct classes are a high risk class and a non-disease class, preferably a high risk cancer class and a non-cancer class. Other preferred distinct classes are distinct cancer classes, such as distinct lung cancer classes, such as NSCLC and SCLC. Other preferred distinct cancer classes are metatstatic cancer and non-metastatic cancer classes.

[0050] In a preferred embodiment, two or more peptides of the epitope microarray correspond to distinct regions of a single protein, preferably non-overlapping regions of the single protein.

[0051] In another preferred embodiment, the invention provides an epitope microarray useful for the diagnosis of lung cancer, particularly NSCLC, which array comprises from 1-25, more preferably from 2-25, more preferably from 5-25, more preferably from 10-25, more preferably from 15-25, more preferably from 20-25 informative epitopes selected from the group consisting of those disclosed in Table 11. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 11. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 11.

[0052] In another preferred embodiment, the invention provides an epitope microarray useful for the diagnosis of lung cancer, particularly NSCLC, which array comprises from 1-51, more preferably from 2-51, more preferably from 5-51, more preferably from 10-51, more preferably from 15-51, more preferably from 20-51, more preferably from 25-51, more preferably from 30-51, more preferably from 35-51, more preferably from 40-51, more preferably from 45-51 informative epitopes selected from the group consisting of those disclosed in Table 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 2.

[0053] In another preferred embodiment, the invention provides an epitope microarray useful for the diagnosis of breast cancer, which array comprises from 1-27, more preferably from 2-27, more preferably from 5-27, more preferably from 10-27, more preferably from 15-27, more preferably from 20-27, more preferably from 25-27 informative epitopes selected from the group consisting of those

disclosed in Figure 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Figure 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Figure 2.

[0054] In another preferred embodiment, the invention provides an epitope microarray useful for distinguishing between NSCLC and SCLC, which array comprises from 1-51, more preferably from 2-51, more preferably from 5-51, more preferably from 10-51, more preferably from 15-51, more preferably from 20-51, more preferably from 25-51, more preferably from 30-51, more preferably from 35-51, more preferably from 40-51, more preferably from 45-51 informative epitopes selected from the group consisting of those disclosed in Table 2. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Table 2. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Table 2.

[0055] In another preferred embodiment, the invention provides an epitope microarray useful for distinguishing between NSCLC and SCLC, which array comprises from 1-28, more preferably from 2-28, more preferably from 5-28, more preferably from 10-28, more preferably from 15-28, more preferably from 20-28, more preferably from 25-28 informative epitopes selected from the group consisting of those disclosed in Figure 3. In a preferred embodiment, the set of informative epitopes comprises those disclosed in Figure 3. In another preferred embodiment, the set of informative epitopes consists essentially of those disclosed in Figure 3.

[0056] In a preferred embodiment, the invention provides an epitope microarray useful for identifying informative epitopes for a particular class distinction. The epitope microarray comprises from 1-1448, more preferably from 2-1448, more preferably from 5-1448, more preferably from 10-1448, more preferably from 25-1448, more preferably from 50-1448, more preferably from 100-1448, more preferably from 250-1448, more preferably from 500-1448, more preferably from 750-1448, more preferably from 1000-1448, more preferably from 1250-1448 peptides selected from the group of peptides disclosed in Table 1, and/or from 1-31, more preferably from 2-31, more preferably from 5-31, more preferably from 10-31, more preferably from 15-31, more preferably from 20-31, more preferably from 25-31 peptides selected from the group of peptides disclosed in Table 10, and/or from 1-83, more preferably 2-83, more preferably 5-83, more preferably 10-83, more preferably 15-83, more preferably 20-83, more preferably 25-83, more preferably 50-83, more preferably 75-83 peptides selected from the group of peptides disclosed in Table 9, and/or from 1-42, more preferably 2-42, more preferably 5-42, more preferably 10-42, more preferably 15-42, more preferably 20-42, more preferably 25-42, more preferably 30-42, more preferably 35-42 peptides selected from the group of peptides disclosed in Table 8, and/or from 1-52, more preferably from 2-52, more preferably from 5-52, more preferably from 10-52, more preferably from 15-52, more preferably from 20-52, more

preferably from 25-52, more preferably from 30-52, more preferably from 35-52, more preferably from 40-52, more preferably from 45-52 peptides selected from the group of peptides disclosed in Table 7.

[0057] In one embodiment, the invention provides an epitope microarray useful for distinguishing between two or more classes and, accordingly, for predicting the classification of a sample, comprising a set of informative epitopes for class distinction that are selected using the methods disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] Figure 1. Epitope microarray design. Both arrays were hybridized with the same serum and the peptide-aAb complexes detected by a secondary anti-Human Ig conjugated to either (A) alkaline phosphatase or (B) Cy3. Similar signal patterns were obtained using these two independent detection methods. Thus, the epitope microarray is compatible with different detection methods. (C) The IgG serial dilutions for data normalization. PC – positive control; NC – negative control.

[0059] Figure 2. Sample set of breast cancer informative epitopes. A set of informative epitopes for breast cancer was determined using two-sided t-test assuming equal variance, and then sorted into two groups based on I/D signal dichotomy. EB and EC were determined as described in the experimental section.

[0060] Figure 3. Sample set of lung cancer informative epitopes. A set of lung cancer informative epitopes was determined using Student t-test, and then sorted into two groups based on I/D signal dichotomy. EN and ES were determined as described in the experimental section.

[0061] Figure 4. Clustering of our results compared with previously published cancer survival data (see Marcus et al., J Natl Cancer Inst. 92:1308-16 (2000).

[0062] Figure 5. Epitope evaluation and signal analysis. Signal strength in each patient and control individual is expressed on a scale of five. A pair-wise epitope signal comparison is then carried out for each individual epitope. Only the epitopes producing a significantly different signal ($p \leq .05$) are then used to compose the marker sets that differentiate between two groups. All epitopes in this figure are considered informative for breast cancer because they all produced a signal that was significantly different in breast cancer compared with non-cancer control.

DETAILED DESCRIPTION

[0063] "Autoantibody binding activity" and "autoantibody binding activity value" refers to the measure of the binding interaction between a given epitope and an autoantibody in a given sample, which is a semiquantifiable measure that is reflective of the amount of epitope-binding autoantibody in the

sample. As used herein, the autoantibody binding activity "of a sample", "in a sample", "with a sample", or "for a sample", refers to the measure of the binding interaction between a given epitope and an autoantibody in the given sample.

[0064] "Epitope binding activity" as used herein refers to an epitope-binding autoantibody in a sample. A "corresponding epitope binding activity" for a particular epitope is an autoantibody that specifically binds the particular epitope.

[0065] "Autoantibodies" ("aABs") specifically bind components of the same body that produces them. Altered serum autoantibody composition has been noted in a number of different cancers including breast (Metcalfe et al., Breast Cancer Res. 2:438-43 (2000)) and lung cancer (Lubin et al., Nat Med. 1:701-2 (1995); Blaes et al., Ann Thorac Surg. 69:254-8 (2000); Gure et al., Cancer Res. 58:1034-41 (1998)), and a variety of other diseases including lupus erythematosus, Sjogren's syndrome, scleroderma, dermato/polymyositis, type I diabetes, paraneoplastic neuronal syndromes, inflammatory bowel disease and thyroid endocrinopathies (see Schwarz, Autoimmunity and Autoimmune Disease, In: Fundamental Immunology, 3rd ed. (Ed. Paul WE) pp. 1033-99 Raven Press, New York, 1993).

[0066] The methods disclosed herein generally relate to two areas: class prediction and class discovery. Class prediction refers to the assignment of particular samples to defined classes which may reflect current states, predispositions, or future outcomes. Class discovery refers to defining one or more previously unrecognized biological classes.

[0067] In one aspect, the invention relates to predicting or determining a classification of a sample, comprising identifying a set of informative epitopes whose autoantibody binding activities correlate with a class distinction among samples. In one embodiment, the method involves sorting epitopes by the degree to which autoantibody binding thereto across all the samples correlates with the class distinction, and then determining whether the correlation is stronger than expected by chance (i.e., statistically significant). If the correlation of autoantibody binding activity with class distinction is statistically significant, that epitope is considered an "informative" or "relevant" epitope.

[0068] Related classification methods based on gene expression profiling have been described previously. See Golub et al., U.S. Patent No. 6,647,341, expressly incorporated herein in its entirety by reference. Notably, the present invention differs from the disclosure of Golub et al. in that the present classification schemes and methods do not involve measurements of gene expression. Rather, the present methods involve measurements of immune status based on the binding of autoantibodies in biological samples to peptide epitopes. The present invention stems from the finding that the immune status evidenced by a sample's autoantibody binding activities is highly informative in respect of biological class distinctions, given an appropriate set of informative epitopes.

[0069] Once a set of informative epitopes is identified, the weight given the information provided by each informative epitope is determined. Each vote is a measure of how much the new sample's level of autoantibody binding activity looks like the typical level of autoantibody binding activity in training samples from a particular class. The more strongly autoantibody binding activity is correlated with a class distinction, the greater the weight given to the information which that epitope provides. In other words, if autoantibody binding to a particular epitope is strongly correlated with a class distinction, that epitope will carry a great deal of weight in determining the class to which a sample belongs. Conversely, if autoantibody binding to a particular epitope is only weakly correlated with a class distinction, that epitope will be given little weight in determining the class to which a sample belongs. Each informative epitope to be used from the set of informative epitopes is assigned a weight. It is not necessary that the complete set of informative epitopes be used; a subset of the total informative epitopes can be used as desired. Using this process, a weighted voting scheme may be determined, and a predictor or model for class distinction may be created from a set of informative epitopes.

[0070] A further aspect of the invention includes assigning a biological sample to a known or putative class (i.e., class prediction) by evaluating the sample's autoantibody binding activity for informative epitopes. For each informative epitope, a vote for one or the other class is determined based on autoantibody binding activity of the sample. Each vote is then weighted in accordance with the weighted voting scheme described above, and the weighted votes are summed to determined the winning class for the sample. The winning class is defined as the class for which the largest vote is cast. Optionally, a prediction strength (PS) for the winning class can also be determined. Prediction strength is the margin of victory of the winning class that ranges from 0 to 1. In one embodiment, a sample can be assigned to the winning class only if the PS exceeds a certain threshold (e.g., 0.3); otherwise the assessment is considered uncertain.

[0071] In another embodiment, a pattern recognition algorithm is used with training samples characteristic of a particular class. The particular class of samples used may be any one of those that are to be distinguished between. For example, samples characteristic of a cancer class, or samples characteristic of a non-cancer class may be used with a pattern recognition algorithm to generate a model useful for distinguishing between cancer and non-cancer samples.

[0072] In one embodiment, a support vector machine algorithm is used. In another embodiment, a neural network algorithm is used. Preferably, if a small number of training samples are used, a support vector machine algorithm is used.

[0073] Another embodiment of the invention relates to a method of discovering or ascertaining two or more classes from samples by clustering the samples based on autoantibody binding activities to obtain putative classes (i.e., class discovery). The putative classes are validated by carrying out the

class prediction steps, as described above. In preferred embodiments, one or more steps of the methods are performed using a suitable processing means, e.g., a computer.

[0074] In one embodiment, the methods of the present invention are used to classify a sample with respect to a specific disease class or a subclass within a specific disease class. The invention is useful in classifying a sample for virtually any disease, condition, or syndrome including, but not limited to, cancer, autoimmune diseases, infectious diseases, neurodegenerative diseases, etc. That is, the invention can be used to determine whether a sample belongs to (is classified as) a specific disease category (e.g., extant lung cancer, as opposed to non-cancer, as opposed to high risk for manifestation of lung cancer) and/or to a class within a specific disease (e.g., small cell lung cancer ("SCLC") class as opposed to non-small cell lung cancer ("NSCLC") class).

[0075] As used herein, the terms "class" and "subclass" are intended to mean a group which shares one or more characteristics. For example, a disease class can be broad (e.g., proliferative disorders), intermediate (e.g., cancer) or narrow (e.g., lung cancer). The term "subclass" is intended to further define or differentiate a class. For example, in the class of lung cancer, NSCLC and SCLC are examples of subclasses; however, NSCLC and SCLC can also be considered as classes in and of themselves. These terms are not intended to impart any particular limitations in terms of the number of group members. Rather, they are intended only to assist in organizing the different sets and subsets of groups as biological distinctions are made.

[0076] The invention can be used to identify classes or subclasses between samples with respect to virtually any category or response, and can be used to classify a given sample with respect to that category or response. In one embodiment the class or subclass is previously known. For example, the invention can be used to classify samples, based on autoantibody binding activities, as being from individuals who are more susceptible to viral (e.g., HIV, human papilloma virus, meningitis) or bacterial (e.g., chlamydial, staphylococcal, streptococcal) infection versus individuals who are less susceptible to such infections. The invention can be used to classify samples based on any phenotypic or physiological trait, including, but not limited to, cancer, obesity, diabetes, high blood pressure, response to chemotherapy, etc. The invention can further be used to identify previously unknown biological classes.

[0077] In particular embodiments, class prediction is carried out using samples from individuals known to have the disease type or class being studied, as well as samples from individuals not having the disease or having a different type or class of the disease. This provides the ability to assess autoantibody binding activity patterns across the full range of phenotypes. Using the methods described herein, a classification model is built with the autoantibody binding activities from these samples.

[0078] In one embodiment, this model is created by identifying a set of informative or relevant epitopes, for which the autoantibody binding activity in samples is correlated with the class distinction to be predicted. For example, the epitopes are sorted by the degree to which their autoantibody binding activities correlate with the class distinction, and this data is assessed to determine whether the observed correlations are stronger than would be expected by chance (e.g., are statistically significant). If the correlation for a particular epitope is statistically significant, then the epitope is considered an informative epitope. If the correlation is not statistically significant, then the epitope is not considered an informative epitope.

[0079] The degree of correlation between autoantibody binding activity and class distinction can be assessed using a number of methods. In a preferred embodiment, each epitope is represented by an autoantibody binding activity vector $v(g)=(a_1, a_2, \ldots, a_n)$, where a_i denotes the autoantibody binding activity of epitope g in i^{th} sample in the initial set (S) of samples. A class distinction is represented by an idealized autoantibody binding activity pattern $c=(c_1, c_2, \ldots, c_n)$, where c_i =+1 or 0 according to whether the i^{th} sample belongs to class 1 or class 2. The correlation between an epitope and a class distinction can be measured in a variety of ways. Suitable methods include, for example, the Pearson correlation coefficient r(g,c) or the Euclidean distance d(g*,c*) between normalized vectors (where the vectors g* and c* have been normalized to have mean 0 and standard deviation 1).

[0080] In a preferred embodiment, the correlation is assessed using a measure of correlation that emphasizes the "signal-to-noise" ratio in using the epitope as a predictor. In this embodiment, (μ_1 (g),.σ_1 (g)) and (μ_2 (g),σ_2 (g)) denote the means and standard deviations of the $\log_{10}$ of the autoantibody binding values of epitope g for the samples in class 1 and class 2, respectively. $P(g,c)=(\mu_1 (g)-\mu_2 (g))/(\sigma_1 (g)+\sigma_2 (g))$, which reflects the difference between the classes relative to the standard deviation within the classes. Large values of $|P(g,c)|$ indicate a strong correlation between the autoantibody binding activity and the class distinction, while small values of $|P(g,c)|$ indicate a weak correlation between autoantibody binding activity and class distinction. The sign of P(g,c) being positive or negative corresponds to g having greater autoantibody binding activity in class 1 or class 2, respectively. Note that P(g,c), unlike a standard Pearson correlation coefficient, is not confined to the range [-1,+1]. If N_1 (c,r) denotes the set of genes such that P(g,c)>=r, and if N_2 (c,r) denotes the set of epitopes such that P(g,c)<=r, N_1 (c,r) and N_2 (c,r) are the neighborhoods of radius r around class 1 and class 2. An unusually large number of epitopes within the neighborhoods indicates that many epitopes have autoantibody binding activity patterns closely correlated with the class vector.

[0081] An assessment of whether the observed correlations are stronger than would be expected by chance is most preferably carried out using a "neighborhood analysis". In this method, an idealized pattern corresponding to autoantibody binding activity that is uniformly high in one class and uniformly low in the other class is defined, and one tests whether there is an unusually high density of

autoantibody binding activities "nearby" or "in the neighborhood of", i.e., more similar to, the idealized pattern than equivalent random patterns. The determination of whether the density of nearby autoantibody binding activities is statistically significantly higher than expected can be carried out using known methods for determining the statistical significance of differences. One preferred method is a permutation test in which the number of autoantibody binding activities in the neighborhood (nearby) is compared to the number of autoantibody binding activities in similar neighborhoods around idealized patterns corresponding to random class distinctions, obtained by permuting the coordinates of c.

[0082] The sample assessed can be any sample that can contain epitope-binding autoantibodies. Preferred samples are serum samples from individuals. Also preferred are samples of synovial fluid and cerebrospinal fluid. Using the methods described herein, the autoantibody binding activities for a plurality of epitopes can be measured simultaneously. The assessment of numerous autoantibody binding activities (autoantibody profiling) provides for a more accurate evaluation of the sample because there are more autoantibody binding activities that can assist in classifying the sample.

[0083] The autoantibody binding activities are obtained, e.g., by contacting the sample with a suitable epitope microarray, and determining the extent of binding of autoantibodies in the sample to the epitopes on the microarray. Once the autoantibody binding activities of the sample are obtained, they are compared or evaluated against the model, and then the sample is classified. The evaluation of the sample determines whether or not the sample should be assigned to the particular class being studied.

[0084] The autoantibody binding activity measured or assessed is the numeric value obtained from an apparatus that can measure autoantibody binding activity levels. Autoantibody binding activity values refer to the amount of autoantibody binding detected for a given epitope, as described herein. The values are raw values from the apparatus, or values that are optionally, rescaled, filtered and/or normalized. Such data is obtained, for example, from an epitope microarray platform using fluorometry-based or colorimetric autoantibody detection techniques.

[0085] The data can optionally be prepared by using a combination of the following: rescaling data, filtering data and normalizing data. The autoantibody binding activity values can be rescaled to account for variables across experiments or conditions, or to adjust for minor differences in overall array intensity. Such variables depend on the experimental design the researcher chooses. The preparation of the data sometimes also involves filtering and/or normalizing the values prior to subjecting the autoantibody binding activity values to clustering.

[0086] Filtering the autoantibody binding activity values involves eliminating any vector in which the autoantibody binding activity value exhibits no change or an insignificant change across samples.

Once the autoantibody binding activities for epitopes are filtered then the subset of epitopes/autoantibody binding activities that remain are referred to herein "working vectors."

[0087] The present invention can also involve normalizing the levels of autoantibody binding activity values. The normalization of autoantibody binding activity values is not always necessary and depends on the type or algorithm used to determine the correlation between autoantibody binding activity and a class distinction. The absolute level of autoantibody binding activity is not as important as the degree of correlation autoantibody binding activity has for a particular class. Normalization occurs using the following equation:

[0088] *NV=(ABV-AABV)/SDV*

[0089] wherein NV is the normalized value, ABV is the autoantibody binding activity value across samples, AABV is the average autoantibody binding activity value across samples, and SDV is the standard deviation of the autoantibody binding activity values.

[0090] Once the autoantibody binding activity values are prepared, then the data is classified or is used to build the model for classification. Epitopes that are relevant for classification are first determined. The term "relevant epitopes" refers to those epitopes for which autoantibody binding activity correlates with a class distinction. The epitopes that are relevant for classification are also referred to herein as "informative epitopes". The correlation between autoantibody binding activity and class distinction can be determined using a variety of methods; for example, a neighborhood analysis can be used. A neighborhood analysis comprises performing a permutation test, and determining probability of number of genes in the neighborhood of the class distinction, as compared to the neighborhoods of random class distinctions. The size or radius of the neighborhood is determined using a distance metric. For example, the neighborhood analysis can employ the Pearson correlation coefficient, the Euclidean distance coefficient, or a signal to noise coefficient. The relevant epitopes are determined by employing, for example, a neighborhood analysis which defines an idealized autoantibody binding activity pattern corresponding to a autoantibody binding activity that is uniformly high in one class and uniformly low in other class(es). A disparity in autoantibody binding activity exists when comparing the level of autoantibody binding activity in one class with other classes. Such epitopes are good indicators for evaluating and classifying a sample based on its autoantibody binding activities. In one embodiment, the neighborhood analysis utilizes the following signal to noise routine:

[0091] $P(g,c)=(\mu_1(g)-\mu_2(g))/(\sigma_1(g)+\sigma_2(g))$,

[0092] wherein g is the autoantibody binding activity value for a given epitope; c is the class distinction, μ_1 (g) is the mean of the autoantibody binding activities for g for a first class; μ_2 (g) is the

mean of the autoantibody binding activities for g for a second class; σ_1 (g) is the standard deviation for g the first class; and σ_2 (g) is the standard deviation for the second class. The invention includes classifying a sample into one of two classes, or into one of multiple (a plurality of) classes.

[0093] Particularly relevant epitopes are those that are best suited for classifying samples. The step of determining the relevant epitopes also provides means for isolating antibodies that can be used to identify immunogenic proteins potentially involved in manifestation of the class, e.g., proteins involved in pathogenesis. Consequently, the methods of the present invention also pertain to determining drug target(s) based on immunogenic proteins that specifically bind to epitope binding autoantibodies and are involved with the class (e.g., disease) being studied, and the drug, itself, as determined by this method.

[0094] The next step for classifying epitopes involves building or constructing a model or predictor that can be used to classify samples to be tested. One builds the model using samples for which the classification has already been ascertained, referred to herein as an "initial dataset." Once the model is built, then a sample to be tested is evaluated against the model (e.g., classified as a function of the relative autoantibody binding activities of the sample with respect to that of the model).

[0095] A portion of the relevant epitopes, determined as described above, can be chosen to build the model. Not all of the epitopes need to be used. The number of relevant epitopes to be used for building the model can be determined by one of skill in the art. For example, out of 1000 epitopes that demonstrate a high correlation of autoantibody binding activity to a class distinction, 25, 50, 75 or 100 or more of these epitopes can be used to build the model.

[0096] The model or predictor is built using a "weighted voting scheme" or "weighted voting routine." A weighted voting scheme allows these informative epitopes to cast weighted votes for one of the classes. The magnitude of the vote is dependant on both the autoantibody binding activity level and the degree of correlation of the autoantibody binding activity with the class distinction. The larger the disparity or difference between autoantibody binding activity from one class and the next, the larger the vote the epitope will cast. An epitope with a larger difference is a better indicator for class distinction, and so casts a larger vote.

[0097] The model is built according to the following weighted voting routine:

[0098] $V_g = a_g (x_g - b_g)$,

[0099] wherein V_g is the weighted vote of the epitope, g; a_g is the correlation between autoantibody binding activity values for the epitope and class distinction, P(g,c), as defined herein; $b_g = (\mu_1 (g) + \mu_2 (g))/2$ which is the average of the mean $\log_{10}$ autoantibody binding activity value in a first class and a

second class; x_g is the $\log_{10}$ autoantibody binding activity value in the sample to be tested. A positive weighted vote is a vote for the new sample's membership in the first class, and a negative weighted vote is a vote for the new sample's membership in the second class. The total vote V_1 for the first class is obtained by summing the absolute values of the positive votes over the informative epitopes, while the total vote V_2 for the second class is obtained by summing the absolute values of the negative votes.

[00100] A prediction strength can also be measured to determine the degree of confidence the model classifies a sample to be tested. The prediction strength conveys the degree of confidence of the classification of the sample and evaluates when a sample cannot be classified. There may be instances in which a sample is tested, but does not belong to a particular class. This is done by utilizing a threshold wherein a sample which scores below the determined threshold is not a sample that can be classified (e.g., a "no call"). For example, if a model is built to determine whether a sample belongs to one of two lung cancer classes, but the sample is taken from an individual who does not have lung cancer, then the sample will be a "no call" and will not be able to be classified. The prediction strength threshold can be determined by the skilled artisan based on known factors, including, but not limited to the value of a false positive classification versus a "no call".

[00101] Once the model is built, the validity of the model can be tested using methods known in the art. One way to test the validity of the model is by cross-validation of the dataset. To perform cross-validation, one of the samples is eliminated and the model is built, as described above, without the eliminated sample, forming a "cross-validation model." The eliminated sample is then classified according to the model, as described herein. This process is done with all the samples of the initial dataset and an error rate is determined. The accuracy the model is then assessed. This model should classify samples to be tested with high accuracy for classes that are known, or classes have been previously ascertained or established through class discovery. Another way to validate the model is to apply the model to an independent data set. Other standard biological or medical research techniques, known or developed in the future, can be used to validate class discovery or class prediction.

[00102] The invention also provides a method for increasing the number of informative epitopes useful for a particular class prediction. The method involves determining the correlation of autoantibody binding activity for an epitope with a class distinction, and determining if the epitope is an informative epitope. In one embodiment, the method involves use of a signal to noise routine. If the epitope is determined to be informative, i.e. as having significant predictive value, it may be combined with other informative epitopes and used in accordance with a weighted voting scheme model as described herein for class prediction.

[00103] The invention also provides alternative means for determining whether epitopes are informative for a particular biological class distinction. For example, in one embodiment, the mean average antibody binding activity (±SEM) for two or more epitopes across samples of a first class is compared to the mean average antibody binding activity (±SEM) for the two or more epitopes across samples of a second class, and a two-sided Student t-test is done to identify informative epitopes.

[00104] An aspect of the invention also includes ascertaining or discovering classes that were not previously known, or validating previously hypothesized classes. This process is referred to herein as "class discovery." This embodiment of the invention involves determining the class or classes not previously known, and then validating the class determination (e.g., verifying that the class determination is accurate).

[00105] To ascertain classes that were not previously known or recognized, or to validate classes which have been proposed on the basis of other findings, the samples are grouped or clustered based on autoantibody binding activities. The autoantibody binding activity pattern (i.e., aAB profile) of a sample and the samples having similar autoantibody binding activity patterns are grouped or clustered together. The group or cluster of samples identifies a class. This clustering methodology can be applied to identify any classes in which the classes differ based on their autoantibody binding activity patterns.

[00106] Determining classes that were not previously known is performed by the present methods using a clustering routine. The present invention can utilize several clustering routines to ascertain previously unknown classes, such as Bayesian clustering, k-means clustering, hierarchical clustering, and Self Organizing Map (SOM) clustering.

[00107] Once the autoantibody binding activity values are prepared, the data is clustered or grouped. One particular aspect of the invention utilizes SOMs, a competitive learning routine, for clustering autoantibody binding activity patterns to ascertain the classes. SOMs impose structure on the data, with neighboring nodes tending to define `related` clusters or classes.

[00108] SOMs are constructed by first choosing a geometry of "nodes". Preferably, a 2 dimensional grid (e.g., a 3x2 grid) is used, but other geometries can be used. The nodes are mapped into k-dimensional space, initially at random and then interactively adjusted. Each iteration involves randomly selecting a vector and moving the nodes in the direction of that vector. The closest node is moved the most, while other nodes are moved by smaller amounts depending on their distance from the closest node in the initial geometry. In this fashion, neighboring points in the initial geometry tend to be mapped to nearby points in k-dimensional space. The process continues for several (e.g., 20,000-50,000) iterations.

[00109] The number of nodes in the SOM can vary according to the data. For example, the user can increase the number of Nodes to obtain more clusters. The proper number of clusters allows for a better and more distinct representation of the particular cluster of samples. The grid size corresponds to the number of nodes. For example a 3x2 grid contains 6 nodes and a 4x5 grid contains 20 nodes. As the SOM algorithm is applied to the samples based on autoantibody binding activity data, the nodes move toward the sample cluster over several iterations. The number of Nodes directly relates to the number of clusters. Therefore, an increase in the number of Nodes results in an increase in the number of clusters. Having too few nodes tends to produce patterns that are not distinct. Additional clusters result in distinct, tight clusters of autoantibody binding activity. The addition of even more clusters beyond this point does not result any fundamentally new patterns. For example, one can choose a 3x2 grid, a 4x5 grid, and/or a 6x7 grid, and study the output to determine the most suitable grid size.

[00110] A variety of SOM algorithms exist that can cluster samples according to autoantibody binding activity vectors. The invention utilizes any SOM routine (e.g., a competitive learning routine that clusters the autoantibody binding activity patterns), and preferably, uses the following SOM routine:

[00111] $f_{i+1}(N)=f_i(N)+\tau(d(N,N_p),i)(P-f_i(N))$,

[00112] wherein i=number of iterations, N=the node of the self organizing map, τ=learning rate, P=the subject working vector, d=distance, N_p =node that is mapped nearest to P, and f_i (N) is the position of N at i.

[00113] Once the samples are grouped into classes using a clustering routine, the putative classes are validated. The steps for classifying samples (e.g., class prediction) can be used to verify the classes. A model based on a weighted voting scheme, as described herein, is built using the autoantibody binding activity data from the same samples for which the class discovery was performed. Such a model will perform well (e.g., via cross validation and via classifying independent samples) when the classes have been properly determined or ascertained. If the newly discovered classes have not been properly determined, then the model will not perform well (e.g., not better than predicting by the majority class). All pairs of classes discovered by the chosen class discovery method may be compared. For each pair C_1, C_2, S is the set of samples in either C_1 or C_2. Class membership (either C_1 or C_2) is predicted for each sample in S by the cross validation method described herein. The median PS (over the $|S|$ predictions) to be a measure of how predictable the class distinction is from the given data. A low median PS value (e.g., near 0.3) indicates either spurious class distinction or an insufficient amount of data to support a real distinction. A high median PS value (e.g., 0.8) indicates a strong, predictable class distinction.

[00114] The class discovery techniques above can be used to identify the fundamental subtypes of any disorder, e.g., cancer. Class discovery methods could also be used to search for fundamental immune mechanisms that cut across distinct types of cancers. For example, one might combine different cancers (for example, breast tumors and prostate tumors) into a single dataset and cluster the samples based on epitope binding activities. Moreover, in a preferred embodiment, the class predictor described herein is adapted to a clinical setting, with an appropriate epitope microarray as described herein.

[00115] Classification of the sample gives a healthcare provider information about a classification to which the sample belongs, based on the analysis or evaluation of autoantibody binding activity for multiple epitopes. The methods provide a more accurate assessment than traditional tests because multiple autoantibody binding activities or markers are analyzed, as opposed to analyzing one or two markers as is done for traditional tests. The information provided by the present invention, alone or in conjunction with other test results, aids the healthcare provider in diagnosing the individual.

[00116] Also, the present invention provides methods for determining a treatment plan. Once the health care provider knows to which disease class the sample, and therefore, the individual belongs, the health care provider can determine an adequate treatment plan for the individual. Different disease classes often require differing treatments. Properly diagnosing and understanding the class of disease of an individual allows for a better, more successful treatment and prognosis.

[00117] Other applications of the invention include ascertaining classes for or classifying persons who are likely to have successful treatment with a particular drug or regimen. Those interested in determining the efficacy of a drug can utilize the methods of the present invention. During a study of the drug or treatment being tested, individuals who have a disease may respond well to the drug or treatment, and others may not. Samples are obtained from individuals who have been subjected to the drug being tested and who have a predetermined response to the treatment. A model can be built from a portion of the relevant epitopes, using the weighted voting scheme described herein. A sample to be tested can then be evaluated against the model and classified on the basis of whether treatment would be successful or unsuccessful. The company testing the drug could provide more accurate information regarding the class of individuals for which the drug is most useful. This information also aids a healthcare provider in determining the best treatment plan for the individual.

[00118] Another application of the present invention is classification of a sample from an individual to determine the likelihood that a particular disease or condition will manifest in an individual. For example, persons who are more likely to contract heart disease or high blood pressure can have autoantibody binding activity profiles different from those who are less likely to suffer from these diseases. A model, using the methods described herein, can be built from individuals who have heart

disease or high blood pressure, and those who do not using a weighted voting scheme. Once the model is built, a sample from an individual can be tested and evaluated with respect to the model to determine to which class the sample belongs. An individual who belongs to the class of individuals who have the disease, can take preventive measures (e.g., exercise, aspirin, etc.). Heart disease and high blood pressure are examples of diseases that can be classified, but the present invention can be used to classify samples for virtually any disease, including predispositions for cancer.

[00119] A preferred embodiment for identifying and predicting predisposition to disease involves building a weighted voting scheme model using the methods described herein with samples from individuals who do not have, but are at high risk for, a particular disease condition. An example of such an individual would be a long term high frequency smoker who has not presented with lung cancer, or a family member whose pedigree predicts occurrence of a familial disease, but who has not presented with the disease. Once the model is built, a sample from an individual can be tested and evaluated with respect to the model to determine to which class the sample belongs. An individual who belongs to the class of individuals predisposed to the disease can take preventive measures (e.g., exercise, aspirin, cessation of smoking, etc.).

[00120] More generally, class predictors may be useful in a variety of settings. First, class predictors can be constructed for known pathological categories, reflecting a tumor's cell of origin, stage or grade. Such predictors could provide diagnostic confirmation or clarify unusual cases. Second, the technique of class prediction can be applied to distinctions relating to future clinical outcome, such as drug response or survival.

Epitope Microarrays

[00121] In one aspect, the invention provides epitope microarrays which are positionally addressable arrays of autoantibody-binding peptides (epitopes) adhered to the array. The array contains from two to thousands of epitopes, more preferably from 10-1,500, more preferably from 20-1000, more preferably from 50-500 epitopes. The epitopes used are preferably from about 3 to about 20, more preferably about 15 amino acids in length, though epitopes of other lengths may be used. A binding agent, preferably a secondary antibody that specifically binds to an autoantibody present in the sample, is used to detect the presence of the autoantibody specifically bound to an epitope of the array. The detection agent is preferably labeled with a detectable label, (e.g., ^{32}P, colorimetric indicator, or a fluorescent label), prior to incubation with the epitope array.

[00122] The choice of epitopes used for autoantibody detection, and for epitope microarrays, may depend on the class distinction desired. Alternatively, a set of random peptides may be used and informative epitopes within the set may be identified using the methods disclosed herein.

[00123] In a preferred embodiment, the invention provides epitope microarrays useful for the diagnosis of cancer, and peptides present on such microarrays are selected from a set designed based on the following scheme. A first group of epitopes of the set corresponds to proteins that are expressed in embryonal tissues, and whose aberrant expression in adult tissues could provoke a humoral immune response. These include transcription factors (TFs) that are active in embryonal development, and also elicit immune responses while expressed in tumor cells. For example, aAbs against the members of SOX-family transcription factors have been identified in the sera of small cell lung cancer (SCLC) patients (Gure et al.. supra). The members of SOX- family TFs are normally expressed in the developing nervous system and their expression has not been documented in normal lung epithelium (Gure et al.. supra). Furthermore, expression of the members of basic helix-loop-helix (bHLH) family TFs that play a role in embryonal nervous system has been documented in NSCLC and SCLC (Chen et al., Proc Natl Acad Sci USA. (1997) 94:5355-60).

[00124] Additionally, the cancer diagnostic epitope microarray preferably incorporates previously published B-cell epitopes and the epitopes predicted to bind various isoforms of class II major histocompatibility complex (MHC). Publicly available MHC II binding algorithms such as ProPred and RankPept may be used. Special attention in epitope design is given to proteins whose autoantibodies have been linked to cancer. These include p53 and various members of SOX, FOX, IMP, ELAV/HU and other families (Tan, J Clin Invest. (2001) 108:1411-5). Also preferably included on the cancer diagnostic microarray are epitopes known to trigger a T-cell response, as an overlap between the T- and B-immunogenicity could be inferred from previous studies (Scanlan et al., Cancer Immun. (2001) 1:4; Chen et al., Proc Natl Acad Sci USA. (1998) 95:6919-23). An excellent collection of known T-cell epitopes exist in Cancer Immunity database. Thus, a highly preferred cancer diagnostic epitope microarray combines previously identified immunogenic sequences with the embryonal factor epitope design described above. The peptides are synthesized and may be printed on a microarray using known methods. For example, see Robinson et al., supra .

[00125] Preferred informative epitopes for the diagnosis of breast cancer include those disclosed in Figure 2.

[00126] Preferred informative epitopes for distinguishing between NSCLC and SCLC include those disclosed in Figures 3, 7, and 13.

[00127] Preferred informative epitopes for the diagnosis of NSCLC include those disclosed in Figures 7 and 13.

[00128] Preferred epitopes from which to select informative epitopes for predicting a class distinction include those disclosed in Figures 6, 7, 9, 10, 11, 12, and 13.

[00129] In one aspect, the invention provides epitope microarrays for distinguishing between a plurality of classes for a biological sample, wherein the microarray comprises a plurality of peptides, each peptide independently having a corresponding epitope binding activity in a sample characteristic of a particular class selected from the plurality of particular classes, wherein taken together, the plurality of peptides have corresponding epitope binding activities in a plurality of samples collectively characteristic of all of the plurality of particular classes, wherein the autoantibody binding activity of each peptide is independently higher in a sample characteristic of one of the plurality of particular classes than in a sample characteristic of another one of the plurality of particular classes.

[00130] In a preferred embodiment, the invention provides epitope microarrays for distinguishing between a first class and a second class for a biological sample. The epitope microarrays comprise a plurality of peptides, each peptide independently having a corresponding epitope binding activity in a sample characteristic of the first class or in a sample characteristic of the second class, wherein taken together, the plurality of peptides have corresponding epitope binding activities in samples collectively characteristic of the first and second classes, wherein the autoantibody binding activity of each peptide is independently higher in a sample characteristic of either the first class or the second class as compared to its autoantibody binding activity in a sample characteristic of the other class.

[00131] In one embodiment, the invention provides epitope microarrays comprising a plurality of peptides, each peptide having a corresponding epitope binding activity in a first sample or a second sample, wherein the autoantibody binding activity of each peptide is higher or lower with the first sample as compared to the second sample, and wherein the first sample and the second sample correspond to distinct classes.

[00132] In a preferred embodiment, at least a first peptide of the epitope microarray has higher autoantibody binding activity with a first sample corresponding to a first class as compared to its autoantibody binding activity with a second sample corresponding to a second class, and at least a second peptide of the epitope microarray has higher autoantibody binding activity with the second sample corresponding to the second class as compared to its autoantibody binding activity with the first sample corresponding to the first class.

[00133] Each peptide included on an epitope microarray displays an autoantibody binding activity that correlates with a class distinction, though the frequency at which autoantibody binding activity for any particular epitope is detected may be low, and the probability of detecting a particular epitope-binding autoantibody in a sample characteristic of a particular class may be low. Such epitopes are nonetheless useful for diagnosis when used in combination, as disclosed herein.

[00134] Preferred distinct classes include a non-disease class and a disease class, more preferably a non-cancer class and a cancer class, the latter preferably being lung cancer, breast cancer,

gastrointestinal cancer, or prostate cancer. Other preferred distinct classes are a high risk class and a non-disease class, preferably a high risk cancer class and a non-cancer class. Other preferred distinct classes are distinct cancer classes, such as distinct lung cancer classes, such as NSCLC and SCLC. Other preferred distinct cancer classes are metastatic cancer and non-metastatic cancer classes.

[00135] In a preferred embodiment, two or more peptides of the epitope microarray correspond to distinct regions of a single protein, preferably non-overlapping regions of the single protein.

[00136] As disclosed herein, epitopes corresponding to different segments of a single protein can exhibit discordant differences in their binding activities between samples from different classes. Without being bound by theory, this discordance of autoantibody binding activities between epitopes corresponding to the same protein may be due, in part, to protein alterations and consequent epitope alterations that contribute to the distinction of the classes. In support, splice variants of a large number of mRNAs, including mRNAs encoding embryonal transcription factors, have been identified in a variety of cancers.

[00137] In one embodiment, one or more peptides of the array is directed to an autoantibody that specifically binds the protein product of an alternatively spliced mRNA that is present or predominant, with respect to transcripts of the particular gene, in a first class, but absent or nondominant in a second class.

[00138] At least a first peptide of an epitope microarray herein has higher autoantibody binding activity with a first sample corresponding to a first class as compared to its autoantibody binding activity with a second sample corresponding to a second class, and at least a second peptide of the epitope microarray has higher autoantibody binding activity with the second sample corresponding to the second class as compared to its autoantibody binding activity with the first sample corresponding to the first class. Thus between two distinct classes, autoantibody binding activity that is higher in each class detectable with the preferred microarrays of the invention. With respect to cancer diagnostics, the preferred cancer diagnostic microarrays include epitopes capable of detecting autoantibody binding activities that are higher in a non-cancer sample than a cancer sample, as well as epitopes that are capable of detecting autoantibody binding activities that are higher in a cancer sample than a non-cancer sample, the latter potentially attributable to the appearance of tumor-associated antigens in an individual with cancer.

[00139] Once binding of autoantibody to array-bound epitope, and binding of detection agent to immobilized autoantibody occurs, the arrays are inserted into a scanner which can detect patterns of binding. The autoantibody binding data may be collected as light emitted from the labeled groups of the detection agents bound to the array. Since the position of each epitope on the array is known,

particular autoantibody binding activities are determined. The amount of light detected by the scanner becomes raw data that the invention applies and utilizes. The epitope array is only one example of obtaining the raw autoantibody binding activity data. Other methods for determining autoantibody binding activity known in the art (eg., ELISA, phage display, etc.), or developed in the future can be used with the present invention.

Peptide Epitopes and Microarray Preparation

[00140] Peptides, as used herein, includes modified peptides, such as phosphopeptides. Peptides may be derived from any of a number of sources, as appreciated by one of skill in the art. For example, random peptides may be generated by expression systems known in the art. Peptides may be generated by extensive protein fragmentation. Preferably, peptides are synthesized according to methods well known in the art. For example, see Methods in Enzymology, Volume 289: Solid-Phase Peptide Synthesis, J. Abelson et al., Academic Press, 1st edition, November 15, 1997, ISBN 0121821900. In a preferred embodiment, a Perkin-Elmer Applied Biosystems 433A Peptide synthesizer is used to synthesize peptides, allowing for synthesis of modified peptides.

[00141] Epitope microarrays may be prepared according to methods well known in the art. For example, see *Protein Microarray Technology*, D. Kambhampati (ed.), John Wiley & Sons, March 5, 2004, ISBN 3527305971; *Protein Microarrays*, M. Schena, Jones & Bartlett Publishers, July, 2004, ISBN 0763731277; and Protein Arrays: Methods and Protocols (Methods in Molecular Biology), E. Fung, Humana Press, April 1, 2004, ISBN 158829255X. In a preferred embodiment, a Piezorray Non-contact Spotting System from Perkin Elmer is used according to the manufacturer's specifications.

Sample Sources and Manipulation

[00142] A sample can be any sample comprising autoantibodies. Preferred samples include blood, plasma, cerebrospinal fluid, and synovial fluid.

[00143] Blood may be collected from each individual by venipuncture. 0.1-0.5 ml may be used to prepare blood serum or plasma. Serum may be prepared just after blood drawing. Tubes may be left at room temperature for 4 hours following centrifugation at 170 x g for 5 minutes after which serum is removed. Serum may be aliquoted and stored at –20 C. Plasma may be prepared by adding EDTA (final concentration of 5 mM) to blood sample. Blood sample may be centrifuged at 170 x g for 5 minutes, supernatant removed and stored at -20°C.

[00144] TABLE 1 – Informative Epitopes - Disclosed are 1,448 peptide epitopes, as well as corresponding protein names, Genbank accession numbers, and peptide sites. These epitopes may

be used as an initial set for autoantibody profiling. Of these, 1,253 were used as an initial set to measure autoantibody binding activities in lung cancer samples. See Experimental.

Gene	Accession #	position	epitope	length
ACADVL - acyl-Coenzyme A dehydrogenase, very long chain	NM_000018			
ACADVL745		745	KHKKGIVNEQFLLQ	14
ACADVL860		860	WQQELYRNFKSISKA	15
ACADVL407		407	KMGIKASNTAEVFFD	15
ACADVL324		324	CGKYYTLNGSKLWIS	15
ACADVL487		487	KAVDHATNRTQFGEK	15
ACADVL257		257	LFGTKAQKEKYLPKL	15
ACADVL661		661	ALKNPFGNAGLLLGE	15
ADSL - adenylosuccinate lyase	NM_000026			
ADSL244		244	DLCMDLQNLKRVRDD	15
ADSL85		85	QIQEMKSNLENIDFK	15
ADSL164		164	TDLIILRNALDLLLP	15
ADSL156		156	TSCYVGDNTDLIILR	15
ADSL476		476	TADTILNTLQNISEG	15
ADSL411		411	RCCSLARHLMTLVMD	15
ADSL97		97	DFKMAAEEEKRLRHD	15
AP1G2 - adaptor-related protein complex 1, gamma 2 subunit	NM_003917			
AP1G2584		584	VRDDAVANLTQLIGG	15
AP1G2497		497	ELSLALVNSSNVRAM	15
AP1G2500		500	LALVNSSNVRAMMQE	15
AP1G2425		425	FLLNSDRNIRYVALT	15
AP1G21020		1020	LFRILNPNKAPLRLK	15
AP1G2656		656	GDLLLAGNCEEIEPL	15
AP1G2938		938	SFIRPPENPALLLIT	15
AP1G2701		701	LLEKVLQSHMSLPAT	15
AP1G2967		967	ICQAAVPKSLQLQLQ	15
AP1G2388		388	DTSRNAGNAVLFETV	15
ASCC3L1 - activating signal cointegrator 1 complex subunit 3-like 1	NM_014014			
ASCC3L1884		884	GLSATLPNYEDVATF	15
ASCC3L12395		2395	RRMTQNPNYYNLQGI	15
ASCC3L11965		1965	RRWKQRKNVQNINLF	15
ASCC3L12472		2472	IAAYYYINYTTIELF	15
ASCC3L1405		405	SDDRECENQLVLLLG	15
ASCC3L11968		1968	KQRKNVQNINLFVVD	15
ASCC3L12519		2519	GLIEIISNAAEYENI	15
ASCC3L1659		659	LYRAALETDENLLLC	15
BAIAP3 - BAI1-associated protein 3	NM_003933			
BAIAP31198		1198	LSPDSIQNDEAVAPL	15
BAIAP31099		1099	ALCVVLNNVELVRKA	15

BAIAP31217		1217	DEKLALLNASLVVRK	15
BAIAP3567		567	EHSAEEPNSSSWRGE	15
BOP1 - block of proliferation 1	NM_015201			
BOP1641		641	LVAAAVEDSVLLLNP	15
BOP1825		825	LTKKLMPNCKWVS	13
Cep290 - Homo sapiens centrosome protein cep290 (Cep290), mRNA.	NM_025114			
Cep290707		707	IDLTEFRNSKHLKQQ	15
Cep2901287		1287	ALQKVVDNSVSLSEL	15
Cep2901345		1345	MLVQRTSNLEHLECE	15
Cep2901423		1423	KAKKSITNSDIVSIS	15
Cep2903023		3023	KLRIAKNNLEILNEK	15
Cep290471		471	QLDADKSNVMALQQG	15
Cep2902537		2537	QGKPLTDNKQSLIEE	15
Cep2902465		2465	RENSLTDNLNDLNNE	15
Cep2901107		1107	RKFAVIRHQQSLLYK	15
CGI-09 - Homo sapiens CGI-09 protein (CGI-09), mRNA.	NM_015939			
CGI-09637		637	ADTSLKSNASTLESH	15
CGI-09169		169	IVQQLIENSTTFRDK	15
CGI-09575		575	LSETWLRNYQVLPDR	15
CGI-09490		490	AALLSERNADGLIVA	15
CGI-0987		87	GTAFEVTSGGSLQPK	15
CGI-63 - Homo sapiens nuclear receptor binding factor 1 (CGI-63)	NM_016011			
CGI-63100		100	KMLAAPINPSDINMI	15
CGI-63156		156	QVVAVGSNVTGLKPG	15
CHTF18 - CTF18, chromosome transmission fidelity factor 18 homolog	NM_022092			
CHTF181110		1110	YIYRLEPNVEELCRF	15
CHTF18882		882	VVQGLFDNFLRLRLR	15
CLK3 - CDC-like kinase 3	NM_001292			
CLK3158		158	RRTRSCSSASSMRLW	15
COTL1 - coactosin-like 1	NM_021149			
COTL1154		154	AKEFVISDRKELEED	15
CSDA				
CSDA - cold shock domain protein A	NM_003651			
CSDA422		422	QQATSGPNQPSVRRG	15
CSDA7		7	AGEATTTTTTTLPQA	15
CSDA175		175	PQARSVGDGETVEFD	15
DKFZp434F054 - Homo sapiens hypothetical protein DKFZp434F054	NM_032259			
DKFZp434F054-113		113	LLATAATNGVVVTW	14
DKFZp434F054-650		650	LPLMNSFNLKDMAPG	15
DKFZp434F054-647		647	SCGLPLMNSFNLKDM	15
DKFZp434F054-26		26	CHLDAPANAISVCRD	15
DKFZp434F054-701		701	SDTVLLDSSATLITN	15

EEF1D - eukaryotic translation elongation factor 1 delta	NM_001960			
EEF1D-37		37	AGASRQENGAS	11
EFHD2 - EF hand domain containing 2	NM_024329			
EFHD2-113		113	FSRKQIKDMEKMFK	14
EXOSC9 - exosome component 9	NM_005033			
EXOSC9-246		246	LILKALENDQKVRKE	15
EXOSC9-24		24	LMERCLRNSKCIDTE	15
FAHD1 - fumarylacetoacetate hydrolase domain containing 1	NM_031208			
FAHD1-104		104	KRCRAVPEAAAMDYV	15
FAHD1-36		36	EMRSAVLSEPVL	12
FAHD1-237		237	YIISYVSKIITLEEG	15
FLJ10385 - Homo sapiens hypothetical protein FLJ10385	NM_018081			
FLJ10385-629		629	LPQKDCTNGVSLHPS	15
FLJ10385-332		332	VASSSRENPIHIWDA	15
FLJ10385-250		250	ILTNSADNILRIYNL	15
FLJ10385-157		157	SLSEEEANGPELGSG	15
FLJ10385-556		556	SLGREVTTNQRIYFD	15
FLJ10385-247		247	GSCILTNSADNILRI	15
FLJ10385-578		578	LVSGSTSGAVSVWDT	15
FLJ10385-557		557	LGREVTTNQRIYFDL	15
FLJ10385-321		321	LMSSAQPDTSYVASS	15
GL009 - Homo sapiens hypothetical protein GL009	NM_032492			
GL009-113		113	LLSFPRNNISYLVL	14
GL009-184		184	LFGFSAVSIMYLVLV	15
GL009-76		76	VAKMSVGHLRLLSHD	15
GL009-15		15	TDGSDFQHRERVAMH	15
GNPTAG - N-acetylglucosamine-1-phosphotransferase, gamma subunit	NM_032520			
GNPTAG-379		379	SNLEHL	12
GNPTAG-263		263	DELITPQGHEKLLRT	15
GNPTAG-109		109	PFHNVTQHEQTFRWN	15
GRINA - glutamate receptor, ionotropic,	XM_291268			
GRINA-299		299	NTEAVIMA	8
GRINA-255		255	FRRKHPWNLVALSVL	15
GRINA-421		421	YVFAALNLYTDIINI	15
GRINA-224		224	FVRENVWTYYVS	12
GRINA-398		398	TCFLAVDTQLLLGNK	15
GTF2H2 - general transcription factor IIH, polypeptide 2	NM_001515			
GTF2H2-240		240	LTTCDPSNIYDLIKT	15
GTF2H2-185		185	HGEPSLYNSLSIAMQ	15
GTF2H2-325		325	PPPASSSSECSLIRM	15
GTF2H2-487		487	YVCAVCQNVFCVDCD	15

GTF2H2-151		151	IIVTKSKRAEKLTEL	15
GTF2H2-193		193	SLSIAMQTLKHMP	13
GTF2H2-462		462	PLEEYNGERFCYG	13
HAGH - hydroxyacylglutathione hydrolase	NM_005326			
HAGH-8		8	VLPALTDNYMYLVID	15
HAGH-238		238	GHEYTINNLKFARHV	15
HAGH-108		108	ALTHKITHLSTLQVG	15
HAGH-80		80	HWDHAGGNEKLVKLE	15
HAGH-105		105	RIGALTHKITHLSTL	15
HAGHL - hydroxyacylglutathione hydrolase-like	NM_032304			
HAGHL-8		8	VIPVLEDNYMYLVIE	15
HAGHL-237		237	GHEHTLSNLEFAQKV	15
HAGHL-190		190	LEGSAQQMYQSLAEL	15
HAGHL-193		193	SAQQMYQSLAELG	13
HAGHL-108		108	SLTRRLAHGEELRFG	15
HDAC5 - histone deacetylase 5	NM_005474			
HDAC5-1027		1027	LYGTSPLNRQKLDSK	15
HDAC5-481		481	LPLDSSPNQFSLYTS	15
HDAC5-1194		1194	GTQQAFYNDPSVLYI	15
HDAC5-1112		1112	VAAGELKNGFAIIRP	15
HDAC5-102		102	QELLALKQQQQLQKQ	15
HDAC5-1136		1136	AMGFCFFNSVAITAK	15
HDAC5-1414		1414	AVLQQKPNINAVATL	15
HDAC5-702		702	QLVMQQQHQQFL	15
HDAC5-175		175	QEMLAAKRQQELEQQ	15
HDAC5-506		506	QATVTVTNSHLTASP	15
HDAC5-426		426	GPSSPNSSHSTIAEN	15
HDAC5-487		487	PNQFSLYTSPSLPNI	15
HDAC5-644		644	TGERVATSMRTVGKL	15
HLA-B - major histocompatibility complex, class I, B	NM_005514			
HLA-B-115		115	YKAQAQTDRESL	12
HLA-B-182		182	HDQYAYDGKDYIALN	15
HLA-C - major histocompatibility complex, class I, C	NM_002117			
HLA-C-479		479	CSNSAQGSDESLITC	15
HLA-C-182		182	YDQSAYDGKDYIALN	15
HLA-C-258		258	LRRYLENGKETLQRA	15
HSPA4 - heat shock 70kDa protein 4	NM_002154			
HSPA4-1022		1022	NNKLNLQNKQSLTMD	15
HSPA4-381		381	MSANASDLPLS	12
HSPA4-76		76	AKSQVISNAKNTVQG	15
HSPA4-873		873	FVSEDDRNSFTLKLE	15
HSPA4-1016		1016	AMEWMNNKLNLQNK	14

HSPA4-966		966	KIISSFKNKEDQYDH	15
HSPA4-806		806	MLNLYIENEGKMIMQ	15
HSPA4-658		658	HGIFSVSSASLVEVH	15
HSPH1 - heat shock 105kDa/110kDa protein 1	NM_006644			
HSPH1-381		381	MSSNSTDLPLN	12
HSPH1-83		83	HANNTVSNFKRFHGR	15
HSPH1-891		891	ICEQDHQNFLRLLTE	15
HSPH1-780		780	IPDADKANEKKVDQP	15
HSPH1-71		71	TIGVAAKNQQITHAN	15
HSPH1-1141		1141	ECYPNEKNSVNMD	13
HSPH1-1107		1107	PKLERTPNGPNIDKK	15
IQWD1 - IQ motif and WD repeats 1				
IQWD1-173		173	LDEQQDNNNEKLSPK	15
IQWD1-315		315	SAENPVENHINITQS	15
IQWD1-655		655	LMLEETRNTITVPAS	15
IQWD1-28		28	RGGTSQSDISTLPTV	15
IQWD1-338		338	DSNSGERNDLNLDRS	15
IQWD1-646		646	ADEVITRNELMLEET	15
IQWD1-395		395	TSTESATNENNTNPE	15
JPH4 - junctophilin 4	NM_032452			
JPH4-498		498	RAVSAARQRQEIAAA	15
KIAA0373/centrosome protein cep290	NM_025114			
KIAA0373-707		707	IDLTEFRNSKHLKQQ	15
KIAA0373-1287		1287	ALQKVVDNSVSLSEL	15
KIAA0373-1345		1345	MLVQRTSNLEHLECE	15
KIAA0373-1410		1410	ETKLGNESSMDKA	13
KIAA0373-1423		1423	KAKKSITNSDIVSIS	15
KIAA0373-3203		3203	KLRIAKNNLEILNEK	15
KIAA0373-271		271	RSQLSKKNYELIQY	14
KIAA0373-471		471	QLDADKSNVMALQQG	15
KIAA0373-113		113	TKVMKLENELEMAQ	14
KIAA0373-2537		2537	QGKPLTDNKQSLIEE	15
KIAA0373-2465		2465	RENSLTDNLNDLNNE	15
KIAA0373-938		938	VNAIESKNAEGIFDA	15
KIAA0373-1107		1107	RKFAVIRHQQSLLYK	15
KIAA0373-807		807	LDLLSLKNMSEAQSK	15
KIAA0373-634		634	VEIKNCKNQIKIRDR	15
KIAA0373-2401		2401	SQKEAHLNVQQIVDR	15
KIAA0373-1203		1203	KITVLQVNEKSLIRQ	15
KIAA0373-1193		1193	MKKILAENSRKITVL	15
KIAA0373-720		720	QQQYRAENQILLKEI	15
KIAA0373-3110		3110	KKNQSITDLKQLVKE	15
KIAA0373-2294		2294	KVKAEVEDLKYLLDQ	15
KIAA0373-1050		1050	ASIINSQNEYLIHLL	15
KIAA0373-64		64	QENVIHLFRI	10

KIAA0373-2692		2692	LGIRALESEKELEEL	15
KIAA0373-1972		1972	DPSLPLPNQLEIALR	15
KIAA0373-3234		3234	GAESTIPDADQLKEK	15
KIAA0373-1210		1210	NEKSLIRQYTTLVEL	15
KIAA0683	NM_016111			
KIAA0683-234		234	GNRLQQENLAEFFPQ	15
KIAA0683-242		242	LAEFFPQNYFRLLGE	15
KIAA0683-868		868	QPGSPSPNTPCLPEA	15
KIAA0683-323		323	PRLAALTQGSYLHQR	15
KRT18 - keratin 18	NM_000224			
KRT18-8		8	TRSTFSTNYRSLGSV	15
KRT18-343		343	YDELARKNREELDKY	15
KRT18-185		185	IFANTVDNARIVLQI	15
KRT18-566		566	GKVVSETNDTKVLRH	15
KRT18-544		544	DALDSSNSMQTIQKT	15
KRT18-252		252	RKVIDDTNITRLQLE	15
KRT18-567		567	KVVSETNDTKVLRH	14
KRT18-484		484	EGQRQAQEYEALLNI	15
KRT18-96		96	AGMGGIQNEKETMQS	15
LDHB - lactate dehydrogenase B	NM_002300			
LDHB-347		347	LIESMLKNLSRIHPV	15
LDHB-18		18	EEATVPNNKITVVGV	15
LDHB-387		387	KGMYGIENEVFLSLP	15
LDHB-177		177	CIIIVVSNPVDILTY	15
LDHB-106		106	KDYSVTANSKIVVVT	15
LDHB-307		307	GTDNDSENWKEVHKM	15
LDHB-17		17	EEEATVPNNKITVVG	15
LGALS4 - lectin, galactoside-binding, soluble, 4 (galectin 4)	NM_006149			
LGALS4-391		391	DRFKVYANGQHLFDF	15
LGALS4-237		237	HCHQQLNSLPTMEGP	15
LGALS4-407		407	HRLSAFQRVDTLEIQ	15
LGALS4-415		415	VDTLEIQGDVTLSYV	15
LGALS4-155		155	EHYKVVVNGNPFYEY	15
LOC162962 - similar to zinc finger protein 616	XM_091886			
LOC162962-177		177	VENKCIENQLTLSFQ	15
LOC162962-232		232	QSEKTVNNSSLVSPL	15
LOC162962-36		36	YWDVMLENYRNL	12
LOC162962-497		497	RQNSNLVNHQRIHTG	15
LOC162962-315		315	RVSSSLINHQMVHTT	15
LOC162962-854		854	LSNHKRIHTG	10
LOC162962-799		799	ECGTVFRNYSCLARH	15
LOC162962-1113		1113	RVRSILVNHQKMHTG	15
LOC162962-231		231	NQSEKTVNNSSLVSP	15
LOC162962-111		111	YLREIQKNLQDLEFQ	15

LOC162962-1189		1189	FGRFSCLNKHQMIHS	15
LOC162962-543		543	KSFSQSSNLATHQTV	15
LOC162962-904		904	DCGKAYTQRSSLT	13
LOC388198 -	XM_373655			
LOC388198-145		145	RSSTGAYALRLC	12
LOC388198-9		9	GAAYSAQRMAGLVLP	15
LOC388561 - similar to zinc finger protein 600	XM_371192			
LOC388561-230		230	NESGKAFNYSSLLRK	15
LOC388561-182		182	NHGNNFWNSSLLTQK	15
LOC388561-7		7	FLSTAQGNREVFHAG	15
LOC388561-461		461	KTFSHKSSLTCH	12
LOC388561-412		412	ECGKTFSHKSSLTCH	15
LOC388561-307		307	ECGKTFSQTSSLTCH	15
LOC388561-874		874	ECGKNFSQKSSLICH	15
LOC401193 - similar to psi neuronal apoptosis inhibitory protein	XM_376391			
LOC401193-87		87	NTASSSLNIFSLLPT	15
LOC401193-77		77	KEPISLNNSINTASS	15
LOC401193-156		156	EFLRSKKSSEEITQY	15
LOC90333	XM_030958			
LOC90333-12		12	IQSFKSFNCSSLLKK	15
LOC90333-398		398	ECGKTFSQMSSLVYH	15
LOC90333-321		321	VCDKAFQRDSHLAQH	15
LSM1 - LSM1 homolog, U6 small nuclear RNA associated	NM_014462			
LSM1-164		164	DRGLSIPRADTLDEY	15
LSM1-33		33	GFLRSIDQFANLVLH	15
LSM1-87		87	IFVVRGENVVLLGEI	15
MAGEA4 - melanoma antigen, family A, 4	NM_002362			
MAGEA4-234		234	KEVDPTSNTYTLVTC	15
MAGEA4-181		181	MLERVIKNYKRCFPV	15
MAGEA4-85		85	GPPQSPQGASALPTT	15
MIF - macrophage migration inhibitory factor	NM_002415			
MIF-141		141	NAANVGWN	8
MIF-92		92	IGGAQNRSYSKLLCG	15
MIF-115		115	SPDRVYINYYDM	12
MSLN - mesothelin	NM_005823			
MSLN-74		74	GVLANPPNISSLSPR	15
MSLN-71		71	PLDGVLANPPNISSL	15
MSLN-186		186	FSRITKANVDLLPRG	15
MSLN-652		652	RLAFQNMNGSEYFVK	15
MSLN-510		510	PEDIRKWNVTSL	12
MSLN-324		324	PSTWSVSTMDALRGL	15
MSLN-259		259	PGRFVAESAEVLLPR	15

NACA - nascent-polypeptide-associated complex alpha	NM_005594			
NACA-261		261	AVRALKNNSNDIVNA	15
NACA-66		66	QATTQQAQLAAA	12
NACA-251		251	MSQANVSRAKAVRAL	15
NISCH - nischarin	NM_007184			
NISCH-428		428	NGLLVVDNLQHLYNL	15
NISCH-478		478	GLHTKLGNIKTLNLA	15
NISCH-805		805	CIGYTATNQDFIQRL	15
NISCH-1764		1764	KTTGKMENYELIHSS	15
NISCH-555		555	EHVSLLNNPLSIIPD	15
NISCH-710		710	ALASSLSSTDSLTPE	15
NISCH-1271		1271	THNCRNRNSFKLSRV	15
NISCH-97		97	PKKIIGKNSRSLVEK	15
NISCH-1360		1360	QLRASLQDLKTVVIA	15
NISCH-465		465	HLDLSYNKLSSLEGL	15
NISCH-333		333	SVRFSATSMKEVLVP	15
NISCH-1105		1105	RSCFAPQHMAMLCSP	15
NUBP2 - nucleotide binding protein 2	NM_012225			
NUBP2-179		179	PPGTSDEHMATIEAL	15
NUBP2-5		5	EAAAEPGNLAGVRHI	15
NUBP2-249		249	RVMGIVENMSGFTCP	15
OGFR - opioid growth factor receptor	NM_007346			
OGFR-165		165	NYDLLEDNHSYIQWL	15
OGFR-639		639	SAAVASGGAQTLALA	15
OGFR-269		269	LNWRSHNNLRITRIL	15
PABPC1 - poly(A) binding protein, cytoplasmic 1	NM_002568			
PABPC1-796		796	GMLLEIDNSELLHML	15
PABPC1-150		150	NLDKSIDNKALYDTF	15
PABPC1-90		90	ERALDTMNFDVIKGK	15
PABPC1-650		650	TQRVANTSTQTMGPR	15
PABPC1-332		332	QKAVDEMNGKELNGK	15
PAI-RBP1 - mRNA-binding protein	NM_015640			
PAI-RBP1-304		304	GTVKDELTDLDQS	13
PAI-RBP1-102		102	RKNPLPPSVGVVDKK	15
PAI-RBP1-158		158	PDQQLQGEGKIIDRR	15
PDXK - pyridoxal (pyridoxine, vitamin B6) kinase	NM_003681			
PDXK-111		111	DKSFLAMVVDIVQEL	15
PDXK-7		7	ECRVLSIQSHVIRGY	15
PDXK-114		114	FLAMVVDIVQELK	13
PDXK-346		346	TVSTLHHVLQRTIQC	15
PDXK-339		339	LKVACEKTVSTLHHV	15
PDXK-89		89	LYEGLRLNNMNKYDY	15
PDXK-263		263	NYLIVLGSQRRRNPA	15

PDXK-101		101	YDYVLTGYTRDKSFL	15
RAB40C- member RAS oncogene family	NM_021168			
RAB40C-310		310	KSFSMANGMNAVMMH	15
RAB40C-319		319	NAVMMHGRSYSLASG	15
RAB40C-225		225	FNVIESFTELSRI	13
RAB40C-164		164	VPRILVGNRLHLAFK	15
RAB40C-78		78	TTILLDGRRVRLELW	15
RAB40C-237		237	SRIVLMRHGMEKIWR	15
RAB40C-340		340	KGNSLKRSKSIRPPQ	15
RAB40C-334		334	AGGGGSKGNSLKRSK	15
RBMS1 - RNA binding motif, single stranded interacting protein 1	NM_002897			
RBMS1-21		21	YPQYLQAKQSLVPAH	15
RBMS1-79		79	GWDQLSKTNLYIRGL	15
RBMS1-462		462	SPLAQQMSHLSLG	13
RBMS1-157		157	SPAAAQKAVSALKAS	15
RBMS1-495		495	QYAHMQTTAVPVEEA	15
RBMS1-108		108	PYGKIVSTKAILDKT	15
RHBDL1 - rhomboid, veinlet-like 1	NM_003961			
RHBDL1-464		464	CPYKLLRMVLALVCM	15
RHBDL1-267		267	ASVTLAQIIVFLCYG	15
RHBDL1-349		349	GFNALLQLMIGVPLE	15
RHBDL1-503		503	FMAHLAGAVVGVSMG	15
RHBDL1-471		471	MVLALVCMSSEVGRA	15
RHBDL1-401		401	LAGSLTVSITDMRAP	15
RHBDL1-555		555	WWVVLLAYGTFLLFA	15
RHBDL1-332		332	AWRFLTYMFMHVGLE	15
RHOT2 - ras homolog gene family, member T2	NM_138769			
RHOT2-309		309	APQALEDVKTVVCRN	15
RHOT2-807		807	LLGVVGAAVAAVLSF	15
RHOT2-815		815	VAAVLSFSLYRVLVK	15
RHOT2-7		7	DVRILLLGEAQVGKT	15
RHOT2-335		335	LDGFLFLNTLFIQRG	15
RHOT2-543		543	QAHAITVTREKRLDQ	15
RHOT2-659		659	VACLMFDGSDPKSFA	15
RNPC2 - RNA-binding region (RNP1, RRM) containing 2	NM_004902			
RNPC2-642		642	KCPSIAAAIAAVNAL	15
RNPC2-701		701	FPDSMTATQLLVPSR	15
RNPC2-231		231	RPRDLEEFFSTVGKV	15
RNPC2-420		420	NGFELAGRPMKVGHV	15
RNPC2-662		662	AGKMITAAYVPLPTY	15
RNPC2-551		551	TEASALAAAASVQPL	15
RNPC2-561		561	SVQPLATQCFQLSNM	15

RNPC2-266		266	EFVDVSSVPLAIGLT	15
ROCK2 - Rho-associated, coiled-coil containing protein kinase 2	NM_004850			
ROCK2-1334		1334	TNRTLTSDVANLANE	15
ROCK2-403		403	YADSLVGTYSKIMDH	15
ROCK2-1517		1517	DIEQLRSQLQALHIG	15
ROCK2-163		163	YAMKLLSKFEMIKRS	15
ROCK2-66		66	SLLDGLNSLVLD	12
ROCK2-1127		1127	ENNHLMEMKMNLEKQ	15
ROCK2-1018		1018	EERTLKQKVENLLLE	15
ROCK2-1296		1296	HKQELTEKDATIASL	15
ROCK2-644		644	VNTRLEKTAKELEEE	15
ROCK2-818		818	KNCLLETAKLKLEKE	15
RPL15-ribosomal protein L15	NM_002948			
RPL15-118		118	FARSLQSVA	9
RPL15-114		114	NQLKFARSLQSVA	12
RPL15-17		17	KQSDVMRFLLRVRCW	15
RUNDC1 - RUN domain containing 1	NM_173079			
RUNDC1-704		704	PKQSLLTAIHMVLTE	15
RUNDC1-795		795	SALNLLSRLSSLKFS	15
RUNDC1-110		110	ERRRLDSALLALSSH	15
RUNDC1-466		466	TGLHLMRRALAVLQI	15
RUNDC1-439		439	NEQRLVSWVNLICKS	15
RUNDC1-316		316	LDMNLNEDISSLSTE	15
RUNDC1-507		507	YSPLLKRLEVSVDRV	15
RUNDC1-332		332	LRQRVDAAVAQIVNP	15
RUNDC1-248		248	QKELILQLKTQLDDL	15
RUNDC1-3		3	MAAIEAAAEPVTVV	15
RUNDC1-576		576	VRKELTVAVRDLLAH	15
RUTBC3 - RUN and TBC1 domain containing 3	NM_015705			
RUTBC3-862		862	PEELLYRAVQSVNVT	15
RUTBC3-386		386	LHWFLTAFASVVDIK	15
RUTBC3-904		904	WLEVLCSSLPTVE	13
RUTBC3-482		482	VAMRLAGSLTDVAVE	15
RUTBC3-475		475	DAELLLGVAMRLAGS	15
RUTBC3-581		581	LVADLREAILRVARH	15
RUTBC3-892		892	ICVGLNEQVLHLWLE	15
RUTBC3-462		462	NTLSDIPSQMEDA	13
RUTBC3-81		81	PGSSLLANSPLMEDA	15
RUTBC3-307		307	AFWMMSAIIEDLLPA	15
RUTBC3-246		246	GVPRLRRVLRALAWL	15
RUTBC3-413		413	GSRVLFQLTLGMLHL	15
RUTBC3-338		338	LRHLIVQYLPRLDKL	15
RUTBC3-740		740	GDDSVTEGVTDLVRG	15
RUTBC3-349		349	LDKLLQEHDIELSLI	15

RUTBC3-502		502	HLAYLIADQGQLLGA	15
SBDS - Shwachman-Bodian-Diamond syndrome	NM_016038			
SBDS-71		71	LDEVLQTHSVFVNVS	15
SBDS-108		108	CKQILTKGEVQVSDK	15
SBDS-252		252	LKEKLKPLIKVIESE	15
SBDS-148		148	QLEQMFRDIATIVAD	15
SCNN1A - sodium channel, nonvoltage-gated 1 alpha	NM_001038			
SCNN1A-732		732	PSVTMVTLLSNLGSQ	15
SCNN1A-346		346	ILSRLPETLPSLEED	15
SCNN1A-786		786	VFDLLVIMFLMLLRR	15
SCNN1A-343		343	YINILSRLPETLPSL	15
SCNN1A-88		88	NNTTIHGAIRLVCSQ	15
SCNN1A-272		272	VASSLRDNNPQVD	13
SCNN1A-166		166	NSDKLVFPAVTICTL	15
SCNN1A-778		778	VEMAELVFDLLVI	13
SCNN1A-471		471	LLSTVTGARVMVHGQ	15
SCNN1A-787		787	FDLLVIMFLMLLRRF	15
SCNN1A-502		502	VETSISMRKETLDRL	15
SCNN1A-745		745	SQWSLWFGSSVLSV	14
SCNN1A-226		226	LYKYSSFTTLVAGS	14
SCNN1A-184		184	RYPEIKEELEELDRI	15
SCP2 - sterol carrier protein 2	NM_002979			
SCP2-330		330	QKYGLQSKAVEILAQ	15
SCP2-318		318	AAAAILASEAFVQKY	15
SCP2-719		719	GNMGLAMKLQNLQLQ	15
SCP2-728		728	QNLQLQPGNAKL	13
SCP2-165		165	GFEKMSKGSLGIKFS	15
SCP2-418		418	TNELLTYEALGLCPE	15
SCP2-153		153	IQGGVAECVLALGFE	16
SCP2-268		268	DEYSLDEVMASKEVF	15
SCP2-233		233	GKEHMEKYGTKIEHF	15
SCP2-100		100	IYHSLGMTGIPIINV	15
SDCCAG1 - serologically defined colon cancer antigen 1 , NY-CO-1	NM_004713			
SDCCAG1-13		13	LRAVLAELNASLLGM	15
SDCCAG1-934		934	LASCTSELISE	13
SDCCAG1-232		232	TLERLTEIVASAPKG	15
SDCCAG1-860		860	TGEYLTTGSFMIRGK	15
SDCCAG1-475		475	LKGELIEMNLQIVDR	15
SDCCAG1-417		417	DLKALQQEKQALKKL	15
SDCCAG1-942		942	TSELISEEMEQLDGG	15
SDCCAG1-9		9	STIDLRAVLAELNAS	15
SDCCAG1-482		482	MNLQIVDRAIQVVRS	15
SDCCAG1-165		165	GNIVLTDYEYVILNI	15

SDCCAG1-71		71	KATLLLESGIRIHTT	15
SDCCAG1-627		627	NKPLLVDVDLSLSAY	15
SDCCAG1-21		21	NASLLGMRVNNVYDV	15
SDCCAG10 - serologically defined colon cancer antigen 10,NY-CO-10	NM_005869			
SDCCAG10-311		311	KRELLAAKQKKVENA	15
SDCCAG10-400		400	FKSKLTQAIAETPEN	15
SDCCAG10-393		393	TLALLNQFKSKLTQA	15
SDCCAG10-159		159	EEEEVNRVSQSMKGK	15
SDCCAG3 -serologically defined colon cancer antigen 3,NY-CO-3	NM_006643			
SDCCAG3-322		322	DYHDLESVVQQVEQN	15
SDCCAG3-350		350	HVVKLKQEISLLQA	14
SDCCAG3-192		192	PSWALSDTDSRVSP	14
SDCCAG3-418		418	LRVVMNSAQASIKQL	15
SDCCAG3-428		428	SIKQLVSGAETLNLV	15
SDCCAG3-262		262	ENSKLRRKLNEVQSF	15
SDCCAG3-255		255	SYDALKDENSKLRRK	15
SDCCAG3-411		411	ADVALQNLRVVMNSA	15
SDCCAG3-462		462	AEILKSIDRISEI	13
SDCCAG3-248		248	HLRTLQISYDALKDE	15
SDCCAG8 - serologically defined colon cancer antigen 8, NY-CO-8	NM_006642			
SDCCAG8-419		419	ERDDLMSALVSVRSS	15
SDCCAG8-557		557	KMLILSQNIAQLEAQ	15
SDCCAG8-815		815	ECCTLAKKLEQISQK	15
SDCCAG8-423		423	LMSALVSVRSSLADT	15
SDCCAG8-945		945	ERQSLSEEVDRLRTQ	15
SDCCAG8-564		564	NIAQLEAQVEKVTKE	15
SDCCAG8-397		397	HEAVLSQTHTNVHMQ	15
SDCCAG8-582		582	AINQLEEIQSQLASR	15
SDCCAG8-798		798	QYLLLTSQNTFLTKL	15
SDCCAG8-776		776	LTQKIQQMEAQ	13
SDCCAG8-589		589	IQSQLASREMDV	13
SDCCAG8-156		156	NMPTMHDLVHTINDQ	15
SDCCAG8-561		561	LSQNIAQLEAQVEKV	15
SDCCAG8-184		184	CKEELSGMKNKIQVV	15
SDCCAG8-35		35	LTCALKEGDVTIG	13
SDCCAG8-28		28	ASRSIHQLTCALKEG	15
SDCCAG8-952		952	EVDRLRTQLPSMPQS	15
SDCCAG8-13		13	LEEILGQYQRSLREH	15
SDCCAG8-550		550	EREYMGSKMLILSQN	15
SEC14L1 - SEC14-like 1	NM_003003			
SEC14L1-488		488	GEEALLRYVLSVNEE	15
SEC14L1-560		560	GVKALLRIIEVVEAN	15
SEC14L1-190		190	EKIAMKQYTSNIKKG	15

SEC14L1-88		88	DAPRLLKKIAGVDYV	15
SEC14L1-730		730	ILIQIVDASSVITWD	15
SEC14L1-106		106	QKNSLNSRERTLHIE	15
SEC14L1-948		948	GFSQLSAATTSSSQS	15
SEC14L1-810		810	KVWQLGRDYSMVESP	15
SEC14L1-803		803	NNVQLIDKVWQLGRD	15
SEC14L1-882		882	SLPRVDDVLASLQVS	15
SEC14L1-579		579	LGRLLILRAPRVFPV	15
SEC14L1-1		1	MVQKYQSPVRVY	12
SEC14L1-493		493	LRYVLSVNEERLRRC	15
SEC14L1-263		263	SKKQAASMAVVIPEA	15
SEC14L1-898		898	HKCKVMYYTEVIGSE	15
SFRS2IP - splicing factor, arginine/serine-rich 2, interacting protein	NM_004719			
SFRS2IP-1417		1417	AAVKLAESKVSVAVE	15
SFRS2IP-339		339	PLSDLSENVESVVNE	15
SFRS2IP-491		491	LEKSLEEKNESLTEH	15
SFRS2IP-336		336	VSCPLSDLSENVESV	15
SFRS2IP-400		400	ESPKLESSEGEIIQT	15
SFRS2IP-1277		1277	LPLHLHTGVPLMQVA	15
SFRS2IP-1206		1206	LPINMMQPQMNVMQQ	15
SFRS2IP-1492		1492	YKEIVRKAVDKVCHS	15
SFRS2IP-1207		1207	PINMMQPQMNVMQQQ	15
SFRS2IP-158		158	DSSNICTVQTHVENQ	15
SFRS2IP-232		232	DLPVLVGEEGEVKKL	15
SFRS2IP-173		173	SANCLKSCNEQIEES	15
SLC2A11 - solute carrier family 2 , member 11 , GLUT10; GLUT11	NM_030807			
SLC2A11-403		403	GNDSVYAYASSVFRK	15
SLC2A11-381		381	LRRQVTSLVVL	12
SLC2A11-147		147	KSLLVNNIFVVSAA	14
SLC2A11-110		110	LFGALLAGPLAITLG	15
SLC2A11-93		93	LVLLMWSLIVSLYPL	15
SLC2A11-501		501	FPWTLYLAMACIFAF	15
SLC2A11-174		174	EMIMLGRLLVGVNAG	15
SLC2A11-151		151	LVNNIFVVSAAILFG	15
SLC2A11-233		233	MSSAIFTALGIVMGQ	15
SLC2A11-229		229	GAVAMSSAIFTALGI	15
SLC2A11-91		91	DHLVLLMWSLIVSLY	15
SLC2A11-237		237	IFTALGIVMGQVVGL	15
SLC2A11-178		178	LGRLLVGVNAGVSMN	15
SLC2A11-567		567	VCGALMWIMLILVGL	15
SOX8 - SRY (sex determining region Y)-box 8	NM_014587			
SOX8-173		173	HNAELSKTLGKLWRL	15
SOX8-349		349	SNVDISELSSEVMGT	15

SOX8-88		88	FPACIRDAVSQVLKG	15
SOX8-161		161	ARRKLADQYPHLHNA	15
SOX8-352		352	DISELSSEVMGT	12
SOX8-263		263	GGGAVYKAEAGLGDG	15
SOX8-17		17	SPSGTASSMSHVEDS	15
SOX8-177		177	LSKTLGKLWRLLSES	15
SOX8-96		96	VSQVLKGYDWSLVPM	15
SSRP1 - structure specific recognition protein 1	NM_003146			
SSRP1-414		414	MSGSLYEMVSRVMKA	15
SSRP1-425		425	VMKALVNRKITVPGN	15
SSRP1-418		418	LYEMVSRVMKALVNR	15
SSRP1-786		786	SITDLSKKAGEIWKG	15
SSRP1-391		391	ISLTLNMNEEEVEKR	15
SSRP1-78		78	RRVALGHGLKLLTKN	15
SSRP1-410		410	LTKNMSGSLYEMVSR	15
SSRP1-84		84	HGLKLLTKNGHVYKY	15
SSTR5 - somatostatin receptor 5	NM_001053			
SSTR5-152		152	FGPVLCRLVMTLDGV	15
SSTR5-100		100	NIYILNLAVADVLYM	15
SSTR5-329		329	SERKVTRMVLVVVLV	15
SSTR5-352		352	FTVNIVNLAVAL	15
SSTR5-230		230	WVLSLCMSLPLLVFA	15
SSTR5-104		104	LNLAVADVLYMLGLP	15
SSTR5-332		332	KVTRMVLVVVLVFAG	15
SSTR5-176		176	TVMSVDRYLAVVHPL	15
SSTR5-75		75	CAAGLGGNTLVIYVV	15
STK16 - serine/threonine kinase 16, MPSK; PKL12	NM_003691			
STK16-351		351	ALRQLLNSMMTVD	13
STK16-390		390	HIPLLLSQLEALQPP	15
STK16-348		348	HSSALRQLLNSMMTV	15
STK16-147		147	RGTLWNEIERLKDK	14
STK16-232		232	DLGSMNQACIHVEGS	15
STK16-304		304	WSLGCVLYAMMFG	13
STUB1 - STIP1 homology and U-Box containing protein 1, NY-CO-7	NM_005861			
STUB1-223		223	LHSYLSRLIAA	12
STUB1-100		100	HEQALADCRRALELD	15
STUB1-93		93	CYLKMQQHEQALADC	15
STUB1-340		340	DRKDIEEHLQRVGHF	15
STUB1-273		273	YMADMDELFSQV	12
TAF10 - TAF10	NM_006284			
TAF10-164		164	FLMQLEDYTPTIPDA	15
TAF10-266		266	LTPALSEYGINVKKP	15
TAF10-157		157	SSTPLVDFLMQLEDY	15

TAF10-112		112	PEGAISNGVYVLPSA	15
TAF10-259		259	YTLTMEDLTPALSEY	15
TP53 - tumor protein p53	NM_000546			
TP53-171		171	YSPALNKMFCQLAKT	15
TP53-348		348	SGNLLGRNSFEVRVC	15
TP53-340		340	TIITLEDSSGNLLGR	15
TP53-224		224	AIYKQSQHMTEV	12
TP53-86		86	EAPRMPEAAPRVAPA	15
TP53-24		24	DLWKLLPENNVLSPL	15
TP53-31		31	ENNVLSPLPSQAMDD	15
TPS1 - tryptase, alpha	NM_003293			
TPS1-1		1	MLSLLLLALPVL	12
TPS1-174		174	EPVNISSRVHTVMLP	15
TPS1-165		165	ADIALLELEEPVNIS	15
TPS1-11		11	ALPVLASRAYAAPAP	15
TPS1-103		103	DVKDLATLRVQLREQ	15
TPS1-237		237	PPFPLKQVKVPIMEN	15
TPSB1 - tryptase beta 1	NM_003294			
TPSB1-174		174	EPVNVSSHVHTVTLP	15
TPSB1-1		1	MLNLLLLALPVL	12
TPSB1-165		165	ADIALLELEEPVNVS	15
TPSB1-103		103	DVKDLAALRVQLREQ	15
TPSB1-11		11	ALPVLASRAYAAPAP	15
TPSB1-159		159	YTAQIGADIALLELE	15
TPSD1 - tryptase delta 1	NM_012217			
TPSD1-3		3	MLLLAPQMLSLLLL	15
TPSD1-181		181	EPVNISSHIHTVTLP	15
TPSD1-149		149	YQDQLLPVSRIIVHP	15
TPSD1-10		10	QMLSLLLLALPVLAS	15
TPSD1-172		172	ADIALLELEEPVNIS	15
UBE2I - ubiquitin-conjugating enzyme E2I	NM_003345			
UBE2I-150		150	PAITIKQILLGIQEL	15
UBE2I-154		154	IKQILLGIQELLNEP	15
UTP14A - UTP14, U3 small nucleolar ribonucleoprot, homA, NY-CO-16	NM_006649			
UTP14A-66		66	KLLEAISSLDGK	12
UTP14A-5		5	TANRLAESLLALSQQ	15
UTP14A-107		107	EKLVLADLLEPVKTS	15
UTP14A-905		905	EKRNIHAAAHQV	12
UTP14A-668		668	EEPLLLQRPERV	12
UTP14A-144		144	VKKQLSRVKSK	12
UTP14A-818		818	IRDFLKEKREAVEAS	15
UTP14A-223		223	LEKEEPAIAPI	12
UTP14A-182		182	TAQVLSKWDPVVLKN	15
UTP14A-89		89	SEASLKVSEFNVSSE	15

UTP14A-627		627	VLSELRVLSQKLKEN	15
UTP14A-254		254	IFNLLHKNKQPVTDP	15
UTP14A-246		246	ARTPLEQEIFNLLHK	15
WFIKKN1 - WAP, follis/kazal, im, kunitz and netrin domain cont. 1	NM_053284			
WFIKKN1-583		583	SDFAIVGRLTEVLEE	15
WFIKKN1-15		15	LLLRLTSGAGLLPGL	15
WFIKKN1-3		3	MPALRPLLPLLLLL	14
WFIKKN1-723		723	ILELLEKQACELLNR	15
WFIKKN1-640		640	GLKFLGTKYLEVTLS	15
WFIKKN1-576		576	LALSLCRSDFAIVGR	15
WFIKKN1-645		645	GTKYLEVTLSGMDWA	15
WFIKKN1-324		324	YGNVVVTSIGQLVLY	15
WFIKKN1-701		701	DGVAVLDAGSYVRAA	15
WFIKKN1-716		716	SEKRVKKILELLEKQ	15
WFIKKN1-506		506	YSPLLQQCHPFVYGG	15
ZNF28 - zinc finger protein 28 (KOX 24)	NM_006969			
ZNF28-15		15	VYDKIFEYNSYLAKH	15
ZNF28-92		92	ECGIVFNQQSHLASH	15
ZNF292 - zinc finger protein 292	XM_048070			
ZNF292-2597		2597	QMMALNSCTTSINSD	15
ZNF292-562		562	PNGKLIEEISEVDCK	15
ZNF292-3236		3236	TPEEIESMTASVDVG	15
ZNF292-1500		1500	TTPLLQSSEVAVSIK	15
ZNF292-2768		2768	SQCVLINTSVTLTPT	15
ZNF292-2630		2630	IKTAMNSQILEVKSG	15
ZNF292-861		861	QCLALMGEEASIVSS	15
ZNF292-662		662	QLSLLTKTVYHIFFL	15
ZNF292-2165		2165	ASMILSTNAVNLQQP	15
ZNF292-1850		1850	FPAHLASVSTPLLSS	15
ZNF292-330		330	PLPLLEVYTVAIQSY	15
ZNF292-659		659	RCRQLSLLTKTVYHI	15
ZNF292-502		502	KTNQLSQATALAKLC	15
ZNF292-2529		2529	LVENLTQKLNNVNNQ	15
ZNF292-2160		2160	QPSLLASMILSTNAV	15
ZNF292-3885		3885	VLKQLQEMKPTVSLK	15
ZNF292-1902		1902	QGGMLCSQMENLPST	15
ZNF292-2479		2479	TTMGLIAKSVEIPTT	15
ZNF292-1105		1105	KKNSLYSTDFIVFND	15
ZNF292-347		347	ARPYLTSECENVALV	15
ZNF292-868		868	EEASIVSSIDELNDS	15
ZNF292-3630		3630	ITKLINEDSTSVETQ	15
ZNF292-1921		1921	QMEDLTKTVLPLNID	15
ZNF292-263		263	LGERLQELELQLRES	15
ZNF292-2553		2553	FKTSLESHTVLAPLT	15
ZNF292-3415		3415	KKNNLENKNAKIVQI	15

ZNF292-1612		1612	TPQNLERQVNNLMTF	15
ZNF292-1597		1597	QNSLVNSETLKIGDL	15
ZNF292-3193		3193	DCSRIFQAITGLIQH	15
ZNF292-3154		3154	HKSDLPAFSAEVEEE	15
ZNF292-2846		2846	TKDALFKHYGKIHQY	15
ZNF292-2533		2533	LTQKLNNVNNQLFMT	15
ZNF292-2163		2163	LLASMILSTNAVNLQ	15
ZNF292-862		862	CLALMGEEASIVSSI	15
AHSA2 - AHA1, activator of heat shock 90 protein ATPase homolog 2	NM_152392			
AHSA2-18		18	VKRKLSGNTLQVQAS	15
AHSA2-7		7	PTKAMATQELTVKRK	15
AHSA2-33		33	SPVALGVRIPTVALH	15
AHSA2-115		115	FVPTLGQTELQL	12
CSNK1G1 - casein kinase 1, gamma 1	NM_022048			
CSNK1G1-189		189	IAIQLLSRMEYVHSK	15
CSNK1G1-183		183	LKTVLMIAIQLLSRM	15
CSNK1G1-342		342	KADTLKERYQKIGDT	15
CSNK1G1-273		273	EHKSLTGTARYM	12
CSNK1G1-390		390	FPEEMATYLRYVRRL	15
CSNK1G1-411		411	DYEYLRTLFTDLFEK	15
CSNK1G1-467		467	GSVHVDSGASAITRE	15
DKFZp451M2119	NM_182585			
DKFZp451M2119-80		80	APTQMSTVPSGLPLP	15
DKFZp451M2119-30		30	DEGLVEGKVVRLGQG	15
DKFZp451M2119-234		234	QILWLYSKSSLAL	13
DKFZP564M182	NM_015659			
DKFZP564M182-309		309	QIEHIIENIVAVTKG	15
DKFZP564M182-77		77	NYGLLLNENESLFLM	15
DKFZP564M182-86		86	ESLFLMVVLWKIPSK	15
DKFZP564M182-344		344	KSAALPIFSSFVSNW	15
DKFZP564M182-190		190	KLRLLSSFDFFLTDA	15
DKFZP564M182-585		585	KEEAVKEKSPSLGKK	15
DKFZP564M182-313		313	IIENIVAVTKGLSEK	15
DKFZP564M182-164		164	NKHGIKTVSQIISLQ	15
DKFZP564M182-260		260	INDCIGGTVLNISKS	15
MAGEA4	NM_002362			
MAGEA4-151		151	FREALSNKVDELAHF	15
MAGEA4-171		171	RAKELVTKAEMLERV	15
MAGEA4-391		391	SYVKVLEHVVRVNAR	15
MAGEA4-265		265	KTGLLIIVLGTIAME	15
MAGEA4-414		414	REAALLEEEEGV	12
MAGEA4-395		395	VLEHVVRVNARVRIA	15
MELK - maternal embryonic leucine zipper kinase	NM_014791			
MELK-783		783	NPDQLLNEIMSILPK	15

MELK-322		322	SSILLLQQMLQVDPK	15
MELK-157		157	VFRQIVSAVAYVHSQ	15
MELK-31		31	ACHILTGEMVAIKIM	15
MELK-784		784	PDQLLNEIMSILPKK	15
MELK-145		145	RLSEEETRVVFR	12
MELK-417		417	QYDHLTATYLLLLAK	15
MELK-722		722	LERGLDKVITVLTRS	15
MELK-234		234	CCGSLAYAAPELIQG	15
MELK-67		67	NTLGSDLPRIKTE	13
MELK-315		315	VPKWLSPSSILLLQQ	15
MELK-718		718	VFGSLERGLDKVITV	15
MELK-95		95	QLYHVLETANKIFMV	15
MELK-74		74	DLPRIKTEIEALKNL	15
MELK-642		642	RNQCLKETPIKIPVN	15
MELK-180		180	PENLLFDEYHKLKLI	15
MELK-241		241	AAPELIQGKSYLGSE	15
NEXN - nexilin (F actin binding protein)	NM_144573			
NEXN-81		81	GDDSLLITVVPVKSY	15
NEXN-34		34	IQRELAKRAEQIED	14
NEXN-382		382	NLKSKFEKIGQL	12
NEXN-340		340	ETFGLSREYEELIKL	15
NEXN-261		261	SQEFLTPGKLEINFE	15
NEXN-661		661	KGSAASTCILTIESK	15
NFE2L2 - nuclear factor (erythroid-derived 2)-like 2	NM_006164			
NFE2L2-409		409	SPATLSHSLSELLNG	15
NFE2L2-741		741	SLHLLKKQLSTLYLE	15
NFE2L2-745		745	LKKQLSTLYLEVFS	14
NFE2L2-164		164	CMQLLAQTFPFVDDN	15
NFE2L2-626		626	TRDELRAKALHIPFP	15
NFE2L2-506		506	EVEELDSAPGSVKQN	15
NFE2L2-249		249	DIEQVWEELLSIPEL	15
NFE2L2-315		315	FYSSIPSMEKEVGNC	15
NFRKB - nuclear factor related to kappa B binding protein	NM_006165			
NFRKB-413		413	GDLTLNDIMTRVNAG	15
NFRKB-559		559	LEILLLESQASLPML	15
NFRKB-1575		1575	SAVSLPSMNAAVSKT	15
NFRKB-1221		1221	TVTSLPATASPV	12
NFRKB-626		626	ALQYLAGESRAVPSS	15
NFRKB-1599		1599	TPISISTGAPTVRQV	15
NFRKB-553		553	SFFSLLLEILLLESQ	15
NFRKB-226		226	KQILASRSDLLEMA	14
NFRKB-1568		1568	GTVHTSAVSLPSM	13
NFRKB-1094		1094	TMLSPASSQTAPS	13
NFRKB-546		546	GINEISSSFFSLLLE	15

NFRKB-88		88	DVVSLSTWQEVLSDS	15
NFRKB-1675		1675	IKGNLGANLSGLGRN	15
NUP107 - nucleoporin 107kDa	NM_020401			
NUP107-413		413	KQRQLTSYVGSVRPL	15
NUP107-577		577	IYAALSGNLKQLLPV	15
NUP107-345		345	QRDSLVRQSQLVVDW	15
NUP107-471		471	DEVRLLKYLFTLIRA	15
NUP107-1218		1218	LLQKLRESSLMLLDQ	15
NUP107-632		632	VEQEIQTSVATLDET	15
NUP107-782		782	SIEVLKTYIQLLIRE	15
NUP107-225		225	SFLKHSSSTVFDL	13
NUP107-1099		1099	WKGHLDALTADVKEK	15
NUP107-734		734	LPGHLLRFMTHLILF	15
NUP107-339		339	VVEALFQRDSLVRQS	15
NUP107-250		250	QVNILSKIVSRATPG	15
NUP107-1110		1110	VKEKMYNVLLFVDGG	15
NUP107-1211		1211	SKEELRKLLQKLRES	15
NUP107-656		656	ANWTLEKVFEELQAT	15
NUP107-811		811	QDLAVAQYALFLESV	15
NUP107-472		472	EVRLLKYLFTLIRAG	15
NUP107-420		420	YVGSVRPLVTELDPD	15
NUP107-940		940	RAEALKQGNAIMRKF	15
RPA2 - replication protein A2, 32kDa	NM_002946			
RPA2-79		79	LSATLVDEVFRIGNV	15
RPA2-322		322	KHMSVSSIKQAVDFL	15
RPA2-267		267	PANGLTVAQNQVLNL	15
RPA2-71		71	VPCTISQLLSATLVD	15
RPA2-325		325	SVSSIKQAVDFLSNE	15
USP34 - ubiquitin specific protease 34	NM_014709			
USP34-3151		3151	FLLSLQAISTMVHFY	15
USP34-1119		1119	QKHALYSHSAEVQVR	15
USP34-1967		1967	QGTSLIQRLMSVAYT	15
USP34-2383		2383	ATCYLASTIQQLYMI	15
USP34-3318		3318	IVSMLFTSIAKLTPE	15
USP34-397		397	PLRHLLNLVSALEPS	15
USP34-4106		4106	FTETLVKLSVLVAYE	15
USP34-1351		1351	CMESLMIASSSLEQE	15
USP34-3874		3874	DLVELLSIFLSVLKS	15
USP34-3310		3310	YNNRLAEHIVSMLFT	15
USP34-2226		2226	GLTGLLRLATSVVKH	15
USP34-4264		4264	NRVEISKASASLNGD	15
USP34-4202		4202	MTHFLLKVQSQVFSE	15
USP34-1961		1961	LVQGTSLIQRL	11
USP34-4518		4518	PSTSISAVLSDLADL	15
USP34-414		414	TEQTLYLASMLIKAL	15

USP34-245		245	RLAGLSQITNQLHTF	15
USP34-4294		4294	LNPALIPTLQELLSK	15
USP34-2529		2529	FGGVITNNVVSLDCE	15
USP34-2517		2517	SPELKNTVKSLFGG	14
USP34-4219		4219	CANLISTLITNLISQ	15
USP34-3226		3226	KMIALVALLVEQ	12
USP34-3875		3875	LVELLSIFLSVLKST	15
USP34-3507		3507	LLGLLSRAKLYVDAA	15
USP34-4593		4593	LCRTIESTIHVVTRI	15
USP34-3106		3106	HSKHLTEYFAFLYEF	15
USP34-2227		2227	LTGLLRLATSVVKHK	15
USP34-2090		2090	NRSFLLLAASTL	12
USP34-1103		1103	FFDNLVYYIQTVREG	15
USP34-416		416	QTLYLASMLIKALWN	15
USP34-3801		3801	CWTTLISAFRILLES	15
USP34-2439		2439	TLLELQKMFTYLMES	15
USP34-465		465	SFASLLNTNIPIGNK	15
USP34-238		238	MSPTLTMRLAGLSQI	15
USP34-3556		3556	MTYCLISKTEKLMFS	15
USP34-3496		3496	TTVVLHQVYNVLLGL	15
USP34-3488		3488	RDLPLSPDTTVVLHQ	15
USP34-3327		3327	KMIALVALLVEQS	13
USP34-2925		2925	DPKAVSLMTAKLSTS	15
AARS - alanyl-tRNA synthetase	NM_001605			
AARS-1289		1289	EALQLATSFAQLRLG	15
AARS-402		402	AYRVLADHARTITVA	15
AARS-1108		1108	QKDELRETLKSLKKV	15
AARS-327		327	TGMGLERLVSVLQNK	15
AARS-889		889	IANEMIEAAKAVYTQ	15
AARS-1046		1046	LKKCLSVMEAKVKAQ	15
AARS-539		539	LDRKIQSLGDS	15
AARS-1115		1115	TLKSLKKVMDDLDRA	15
AARS-1042		1042	KAESLKKCLSVMEAK	15
AARS-1017		1017	TEEAIAKGIRRIVAV	15
AARS-820		820	ATHILNFALRSVLGE	15
AARS-482		482	VVQSLGDAFPELKKD	15
AARS-658		658	YNYHLDSSGSYVFEN	15
AARS-1135		1135	QKRVLEKTKQFIDSN	15
ABL1 - v-abl Abelson murine leukemia viral oncogene homolog 1	NM_005157			
ABL1-1515		1515	DFSKLLSSVKEISDI	15
ABL1-1342		1342	PLSTLPSASSALAGD	15
ABL1-349		349	KKYSLTVAVKTLKED	15
ABL1-465		465	NAVVLLYMATQISSA	15
ABL1-1427		1427	NSEQMASHSAVLEAG	15
ABL1-472		472	MATQISSAMEYLEKK	15

ABL1-937		937	SPHLWKKSSTLTSS	14
ABL1-1488		1488	KLENNLRELQIC	12
ABL1-1362		1362	AFIPLISTRVSLRKT	15
ABL1-260		260	TLAELVHHHSTVADG	15
ABL1-1409		1409	VVLDSTEALCLA	12
ABL1-557		557	APESLAYNKFSIKSD	15
ACAT2 - acetyl-Coenzyme A acetyltransferase 2 (	NM_005891			
ACAT2-488		488	GCRILVTLLHTLERM	15
ACAT2-9		9	DPVVIVSAARTIIGS	15
ACAT2-424		424	DIFEINEAFAAVSAA	15
ACAT2-322		322	KPYFLTDGTGTVTPA	15
ACAT2-428		428	INEAFAAVSAAIVKE	15
ACAT2-491		491	ILVTLLHTLERMGRS	15
ACAT2-337		337	NASGINDGAAAVALM	15
AKAP13 - A kinase (PRKA) anchor protein 13	NM_006738			
AKAP13-2954		2954	EQEDLAQSLSLVKDV	15
AKAP13-3489		3489	LTRSLSRPSSLIEQE	15
AKAP13-3096		3096	IFASLDQKSTVISLK	15
AKAP13-229		229	PRETLMHFAVRLGLL	15
AKAP13-3077		3077	QAVLLTDILVFLQEK	15
AKAP13-1520		1520	PNVLLSQEKNAVLGL	15
AKAP13-585		585	DQESLSSGDAVLQRD	15
AKAP13-3420		3420	LVFMLKRNSEQVVQS	15
AKAP13-3306		3306	PLMKSAINEVEIL	13
AKAP13-3069		3069	GRLKEVQAVLLTD	13
AKAP13-1688		1688	GADLIEEAASRIVDA	15
AKAP13-1052		1052	DQAVISDSTFSLANS	15
AKAP13-383		383	FKLMNIQQQLMKT	13
AKAP13-1024		1024	LDKPLTNMLEVVSHP	15
AKAP9 - A kinase (PRKA) anchor protein (yotiao) 9	NM_005751			
AKAP9-5282		5282	DRALTDYITRLEAL	14
AKAP9-4202		4202	DRRSLLSEIQALHAQ	15
AKAP9-1964		1964	QEQLEEEVAKVIVS	14
AKAP9-3115		3115	EIDQLNEQVTKLQQ	14
AKAP9-1825		1825	QVQELESLISSLQQQ	15
AKAP9-3715		3715	NMTSLQKDLSQVRDH	15
AKAP9-2532		2532	LLEAISETSSQLEHA	15
AKAP9-4287		4287	LQEQLSSEKMVVAEL	15
AKAP9-2360		2360	ANNRLLKILLEVVKT	15
AMOTL2 - angiomotin like 2	NM_016201			
AMOTL2-415		415	GSAHLAQMEAVLREN	15
AMOTL2-583		583	EQEKLEREMALLRGA	15
AMOTL2-473		473	RIEKLESEIQRLSEA	15

AMOTL2-656		656	KVERLQQALGQLQAA	15
AMOTL2-480		480	EIQRLSEAHESLTRA	15
AMOTL2-330		330	EVRILQAQVPPVFLQ	15
ANKHD1 - ankyrin repeat and KH domain containing 1	NM_017747			
ANKHD1-245		245	VSCALDEAAAALTRM	15
ANKHD1-2244		2244	TPNSLSTSYKTVSLP	15
ANKHD1-1352		1352	LTDTLDDLIAAVSTR	15
ANKHD1-234		234	DPEVLRRLTSSVSCA	15
ANKHD1-2955		2955	AAVQLSSAVNIMNGS	15
ANKHD1-1356		1356	LDDLIAAVSTRVPTG	15
ANKHD1-1061		1061	KLNELGQRISAIEK	14
ANKHD1-336		336	GYYELAQVLLAMHAN	15
ANKHD1-340		340	LAQVLLAMHANVEDR	15
ANKHD1-3006		3006	GPATLFNHFSSLFDS	15
ANKHD1-2308		2308	RSKKLSVPASVVSRI	15
ANKRD11 - ankyrin repeat domain 11	NM_013275			
ANKRD11-3272		3272	TREVIQQTLAAIVDA	15
ANKRD11-304		304	KQLLAAGAEVNTK	13
ANKRD11-3400		3400	PPPSLAEPLKELFRQ	15
ANKRD11-822		822	KSPFLSSAEGAVPKL	15
ANKRD11-2154		2154	FERMLSQKDLEIEER	15
ANKRD11-3407		3407	PLKELFRQQEAVRGK	15
ANKRD13 - ankyrin repeat domain 13	NM_033121			
ANKRD13-499		499	FPLSLVEQVIPIIDL	15
ANKRD13-720		720	IQESLLTSTEGLCPS	15
ANKRD13-781		781	WELRLQEEEAELQQV	15
ANKRD13-266		266	ERFDLSQEMERLTLD	15
ANKRD13-74		74	SLGHLESARVLLRHK	15
ANKRD13-404		404	DRNPLESLLGTVEHQ	15
ANKRD17 - ankyrin repeat domain 17	NM_032217			
ANKRD17-1379		1379	LNDTLDDIMAAV	12
ANKRD17-263		263	DPEVLRRLTSSVSCA	15
ANKRD17-3102		3102	PESMLSGKSSYLPNS	15
ANKRD17-386		386	GYYELAQVLLAMHAN	15
ANKRD17-1667		1667	MLAAMNGHTAAVKLL	15
ANKRD17-478		478	VVKVLLESGASIEDH	15
ANKRD17-390		390	LAQVLLAMHANVEDR	15
ANKRD17-188		188	ENPMLETASKLLLSG	15
ANKRD30A - ankyrin repeat domain 30A	NM_052997			
ANKRD30A-577		577	DSRSLFESSAKIQVC	15
ANKRD30A-158		158	NKASLTPLLLSITKR	15
ANKRD30A-1219		1219	DSTSLSKILDTVHS	14
ANKRD30A-1428		1428	ENCMLKKEIAMLKLE	15
ANKRD30A-115		115	VYSEILSVVAKL	12

ANKRD30A-1435		1435	EIAMLKLEIATLKHQ	15
ANKRD30A-230		230	IVGMLLQQNVDVFAA	15
APEX2 - APEX nuclease	NM_014481			
APEX2-76		76	TRDALTEPLAIVEGY	15
APEX2-247		247	RAEALLAAGSHVIIL	15
APEX2-384		384	DYVLGDRTLVIDTF	14
APEX2-240		240	FYRLLQIRAEALLAA	15
ARID4B - AT rich interactive domain 4B, BCAA; BRCAA1; SAP180	NM_016374			
ARID4B-1690		1690	HYLSLKSEVASIDRR	15
ARID4B-1676		1676	RITILQEKLQEIRKH	15
ARID4B-468		468	NLFKLFRLVHKLGGF	15
ARID4B-234		234	QIDELLGKVVCVDYI	15
ARNTL - aryl hydrocarbon receptor nuclear translocator-like	NM_001178			
ARNTL-665		665	IGRMIAEEIMEIHRI	15
ARNTL-808		808	DEAAMAVIMSLLEAD	15
ARNTL-579		579	EVEYIVSTNTVVLAN	15
ARNTL-153		153	KLDKLTVLRMAVQHM	15
ARNTL-814		814	VIMSLLEADAGLGGP	15
ARNTL-234		234	KILFVSESVFKILNY	15
ASPSCR1 -alveolar soft part sarcoma chromosome region, candidate 1	NM_024083			
ASPSCR1-345		345	PTRPLTSSSAKLPKS	15
ASPSCR1-223		223	LTGGSATIRFV	12
ASPSCR1-648		648	LEHAISPSAADVLVA	15
ASPSCR1-158		158	TLWELLSHFPQIREC	15
ATF3 - activating transcription factor 3	NM_001674			
ATF3-78		78	LCHRMSSALESVTVS	15
ATF3-162		162	ESEKLESVNAELKAQ	15
ATF3-169		169	VNAELKAQIEELKNE	15
ATXN3 - ataxin 3	NM_004993			
ATXN3-32		32	SPVELSSIAHQLDEE	15
ATXN3-189		189	SDTYLALFLAQLQQE	15
ATXN3-469		469	LQAAVTMSLETVRND	15
ATXN3-254		254	RPKLIGEELAQLKEQ	15
ATXN3-99		99	FSIQVISNALKVWGL	15
B3GALT4 - UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase	NM_003782			
B3GALT4-352		352	TGYVLSASAVQL	12
B3GALT4-9		9	FRRLLLAALLLVIVW	15
B3GALT4-32		32	GEELLSLSLASLLPA	15
BAIAP3 - BAI1-associated protein 3	NM_003933			
BAIAP3-227		227	DEEALLSYLQQVFGT	15
BAIAP3-578		578	WRGELSTPAATILCL	15
BAIAP3-239		239	FGTSLEEHTEAIERV	15

BAIAP3-1261		1261	WELLLQAILQALGAN	15
BAIAP3-555		555	SHLLLLSHLLRLEHS	15
BAIAP3-1212		1212	LMKYLDEKLALLNAS	15
BAIAP3-406		406	DDVSLVEACRKLNEV	15
BCR - breakpoint cluster region	NM_004327			
BCR-265		265	RISSLGSQAMQMERK	15
BCR-1196		1196	ELQMLTNSCVKLQTV	15
BCR-1111		1111	LKKKLSEQESLLLLM	15
BCR-1188		1188	RSFSLTSVELQMLTN	15
BCR-1059		1059	ELDALKIKISQIKSD	15
BDP1 - TFIIIB150; TFIIIB90	NM_018429			
BDP1-145		145	SLVKSSVSVPSE	12
BDP1-2842		2842	TRNTISKVTSNLRIR	15
BDP1-341		341	GSIILDEESLTVEVL	15
BDP1-2385		2385	KESALAKIDAELEEV	15
BDP1-1837		1837	DIQNISSEVLSMMHT	15
BDP1-2205		2205	EKKVLTVSNSQIETE	15
BDP1-2358		2358	QLLLKEKAELLTS	13
BRD2 - bromodomain containing 2, NAT; RING3	NM_005104			
BRD2-711		711	RLAELQEQLRAVHEQ	15
BRD2-410		410	PPGSLEPKAARLPPM	15
BRD2-267		267	KLAALQGSVTSAHQV	15
BRD2-227		227	DIVLMAQTLEKIFLQ	15
BRD2-718		718	QLRAVHEQLAALSQG	15
BRD2-708		708	RAHRLAELQEQLRAV	15
BZW2 - basic leucine zipper and W2 domains 2	NM_014038			
BZW2-426		426	ALKHLKQYAPLLAVF	15
BZW2-65		65	LEAVAKFLDST	12
CHTF18 - chromosome transmission fidelity factor 18 homolog	NM_022092			
CHTF18-328		328	EAQKLSDTLHSLRSG	15
CHTF18-306		306	LGVSLASLKKQVDGE	15
CHTF18-706		706	LPSRLVQRLQEVSLR	15
CHTF18-1061		1061	EKQQLASLVGTMLA	15
CHTF18-896		896	RDSSLGAVCVALDWL	15
CHTF18-321		321	RRERLLQEAQKLSDT	15
CHTF18-1045		1045	LAPKLRPVSTQLYST	15
CHTF18-1030		1030	PQALLLDALCLLLDI	15
CLIC6 - chloride intracellular channel 6	NM_053277			
CLIC6-408		408	GDGSLSPQAEAIEVA	15
CLIC6-787		787	HEKNLLKALRKLDNY	15
CTNNA1 - catenin (cadherin-associated protein), alpha 1, 102kDa	NM_001903			
CTNNA1-172		172	AARALLSAVTRLLIL	15

CTNNA1-331		331	IYKQLQQAVTGISNA	15
CTNNA1-28		28	VERLLEPLVTQVTTL	15
CTNNA1-966		966	DIIVLAKQMCMIMME	15
CTNNA1-409		409	FRPSLEERLESIISG	15
CTNNA1-1119		1119	AKNLMNAVVQTVKAS	15
CTNNA1-1111		1111	SAMSLIQAAKNLMNA	15
CTTN - cortactin	NM_005231			
CTTN-149		149	YQSKLSKHCSQVDSV	15
CTTN-468		468	PVEAVTSKTSNIRAN	15
CTTN-629		629	SQQGLAYATEAVYES	15
CTTN-706		706	DPDDIITNIEMIDDG	15
CTTN-660		660	YENDLGITAVALYDY	15
CTTN-427		427	KNASTFEDVTQVSSA	15
CTTNBP2 - cortactin binding protein 2	NM_033427			
CTTNBP2-1035		1035	CVRLLLSAEAQVNAA	15
CTTNBP2-2134		2134	NNPVLSATINNLRMP	15
CTTNBP2-254		254	EAQKLEDVMAKLEEE	15
CTTNBP2-1373		1373	VSQALTNHFQAISSD	15
CTTNBP2-1901		1901	GQQAVVKAALSILLN	15
CTTNBP2-1296		1296	DCKHLLENLNALKIP	15
DAD1 - defender against cell death 1	NM_001344			
DAD1-26		26	RLKLLDAYLLYILLT	15
DAD1-77		77	FNSFLSGFISCVGSF	15
DAD1-16		16	LEEYLSSTPQRLKLL	15
DDX5 - DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	NM_004396			
DDX5-241		241	PTRELAQQVQQVAAE	15
DDX5-190		190	TLSYLLPAIVHINHQ	15
DDX5-627		627	LISVLREANQAINPK	15
DDX5-322		322	GKTNLRRTTYLVLDE	15
DDX5-620		620	KQVSDLISVLREA	13
DDX5-634		634	ANQAINPKLLQLVED	15
DDX58 - DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	NM_014314			
DDX58-488		488	TIPSLSIFTLMIFDE	15
DDX58-965		965	NLVILYEYVGNVIKM	15
DDX58-1109		1109	KCKALACYTADVRVI	15
DDX58-1013		1013	LTSNAGVIEKE	12
DDX58-726		726	ICKALFLYTSHLRKY	15
DDX58-645		645	IIAQLMRDTESLAKR	15
DNAJA1 - DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001539			
DNAJA1-384		384	ISTLDNRTIVITSH	14
DNAJA1-231		231	IGPGMVQQIQSVCME	15
DNAJA1-152		152	VVHQLSVTLEDLYNG	15
DNAJA1-68		68	FKQISQAYEVLSDA	14

DNAJA1-21		21	TQEELKKAYRKLALK	15
DNAJA2 - DnaJ (Hsp40) homolog, subfamily A, member 2	NM_005880			
DNAJA2-240		240	LAPGMVQQMQSVCSD	15
DNAJA2-335		335	IVLLLQEKEHEVFQR	15
DNAJA2-473		473	NPDKLSELEDLLPSR	15
DNAJA2-23		23	SENELKKAYRKLAKE	15
DNAJA2-489		489	EVPNIIGETEEVELQ	15
DNAJB1 - DnaJ (Hsp40) homolog, subfamily B, member 1	NM_006145			
DNAJB1-349		349	LREALCGCTVNVPTL	15
DNAJB1-430		430	FPERIPQTSRTVL	13
DNAJB1-338		338	GSDVIYPARISLREA	15
DNAJB1-230		230	VTHDLRVSLEEIYSG	15
DNM1L - dynamin 1-like , DRP1; DVLP; DYMPLE; HDYNIV; VPS	NM_005690			
DNM1L-627		627	RFPKLHDAIVEVVTC	15
DNM1L-415		415	RINVLAAQYQSLLNS	15
DNM1L-389		389	GTKYLARTLNRLLMH	15
DNM1L-313		313	AMDVLMGRVIPVKLG	15
DNM1L-3		3	MEALIPVINKLQDV	14
DNM1L-10		10	VINKLQDVFNTVGAD	15
DRCTNNB1A - down-regulated by Ctnnb1, a (DRCTNNB1A)	NM_032581			
DRCTNNB1A-36		36	DKSSLVSSLYKV	12
DRCTNNB1A-588		588	SSHGLAKTAATVF	13
DRCTNNB1A-23		23	PETSLPNYATNLKDK	15
DRCTNNB1A-265		265	SLQSLCQICSRICVC	15
DRCTNNB1A-164		164	HTKVLSFTIPSLSKP	15
DUSP12 - dual specificity phosphatase 12	NM_007240			
DUSP12-311		311	CRRSLFRSSSILDHR	15
DUSP12-259		259	ELQNLPQELFAVDPT	15
DUSP12-160		160	CHAGVSRSVAIITAF	15
DUSP12-114		114	LLSHLDRCVAFIG	13
ELKS - Rab6-interacting protein 2 (ELKS)	NM_015064			
ELKS-241		241	KESKLSSSMNSIKTF	15
ELKS-1120		1120	MKAKLSSTQQSLAEK	15
ELKS-778		778	SSLKERVKSLQAD	13
ELKS-984		984	EVDRLLEILKEV	12
ELKS-624		624	ELLALQTKLETLTNQ	15
ELKS-1102		1102	QVEELLMAMEKVKQE	15
ELKS-1113		1113	VKQELESMKAKLSST	15
ELKS-803		803	LEEALAEKERTIERL	15
EXOSC6 - exosome component 6	NM_058219			
EXOSC6-224		224	ALTAAALALADA	12

EXOSC6-273		273	AAAGLTVALMPV	12
EXOSC6-185		185	PRAQLEVSALLLEDG	15
EXOSC6-302		302	LNQVAGLLGSG	12
EXOSC6-338		338	LYPVLQQSLVRAARR	15
EXOSC6-231		231	AALALADAGVEMYDL	15
EXOSC6-229		229	TAAALALADAGVEMY	15
EXOSC10 - exosome component 10	NM_001001998			
EXOSC10-883		883	TTCLIATAVITLFNE	15
EXOSC10-100		100	QGDRLLQCMSRVMQY	15
EXOSC10-168		168	RVGILLDEASGVNKN	15
EXOSC10-876		876	KEDNLLGTTCLIATA	15
EXOSC10-725		725	PNHMMLKIAEELPKE	15
FAHD1 - fumarylacetoacetate hydrolase domain containing 1	NM_031208			
FAHD1-234		234	SIPYIISYVSKIITL	15
FAHD1-228		228	TSSMIFSIPYIISYV	15
FAHD1-251		251	GDIILTGTPKGVGPV	15
FRS2 - fibroblast growth factor receptor substrate 2	NM_006654			
FRS2-32		32	DGNELGSGIMELTDT	15
FRS2-649		649	RTAAMSNLQKALPRD	15
FRS2-497		497	EDDNLGPKTPSLNGY	15
FRS2-146		146	EIMQNNSINVVEE	13
FRS2-504		504	KTPSLNGYHNNLDPM	15
FRS2-539		539	VNTENVTVPAS	12
GLIPR1 - GLI pathogenesis-related 1 (glioma)	NM_006851			
GLIPR1-329		329	SVILILSVIITILVQ	15
GLIPR1-330		330	VILILSVIITILVQL	15
GLIPR1-319		319	RYTSLFLIVNSVILI	15
GLIPR1-4		4	MRVTLATIAWMVSFV	15
GLIPR1-227		227	GFDALSNGAHFICNY	15
GMRP-1 - K+ channel tetramerization protein	NM_032320			
GMRP-1-574		574	SITNLAAAAADIPQD	15
GMRP-1-393		393	FEFYLEEMILPLMVA	15
GMRP-1-352		352	KCRDLSALMHEL	12
GMRP-1-467		467	YSTKLYRFFKYIENR	15
GMRP-1-571		571	KSKSITNLAAAAADI	15
GNPTAG - N-acetylglucosamine-1-phosphotransferase, gamma subunit	NM_032520			
GNPTAG-335		335	AHKELSKEIKRLKGL	15
GNPTAG-4		4	MAAGLARLLLLLGLS	15
GNPTAG-87		87	HLFRLSGKCFSLVES	15
GOLGA1 - golgi autoantigen, golgin subfamily a, 1	NM_002077			
GOLGA1-561		561	RTQALEAQIVALERT	15

GOLGA1-400		400	VITHLQEKVASLEKR	15
GOLGA1-967		967	EAFHLIKAVSVLLNF	15
GOLGA1-94		94	LEARLSDYAEQVRNL	15
GOLGA1-649		649	VSVAMAQALEEVRKQ	15
GOLGA1-351		351	KEQELQALIQQLS	13
GOLGA1-743		743	ALRTLKAEEAAVVAE	15
GOLGA1-733		733	QIHQLQAELEALRTL	15
GOLGA1-785		785	LRGPLQAEALSVNES	15
GOLGA1-904		904	PGPEMANMAPSVT	13
GOLGA2 - golgi autoantigen, golgin subfamily a, 2	NM_004486			
GOLGA2-339		339	RVGELERALSAVSTQ	15
GOLGA2-1130		1130	EYIALYQSQRAVLKE	15
GOLGA2-492		492	LEAHLGQVMESVRQL	15
GOLGA2-1187		1187	KLLELQELVLRLVGD	15
GOLGA2-1061		1061	THRALQGAMEKLQS	14
GOLGA2-569		569	RVQELETSLAELRNQ	15
GOLGA2-788		788	LQEKLSELKETVELK	15
GOLGA2-721		721	QNRELKEQLAELQSG	15
GOLGA2-156		156	STESLRQLSQQLNGL	15
GOLGA4 - golgi autoantigen, golgin subfamily a, 4	NM_002078			
GOLGA4-940		940	ELESLSSELSEVLKA	15
GOLGA4-1131		1131	ERILLTKQVAEVEAQ	15
GOLGA4-2867		2867	LQTQLAQKTTLISDS	15
GOLGA4-622		622	ERISLQQELSRVKQE	15
GOLGA4-2991		2991	TKTMAKVITTVLKF	14
GOLGA4-1892		1892	NSISLSEKEAAISSL	15
GOLGA4-307		307	YISVLQTQVSLLKQR	15
GOLGA4-2065		2065	LETELKSQTARIMEL	15
GOLGA4-1830		1830	LKKELSENINAVTLM	15
GOLGA4-1572		1572	ENTFLQEQLVELKML	15
GOLGA4-2299		2299	EVHILEEKLKSVESS	15
GOLGA4-954		954	ARHKLEEELSVLKDQ	15
GOLGA4-937		937	QTELESLSSELSEV	14
GOLGB1 - golgi autoantigen, golgin subfamily b, macrogolgin	NM_004487			
GOLGB1-3907		3907	EVQSLKKAMSSL	12
GOLGB1-3322		3322	KTNQLMETLKTIKKE	15
GOLGB1-3558		3558	SISQLTRQVTALQEE	15
GOLGB1-2956		2956	LQENLDSTVTQLAAF	15
GOLGB1-2618		2618	LEERLMNQLAELNGS	15
GOLGB1-2131		2131	ENQSLSSSCESLKLA	15
GOLGB1-640		640	NIASLQKRVVELENE	15
GOLGB1-2065		2065	LTKSLADVESQVSAQ	15
GOLGB1-1925		1925	KEAALTKIQTEIIEQ	15

GOLGB1-1021		1021	ERDQLLSQVKELSMV	15
GOLGB1-2381		2381	EKDSLSEEVQDLKHQ	15
GOLGB1-3551		3551	EIESLKVSISQLTRQ	15
GOLGB1-2772		2772	KISALERTVKALEFV	15
GRASP - GRP1-associated scaffold protein	NM_181711			
GRASP-319		319	KDPSIYDTLESVRSC	15
GRASP-502		502	FRRRLLKFIPGLNRS	15
GRASP-259		259	RKAELEARLQYLKQT	15
GRASP-323		323	IYDTLESVRSCLYGA	15
GRIM19 - cell death-regulatory protein GRIM19 (GRIM19)	NM_015965			
GRIM19-76		76	VPRTISSASATLIMA	15
GRIM19-20		20	KTPQLQPGSAFLPRV	15
GRIM19-236		236	LRENLEEEAIIMKDV	15
GRIM19-160		160	GYSMLAIGIGTLIYG	15
GSPT1 - G1 to S phase transition 1	NM_002094			
GSPT1-267		267	REHAMLAKTAGVKHL	15
GSPT1-324		324	CKEKLVPFLKKVGFN	15
GSPT1-655		655	KTIAIGKVLKLVPEK	15
HAGH - hydroxyacylglutathione hydrolase	NM_005326			
HAGH-105		105	RIGALTHKITHLSTL	15
HAGH-8		8	VLPALTDNYMYLVID	15
HAGH-115		115	HLSTLQVGSLNV	12
HNRPAB - heterogeneous nuclear ribonucleoprotein A/B	NM_004499			
HNRPAB-156		156	FGFILFKDAASVEKV	15
HNRPAB-273		273	VKKVLEKKFHTV	12
HNRPAB-167		167	VEKVLDQKEHRLDGR	15
HNRPAB-252		252	MDPKLNKRRGFVFIT	15
HSPCA - heat shock 90kDa protein 1, alpha	NM_005348			
HSPCA-184		184	YSAYLVAEKVTVITK	15
HSPCA-25		25	FQAEIAQLMSLIINT	15
HSPCA-788		788	MKDILEKKVEKVVVS	15
HSPCA-901		901	YETALLSSGFSLEDP	15
HSPCA-895		895	DLVILLYETALLSSG	15
HSPD1 - heat shock 60kDa protein 1	NM_002156			
HSPD1-726		726	GVASLLTTAEVVVTE	15
HSPD1-543		543	RLAKLSDGVAVLKVG	15
HSPD1-571		571	VTDALNATRAAVEEG	15
HSPD1-661		661	IVEKIMQSSSEVGYD	15
HSPD1-337		337	KISSIQSIVPALEIA	15
HSPD1-248		248	IGNIISDAMKKVGRK	15
HUMAUANTIG - nucleolar GTPase	NM_013285			
HUMAUANTIG-641		641	APQLLPSSSLEVVPE	15

HUMAUANTIG-478		478	QYITLMRRIFLIDCP	15
HUMAUANTIG-710		710	ANTEMQQILTRVRQN	15
HUMAUANTIG-502		502	ETDIVLKGVVQVEKI	15
IFI16 - interferon, gamma-inducible protein 16	NM_005531			
IFI16-95		95	DIPTLEDLAETLKKE	15
IFI16-9		9	KNIVLLKGLEVINDY	15
IFI16-715		715	EVMVLNATESFVYEP	15
IFI16-500		500	KKNQMSKLISEMHSF	15
IKBKAP - inhibitor of kappa light polypeptide gene enhancer	NM_003640			
IKBKAP-1658		1658	EDLALLEALSEVVQN	15
IKBKAP-1584		1584	QESDLFSETSSVVSG	15
IKBKAP-313		313	REFALQSTSEPVAGL	15
IKBKAP-719		719	VIHHLTAASSEMDEE	15
IKBKAP-1116		1116	VCDAMRAVMESINPH	15
ILF3 - interleukin enhancer binding factor 3, 90kDa	NM_004516			
ILF3-246		246	MEKVLAGETLSVNDP	15
ILF3-173		173	VADNLAIQLAAVTED	15
ILF3-622		622	KTAKLHVAVKVLQDM	15
ILF3-566		566	LQYKLVSQTGPVHAP	15
IQWD1 - IQ motif and WD repeats 1	NM_018442			
IQWD1-667		667	PASFMLRMLASLN	13
IQWD1-67		67	LEVSETAMEVDTP	13
IQWD1-653		653	NELMLEETRNTITVP	15
IQWD1-237		237	EWSSIASSSRGIGSH	15
IQWD1-575		575	EHLMLLEADNHVVNC	15
KLHL2 - kelch-like 2,	NM_007246			
KLHL2-661		661	GVGVLNNLLYAVGGH	15
KLHL2-544		544	GAAVLNGLLYAVGGF	15
KLHL2-409		409	TPMNLPKLMVVVGGQ	15
KLHL2-252		252	ADVVLSEEFLNLGIE	15
LIMS1 - LIM and senescent cell antigen-like domains 1	NM_004987			
LIMS1-419		419	LKKRLKKLAETLGRK	15
LIMS1-230		230	CGKELTADARELKGE	15
LIMS1-182		182	KCHAIIDEQPLIFKN	15
LMNA - lamin A/C	NM_005572			
LMNA-406		406	RIDSLSAQLSQLQKQ	15
LMNA-731		731	AMRKLVRSVTVVEDD	15
LMNA-324		324	FESRLADALQELRAQ	15
LMNA-182		182	LEALLNSKEAALSTA	15
LMNA-410		410	LSAQLSQLQKQLAAK	15
LMNA-417		417	LQKQLAAKEAKLRDL	15
LMNA-403		403	SRIRIDSLSAQLSQL	15

LMNA-238		238	LEAALGEAKKQLQDE	15
LMNA-487		487	EYQELLDIKLALDME	15
MED6 - mediator of RNA polymerase II transcription, subunit 6	NM_005466			
MED6-77		77	QRLTLEHLNQMVGIE	15
MED6-91		91	EYILLHAQEPILFII	15
MED6-160		160	INSRVLTAVHGIQSA	15
MED6239		239	QRQRVDALLLDLRQK	15
MKRN1 - makorin, ring finger protein, 1	NM_013446			
MKRN1-175		175	ASSSLSSIVGPLVEM	15
MKRN1-101		101	YSHDLSDSPYSVVCK	15
MKRN1-163		163	TATELTTKSSLAASS	15
MKRN1-483		483	KQKLILKYKEAMSNK	15
NAP1L3 - nucleosome assembly protein 1-like 3	NM_004538			
NAP1L3-145		145	AVRNRVQALRNI	12
NAP1L3-648		648	ILKSIYYYTGEVNGT	15
NAP1L3-173		173	AIHDLERKYAELNKP	15
NEDD9 - neural precursor cell expressed, dev. down-regulated 9	NM_006403			
NEDD9-1100		1100	STTALQEMVHQVTDL	15
NEDD9-973		973	HFISLLNAIDALFSC	15
NEDD9-566		566	LQQALEMGVSSLMAL	15
NEDD9-1055		1055	SSNQLCEQLKTIVMA	15
NEDD9-980		980	AIDALFSCVSSAQPP	15
NEDD9-626		626	VELFLKEYLHFVKGA	15
NS - nucleostemin	NM_014366			
NS-392		392	VSMGLTRSMQVVPLD	15
NS-257		257	WLNYLKKELPTVVFR	15
NS-401		401	QVVPLDKQITIIDSP	15
NS-250		250	PKENLESWLNYLKKE	15
NUBP2 - nucleotide binding protein 2	NM_012225			
NUBP2-338		338	AFAALTSIAQKILDA	15
NUBP2-109		109	QSISLMSVGFLLEKP	15
NUBP2-155		155	KNALIKQFVSDVAWG	15
OGFR - opioid growth factor receptor	NM_007346			
OGFR-570		570	SQGSLRTGTQEVGGQ	15
OGFR-337		337	RQSALDYFMFAVRCR	15
OGFR-565		565	EGCALSQGSLRTGTQ	15
PARC - p53-associated parkin-like cytoplasmic protein	NM_015089			
PARC-956		956	GLSALSQAVEEVTER	15
PARC-722		722	GEKALGEISVSVEMA	15
PARC-981		981	LREKLVKMLVELLTN	15
PARC-1368		1368	NKTLLLSVLRVITRL	15
PARC-1140		1140	SESLLLTVPAAVIL	14

PARC-3152		3152	FAVNLRNRVSAIHEV	15
PARC-2454		2454	SPELLLQALVPLTSG	15
PARC-1654		1654	HRGVLVRQLTLLVAS	15
PARC-731		731	VSVEMAESLLQVLSS	15
PIAS1 - protein inhibitor of activated STAT, 1	NM_016166			
PIAS1-338		338	NITSLVRLSTTVPNT	15
PIAS1-6		6	DSAELKQMVMSLRVS	15
PIAS1-166		166	ELPHLTSALHPVHPD	15
PIAS1-428		428	PDSEIATTSLRVSLL	15
PPIL4 - peptidylprolyl isomerase (cyclophilin)-like 4	NM_139126			
PPIL4-8		8	LETTLGDVVIDLYTE	15
PPIL4-306		306	TQAILLEMVGDLPDA	15
PPIL4-419		419	IHVDFSQSVAKVKWK	15
PPIL4-150		150	GSQFLITTGENLDYL	15
PSME3 - proteasome (prosome, macropain) activator subunit 3	NM_005789			
PSME3-156		156	SNQQLVDIIEKVKPE	15
PSME3-150		150	PNGMLKSNQQLVDII	15
PSME3-3		3	MASLLKVDQEVKLK	14
PSME3-318		318	LHDMILKNIEKIKRP	15
RAB40C - member RAS oncogene family	NM_021168			
RAB40C-310		310	KSFSMANGMNAVMMH	15
RAB40C-319		319	NAVMMHGRSYSLASG	15
RAB40C-225		225	FNVIESFTELSRI	13
RABEP1 - rabaptin, RAB GTPase binding effector protein 1	NM_004703			
RABEP1-13		13	PDVSLQQRVAELEKI	15
RABEP1-810		810	SALVLRAQASEILLE	15
RABEP1-1044		1044	QLESLQEIKISLEEQ	15
RABEP1-1016		1016	ISSLKAELERIKVE	14
RABEP1-861		861	QMAVLMQSREQVSEE	15
RABEP1-657		657	TASLLSSVTQGMESA	15
RABEP1-1034		1034	LESTLREKSQQLESL	15
RABEP1-246		246	DAEKLRSVVMPMEKE	15
RBM25 - RNA binding motif protein 25	XM_027330			
RBM25-34		34	VPMSIMAPAPTVLV	14
RBM25-978		978	KRKHIKSLIEKIPTA	15
RBM25-266		266	IEVLIREYSSELNAP	15
RBM25-258		258	RDQMIKGAIEVLIRE	15
RBPSUH - recombining binding protein suppressor of hairless	NM_005349			
RBPSUH-658		658	NSTSVTSSTATVVS	14
RBPSUH-628		628	AGAILRANSSQVPPN	15
RBPSUH-255		255	LFNRLRSQTVSTRYL	15

RBPSUH-659		659	STSVTSSTATVVS	13
RBPSUH-350		350	IIRKVDKQTALLDA	14
RBPSUH-236		236	KKQSLKNADLCIASG	15
SDCCAG1 - serologically defined colon cancer antigen 1 , NY-CO-1	NM_004713			
SDCCAG1-13		13	LRAVLAELNASLLGM	15
SDCCAG1-934		934	LASCTSELISE	12
SDCCAG1-232		232	TLERLTEIVASAPKG	15
SDCCAG1-860		860	TGEYLTTGSFMIRGK	15
SDCCAG1-475		475	LKGELIEMNLQIVDR	15
SDCCAG1-229		229	PLLTLERLTEIVASA	15
SR-A1 - serine arginine-rich pre-mRNA splicing factor	NM_021228			
SR-A1-1126		1126	RKVKLQSKVAVLIRE	15
SR-A1-394		394	EEEGLSQSISRISET	15
SR-A1-1525		1525	KAQELIQATNQILSH	15
SR-A1-1683		1683	YKDILRKAVHKICHS	15
SR-A1-1504		1504	GVLALTALLFKMEEA	15
HUB - Hu antigen B (ELAVL2)	NM_004432			
HUB-146		146	LRLQTKTIKVSYA	13
HUB-467		467	NGYRLGDRVLQVSFK	15
HUB-78		78	ELKSLFGSIGEIESC	15
HUB-325		325	RLDNLLNMAYGVKRF	15
HUB-185		185	ELEQLFSQYGRIITS	15
HUB-75		75	TQEELKSLFGSIGEI	15
HUC - Hu antigen C (ELAVL3)	NM_001420			
HUC-146		146	LKLQTKTIKVSYA	13
HUC-475		475	NGYRLGERVLQVSFK	15
HUC-5		5	VTQILGAMESQVGGG	15
HUC-338		338	SPLSLIARFSPIAID	15
HUC-325		325	RLDNLLNMAYGVKSP	15
HUC-78		78	EFKSLFGSIGDIESC	15
HUD - Hu antigen D (ELAVL4)	NM_021952			
HUD-153		153	NGLRLQTKTIKVSYA	15
HUD-226		226	SRILVDQVTGVSRG	15
HUD-488		488	NGYRLGDRVLQVSFK	15
HUD-85		85	EFRSLFGSIGEIESC	15
HUR - Hu antigen R (ELAVL1)	NM_001419			
HUR-106		106	NGLRLQSKTIKVSYA	15
HUR-35		35	TQDELRSLFSSIG	13
HUR-414		414	NGYRLGDKILQVSFK	15
HUR-186		186	QTTGLSRGVAFIRFD	15
HUR-179		179	NSRVLVDQTTGLSRG	15
CRMP5 - colapsin rec. dihydropyrimidinase-like 5 (DPYSL5)	NM_020134			
CRMP5-110		110	TKAALVGGTTMIIGH	15

CRMP5-660		660	RTPYLGDVAVVVHPG	15
CRMP5-418		418	LMSLLANDTLNIVAS	15
CRMP5-716		716	GMRDLHESSFSLSGS	15
CRMP5-642		642	VYKKLVQREKTLKVR	15
CRMP5-111		111	KAALVGGTTMIIGHV	15
CRMP5-558		558	EATKTISASTQVQGG	15
EXOSC1 hRrp46p	NM_016046			
EXOSC1-98		98	KVSSINSRFAKVHIL	15
EXOSC1-185		185	SNYLLTTAENELGVV	15
EXOSC1-169		169	PGDIVLAKVISLGDA	15
EXOSC1-83		83	TESQLLPDVGAIVTC	15
EXOSC7	NM_015004			
EXOSC7-306		306	EACSLASLLVSVTSK	15
EXOSC7-349		349	VGKVLHASLQSVLHK	15
EXOSC7-176		176	HCWVLYVDVLLLECG	15
EXOSC5	NM_020158			
EXOSC5-255		255	ERKLLMSSTKGLYSD	15
EXOSC5-157		157	PRTSITVVLQVVSDA	15
EXOSC5-175		175	LACCLNAACMALVDA	15
EXOSC5-243		243	ARAVLTFALDSVERK	15
PGP 9.5 ubiquitin carboxyl-terminal hydrolase UCH-L3	M30496			
PGP 9.5-263		263	SDETLLEDAIEVCKK	15
PGP 9.5-111		111	MKQTISNACGTIGLI	15
GAD2 - glutamate decarboxylase 2	NM_000818			
GAD2-714		714	RMSRLSKVAPVIKAR	15
GAD2-389		389	SHFSLKKGAAALGIG	15
GAD2-644		644	KCLELAEYLYNIIKN	15
GAD2-244		244	YFNQLSTGLDMVGLA	15
GAD2-328		328	PGGAISNMYAMMIAR	15
GAD2-152		152	TLAFLQDVMNILLQY	15
GAD2-783		783	DIDFLIEEIERLGQD	15
GAD2-304		304	VTLKKMREIIGWP	13

[00145] Table 2 - Disclosed are 51 peptide epitopes, from the set of 1,448 peptide epitopes in Table 1, which were determined to be informative for distinguishing between NSCLC, SCLC, and control. See Experimental.

Number	Gene/epitope	peptide	mer
	TRP-2/ 4	ANDPIFVVL	9
	HAGHL-237	GHEHTLSNLEFAQKV	15
14	IQWD1-315	SAENPVENHINITQS	15
33	KIAA0373-1107	RKFAVIRHQQSLLYK	15
38	KIAA0373-1193	MKKILAENSRKITVL	15

88	LOC401193-156	EFLRSKKSSEEITQY	15
103	MSLN-186	FSRITKANVDLLPRG	15
108	NACA-261	AVRALKNNSNDIVNA	15
113	NISCH-805	CIGYTATNQDFIQRL	15
114	NISCH-1764	KTTGKMENYELIHSS	15
117	NISCH-1271	THNCRNRNSFKLSRV	15
122	NISCH-1105	RSCFAPQHMAMLCSP	15
158	RBMS1-108	PYGKIVSTKAILDKT	15
189	ROCK2-1296	HKQELTEKDATIASL	15
272	SDCCAG3-255	SYDALKDENSKLRRK	15
274	SDCCAG3-462	AEILKSIDRISEI	13
278	SDCCAG8-815	ECCTLAKKLEQISQK	15
377	TP53-171	YSPALNKMFCQLAKT	15
409	UTP14A-818	IRDFLKEKREAVEAS	15
411	UTP14A-182	TAQVLSKWDPVVLKN	15
454	ZNF292-3415	KKNNLENKNAKIVQI	15
455	ZNF292-1612	TPQNLERQVNNLMTF	15
458	ZNF292-3154	HKSDLPAFSAEVEEE	15
501	MELK-67	NTLGSDLPRIKTE	13
508	MELK-241	AAPELIQGKSYLGSE	15
525	NFRKB-1575	SAVSLPSMNAAVSKT	15
608	AARS-1017	TEEAIAKGIRRIVAV	15
616	ABL1-465	NAVVLLYMATQISSA	15
625	ACAT2-488	GCRILVTLLHTLERM	15
780	CTTNBP2-254	EAQKLEDVMAKLEEE	15
788	DDX5-190	TLSYLLPAIVHINHQ	15
803	DNAJA1-21	TQEELKKAYRKLALK	15
817	DNM1L-3	MEALIPVINKLQDV	14
820	DRCTNNB1A-588	SSHGLAKTAATVF	13
828	ELKS-241	KESKLSSSMNSIKTF	15
843	EXOSC10-883	TTCLIATAVITLFNE	15
884	GOLGA2-1061	THRALQGAMEKLQS	14
965	IQWD1-575	EHLMLLEADNHVVNC	15
972	LIMS1-182	KCHAIIDEQPLIFKN	15
978	LMNA-417	LQKQLAAKEAKLRDL	15
989	MKRN1-483	KQKLILKYKEAMSNK	15
990	NAP1L3-145	AVRNRVQALRNI	12
1042	RBM25-978	KRKHIKSLIEKIPTA	15
1049	RBPSUH-350	IIRKVDKQTALLDA	14
1050	RBPSUH-236	KKQSLKNADLCIASG	15
1053	SDCCAG1-232	TLERLTEIVASAPKG	15
1057	SR-A1-1126	RKVKLQSKVAVLIRE	15
1115	SOX1/17	HPHAHPHNPQPMHRY	15
1145	NY-ESO-1/2	GDADGPGGPGIPDGP	15
1146	NY-ESO-1/6	PRGPHGGAASGLNGC	15

1149	SSX1/11	SGPQNDGKQLHPPGK	15

[00146] Tables 3-6 disclose the results of autoantibody profiling using 51 epitopes of Table 2 in NSCLC, SCLC and control samples. See Experimental.

[00147] Table 3

Classifier: NON-SMALL CELL LUNG CANCER SAMPLES as training group					
Number of markers in training group: 1253					
Method: Neural Network					Statistical match
Plasma sample	Statistical match	Plasma sample	Statistical match	Plasma sample	
NSCLC	0%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	60%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	100%
NSCLC	0%	Control	0%	SCLC	100%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	56%
NSCLC	100%	Control	100%	SCLC	1%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	7%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	2%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	0%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	0%	SCLC	0%
NSCLC	100%	Control	65%	SCLC	0%
NSCLC	100%	Control	0%		
NSCLC	100%	Control	0%		
NSCLC	100%	Control	0%		
NSCLC	0%	Control	0%		

NSCLC	100%	Control	9%		
NSCLC	100%	Control	0%		
NSCLC	100%	Control	0%		
NSCLC	0%				
NSCLC	100%				
NSCLC	100%				
NSCLC	0%				
Mean	0.837837838		0.054848485		0.315
Standard Error	0.061433251		0.035571953		0.08852857
Median	1		0		0
Mode	1		0		0
Standard Deviation	0.373683877		0.204345315		0.451408906
Sample Variance	0.13963964		0.041757008		0.20377
Kurtosis	1.745188398		16.66992414		-1.295276226
Skewness	-1.911470521		4.095015871		0.831444585
Range	1		1		1
Minimum	0		0		0
Maximum	1		1		1
Sum	31		1.81		8.19
Count	37		33		26

[00148] Table 4

Method: Support Vector Machine: Radial Base Function kernel.					
Plasma sample	Statistical match	Plasma sample	Statistical match	Plasma sample	Statistical match
NSCLC	81%	Control	41%	SCLC	35%
NSCLC	98%	Control	1%	SCLC	58%
NSCLC	98%	Control	0%	SCLC	30%
NSCLC	100%	Control	3%	SCLC	6%
NSCLC	101%	Control	-2%	SCLC	32%
NSCLC	100%	Control	-3%	SCLC	91%
NSCLC	86%	Control	1%	SCLC	13%
NSCLC	102%	Control	2%	SCLC	4%
NSCLC	90%	Control	1%	SCLC	43%
NSCLC	88%	Control	2%	SCLC	21%
NSCLC	90%	Control	-2%	SCLC	4%
NSCLC	66%	Control	-21%	SCLC	4%
NSCLC	100%	Control	2%	SCLC	4%
NSCLC	97%	Control	4%	SCLC	43%
NSCLC	92%	Control	-12%	SCLC	22%
NSCLC	78%	Control	-20%	SCLC	19%
NSCLC	92%	Control	0%	SCLC	3%
NSCLC	42%	Control	1%	SCLC	5%

NSCLC	102%	Control	-1%	SCLC	5%
NSCLC	100%	Control	5%	SCLC	2%
NSCLC	98%	Control	-2%	SCLC	12%
NSCLC	98%	Control	-6%	SCLC	13%
NSCLC	59%	Control	1%	SCLC	3%
NSCLC	36%	Control	-5%	SCLC	-2%
NSCLC	97%	Control	23%	SCLC	3%
NSCLC	90%	Control	4%	SCLC	-3%
NSCLC	97%	Control	1%		
NSCLC	87%	Control	-9%		
NSCLC	97%	Control	-15%		
NSCLC	23%	Control	1%		
NSCLC	82%	Control	1%		
NSCLC	100%	Control	3%		
NSCLC	81%	Control	1%		
NSCLC	101%				
NSCLC	83%				
NSCLC	60%				
NSCLC	56%				
Mean	0.850810811		-0.0003125		0.180769231
Standard Error	0.032816668		0.019257824		0.042891359
Median	0.92		0.01		0.09
Mode	1		0.01		0.04
Standard Deviation	0.199615998		0.108938704		0.218703874
Sample Variance	0.039846547		0.011867641		0.047831385
Kurtosis	2.220723288		6.551736654		3.841127046
Skewness	-1.669600142		1.551257739		1.830688658
Range	0.79		0.62		0.94
Minimum	0.23		-0.21		-0.03
Maximum	1.02		0.41		0.91
Sum	31.48		-0.01		4.7
Count	37		32		26

[00149] Table 5

Classifier of the Arrays: NSCLC samples on 50 marker set					
Method: Support Vector Machine: Radial Base Function kernel.					
Plasma sample	Statistical match	Plasma sample	Statistical match	Plasma sample	Statistical match
NSCLC	102%	Control	51%	SCLC	3%
NSCLC	89%	Control	-2%	SCLC	2%
NSCLC	85%	Control	12%	SCLC	15%
NSCLC	98%	Control	-5%	SCLC	30%
NSCLC	76%	Control	-14%	SCLC	53%
NSCLC	102%	Control	-2%	SCLC	88%

NSCLC	94%	Control	0%		SCLC	-3%	
NSCLC	99%	Control	10%		SCLC	4%	
NSCLC	77%	Control	-6%		SCLC	20%	
NSCLC	82%	Control	4%		SCLC	17%	
NSCLC	71%	Control	-1%		SCLC	3%	
NSCLC	62%	Control	-22%		SCLC	4%	
NSCLC	63%	Control	5%		SCLC	2%	
NSCLC	57%	Control	2%		SCLC	21%	
NSCLC	101%	Control	2%		SCLC	3%	
NSCLC	100%	Control	-30%		SCLC	11%	
NSCLC	64%	Control	4%		SCLC	0%	
NSCLC	11%	Control	-13%		SCLC	0%	
NSCLC	101%	Control	-15%		SCLC	2%	
NSCLC	97%	Control	3%		SCLC	7%	
NSCLC	97%	Control	-4%		SCLC	6%	
NSCLC	82%	Control	-14%		SCLC	-1%	
NSCLC	68%	Control	0%		SCLC	4%	
NSCLC	34%	Control	-17%		SCLC	10%	
NSCLC	98%	Control	20%		SCLC	-2%	
NSCLC	79%	Control	34%		SCLC	2%	
NSCLC	76%	Control	3%				
NSCLC	98%	Control	-15%				
NSCLC	85%	Control	-1%				
NSCLC	17%	Control	3%				
NSCLC	43%	Control	-32%				
NSCLC	71%	Control	4%				
NSCLC	45%	Control	-4%				
NSCLC	82%						
NSCLC	98%						
NSCLC	26%						
NSCLC	75%						
Mean	0.758108		-0.012121212			0.115769231	
Standard Error	0.040918		0.027987272			0.03869873	
Median	0.82		-0.01			0.04	
Mode	0.98		0.04			0.02	
Standard Deviation	0.248896		0.16077464			0.19732558	
Sample Variance	0.061949		0.025848485			0.038937385	
Kurtosis	0.581168		3.018160625			9.147145282	
Skewness	-1.1099		0.984452432			2.863009047	
Range	0.91		0.83			0.91	
Minimum	0.11		-0.32			-0.03	
Maximum	1.02		0.51			0.88	
Sum	28.05		-0.4			3.01	
Count	37		33			26	

[00150] Table 6

Classifier: NON-SMALL CELL LUNG CANCER SAMPLES as training group								
Number of markers in training group: entire peptide library								
METHOD 1								
Method: Neural Network								
	NSCLC			NON-CANCER Control			SCLC	
	Statistical match			Statistical match			Statistical match	
Mean	0.837837838			0.054848485			0.315	
Standard Error	0.061433251			0.035571953			0.08852857	
number of samples	37			33			26	
METHOD 2								
Support Vector Machine: Radial Base Function kernel								
NSCLC			NON-CANCER Control			SCLC		
Statistical match			Statistical match			Statistical match		
0.850810811			-0.0003125			0.180769		
0.032816668			0.019257824			0.042891		
37			32			26		
Classifier: NSCLC samples as training group								
Number of markers: 50 peptides								
Support Vector Machine: Radial Base Function kernel								
	NSCLC			NON-CANCER Control			SCLC	
	Statistical match			Statistical match			Statistical match	
Mean	0.758108108			-0.012121212			0.115769231	
Standard Error	0.040918211			0.027987272			0.03869873	
number of samples	37			33			26	

Abbreviations:
NSCLC - non-small cell lung cancer
SCLC - small cell lung cancer

[00151] Table 7 discloses additional epitopes, corresponding to differentiation antigens, that may be used for autoantibody profiling.

Differentiation antigens	
CEA	YLSGANLNL
	IMIGVLVGV
	HLFGYSWYK
	YACFVSNLATGRNNS
	LWWVNNQSLPVSP
gp100/Pmel17	KTWGQYWQV
	AMLGTHTMEV
	ITDQVPFSV
	YLEPGPVTA
	LLDGTATLRL
	VLYRYGSFSV
	SLADTNSLAV
	RLMKQDFSV
	RLPRIFCSC
	LIYRRRLMK
	ALLAVGATK
	IALNFPGSQK
	ALNFPGSQK
	VYFFLPDHL
	RTKQLYPEW
	HTMEVTVYHR
	VPLDCVLYRY
	SNDGPTLI
Kallikrein4	SVSESDTIRSISIAS
	LLANGRMPTVLQCVN
	RMPTVLQCVNVSVVS
mammaglobin-A	PLLENVISK
Melan-A/MART-1	EAAGIGILTV
	ILTVILGVL
	AEEAAGIGILT
	RNGYRALMDKSLHVGTQCALTRR
PSA	FLTPKKLQCV
	VISNDVCAQV
TRP-1/gp75	MSLQRQFLR
	SLPYWNFATG
TRP-2	SVYDFFVWL
	TLDSQVMSL
	LLGPGRPYR
	ANDPIFVVL
	ALPYWNFATG
tyrosinase	KCDICTDEY

	SSDYVIPIGTY
	MLLAVLYCL
	CLLWSFQTSA
	YMDGTMSQV
	AFLPWHRLF
	TPRLPSSADVEF
	LPSSADVEF
	SEIWRDIDFd
	QNILLSNAPLGPQFP
	SYLQDSDPDSFQD
	FLLHHAFVDSIFEQWLQRHRP

[00152] Table 8 discloses additional epitopes, corresponding to antigens overexpressed in tumors, that may be used for autoantibody profiling.

ANTIGENS OVEREXPRESSED IN TUMORS	
adipophilin	SVASTITGV
CPSF	KVHPVIWSL
	LMLQNALTTM
EphA3	DVTFNIICKKCG
G250/MN/CAIX	HLSTAFARV
HER-2/neu	KIFGSLAFL
	IISAVVGIL
	ALCRWGLLL
	ILHNGAYSL
	RLLQETELV
	VVLGVVFGI
	YMIMVKCWMI
	HLYQGCQVV
	YLVPQQGFFC
	PLQPEQLQV
	TLEEITGYL
	ALIHHNTHL
	PLTSIISAV
	VLRENTSPK
Intestinalcarboxylesterase	SPRWWPTCL
alpha-foetoprotein	GVALQTMKQ
M-CSF	LPAVVGLSPGEQEY
MUC1	STAPPVHNV
	LLLLTVLTV

	PGSTAPPAHGVT
p53	LLGRNSFEV
	RMPEAAPPV
	SQKTYQGSY
PRAME	VLDGLDVLL
	SLYSFPEPEA
	ALYVDSLFFL
	SLLQHLIGL
	LYVDSLFFL
PSMA	NYARTEDFF
RAGE-1	SPSSNRIRNT
RU2AS	LPRWPPPQL
survivin	ELTLGEFLKL
Telomerase	ILAKFLHWL
	RLVDDFLLV
	RPGLLGASVLGLDDI
	LTDLQPYMRQFVAHL
WT1	CMTWNQMNL

[00153] Table 9 discloses additional epitopes, corresponding to antigens expressed in multiple tumor types, that may be used for autoantibody profiling.

SHARED TUMOR SPECIFIC ANTIGENS	
BAGE-1	AARAVFLAL
GAGE-1,2,8	YRPRPRRY
GAGE-3,4,5,6,7	YYWPRPRRY
GnTVf	VLPDVFIRCV
HERV-K-MEL	MLAVISCAV
LAGE-1	MLMAQEALAFL
	SLLMWITQC
	LAAQERRVPR
	SLLMWITQCFLPVF
	QGAMLAAQERRVPRAAEVPR
	AADHRQLQLSISSCLQQL
	CLSRRPWKRSWSAGSCPGMPHL
	ILSRDAAPLPRPG
MAGE-A1	EADPTGHSY
	SLFRAVITK
	EVYDGREHSA

	RVRFFFPSL
	EADPTGHSY
	REPVTKAEML
	DPARYEFLW
	ITKKVADLVGF
	SAFPTTINF
	SAYGEPRKL
	LLKYRAREPVTKAE
	EYVIKVSARVRF
MAGE-A2	YLQLVFGIEV
	EYLQLVFGI
	REPVTKAEML
	EGDCAPEEK
	LLKYRAREPVTKAE
MAGE-A3	EVDPIGHLY
	FLWGPRALV
	KVAELVHFL
	TFPDLESEF
	MEVDPIGHLY
	EVDPIGHLY
	REPVTKAEML
	AELVHFLLL
	MEVDPIGHLY
	WQYFFPVIF
	EGDCAPEEK
	KKLLTQHFVQENYLEY
	ACYEFLWGPRALVETS
	VIFSKASSSLQL
	GDNQIMPKAGLLIIV
	TSYVKVLHHMVKISG
	AELVHFLLLKYRAR
	LLKYRAREPVTKAE
MAGE-A4	EVDPASNTY
	GVYDGREHTV
	SESLKMIF
MAGE-A6	MVKISGGPR
	EVDPIGHVY
	REPVTKAEML
	EGDCAPEEK

	LLKYRAREPVTKAE
MAGE-A10	GLYDGMEHL
	DPARYEFLW
MAGE-A12	FLWGPRALV
	VRIGHLYIL
	EGDCAPEEK
	AELVHFLLLKYRAR
MAGE-C2	LLFGLALIEV
	ALKDVEERV
NA-88	QGQHFLQKV
NY-ESO-1/LAGE-2	SLLMWITQC
	ASGPGGGAPR
	LAAQERRVPR
	MPFATPMEA
	MPFATPMEA
	LAMPFATPM
	ARGPESRLL
	SLLMWITQCFLPVF
	QGAMLAAQERRVPRAAEVPR
	PGVLLKEFTVSGNILTIRLT
	VLLKEFTVSG
	AADHRQLQLSISSCLQQL
	PGVLLKEFTVSGNILTIRLTAADHR
Sp17	ILDSSEEDK
SSX-2	KASEKIFYV
	EKIQKAFDDIAKYFSK
	KIFYVYMKRKYEAM
TRP2-INT2g	EVISCKLIKR

[00154] Table 10 discloses additional epitopes, corresponding to tumor antigens that arise through mutation, that may be used for autoantibody profiling.

Tumor antigens resulting from mutations	
alpha-actinin-4	FIASNGVKLV
BCR-ABLfusionprotein(b3a2)	SSKALQRPV
	GFKQSSKAL
	ATGFKQSSKALQRPVAS
CASP-8	FPSDSWCYF
beta-catenin	SYLDSGIHF

Cdc27	FSWAMDLDPKGA
CDK4	ACDPHSGHFV
CDKN2A	AVCPWTWLR
COA-1f	TLYQDDTLTLQAAG
dek-canfusionprotein	TMKQICKKEIRRLHQY
Elongationfactor2	ETVSEQSNV
ETV6-AML1fusionprotein	RIAECILGM
	IGRIAECILGMNPSR
LDLR-fucosyltransferaseASfusionprotein	WRRAPAPGA
	PVTWRRAPA
hsp70-2	SLFEGIDIYT
KIAAO205	AEPINIQTW
MART2	FLEGNEVGKTY
MUM-1f	EEKLIVVLF
MUM-2	SELFRSGLDSY
	FRSGLDSYV
MUM-3	EAFIQPITR
neo-PAP	RVIKNSIRLTL
Myosinclassl	KINKNPKYK
OS-9g	KELEGILLL
pml-RARalphafusionprotein	NSNHVASGAGEAAIETQSSSSEEIV
PTPRK	PYYFAAELPPRNLPEP
K-ras	VVVGAVGVG
N-ras	ILDTAGREEY
Triosephosphatelsomerase	GELIGILNAAKVPAD

[00155] Table 11 discloses are 25 preferred lung cancer deterministic epitopes from the set of 1,448 peptide epitopes in Table 1. See Experimental.

1	GRINA-398	TCFLAVDTQLLLGNK	15
2	AP1G21020	LFRILNPNKAPLRLK	15
14	IQWD1-315	SAENPVENHINITQS	15
33	KIAA0373-1107	RKFAVIRHQQSLLYK	15
38	KIAA0373-1193	MKKILAENSRKITVL	15
88	LOC401193-156	EFLRSKKSSEEITQY	15
103	MSLN-186	FSRITKANVDLLPRG	15

108	NACA-261	AVRALKNNSNDIVNA	15
114	NISCH-1764	KTTGKMENYELIHSS	15
117	NISCH-1271	THNCRNRNSFKLSRV	15
122	NISCH-1105	RSCFAPQHMAMLCSP	15
158	RBMS1-108	PYGKIVSTKAILDKT	15
274	SDCCAG3-462	AEILKSIDRISEI	13
411	UTP14A-182	TAQVLSKWDPVVLKN	15
454	ZNF292-3415	KKNNLENKNAKIVQI	15
455	ZNF292-1612	TPQNLERQVNNLMTF	15
525	NFRKB-1575	SAVSLPSMNAAVSKT	15
608	AARS-1017	TEEAIAKGIRRIVAV	15
616	ABL1-465	NAVVLLYMATQISSA	15
828	ELKS-241	KESKLSSSMNSIKTF	15
965	IQWD1-575	EHLMLLEADNHVVNC	15
972	LIMS1-182	KCHAIIDEQPLIFKN	15
1050	RBPSUH-236	KKQSLKNADLCIASG	15
1057	SR-A1-1126	RKVKLQSKVAVLIRE	15
1146	NY-ESO-1/6	PRGPHGGAASGLNGC	15

[00156] Table 12 discloses the results of autoantibody profiling using 25 epitopes of Table 11 in NSCLC control samples. See Experimental.

Support Vector Machine: Radial Base Function kernel
Layer: RawData
Subset: Complete set

	Statistical match to NSCLC Classifier	
	NSCLC	CONTROL
Mean	0.948275862	0.124516129
Standard Error	0.020541134	0.037884484

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0.948275862	0.124516129
Variance	0.012236207	0.044492258
Observations	29	31
Pooled Variance	0.028920371	
Hypothesized Mean Difference	0	
df	58	
t Stat	18.75006802	
P(T<=t) one-tail	1.35315E-26	
t Critical one-tail	1.671552763	
P(T<=t) two-tail	*2.70629E-26*	
t Critical two-tail	2.001717468	

NSCLC = NON-SMALL LUNG CANCER

We tested an array that contained 25 of our best markers (the ones that scored the best among the entire peptide library)
We tested these 25-marker arrays with 29 NSCLC and 31 non-cancer control markers
We carried out the pattern recognition using Support Vector Machine (available in

GeneMath XT bioinformatics package)

EXPERIMENTAL

[00157] We have carried out pilot studies on breast and lung cancer. In our breast cancer study, we determined the serum aAB composition in 16 breast cancer patients and 16 gender-matched non-cancer control individuals. The lung cancer study was carried out as a comparative study on NSCLC and SCLC sera in order to detect differences between these two predominant types of lung caner. Both of these pilot studies were carried out simultaneously with the same set of epitopes. This set included 428 different epitopes representing 135 different proteins. The informative epitopes were sorted into two groups based on an increased/decreased (I/D) signal dichotomy. Briefly, we carried out a cancer vs. non-cancer comparison for breast cancer, and an NSCLC vs. SCLC for lung cancer using the neighborhood analysis. This method, adopted from large-scale gene-expression studies (Golub et al., Science (1999) 286:531-7) identifies informative peptide epitopes. Informative epitopes are the epitopes that produce a significantly different signal in one group of patient sera compared with another group of patient sera.

Breast Cancer : Informative Epitopes

[00158] The breast cancer pilot study produced a set of 27 informative epitopes exhibiting an increased/decreased (I/D) dichotomy (Fig. 2). Intriguingly, the subset of epitopes that produced a decreased signal was greater than the subset of epitopes which produced an increased signal in breast cancer compared with non-cancer control. For both subsets of informative epitopes, the highly significant p-values were determined in the EB vs. EC comparison (Fig. 2).

[00159] The I/D-dichotomy for informative breast cancer epitopes is significantly disproportional. Determined on unsorted informative epitopes, EB was significantly smaller than EC (22 ± 0.8 vs. 30 ± 1.3, respectively; $p = 0.00000183$). Thus, as demonstrated by informative breast cancer epitopes, the capacity of peptide epitopes to produce an in vitro immune reaction with serum aAB is smaller in breast cancer compared with non-cancer control (Fig. 2). We interpret this result as an indication that breast cancer sera contain either lower titer aAB or lower affinity aAB than control sera. In fact, we hypothesize that this “fading” of the "in vitro immune reaction" in breast cancer points to a weakened

B-cell immunity. Nevertheless, we believe that also the anti-tumor humoral immune response is manifest in breast cancer because we detected a sub-set of informative epitopes that produced a significantly increased in vitro immune reaction in breast cancer sera (Fig. 2).

Lung Cancer: NSCLC vs. SCLC : Informative Epitopes

[00160] The lung cancer pilot study produced 28 informative epitopes that characterize the serum aAB difference between NSCLC and SCLC. Similar to the informative breast cancer epitopes, the informative lung cancer epitopes exhibited a significantly disproportional I/D-dichotomy (Fig. 3). Specifically, ES was significantly smaller than EN (28.4 ± 1.0 vs. 32.5 ± 0.9; p = 0.006). Considering also our breast cancer study, and the published data about cancer survival, the following hypothesis can be put forward: Decreased average informative epitope strength [E] in breast cancer and SCLC indicate a compromised immune status of breast cancer and SCLC patients compared with their reference groups. This weakened immune status explains poorer survival in breast cancer and SCLC relative to non-cancer controls and NSCLC patients, respectively. As demonstrated by the Mayo Lung Project, the median survival is shorter and the 5-year survival poorer in SCLC compared with NSCLC (Marcus et al., J Natl Cancer Inst. (2000) 92:1308-16). Furthermore, in view of the above hypothesis, it is reasonable that a smaller difference emerged between ES and EN compared with EB and EC because non-cancer individuals generally have a better life expectancy than cancer patients.

Epitope Microarrray Reveals Higher Order Among Informative Cancer Epitopes: (i) Overlapping Informative Epitopes

[00161] The two above pilot studies revealed an overlap (Fig. 4). We detected three epitopes that were informative for both breast and lung cancer (Fig. 4). Intriguingly, all three of these overlapping epitopes exhibited the same I/D-dichotomy in regard to the published knowledge about cancer survival. Specifically, ZFP-200 produced an increased signal in both breast cancer and SCLC relative to the non-cancer control and NSCLC, respectively; MAGE4a/14 and SOX2/5 produced a decreased signal in breast cancer and SCLC relative to the non-cancer control and NSCLC.

(ii) Overlapping Informative Proteins

[00162] We also detected informative epitopes that did not overlap but represented the same protein (Fig. 4). Non-overlapping epitopes from four proteins, MAGE4a, NY-ESO, SOX-1 and SOX-2, produced an informative signal for both breast and lung cancer. The I/D-dichotomy of all four of these proteins in regard to the published cancer survival data (Marcus et al., J Natl Cancer Inst. (2000) 92:1308-16) was the same in that they all exhibited a decreased in vitro immune reactivity in the poorer survival group (Fig. 4). Thus, clustering of both informative epitopes and proteins to reveal

aAB associations between cancer types, and potentially common pathogenic mechanisms, appears to be possible using an epitope microarray.

Epitope Validation

[00163] With our cancer epitope microarrays, we have focused on (1) transcription factors expressed in embryonal tissues (Gure et al. supra; Chen et al., (1997) supra), (2) proteins known to trigger B-cell response in cancer (Tan, supra, Lubin, supra), and (3) proteins with embryo/testis/tumor specificity known to activate tumor specific cytolytic T-cells (Van Der Bruggen et al., Immunol Rev. (2002) 188:51-64; Boon et al., Annu Rev Immunol. (1994) 12:337-65). As our pilot studies indicate, this approach appears to bear fruit in that the informative epitopes for both breast and lung cancer include members of the SOX-family (embryo specific transcription factor), p53, members of IMP and HuD-family (known inducers of B-cell response in cancer), and tumor/testis/cancer proteins such as members of MAGE and NY-ESO family (Figs. 2-4).

Epitope Signal Analysis

[00164] We used the neighborhood analysis (Golub et al., supra) in order to determine informative epitopes. We included both signal frequency and intensity in data analysis. Mean average ± SEM of signal intensity per a specific epitope in a group is referred to as an epitope signal. In order to evaluate epitopes, we carried out a two-sided Student t-test assuming equal variance (Fig. 5) on epitope signals. All epitopes that produce a significantly different epitope signal in a two-way comparison were considered informative epitopes. The example in Fig. 5 illustrates the evaluation of epitopes. In addition to epitope signal, the following endpoints were calculated and evaluated in data analysis:

[00165] ΣP – composite signal strength for all informative epitopes per an individual test subject;

[00166] E - Average Informative Epitope Strength per group of patients;

[00167] $E = [\Sigma P1 + \ldots + \Sigma Pn \ / \ N] \pm SEM$, where N denotes a number of patients in a group (Fig. 5). This parameter is calculated for both unsorted and sorted data.

Signal Detection and Quantification

[00168] Our preliminary comparative experiments on alkaline phosphatase- ("AP") based colorimetry and Cy3-based fluorimetry indicate that the signal over background ratio is up to an order of magnitude greater when Cy3 in place of AP is used (data not shown). This result is in agreement with previous studies indicating that fluorescence-based labeling produces a superior dynamic signal range over traditional color-producing labeling (Boon et al., supra).

[00169] Our existing, colorimetry-based data have the maximum range of 3 in 99% cases. Cy3-fluoresence-based experiments are done using neighborhood analysis in order decrease underestimates and overestimates of epitope importance based on colorimetric data. Somewhat different informative epitope sets may emerge. Because of greater sensitivity, the smaller quantities of sera required per assay are envisioned as a very relevant benefit of the fluorimetry-based visualization platform; a benefit that will increase in importance as the density of epitopes on the microarray increases.

Data Normalization

[00170] As depicted in Fig. 1, signal quantification and normalization is improved by implementing an internal control that is based on serial dilutions of human IgG. This internal control enables a more accurate normalization of each one of the individual peptide:aAB interactions as compared to single-concentration based signal quantification. As a result, the individual peptide epitope/aAB-binding activities may be expressed as equivalents of immunoreactivity of x-amount of human IgG. Introducing this specific normalization feature will improve the compatibility of the data from different experiments and test sites.

Data Analysis

[00171] Epitopes that produce the greatest variance in the t-test are sorted in order determine the value of the most deviating epitopes. As our preliminary data indicate, approximately 1% of all individual peptide/autoantibody binding reactions produce a very strong signal, which in some cases exceeds even the positive control (data not shown). These rare, very strong signals may represent the cases in which a certain epitope detects a specific high-affinity anti-tumor serum aAB. Cy3-based fluorimetric detection is validated because it produces a greater dynamic range for the epitope microarray. Use of Cy3 reveals epitopes that identify high titer and high affinity anti-tumor serum aAB. Both colorimetry- and fluorimetry-produced data are analyzed and cross-validated. Cross-validation includes both p-value and variance-based analyses.

Power of Individual aABs and aAB Patterns

[00172] The system used determines (1) the individual diagnostic powers of each one of the informative epitopes, and (2) validates the diagnostic power of various combinations of informative epitopes (aAB patterns). The former can be achieved using the principles of "weighted votes" described by Golub et al., supra, whereas the latter can be accomplished using various pattern recognition algorithms, and then validating the resulting patterns individually. Briefly, in order to elucidate the diagnostic power of individual epitopes, a system of "weighted votes" may be used. In this type of system, the capacity of an informative epitope to predict a certain tumor is dependent on

(1) its ability to alter the diagnostic power of a group of informative epitopes, and (2) to predict a tumor class in a blinded study. Specifically, the greater the capacity of an individual epitope to alter the diagnostic power of a group of epitopes, the more likely this epitope is to predict a certain tumor. The epitopes with the greatest individual predictive power will also be the most valuable markers in a blinded study. Because of enormous genetic complexity of cancer, and the variability of immune responses and antigen presentation, the diagnostic utility of various aAB patterns surpasses the diagnostic utility of individual epitopes.

Different Epitopes Corresponding to Same Antigen Have Different Diagnostic Values

[00173] Proteins as antigens carry large number of epitopes that are not equally immunogenic and are not equally presented by antigen presenting and tumor cells.

[00174] For example from twenty-two KIA0373 epitopes, only two (KIAA0373-1107-RKFAVIRHQQSLLYK; and KIAA0373-1193- MKKILAENSRKITVL) exhibit consistent autoantibody binding activity and strong diagnostic value for NSCLC. Similar distinctions in diagnostic value between individual epitopes are observed for NISCH, SDCCAG3, ZNF292, RBPSUH and many other proteins.

[00175] In conclusion, our analysis has demonstrated that different epitopes from the same protein antigen may have different and even opposite diagnostic values. For example antibodies recognizing epitope SOX3/7 (peptide – PAMYSLLETELKNPV) are present and characteristic for NSCLC and epitope SOX3/14 (peptide – DEAKRLRAVHMKEYP) is characteristic for SCLC.

Large Scale Autoantibody Profiling of Lung Cancer Patients: Diagnostic Value of Autoantibody Patterns

[00176] This study has three groups of patients:

[00177] 1. healthy patients with history of heavy smoking (32 patients)

[00178] 2. non small cell lung cancer patients (36 patients)

[00179] 3. small cell lung cancer patients (26 patients)

[00180] Blood serum from all study individuals was analyzed using a peptide epitope array with 1,253 of the 1,448 peptide epitopes disclosed in Table 1.

[00181] Array images were analyzed using Array-Pro Analyzer (Media Cybernetics) and image data were analyzed using GeneMaths XT (Applied Maths) to obtain patterns of autoantibody binding activities that are characteristic for cancer patients and can be used as diagnostic tools. (Tables 3-6)

[00182] Analysis using Neural Networks and Support Vector Machine software demonstrated that discrete groups of autoantibodies are present in each patient category. In this specific set of study individuals, non small cell cancer patients can be grouped together with 83-85 % specificity, whereas control patients belong to this group with less than 5% probability. (Tables 3-6)

Autoantibody Profiling of Lung Cancer Patients: Lung Cancer Deterministic Peptides

[00183] A peptide array containing 25 of the most informative epitopes (Table 11) was used with the samples described above. This array contained the peptides that produced the best discrimination between non-small cell lung cancer (NSCLC) and control samples in the large-scale screening with 1,253 of the 1,448 peptide epitopes disclosed in Table 1. We refer to these as 'lung cancer deterministic peptides', which can be used as a highly accurate set of lung cancer diagnostic epitopes. We used Support Vector Machine as a pattern recognition algorithm. First, we used all of the NSCLC samples to compose a classifier and then we applied this classifier on both NSCLC and control samples. The average similarity of an NSCLC sample to the NSCLC classifier turned out to be ~95%, and that of a control sample, 12.5%. (Table 12)

Detection of Auto-antibodies: Peptide Microarray Protocol Using Nitrocellulose Pads on Coverslips

[00184] Microarray slides are commercially available, for example from Schleicher & Schuell. The protocol is a follows:

[00185] 1. Blocking with Superblock, TBS based (pH 7.4), (Pierce Cat# 37535), 0.05% Tween 20 for 1 h at room temperature. Use 100-150 µl of blocking solution per well (16 pad slides)

[00186] 2. Wash twice with TBS, pH 7.4 and 0.05% Tween 20 at room temperature 2 min each wash. Each wash 150 µl.

[00187] 3. Dilute serum1:15 with TBS, pH 7.4 containing Superblock diluted 1:10 and 0.05% Tween 20.

[00188] 4. Incubate array with 150 µl of diluted serum overnight at +4 0C (minimum 16 hours).

[00189] 5. Wash 5 times using TBS, pH 7.4 containing 0.05% Tween 20 at room temperature 5 min each wash. Each wash 150 µl.

[00190] 6. Incubate with secondary antibody (alkaline phosphatase conjugated anti human IgA, IgM, IgG; ChemiconAP120A, lot 23091469) diluted 1:3000 with TBS, pH 7.4 containing Superblock diluted 1:10 and 0.05% Tween 20 for 1 hour at room temperature. Volume 150 µl.

[00191] 7. Wash 5 times using TBS, pH 7.4 containing 0.05% Tween 20 at room temperature 5 min each wash. Each wash 150 µl.

[00192] 8. Visualize auto-antibody binding using alkaline phosphatase substrate (Pierce 1-Step NBT/BCIP, product # 34042). It will take 15–30 minutes to see reaction products. Do not over incubate. Long incubation time will result in high background.

[00193] 9. Stop reaction by rinsing with water

[00194] 10. Dry slides and analyze.

Peptide Printing Protocol using Perkin Elmer Piezzo Arrayer

[00195] Preparation:

[00196] 0.1% Tween in PBS Buffer

[00197] HPLC Grade Water

[00198] 50mM NaOH

[00199] Repel-Silane ES

[00200] HPLC Methanol

[00201] Method:

[00202] Before any run do the following:

[00203] 1) Prime the tips using the Prime Utility;

[00204] 2) Clean the tips with 50mM NaOH, using the advance NaOH cleaning utility;

[00205] 3) Prime the tips using the Prime Utility;

[00206] 4) Silanate the tips using the Silanate Utility, the first four wells should be filled with 100% HPLC Grade Methanol; protein precipitation should not occur due to the NaOH cleaning; the last four wells will contain the Repel-Silane ES solution;

[00207] 5) Prime the tips using the Prime Utility;

[00208] 6) Tune the tips using the Tuning Utility;

[00209] 7) Do a Standard Wash.

[00210] Setting up the protocol:

[00211] 1) The Wash settings tab should be set to the following: syringe wash volume is 400µl, Peripump on time is 10 seconds, and Sonication is set to yes;

[00212] 2) Protocol Setup should implement the cleaning solution; the solution should be 1% Tween in PBS; the contact time should be 35 seconds, the flush volume 400µl, and the aspirate volume is 15µl;

[00213] 3) The arrays should print 55 samples in duplicate or 110 spots on a 16 Pad Fast Slide;

[00214] 4) Upon Error, a retry should be attempted once before ignoring.

[00215] Printing:

[00216] 1) Peptide Samples (2mg/ml in H_2O) along with controls arrive in 96 well plates and only need to be properly positioned in the source holder;

[00217] 2) After printing, all slides need to be properly labeled.

[00218] Repeat above to clean for next printing.

[00219] All references and patents cited herein are expressly incorporated herein in their entirety by reference.

We Claim:

1. A method for identifying a set of informative epitopes having autoantibody binding activities that correlate with a class distinction between samples, comprising the steps of:

a) determining autoantibody binding activities for a plurality of epitopes in a plurality of samples for each of two or more classes;

b) sorting said epitopes by degree to which their autoantibody binding activity in said plurality of samples correlates with a class distinction; and

c) determining whether said correlation is stronger than expected by chance;

wherein epitopes having autoantibody binding activity that correlates with a class distinction more strongly than expected by chance are informative epitopes, thereby identifying a set of informative epitopes.

2. A method for identifying a set of informative epitopes having autoantibody binding activities that correlate with a class distinction between samples, comprising the steps of:

a) determining autoantibody binding activities for a plurality of epitopes in a plurality of samples for each of two or more classes;

b) identifying clusters of epitopes from said plurality of epitopes which have autoantibody binding activities in samples of the same class from said plurality of samples, wherein said clusters of epitopes have autoantibody binding activities that correlate with a class distinction between samples of different classes from said plurality of samples; and

c) determining whether said correlation is stronger than expected by chance;

wherein a cluster of epitopes having autoantibody binding activities that correlate with a class distinction more strongly than expected by chance are a set of informative epitopes.

3. An epitope microarray for distinguishing between a plurality of classes for a biological sample, comprising a plurality of peptides, each of said peptides independently having a corresponding epitope binding activity in a sample characteristic of a particular class selected from a plurality of particular classes, wherein taken together, said plurality of peptides have corresponding epitope binding activities in a plurality of samples collectively characteristic of all of said plurality of particular classes, wherein the autoantibody binding activity of each of said peptides is independently higher in

a sample characteristic of one of said plurality of particular classes than in a sample characteristic of another one of said plurality of particular classes.

FIGURE 1

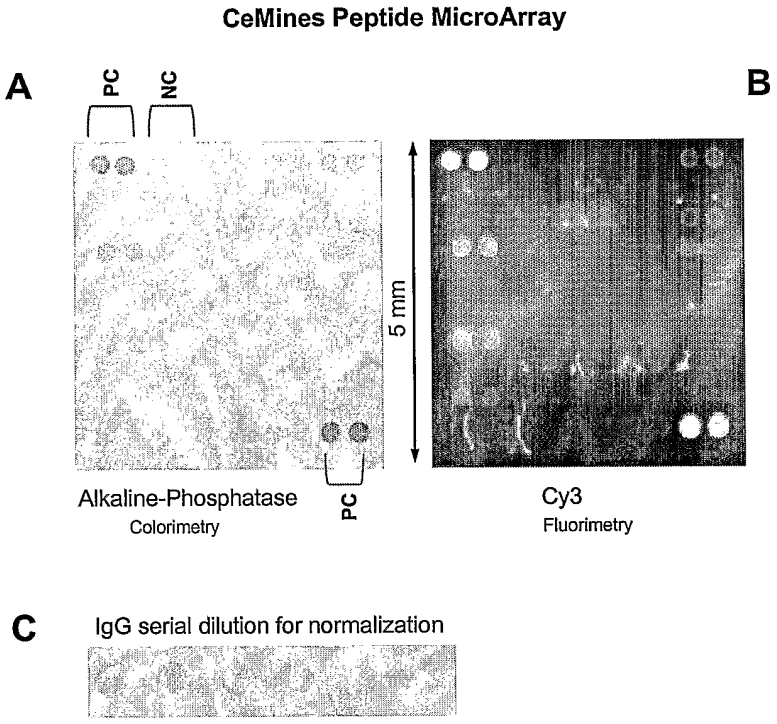

CeMines Peptide MicroArray
A
PC
NC
B
5 mm
PC
Alkaline-Phosphatase
Colorimetry
Cy3
Fluorimetry
C
IgG serial dilution for normalization

FIGURE 2

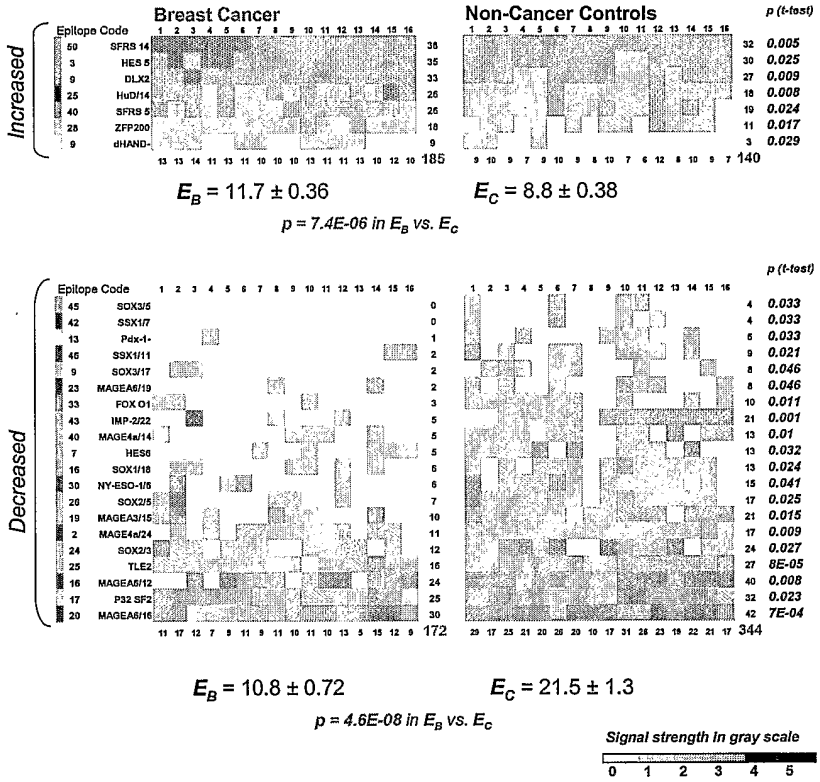

Breast Cancer
Non-Cancer Controls
p (t-test)
Increased
Epitope Code
50 SFRS 14 0.005
3 HES 5 0.025
9 DLX2 0.009
25 HuD/14 0.008
40 SFRS 5 0.024
28 ZFP200 0.017
9 dHAND- 0.029
E_B = 11.7 ± 0.36
E_C = 8.8 ± 0.38
p = 7.4E-06 in E_B vs. E_C
Decreased
45 SOX3/5 0.033
42 SSX1/7 0.033
13 Pdx-1- 0.033
46 SSX1/11 0.021
9 SOX3/17 0.046
23 MAGEA6/19 0.046
33 FOX O1 0.011
43 IMP-2/22 0.001
40 MAGE4a/14 0.01
7 HES6 0.032
16 SOX1/18 0.024
30 NY-ESO-1/5 0.041
26 SOX2/5 0.025
19 MAGEA3/15 0.015
2 MAGE4a/24 0.009
24 SOX2/3 0.027
25 TLE2 8E-05
16 MAGEA6/12 0.008
17 P32 SF2 0.023
20 MAGEA6/16 7E-04
E_B = 10.8 ± 0.72
E_C = 21.5 ± 1.3
p = 4.6E-08 in E_B vs. E_C
Signal strength in gray scale
0 1 2 3 4 5

FIGURE 3

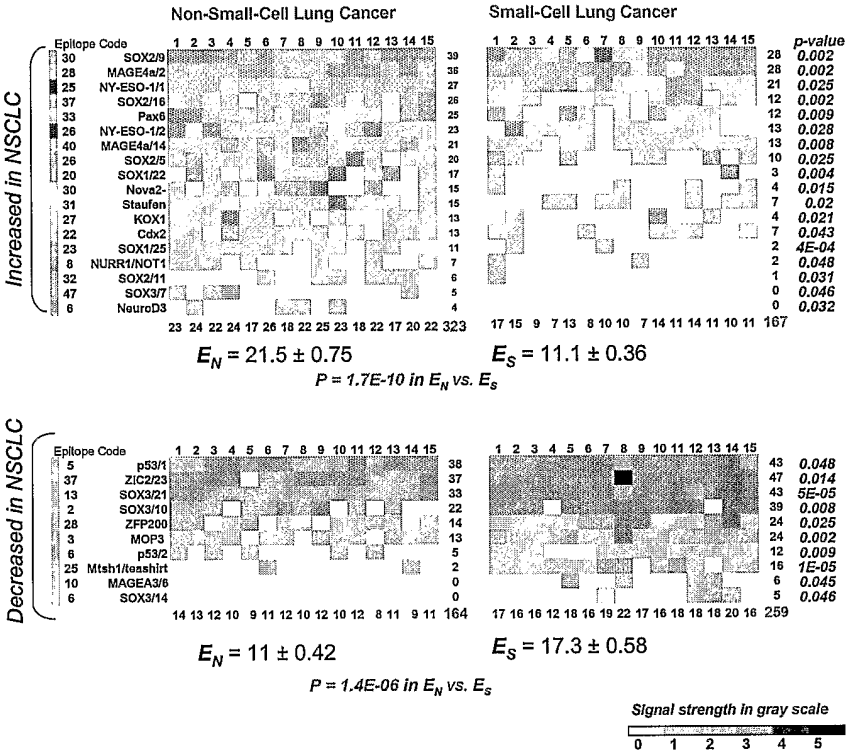

Non-Small-Cell Lung Cancer
Small-Cell Lung Cancer
Increased in NSCLC
Epitope Code
SOX2/9
MAGE4a/2
NY-ESO-1/1
SOX2/16
Pax6
NY-ESO-1/2
MAGE4a/14
SOX2/5
SOX1/22
Nova2-
Staufen
KOX1
Cdx2
SOX1/25
NURR1/NOT1
SOX2/11
SOX3/7
NeuroD3
p-value
0.002
0.002
0.025
0.002
0.009
0.028
0.008
0.025
0.004
0.015
0.02
0.021
0.043
4E-04
0.048
0.031
0.046
0.032
E_N = 21.5 ± 0.75
E_S = 11.1 ± 0.36
P = 1.7E-10 in E_N vs. E_S
Decreased in NSCLC
p53/1
ZIC2/23
SOX3/21
SOX3/10
ZFP200
MOP3
p53/2
Mtsh1/teashirt
MAGEA3/6
SOX3/14
0.048
0.014
5E-05
0.008
0.025
0.002
0.009
1E-05
0.045
0.046
E_N = 11 ± 0.42
E_S = 17.3 ± 0.58
P = 1.4E-06 in E_N vs. E_S
Signal strength in gray scale
0 1 2 3 4 5

FIGURE 4

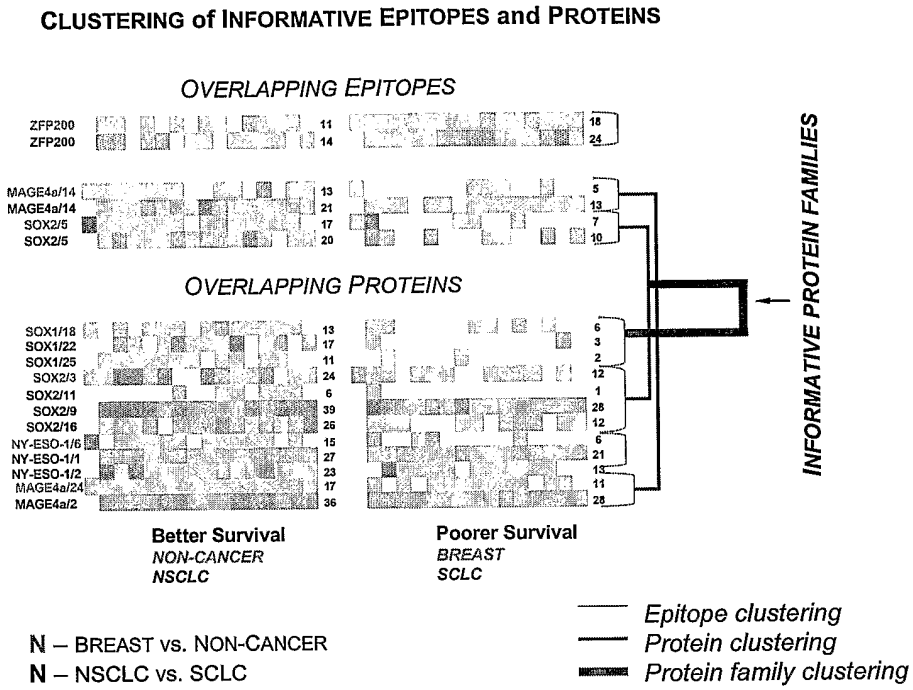
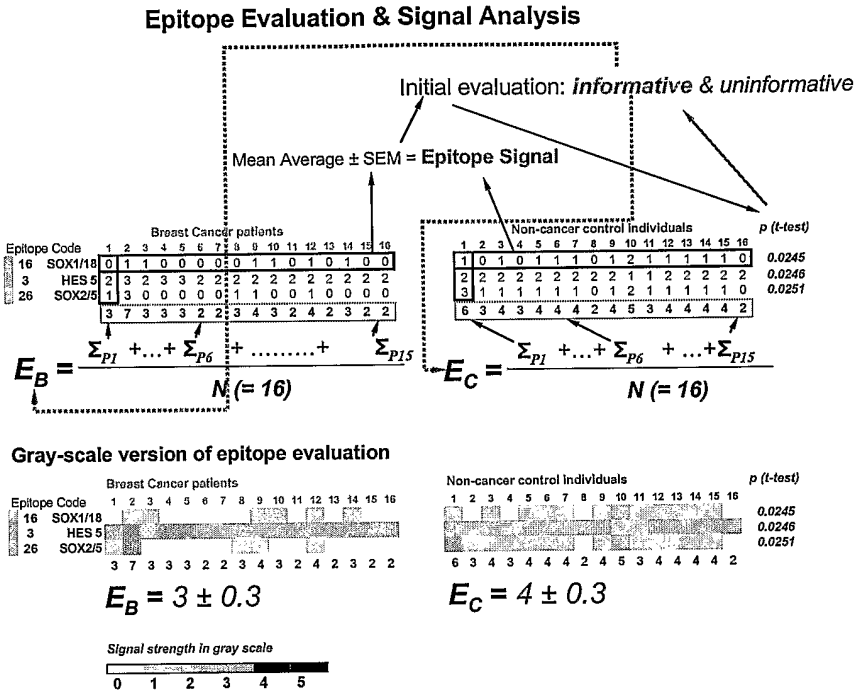

CLUSTERING of INFORMATIVE EPITOPES and PROTEINS
OVERLAPPING EPITOPES
ZFP200
ZFP200
MAGE4a/14
MAGE4a/14
SOX2/5
SOX2/5
OVERLAPPING PROTEINS
SOX1/18
SOX1/22
SOX1/25
SOX2/3
SOX2/11
SOX2/9
SOX2/16
NY-ESO-1/6
NY-ESO-1/1
NY-ESO-1/2
MAGE4a/24
MAGE4a/2
INFORMATIVE PROTEIN FAMILIES
Better Survival
NON-CANCER
NSCLC
Poorer Survival
BREAST
SCLC
N – BREAST vs. NON-CANCER
N – NSCLC vs. SCLC
Epitope clustering
Protein clustering
Protein family clustering

FIGURE 5


Epitope Evaluation & Signal Analysis
Initial evaluation: informative & uninformative
Mean Average ± SEM = Epitope Signal
Breast Cancer patients
Non-cancer control individuals
p (t-test)
Epitope Code
16 SOX1/18
3 HES 5
26 SOX2/5
0.0245
0.0246
0.0251
$E_B = \frac{\Sigma_{P1} + \ldots + \Sigma_{P6} + \ldots\ldots\ldots + \Sigma_{P15}}{N (= 16)}$
$E_C = \frac{\Sigma_{P1} + \ldots + \Sigma_{P6} + \ldots + \Sigma_{P15}}{N (= 16)}$
Gray-scale version of epitope evaluation
Breast Cancer patients
Non-cancer control individuals
p (t-test)
$E_B = 3 \pm 0.3$
$E_C = 4 \pm 0.3$
Signal strength in gray scale
0 1 2 3 4 5