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(54) Titre : BIOMARQUEURS DU CANCER DU PANCREAS ET LEURS UTILISATIONS
(54) Title: PANCREATIC CANCER BIOMARKERS AND USES THEREOF

(57) Abrégé/Abstract:
The present disclosure includes biomarkers, methods, devices, reagents, systems, and kits for the detection and diagnosis of cancer generally and pancreatic cancer specifically. In one aspect, the disclosure provides biomarkers that can be used alone or in various combinations to diagnose cancer generally or pancreatic cancer specifically. In another aspect, methods are provided for diagnosing pancreatic cancer in an individual, where the methods include detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 1, wherein the individual is classified as having pancreatic cancer, or the likelihood of the individual having pancreatic cancer is determined, based on the at least one biomarker value. In a further aspect, methods are provided for diagnosing cancer generally in an individual, where the methods include detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 19, wherein the individual is classified as having cancer generally, or the likelihood of the individual having cancer is determined, based on the at least one biomarker value.

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(57) Abstract: The present disclosure includes biomarkers, methods, devices, reagents, systems, and kits for the detection and diagnosis of cancer generally and pancreatic cancer specifically. In one aspect, the disclosure provides biomarkers that can be used alone or in various combinations to diagnose cancer generally or pancreatic cancer specifically. In another aspect, methods are provided for diagnosing pancreatic cancer in an individual, where the methods include detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 1, wherein the individual is classified as having pancreatic cancer, or the likelihood of the individual having pancreatic cancer is determined, based on the at least one biomarker value. In a further aspect, methods are provided for diagnosing cancer generally in an individual, where the methods include detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 19, wherein the individual is classified as having cancer generally, or the likelihood of the individual having cancer is determined, based on the at least one biomarker value.

Pancreatic Cancer Biomarkers and Uses Thereof

RELATED APPLICATIONS

[0001]

FIELD OF THE INVENTION

[0002] The present application relates generally to the detection of biomarkers and the diagnosis of cancer in an individual and, more specifically, to one or more biomarkers, methods, devices, reagents, systems, and kits for diagnosing cancer, more particularly pancreatic cancer, in an individual.

BACKGROUND

[0003] The following description provides a summary of information relevant to the present application and is not an admission that any of the information provided or publications referenced herein is prior art to the present application.

[0004] Pancreatic cancer is the fourth leading cause of cancer-related death in the USA. While the 5-year survival is only 5%, this has been shown to increase with early surgical intervention: in the 20% of subjects eligible for a "curative" resection the survival increases to 15-20%. At the time of diagnosis, more than half the patients have distant disease and another 25% have regional spread. This is because the disease is notoriously difficult to diagnose in its early stages. About 20 percent of patients with "operable" disease [stage IIb or less] undergo a "curative" resection and the 5-year survival increases from less than 5% to 15-20%.

[0005] Pancreatic cancers can arise from both the exocrine and endocrine portions of the pancreas. Of pancreatic tumors, 95% develop from the exocrine portion of the pancreas,

including the ductal epithelium, acinar cells, connective tissue, and lymphatic tissue. Approximately 75% of all pancreatic carcinomas occur within the head or neck of the pancreas, 15-20% occur in the body of the pancreas, and 5-10% occur in the tail.

[0006] Recurrence can be local (in or near the same place it started) or distant (spread to organs such as the liver, lungs, or bone). When pancreatic exocrine cancer recurs, it is essentially treated the same way as metastatic cancer, and is likely to include chemotherapy if the patient can tolerate it. Typically, pancreatic cancer first metastasizes to regional lymph nodes, then to the liver, and, less commonly, to the lungs. It can also directly invade surrounding visceral organs such as the duodenum, stomach, and colon or metastasize to any surface in the abdominal cavity via peritoneal spread. Ascites may result, and this has an ominous prognosis. Pancreatic cancer may spread to the skin as painful nodular metastases. Pancreatic cancer uncommonly metastasizes to bone.

[0007] Two clinical applications for a blood based pancreatic cancer test are for preclinical diagnosis in the asymptomatic, high-risk population and differential diagnosis in the symptomatic population. The clinical utility for both of these indications is outlined below.

[0008] **Screening in an asymptomatic, high risk population**: There were an estimated 43,140 new cases of pancreatic cancer in the USA in 2010, and 36,800 deaths. Genetics, family history, chronic pancreatitis, smoking and high alcohol consumption increase the risk of pancreatic cancer, as does cystic fibrosis. The increase in risk has been reported as:

- Cigarette smoking: <25 per day is 2x risk, >25 per day is 3x risk
- Alcohol: more than 3 drinks per day gives a 1.6 fold risk increase
- Family history: a first degree relative with the disease gives a 5x increase
- Adults with cystic fibrosis: 31x risk
- BRCA2 genetic mutations: 10x risk

[0009] In the asymptomatic but at-risk population in the absence of an effective screening paradigm the cancer is simply detected at the time of symptomatic presentation. This is likely to be late. The existence of an early detection test would increase the proportion of patients eligible for curative surgery. The current cure rate of 20% in the 20% of early detection subjects is only 4% of the total population. If the eligibility for curative surgery increased - by early detection in the asymptomatic population - from the current 20%, then the curable total would increase, as would the number of lives saved per year. Since pancreatic cancer

is a low prevalence disease, even in this high-risk population, high specificity is an important attribute of a screening test. A low false positive rate is essential to reduce the cost incurred by unnecessary follow-up procedures and reduce anxiety for the patient.

[0010] **Differential diagnosis in the symptomatic patient**. Pancreatic cancer may be difficult to distinguish from benign conditions such as pancreatitis or gastro-intestinal disorders. The differential diagnosis of a primary exocrine pancreatic cancer includes chronic pancreatitis, pancreatic endocrine tumors, autoimmune pancreatitis, lymphoma, and a variety of other rare conditions. Common but non-specific symptoms associated with pancreatic cancer include:

- Abdominal pain - particularly when radiating to the back
- Obstructive jaundice
- Sudden unexplained diabetes
- Weight loss
- Anorexia, fatigue
- Nausea, vomiting
- Acute or chronic pancreatitis

[0011] The table below shows the numbers of patients presenting to emergency rooms and to hospitals with at least two of these relevant symptoms; the first symptom is any one of the listed symptoms, and the second symptom is the one listed in the table. The emergency room data was from: (http://hcupnet.ahrq.gov/) while the ambulatory data was from the CDC 2008 National Ambulatory Medical Care Survey 2006 (number 8).

Relevant Symptoms by Age					
		Emergency Dept		**All Ambulatory**	
	Percentage	64-84	45-84	64-84	45-84
Abdominal pain	10.6%	39,922	142,188	106,458	379,168
Jaundice	11.0%	1,405	3,429	3,746	9,145
Weight loss	11.0%	5,465	9,860	14,574	26,294
Malaise and fatigue	4.1%	18,200	32,241	48,533	85,977
Acute pancreatitis	18.6%	9,807	31,080	26,152	82,879
Chronic pancreatitis	43.2%	1,061	7,966	2,829	21,242
All		75,860	226,765	202,293	604,705

[0012] Sensitive detection of resectable disease is essential for the clinical utility of this indication. Prompt detection of pancreatic cancer increases the chances of diagnosis of curable disease. The diagnosis of pancreatic cancer is typically made radiographically by the finding of a mass within the pancreas, which often obstructs the pancreatic duct or biliary tree. However, imaging can be invasive and costly. A blood test that determines which patients require follow-up, including diagnostic imaging, would benefit the patients and simplify the diagnosis.

[0013] Biomarker selection for a specific disease state involves first the identification of markers that have a measurable and statistically significant difference in a disease population compared to a control population for a specific medical application. Biomarkers can include secreted or shed molecules that parallel disease development or progression and readily diffuse into the blood stream from pancreatic cancer tissue or from surrounding tissues and circulating cells in response to a tumor. The biomarker or set of biomarkers identified are generally clinically validated or shown to be a reliable indicator for the original intended use for which it was selected. Biomarkers can include small molecules, peptides, proteins, and nucleic acids. Some of the key issues that affect the identification of biomarkers include over-fitting of the available data and bias in the data.

[0014] A variety of methods have been utilized in an attempt to identify biomarkers and diagnose disease. For protein-based markers, these include two-dimensional electrophoresis, mass spectrometry, and immunoassay methods. For nucleic acid markers, these include mRNA expression profiles, microRNA profiles, FISH, serial analysis of gene expression (SAGE), and large scale gene expression arrays.

[0015] The utility of two-dimensional electrophoresis is limited by low detection sensitivity; issues with protein solubility, charge, and hydrophobicity; gel reproducibility; and the possibility of a single spot representing multiple proteins. For mass spectrometry, depending on the format used, limitations revolve around the sample processing and separation, sensitivity to low abundance proteins, signal to noise considerations, and inability to immediately identify the detected protein. Limitations in immunoassay approaches to biomarker discovery are centered on the inability of antibody-based multiplex assays to measure a large number of analytes. One might simply print an array of high-quality antibodies and, without sandwiches, measure the analytes bound to those antibodies. (This would be the formal equivalent of

using a whole genome of nucleic acid sequences to measure by hybridization all DNA or RNA sequences in an organism or a cell. The hybridization experiment works because hybridization can be a stringent test for identity. Even very good antibodies are not stringent enough in selecting their binding partners to work in the context of blood or even cell extracts because the protein ensemble in those matrices have extremely different abundances.) Thus, one must use a different approach with immunoassay-based approaches to biomarker discovery - one would need to use multiplexed ELISA assays (that is, sandwiches) to get sufficient stringency to measure many analytes simultaneously to decide which analytes are indeed biomarkers. Sandwich immunoassays do not scale to high content, and thus biomarker discovery using stringent sandwich immunoassays is not possible using standard array formats. Lastly, antibody reagents are subject to substantial lot variability and reagent instability. The instant platform for protein biomarker discovery overcomes this problem.

[0016] Many of these methods rely on or require some type of sample fractionation prior to the analysis. Thus the sample preparation required to run a sufficiently powered study designed to identify and discover statistically relevant biomarkers in a series of well-defined sample populations is extremely difficult, costly, and time consuming. During fractionation, a wide range of variability can be introduced into the various samples. For example, a potential marker could be unstable to the process, the concentration of the marker could be changed, inappropriate aggregation or disaggregation could occur, and inadvertent sample contamination could occur and thus obscure the subtle changes anticipated in early disease.

[0017] It is widely accepted that biomarker discovery and detection methods using these technologies have serious limitations for the identification of diagnostic biomarkers. These limitations include an inability to detect low-abundance biomarkers, an inability to consistently cover the entire dynamic range of the proteome, irreproducibility in sample processing and fractionation, and overall irreproducibility and lack of robustness of the method. Further, these studies have introduced biases into the data and not adequately addressed the complexity of the sample populations, including appropriate controls, in terms of the distribution and randomization required to identify and validate biomarkers within a target disease population.

[0018] Although efforts aimed at the discovery of new and effective biomarkers have gone on for several decades, the efforts have been largely unsuccessful. Biomarkers for various diseases typically have been identified in academic laboratories, usually through an accidental

discovery while doing basic research on some disease process. Based on the discovery and with small amounts of clinical data, papers were published that suggested the identification of a new biomarker. Most of these proposed biomarkers, however, have not been confirmed as real or useful biomarkers, primarily because the small number of clinical samples tested provide only weak statistical proof that an effective biomarker has in fact been found. That is, the initial identification was not rigorous with respect to the basic elements of statistics. In each of the years 1994 through 2003, a search of the scientific literature shows that thousands of references directed to biomarkers were published. During that same time frame, however, the FDA approved for diagnostic use, at most, three new protein biomarkers a year, and in several years no new protein biomarkers were approved.

[0019] Based on the history of failed biomarker discovery efforts, mathematical theories have been proposed that further promote the general understanding that biomarkers for disease are rare and difficult to find. Biomarker research based on 2D gels or mass spectrometry supports these notions. Very few useful biomarkers have been identified through these approaches. However, it is usually overlooked that 2D gel and mass spectrometry measure proteins that are present in blood at approximately 1 nM concentrations and higher, and that this ensemble of proteins may well be the least likely to change with disease. Other than the instant biomarker discovery platform, proteomic biomarker discovery platforms that are able to accurately measure protein expression levels at much lower concentrations do not exist.

[0020] Much is known about biochemical pathways for complex human biology. Many biochemical pathways culminate in or are started by secreted proteins that work locally within the pathology, for example growth factors are secreted to stimulate the replication of other cells in the pathology, and other factors are secreted to ward off the immune system, and so on. While many of these secreted proteins work in a paracrine fashion, some operate distally in the body. One skilled in the art with a basic understanding of biochemical pathways would understand that many pathology-specific proteins ought to exist in blood at concentrations below (even far below) the detection limits of 2D gels and mass spectrometry. What must precede the identification of this relatively abundant number of disease biomarkers is a proteomic platform that can analyze proteins at concentrations below those detectable by 2D gels or mass spectrometry.

[0021] Accordingly, a need exists for biomarkers, methods, devices, reagents, systems, and

kits that enable (a) the differentiation of pancreatic cancer from benign conditions; (b) screening of asymptomatic, high risk individuals for pancreatic cancer; (c) the detection of pancreatic cancer biomarkers; and (d) the diagnosis of pancreatic cancer.

SUMMARY

[0022] The present application includes biomarkers, methods, reagents, devices, systems, and kits for the detection and diagnosis of cancer and more particularly, pancreatic cancer. The biomarkers of the present application were identified using a multiplex aptamer-based assay which is described in detail in Example 1. By using the aptamer-based biomarker identification method described herein, this application describes a surprisingly large number of pancreatic cancer biomarkers that are useful for the detection and diagnosis of pancreatic cancer as well as a large number of cancer biomarkers that are useful for the detection and diagnosis of cancer more generally. In identifying these biomarkers, over 800 proteins from hundreds of individual samples were measured, some of which were at concentrations in the low femtomolar range. This is about four orders of magnitude lower than biomarker discovery experiments done with 2D gels and/or mass spectrometry.

[0023] While certain of the described pancreatic cancer biomarkers are useful alone for detecting and diagnosing pancreatic cancer, methods are described herein for the grouping of multiple subsets of the pancreatic cancer biomarkers that are useful as a panel of biomarkers. Once an individual biomarker or subset of biomarkers has been identified, the detection or diagnosis of pancreatic cancer in an individual can be accomplished using any assay platform or format that is capable of measuring differences in the levels of the selected biomarker or biomarkers in a biological sample.

[0024] However, it was only by using the aptamer-based biomarker identification method described herein, wherein over 800 separate potential biomarker values were individually screened from a large number of individuals having previously been diagnosed either as having or not having pancreatic cancer that it was possible to identify the pancreatic cancer biomarkers disclosed herein. This discovery approach is in stark contrast to biomarker discovery from conditioned media or lysed cells as it queries a more patient-relevant system that requires no translation to human pathology.

[0025] Thus, in one aspect of the instant application, one or more biomarkers are provided for use either alone or in various combinations to diagnose pancreatic cancer or permit the differential diagnosis of of pancreatic cancer from benign gastrointestinal (GI) conditions such as acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign. Exemplary embodiments include the biomarkers provided in Table 1, Col. 2, which as noted above, were identified using a multiplex aptamer-based assay, as described generally in Example 1 and more specifically in Example 2. The markers provided in Table 1 are useful in diagnosing pancreatic cancer in a high risk, asymptomatic population and for distinguishing acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign from pancreatic cancer.

[0026] While certain of the described pancreatic cancer biomarkers are useful alone for detecting and diagnosing pancreatic cancer, methods are also described herein for the grouping of multiple subsets of the pancreatic cancer biomarkers that are each useful as a panel of two or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least two biomarkers. In other embodiments, N is selected to be any number from 2-65 biomarkers.

[0027] In yet other embodiments, N is selected to be any number from 2-7, 2-10, 2-15, 2-20, 2-25, 2-30, 2-35, 2-40, 2-45, 2-50, 2-55, or 2-65. In other embodiments, N is selected to be any number from 3-7, 3-10, 3-15, 3-20, 3-25, 3-30, 3-35, 3-40, 3-45, 3-50, 3-55, or 3-65. In other embodiments, N is selected to be any number from 4-7, 4-10, 4-15, 4-20, 4-25, 4-30, 4-35, 4-40, 4-45, 4-50, 4-55, or 4-65. In other embodiments, N is selected to be any number from 5-7, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, or 5-65. In other embodiments, N is selected to be any number from 6-10, 6-15, 6-20, 6-25, 6-30, 6-35, 6-40, 6-45, 6-50, 6-55, or 6-65. In other embodiments, N is selected to be any number from 7-10, 7-15, 7-20, 7-25, 7-30, 7-35, 7-40, 7-45, 7-50, 7-55, or 7-65. In other embodiments, N is selected to be any number from 8-10, 8-15, 8-20, 8-25, 8-30, 8-35, 8-40, 8-45, 8-50, 8-55, or 8-65. In other embodiments, N is selected to be any number from 9-15, 9-20, 9-25, 9-30, 9-35, 9-40, 9-45, 9-50, 9-55, or 9-65. In other embodiments, N is selected to be any number from 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, 10-45, 10-50, 10-55, or 10-65. It will be appreciated that N can be selected to encompass similar, but higher order, ranges.

[0028] In another aspect, a method is provided for diagnosing pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 1, Col. 2, wherein the individual is classified as having pancreatic cancer based on the at least one biomarker value.

[0029] In another aspect, a method is provided for diagnosing pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the likelihood of the individual having pancreatic cancer is determined based on the biomarker values.

[0030] In another aspect, a method is provided for diagnosing pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the individual is classified as having pancreatic cancer based on the biomarker values, and wherein N = 2-10.

[0031] In another aspect, a method is provided for diagnosing pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the likelihood of the individual having pancreatic cancer is determined based on the biomarker values, and wherein N = 2-10.

[0032] In another aspect, a method is provided for diagnosing that an individual does not have pancreatic cancer, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the individual is classified as not having pancreatic cancer based on the at least one biomarker value.

[0033] In another aspect, a method is provided for diagnosing that an individual does not have pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each corresponding to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the individual is classified as not having pancreatic cancer based on the biomarker values, and wherein N = 2-10.

[0034] In another aspect, a method is provided for diagnosing pancreatic cancer, the method

including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, Col. 2, wherein a classification of the biomarker values indicates that the individual has pancreatic cancer, and wherein N = 3-10.

[0035] In another aspect, a method is provided for diagnosing pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, Col. 2, wherein a classification of the biomarker values indicates that the individual has pancreatic cancer, and wherein N = 3-10.

[0036] In another aspect, a method is provided for diagnosing pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels set forth in Tables 2-11, wherein a classification of the biomarker values indicates that the individual has pancreatic cancer.

[0037] In another aspect, a method is provided for diagnosing an absence of pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, Col. 2, wherein a classification of the biomarker values indicates an absence of pancreatic cancer in the individual, and wherein N = 3-10.

[0038] In another aspect, a method is provided for diagnosing an absence of pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, Col. 2, wherein a classification of the biomarker values indicates an absence of pancreatic cancer in the individual, and wherein N = 3-10.

[0039] In another aspect, a method is provided for diagnosing an absence of pancreatic cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels provided in Tables 2-11, wherein a classification of the biomarker values indicates an absence of pancreatic cancer in the individual.

[0040] In another aspect, a method is provided for diagnosing pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein the individual is classified as having pancreatic cancer based on a classification score that deviates from a predetermined threshold, and wherein N=2-10.

[0041] In another aspect, a method is provided for diagnosing an absence of pancreatic cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, Col. 2, wherein said individual is classified as not having pancreatic cancer based on a classification score that deviates from a predetermined threshold, and wherein N=2-10.

[0042] In another aspect, a computer-implemented method is provided for indicating a likelihood of pancreatic cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers, wherein N is as defined above, selected from the group of biomarkers set forth in Table 1, Col. 2; performing with the computer a classification of each of the biomarker values; and indicating a likelihood that the individual has pancreatic cancer based upon a plurality of classifications.

[0043] In another aspect, a computer-implemented method is provided for classifying an individual as either having or not having pancreatic cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers provided in Table 1, Col. 2; performing with the computer a classification of each of the biomarker values; and indicating whether the individual has pancreatic cancer based upon a plurality of classifications.

[0044] In another aspect, a computer program product is provided for indicating a likelihood of pancreatic cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of

at least N biomarkers, wherein N is as defined above, in the biological sample selected from the group of biomarkers set forth in Table 1, Col. 2; and code that executes a classification method that indicates a likelihood that the individual has pancreatic cancer as a function of the biomarker values.

[0045] In another aspect, a computer program product is provided for indicating a pancreatic cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers in the biological sample selected from the group of biomarkers provided in Table 1, Col. 2; and code that executes a classification method that indicates a pancreatic cancer status of the individual as a function of the biomarker values.

[0046] In another aspect, a computer-implemented method is provided for indicating a likelihood of pancreatic cancer. The method comprises retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers set forth in Table 1, Col. 2; performing with the computer a classification of the biomarker value; and indicating a likelihood that the individual has pancreatic cancer based upon the classification.

[0047] In another aspect, a computer-implemented method is provided for classifying an individual as either having or not having pancreatic cancer. The method comprises retrieving from a computer biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers provided in Table 1, Col. 2; performing with the computer a classification of the biomarker value; and indicating whether the individual has pancreatic cancer based upon the classification.

[0048] In still another aspect, a computer program product is provided for indicating a likelihood of pancreatic cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers set forth in Table 1, Col. 2; and

code that executes a classification method that indicates a likelihood that the individual has pancreatic cancer as a function of the biomarker value.

[0049] In still another aspect, a computer program product is provided for indicating a pancreatic cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers provided in Table 1, Col. 2; and code that executes a classification method that indicates a pancreatic cancer status of the individual as a function of the biomarker value.

[0050] While certain of the described cancer biomarkers are useful alone for detecting and diagnosing cancer, methods are described herein for the grouping of multiple subsets of the cancer biomarkers that are useful as a panel of biomarkers. Once an individual biomarker or subset of biomarkers has been identified, the detection or diagnosis of cancer in an individual can be accomplished using any assay platform or format that is capable of measuring differences in the levels of the selected biomarker or biomarkers in a biological sample.

[0051] However, it was only by using the aptamer-based biomarker identification method described herein, wherein over 800 separate potential biomarker values were individually screened from a large number of individuals having previously been diagnosed either as having or not having cancer that it was possible to identify the cancer biomarkers disclosed herein. This discovery approach is in stark contrast to biomarker discovery from conditioned media or lysed cells as it queries a more patient-relevant system that requires no translation to human pathology.

[0052] Thus, in one aspect of the instant application, one or more biomarkers are provided for use either alone or in various combinations to diagnose cancer. Exemplary embodiments include the biomarkers provided in Table 19, which were identified using a multiplex aptamer-based assay, as described generally in Example 1 and more specifically in Example 7. The markers provided in Table 19 are useful in distinguishing individuals who have cancer from those who do not have cancer.

[0053] While certain of the described cancer biomarkers are useful alone for detecting and diagnosing cancer, methods are also described herein for the grouping of multiple subsets

of the cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. In other embodiments, N is selected to be any number from 3-65 biomarkers.

[0054] In yet other embodiments, N is selected to be any number from 3-7, 3-10, 3-15, 3-20, 3-25, 3-30, 3-35, 3-40, 3-45, 3-50, 3-55, 3-60, or 3-65. In other embodiments, N is selected to be any number from 4-7, 4-10, 4-15, 4-20, 4-25, 4-30, 4-35, 4-40, 4-45, 4-50, 4-55, 4-60, or 4-65. In other embodiments, N is selected to be any number from 5-7, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, 5-60, or 5-65. In other embodiments, N is selected to be any number from 6-10, 6-15, 6-20, 6-25, 6-30, 6-35, 6-40, 6-45, 6-50, 6-55, 6-60, or 6-65. In other embodiments, N is selected to be any number from 7-10, 7-15, 7-20, 7-25, 7-30, 7-35 7-40, 7-45, 7-50, 7-55, 7-60, or 7-65. In other embodiments, N is selected to be any number from 8-10, 8-15, 8-20, 8-25, 8-30, 8-35, 8-40, 8-45, 8-50, 8-55, 8-60, or 8-65. In other embodiments, N is selected to be any number from 9-15, 9-20, 9-25, 9-30, 9-35, 9-40, 9-45, 9-50, 9-55, 9-60, or 9-65. In other embodiments, N is selected to be any number from 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, 10-45, 10-50, 10-55, 10-60, or 10-65. It will be appreciated that N can be selected to encompass similar, but higher order, ranges.

[0055] In another aspect, a method is provided for diagnosing cancer in an individual, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 19, wherein the individual is classified as having cancer based on the at least one biomarker value.

[0056] In another aspect, a method is provided for diagnosing cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein the likelihood of the individual having cancer is determined based on the biomarker values.

[0057] In another aspect, a method is provided for diagnosing cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein the individual is classified as having cancer based on the biomarker

values, and wherein N = 3-10.

[0058] In another aspect, a method is provided for diagnosing cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein the likelihood of the individual having cancer is determined based on the biomarker values, and wherein N = 3-10.

[0059] In another aspect, a method is provided for diagnosing that an individual does not have cancer, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers set forth in Table 19, wherein the individual is classified as not having cancer based on the at least one biomarker value.

[0060] In another aspect, a method is provided for diagnosing that an individual does not have cancer, the method including detecting, in a biological sample from an individual, biomarker values that each corresponding to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein the individual is classified as not having cancer based on the biomarker values, and wherein N = 3-10.

[0061] In another aspect, a method is provided for diagnosing cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 19, wherein a classification of the biomarker values indicates that the individual has cancer, and wherein N = 3-10.

[0062] In another aspect, a method is provided for diagnosing cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 19, wherein a classification of the biomarker values indicates that the individual has cancer, and wherein N = 3-10.

[0063] In another aspect, a method is provided for diagnosing cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels set forth in Tables 20-29 wherein a classification of the biomarker values indicates that the individual has cancer.

[0064] In another aspect, a method is provided for diagnosing an absence of cancer, the

method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 19, wherein a classification of the biomarker values indicates an absence of cancer in the individual, and wherein N = 3-10.

[0065] In another aspect, a method is provided for diagnosing an absence of cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 19, wherein a classification of the biomarker values indicates an absence of cancer in the individual, and wherein N = 3-10.

[0066] In another aspect, a method is provided for diagnosing an absence of cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels provided in Tables 20-29, wherein a classification of the biomarker values indicates an absence of cancer in the individual.

[0067] In another aspect, a method is provided for diagnosing cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein the individual is classified as having cancer based on a classification score that deviates from a predetermined threshold, and wherein N—3-10.

[0068] In another aspect, a method is provided for diagnosing an absence of cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 19, wherein said individual is classified as not having cancer based on a classification score that deviates from a predetermined threshold, and wherein N—3-10.

[0069] In another aspect, a computer-implemented method is provided for indicating a likelihood of cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers, wherein N is as defined above, selected from the group of biomarkers set forth in Table 19; performing with the computer a classification of each of the biomarker values; and indicating a likelihood that the individual has cancer based

upon a plurality of classifications.

[0070] In another aspect, a computer-implemented method is provided for classifying an individual as either having or not having cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers provided in Table 19; performing with the computer a classification of each of the biomarker values; and indicating whether the individual has cancer based upon a plurality of classifications.

[0071] In another aspect, a computer program product is provided for indicating a likelihood of cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers, wherein N is as defined above, in the biological sample selected from the group of biomarkers set forth in Table 19; and code that executes a classification method that indicates a likelihood that the individual has cancer as a function of the biomarker values.

[0072] In another aspect, a computer program product is provided for indicating a cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers in the biological sample selected from the group of biomarkers provided in Table 19; and code that executes a classification method that indicates a cancer status of the individual as a function of the biomarker values.

[0073] In another aspect, a computer-implemented method is provided for indicating a likelihood of cancer. The method comprises retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers set forth in Table 19; performing with the computer a classification of the biomarker value; and indicating a likelihood that the individual has cancer based upon the classification.

[0074] In another aspect, a computer-implemented method is provided for classifying an

individual as either having or not having cancer. The method comprises retrieving from a computer biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers provided in Table 19; performing with the computer a classification of the biomarker value; and indicating whether the individual has cancer based upon the classification.

[0075] In still another aspect, a computer program product is provided for indicating a likelihood of cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers set forth in Table 19; and code that executes a classification method that indicates a likelihood that the individual has cancer as a function of the biomarker value.

[0076] In still another aspect, a computer program product is provided for indicating a cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers provided in Table 19; and code that executes a classification method that indicates a cancer status of the individual as a function of the biomarker value.

[0077] In still another aspect, a method is provided for diagnosing pancreatic cancer, the method including detecting, in a biological sample from an individual, the tumor marker CA 19-9 in addition to biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels set forth in Table 1 wherein a classification of the combined CA 19-9 and biomarker values indicates that the individual has pancreatic cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078] Figure 1A is a flowchart for an exemplary method for detecting pancreatic cancer in a biological sample.

[0079] Figure 1B is a flowchart for an exemplary method for detecting pancreatic cancer in a biological sample using a naïve Bayes classification method.

[0080] Figure 2 shows a ROC curve for a single biomarker, CTSB, using a naïve Bayes classifier for a test that detects pancreatic cancer.

[0081] Figure 3 shows ROC curves for biomarker panels of from two to ten biomarkers using naïve Bayes classifiers for a test that detects pancreatic cancer.

[0082] Figure 4 illustrates the increase in the classification score (AUC) as the number of biomarkers is increased from one to ten using naïve Bayes classification for a pancreatic cancer panel.

[0083] Figure 5 shows the measured biomarker distributions for CTSB as a cumulative distribution function (cdf) in log-transformed RFU for the GI and normal controls combined (solid line) and the pancreatic cancer disease group (dotted line) along with their curve fits to a normal cdf (dashed lines) used to train the naïve Bayes classifiers.

[0084] Figure 6 illustrates an exemplary computer system for use with various computer-implemented methods described herein.

[0085] Figure 7 is a flowchart for a method of indicating the likelihood that an individual has pancreatic cancer in accordance with one embodiment.

[0086] Figure 8 is a flowchart for a method of indicating the likelihood that an individual has pancreatic cancer in accordance with one embodiment.

[0087] Figure 9 illustrates an exemplary aptamer assay that can be used to detect one or more pancreatic cancer biomarkers in a biological sample.

[0088] Figure 10 shows a histogram of frequencies for which biomarkers were used in building classifiers to distinguish between pancreatic cancer and the GI and normal controls from an aggregated set of potential biomarkers.

[0089] Figure 11A shows a pair of histograms summarizing all possible single protein naïve Bayes classifier scores (AUC) using the biomarkers set forth in Table 1 (solid) and a set of random markers (dotted).

[0090] Figure 11B shows a pair of histograms summarizing all possible two-protein protein naïve Bayes classifier scores (AUC) using the biomarkers set forth in Table 1 (solid) and a set of random markers (dotted).

[0091] Figure 11C shows a pair of histograms summarizing all possible three-protein naïve

Bayes classifier scores (AUC) using the biomarkers set forth in Table 1 (solid) and a set of random markers (dotted).

[0092] Figure 12 shows the AUC for naïve Bayes classifiers using from 2-10 markers selected from the full panel (diamond) and the scores obtained by dropping the best 5, 10, and 15 markers during classifier generation.

[0093] Figure 13 shows the performance of three different classifiers: CA19-9 alone, the SOMAmer panel and the combination of SOMAmers and CA19-9.

[0094] Figure 14 shows the performance of CA19-9 plus one (HAMP) or two (HAMP and CTSB) SOMAmer biomarkers.

[0095] Figure 15 shows the performance of the 10 marker random forest classifier.

[0096] Figure 16A shows a set of ROC curves modeled from the data in Table 14 for panels of from one to five markers.

[0097] Figure 16B shows a set of ROC curves computed from the training data for panels of from one to five markers as in Figure 12A.

[0098] Figures 17A and 17B show a comparison of performance between ten biomarkers selected by a greedy selection procedure (Table 19) and 1,000 randomly sampled sets of ten "non marker" biomarkers. The mean AUC for the ten biomarkers in Table 19 is shown as a dotted vertical line. In Figure 17A, sets of ten biomarkers were randomly selected from all 10 analytes present in all 3 cancer studies that were not selected by the greedy procedure. In Figure 17B, the same procedure as 17A was used; however, the sampling was restricted to the remaining 55 biomarkers from Table 1 that were not selected by the greedy procedure.

[0099] Figure 18 shows receiver operating characteristic (ROC) curves for the 3 naïve Bayes classifiers set forth in Table 19. For each study, the area under the curve (AUC) is also displayed next to the legend.

DETAILED DESCRIPTION

[0100] Reference will now be made in detail to representative embodiments of the invention. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that the invention is not intended to be limited to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents

that may be included within the scope of the present invention as defined by the claims.

[0101] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in and are within the scope of the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[0102] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[0103]

[0104] As used in this application, including the appended claims, the singular forms "a," "an," and "the" include plural references, unless the content clearly dictates otherwise, and are used interchangeably with "at least one" and "one or more." Thus, reference to "an aptamer" includes mixtures of aptamers, reference to "a probe" includes mixtures of probes, and the like.

[0105] As used herein, the term "about" represents an insignificant modification or variation of the numerical value such that the basic function of the item to which the numerical value relates is unchanged.

[0106] As used herein, the terms "comprises," "comprising," "includes," "including," "contains," "containing," and any variations thereof, are intended to cover a non-exclusive inclusion, such that a process, method, product-by-process, or composition of matter that comprises, includes, or contains an element or list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, product-by-process, or composition of matter.

[0107] The present application includes biomarkers, methods, devices, reagents, systems,

and kits for the detection and diagnosis of pancreatic cancer and cancer more generally.

[0108] In one aspect, one or more biomarkers are provided for use either alone or in various combinations to diagnose pancreatic cancer, permit the differential diagnosis of pancreatic cancer from non-malignant GI conditions including acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign, monitor pancreatic cancer recurrence, or address other clinical indications. As described in detail below, exemplary embodiments include the biomarkers provided in Table 1, Col. 2, which were identified using a multiplex aptamer-based assay that is described generally in Example 1 and more specifically in Example 2.

[0109] Table 1, Col. 2 sets forth the findings obtained from analyzing hundreds of individual blood samples from pancreatic cancer cases, and hundreds of equivalent individual blood samples from GI and normal controls. The GI and normal controls group was designed to match the populations with which a pancreatic cancer diagnostic test can have the most benefit, including asymptomatic individuals and symptomatic individuals. The normal control group represents asymptomatic individuals with a high risk of pancreatic cancer. High risk for pancreatic cancer includes family history of pancreatic cancer, obesity, smoking, diabetes, cystic fibrosis, chronic or hereditary pancreatitis, BRCA mutation carrier, p16 mutation, and Peutz-Jeghers syndrome (Brand E et al. Gut 2007:56:1460). The GI control group includes nonspecific abdominal symptoms such as acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign. Samples from the normal controls were combined with the GI controls to discover biomarkers useful for both screening high risk asymptomatic individuals and differential diagnosis in symptomatic individuals. The potential biomarkers were measured in individual samples rather than pooling the disease and control blood; this allowed a better understanding of the individual and group variations in the phenotypes associated with the presence and absence of disease (in this case pancreatic cancer). Since 823 protein measurements were made on each sample, and several hundred samples from each of the disease and the control populations were individually measured, Table 1, Col. 2 resulted from an analysis of an uncommonly large set of data. The measurements were analyzed using the methods described in the section, "Classification of Biomarkers and Calculation of Disease Scores" herein. Table 1, Col. 2 lists the 65 biomarkers found to be useful in distinguishing samples obtained from individuals with pancreatic cancer

from "control" samples obtained from GI and normal controls. GI controls include subjects with acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign.

[0110] While certain of the described pancreatic cancer biomarkers are useful alone for detecting and diagnosing pancreatic cancer, methods are also described herein for the grouping of multiple subsets of the pancreatic cancer biomarkers, where each grouping or subset selection is useful as a panel of three or more biomarkers, interchangeably referred to herein as a "biomarker panel" and a panel. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least two biomarkers. In other embodiments, N is selected from 2-65 biomarkers.

[0111] In yet other embodiments, N is selected to be any number from 2-7, 2-10, 2-15, 2-20, 2-25, 2-30, 2-35, 2-40, 2-45, 2-50, 2-55, or 2-65. In other embodiments, N is selected to be any number from 3-7, 3-10, 3-15, 3-20, 3-25, 3-30, 3-35, 3-40, 3-45, 3-50, 3-55, or 3-65. In other embodiments, N is selected to be any number from 4-7, 4-10, 4-15, 4-20, 4-25, 4-30, 4-35, 4-40, 4-45, 4-50, 4-55, or 4-65. In other embodiments, N is selected to be any number from 5-7, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, or 5-65. In other embodiments, N is selected to be any number from 6-10, 6-15, 6-20, 6-25, 6-30, 6-35, 6-40, 6-45, 6-50, 6-55, or 6-65. In other embodiments, N is selected to be any number from 7-10, 7-15, 7-20, 7-25, 7-30, 7-35, 7-40, 7-45, 7-50, 7-55, or 7-65. In other embodiments, N is selected to be any number from 8-10, 8-15, 8-20, 8-25, 8-30, 8-35, 8-40, 8-45, 8-50, 8-55, or 8-65. In other embodiments, N is selected to be any number from 9-15, 9-20, 9-25, 9-30, 9-35, 9-40, 9-45, 9-50, 9-55, or 9-65. In other embodiments, N is selected to be any number from 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, 10-45, 10-50, 10-55, or 10-65. It will be appreciated that N can be selected to encompass similar, but higher order, ranges.

[0112] In one embodiment, the number of biomarkers useful for a biomarker subset or panel is based on the sensitivity and specificity value for the particular combination of biomarker values. The terms "sensitivity" and "specificity" are used herein with respect to the ability to correctly classify an individual, based on one or more biomarker values detected in their biological sample, as having pancreatic cancer or not having pancreatic cancer. "Sensitivity" indicates the performance of the biomarker(s) with respect to correctly classifying individuals that have pancreatic cancer. "Specificity" indicates the performance of the biomarker(s) with

respect to correctly classifying individuals who do not have pancreatic cancer. For example, 85% specificity and 90% sensitivity for a panel of markers used to test a set of control samples and pancreatic cancer samples indicates that 85% of the control samples were correctly classified as control samples by the panel, and 90% of the pancreatic cancer samples were correctly classified as pancreatic cancer samples by the panel. The desired or preferred minimum value can be determined as described in Example 3. Representative panels are set forth in Tables 4-11, which set forth a series of 100 different panels of 3-10 biomarkers, which have the indicated levels of specificity and sensitivity for each panel. The total number of occurrences of each marker in each of these panels is indicated at the bottom of each Table.

[0113] In one aspect, pancreatic cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to at least one of the biomarkers CTSB, C5a or C5 and at least N additional biomarkers selected from the list of biomarkers in Table 1, Col. 2, wherein N equals 2, 3, 4, 5, 6, 7, 8, or 9. In a further aspect, pancreatic cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarkers CTSB, C5a or C5 and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, Col. 2, wherein N equals 1, 2, 3, 4, 5, 6, or 7. In a further aspect, pancreatic cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker CTSB and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, Col. 2, wherein N equals 2, 3, 4, 5, 6, 7, 8, or 9. In a further aspect, pancreatic cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker C5a and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, Col. 2, wherein N equals 2, 3, 4, 5, 6, 7, 8, or 9. In a further aspect, pancreatic cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker C5 and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, Col. 2, wherein N equals 2, 3, 4, 5, 6, 7, 8, or 9.

[0114] The pancreatic cancer biomarkers identified herein represent a relatively large number of choices for subsets or panels of biomarkers that can be used to effectively detect or diagnose

pancreatic cancer. Selection of the desired number of such biomarkers depends on the specific combination of biomarkers chosen. It is important to remember that panels of biomarkers for detecting or diagnosing pancreatic cancer may also include biomarkers not found in Table 1, Col. 2, and that the inclusion of additional biomarkers not found in Table 1, Col. 2 may reduce the number of biomarkers in the particular subset or panel that is selected from Table 1, Col. 2. The number of biomarkers from Table 1, Col. 2 used in a subset or panel may also be reduced if additional biomedical information is used in conjunction with the biomarker values to establish acceptable sensitivity and specificity values for a given assay.

[0115] Another factor that can affect the number of biomarkers to be used in a subset or panel of biomarkers is the procedures used to obtain biological samples from individuals who are being diagnosed for pancreatic cancer. In a carefully controlled sample procurement environment, the number of biomarkers necessary to meet desired sensitivity and specificity values will be lower than in a situation where there can be more variation in sample collection, handling and storage. In developing the list of biomarkers set forth in Table 1, Col. 2, multiple sample collection sites were utilized to collect data for classifier training. This provides for more robust biomarkers that are less sensitive to variations in sample collection, handling and storage, but can also require that the number of biomarkers in a subset or panel be larger than if the training data were all obtained under very similar conditions.

[0116] One aspect of the instant application can be described generally with reference to Figures 1A and 1B. A biological sample is obtained from an individual or individuals of interest. The biological sample is then assayed to detect the presence of one or more (N) biomarkers of interest and to determine a biomarker value for each of said N biomarkers (referred to in Figure 1B as marker RFU). Once a biomarker has been detected and a biomarker value assigned each marker is scored or classified as described in detail herein. The marker scores are then combined to provide a total diagnostic score, which indicates the likelihood that the individual from whom the sample was obtained has pancreatic cancer.

[0117] "Biological sample", "sample", and "test sample" are used interchangeably herein to refer to any material, biological fluid, tissue, or cell obtained or otherwise derived from an individual. This includes blood (including whole blood, leukocytes, peripheral blood mononuclear cells, buffy coat, plasma, and serum), sputum, tears, mucus, nasal washes, nasal aspirate, breath, urine, semen, saliva, peritoneal washings, ascites, cystic fluid, meningeal fluid, amni-

otic fluid, glandular fluid, pancreatic fluid, lymph fluid, pleural fluid, nipple aspirate, bronchial aspirate, bronchial brushing, synovial fluid, joint aspirate, organ secretions, cells, a cellular extract, and cerebrospinal fluid. This also includes experimentally separated fractions of all of the preceding. For example, a blood sample can be fractionated into serum, plasma or into fractions containing particular types of blood cells, such as red blood cells or white blood cells (leukocytes). If desired, a sample can be a combination of samples from an individual, such as a combination of a tissue and fluid sample. The term "biological sample" also includes materials containing homogenized solid material, such as from a stool sample, a tissue sample, or a tissue biopsy, for example. The term "biological sample" also includes materials derived from a tissue culture or a cell culture. Any suitable methods for obtaining a biological sample can be employed; exemplary methods include, e.g., phlebotomy, swab (e.g., buccal swab), and a fine needle aspirate biopsy procedure. Exemplary tissues susceptible to fine needle aspiration include lymph node, lung, lung washes, BAL (bronchoalveolar lavage),thyroid, breast, pancreas and liver. Samples can also be collected, e.g., by micro dissection (e.g., laser capture micro dissection (LCM) or laser micro dissection (LMD)), bladder wash, smear (e.g., a PAP smear), or ductal lavage. A "biological sample" obtained or derived from an individual includes any such sample that has been processed in any suitable manner after being obtained from the individual.

[0118] Further, it should be realized that a biological sample can be derived by taking biological samples from a number of individuals and pooling them or pooling an aliquot of each individual's biological sample. The pooled sample can be treated as a sample from a single individual and if the presence of cancer is established in the pooled sample, then each individual biological sample can be re-tested to determine which individual/s have pancreatic cancer.

[0119] For purposes of this specification, the phrase "data attributed to a biological sample from an individual" is intended to mean that the data in some form derived from, or were generated using, the biological sample of the individual. The data may have been reformatted, revised, or mathematically altered to some degree after having been generated, such as by conversion from units in one measurement system to units in another measurement system; but, the data are understood to have been derived from, or were generated using, the biological sample.

[0120] "Target", "target molecule", and "analyte" are used interchangeably herein to refer to any molecule of interest that may be present in a biological sample. A "molecule of interest" includes any minor variation of a particular molecule, such as, in the case of a protein, for example, minor variations in amino acid sequence, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component, which does not substantially alter the identity of the molecule. A "target molecule", "target", or "analyte" is a set of copies of one type or species of molecule or multi-molecular structure. "Target molecules", "targets", and "analytes" refer to more than one such set of molecules. Exemplary target molecules include proteins, polypeptides, nucleic acids, carbohydrates, lipids, polysaccharides, glycoproteins, hormones, receptors, antigens, antibodies, affybodies, antibody mimics, viruses, pathogens, toxic substances, substrates, metabolites, transition state analogs, cofactors, inhibitors, drugs, dyes, nutrients, growth factors, cells, tissues, and any fragment or portion of any of the foregoing.

[0121] As used herein, "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. Polypeptides can be single chains or associated chains. Also included within the definition are preproteins and intact mature proteins; peptides or polypeptides derived from a mature protein; fragments of a protein; splice variants; recombinant forms of a protein; protein variants with amino acid modifications, deletions, or substitutions; digests; and post-translational modifications, such as glycosylation, acetylation, phosphorylation, and the like.

[0122] As used herein, "marker" and "biomarker" are used interchangeably to refer to a target molecule that indicates or is a sign of a normal or abnormal process in an individual or of a disease or other condition in an individual. More specifically, a "marker" or "biomarker" is an anatomic, physiologic, biochemical, or molecular parameter associated with the presence of a

specific physiological state or process, whether normal or abnormal, and, if abnormal, whether chronic or acute. Biomarkers are detectable and measurable by a variety of methods including laboratory assays and medical imaging. When a biomarker is a protein, it is also possible to use the expression of the corresponding gene as a surrogate measure of the amount or presence or absence of the corresponding protein biomarker in a biological sample or methylation state of the gene encoding the biomarker or proteins that control expression of the biomarker.

[0123] As used herein, "biomarker value", "value", "biomarker level", and "level" are used interchangeably to refer to a measurement that is made using any analytical method for detecting the biomarker in a biological sample and that indicates the presence, absence, absolute amount or concentration, relative amount or concentration, titer, a level, an expression level, a ratio of measured levels, or the like, of, for, or corresponding to the biomarker in the biological sample. The exact nature of the "value" or "level" depends on the specific design and components of the particular analytical method employed to detect the biomarker.

[0124] When a biomarker indicates or is a sign of an abnormal process or a disease or other condition in an individual, that biomarker is generally described as being either over-expressed or under-expressed as compared to an expression level or value of the biomarker that indicates or is a sign of a normal process or an absence of a disease or other condition in an individual. "Up-regulation", "up-regulated", "over-expression", "over-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

[0125] "Down-regulation", "down-regulated", "under-expression", "under-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

[0126] Further, a biomarker that is either over-expressed or under-expressed can also be referred to as being "differentially expressed" or as having a "differential level" or "differential value" as compared to a "normal" expression level or value of the biomarker that indicates or is a sign of a normal process or an absence of a disease or other condition in an individual. Thus, "differential expression" of a biomarker can also be referred to as a variation from a "normal" expression level of the biomarker.

[0127] The term "differential gene expression" and "differential expression" are used interchangeably to refer to a gene (or its corresponding protein expression product) whose expression is activated to a higher or lower level in a subject suffering from a specific disease, relative to its expression in a normal or control subject. The terms also include genes (or the corresponding protein expression products) whose expression is activated to a higher or lower level at different stages of the same disease. It is also understood that a differentially expressed gene may be either activated or inhibited at the nucleic acid level or protein level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a variety of changes including mRNA levels, surface expression, secretion or other partitioning of a polypeptide. Differential gene expression may include a comparison of expression between two or more genes or their gene products; or a comparison of the ratios of the expression between two or more genes or their gene products; or even a comparison of two differently processed products of the same gene, which differ between normal subjects and subjects suffering from a disease; or between various stages of the same disease. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a gene or its expression products among, for example, normal and diseased cells, or among cells which have undergone different disease events or disease stages.

[0128] As used herein, "individual" refers to a test subject or patient. The individual can be a mammal or a non-mammal. In various embodiments, the individual is a mammal. A mammalian individual can be a human or non-human. In various embodiments, the individual is a human. A healthy or normal individual is an individual in which the disease or condition of interest (including, for example, pancreatic diseases, pancreatic-associated diseases, or other pancreatic conditions) is not detectable by conventional diagnostic methods.

[0129] "Diagnose", "diagnosing", "diagnosis", and variations thereof refer to the detection,

determination, or recognition of a health status or condition of an individual on the basis of one or more signs, symptoms, data, or other information pertaining to that individual. The health status of an individual can be diagnosed as healthy / normal (i.e., a diagnosis of the absence of a disease or condition) or diagnosed as ill / abnormal (i.e., a diagnosis of the presence, or an assessment of the characteristics, of a disease or condition). The terms "diagnose", "diagnosing", "diagnosis", etc., encompass, with respect to a particular disease or condition, the initial detection of the disease; the characterization or classification of the disease; the detection of the progression, remission, or recurrence of the disease; and the detection of disease response after the administration of a treatment or therapy to the individual. The diagnosis of pancreatic cancer includes distinguishing individuals who have cancer from individuals who do not. It further includes distinguishing GI and normal controls from pancreatic cancer.

[0130] "Prognose", "prognosing", "prognosis", and variations thereof refer to the prediction of a future course of a disease or condition in an individual who has the disease or condition (e.g., predicting patient survival), and such terms encompass the evaluation of disease response after the administration of a treatment or therapy to the individual.

[0131] "Evaluate", "evaluating", "evaluation", and variations thereof encompass both "diagnose" and "prognose" and also encompass determinations or predictions about the future course of a disease or condition in an individual who does not have the disease as well as determinations or predictions regarding the likelihood that a disease or condition will recur in an individual who apparently has been cured of the disease. The term "evaluate" also encompasses assessing an individual's response to a therapy, such as, for example, predicting whether an individual is likely to respond favorably to a therapeutic agent or is unlikely to respond to a therapeutic agent (or will experience toxic or other undesirable side effects, for example), selecting a therapeutic agent for administration to an individual, or monitoring or determining an individual's response to a therapy that has been administered to the individual. Thus, "evaluating" pancreatic cancer can include, for example, any of the following: prognosing the future course of pancreatic cancer in an individual; predicting the recurrence of pancreatic cancer in an individual who apparently has been cured of pancreatic cancer; or determining or predicting an individual's response to a pancreatic cancer treatment or selecting a pancreatic cancer treatment to administer to an individual based upon a determination of the biomarker values derived from the individual's biological sample.

[0132] Any of the following examples may be referred to as either "diagnosing" or "evaluating" pancreatic cancer: initially detecting the presence or absence of pancreatic cancer; determining a specific stage, type or sub-type, or other classification or characteristic of pancreatic cancer; determining whether a suspicious mass is a benign lesion or a malignant pancreatic tumor; or detecting/monitoring pancreatic cancer progression (e.g., monitoring tumor growth or metastatic spread), remission, or recurrence.

[0133] As used herein, "additional biomedical information" refers to one or more evaluations of an individual, other than using any of the biomarkers described herein, that are associated with cancer risk or, more specifically, pancreatic cancer risk. "Additional biomedical information" includes any of the following: physical descriptors of an individual, including a pancreatic mass observed by any of contrast-enhanced multislice (multidetector) helical computed tomography (CT) scanning with three dimensional reconstruction ,transcutaneous or endoscopic ultrasound (US or EUS), endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance imaging (MRI), MR cholangiopancreatography (MRCP), or abdominal ultrasound; the height and/or weight of an individual; change in weight; the ethnicity of an individual; occupational history; family history of pancreatic cancer (or other cancer); the presence of a genetic marker(s) correlating with a higher risk of pancreatic cancer (or other cancer) in the individual or a family member; the presence or absence of a pancreatic mass or other abdominal mass; size of mass; location of mass; morphology of mass and associated abdominal region (e.g., as observed through imaging); clinical symptoms such as abdominal pain, weight loss, anorexia, early satiety, diarrhea, or steatorrhea, jaundice, recent onset of atypical diabetes mellitus, a history of recent but unexplained thrombophlebitis, or previous attack of pancreatitis, and the like; gene expression values; physical descriptors of an individual, including physical descriptors observed by radiologic imaging; the height and/or weight of an individual;, the gender of an individual; the ethnicity of an individual; smoking history; alcohol use history; occupational history; exposure to known carcinogens (e.g., exposure to any of asbestos, radon gas, chemicals, smoke from fires, and air pollution, which can include emissions from stationary or mobile sources such as industrial/factory or auto/marine/aircraft emissions); exposure to second-hand smoke; and family history of pancreatic cancer or other cancer. Testing of biomarker levels in combination with an evaluation of any additional biomedical information, including other laboratory tests (e.g., CA 19-9 testing, serum bilirubin concentration, alkaline

phosphatase activity, presence of anemia), may, for example, improve sensitivity, specificity, and/or AUC for detecting pancreatic cancer (or other pancreatic cancer-related uses) as compared to biomarker testing alone or evaluating any particular item of additional biomedical information alone (e.g., ultrasound imaging alone). Additional biomedical information can be obtained from an individual using routine techniques known in the art, such as from the individual themselves by use of a routine patient questionnaire or health history questionnaire, etc., or from a medical practitioner, etc. Testing of biomarker levels in combination with an evaluation of any additional biomedical information may, for example, improve sensitivity, specificity, and/or AUC for detecting pancreatic cancer (or other pancreatic cancer-related uses) as compared to biomarker testing alone or evaluating any particular item of additional biomedical information alone (e.g., CT imaging alone).

[0134] Cancer associated antigen 19-9 (CA 19-9) is a known blood marker for pancreatic cancer. The reported sensitivity and specificity of CA 19-9 for pancreatic cancer are 80 to 90 percent, respectively. However, these values are closely related to tumor size. The accuracy of CA 19-9 to identify patients with small surgically resectable cancers is limited. CA 19-9 requires the presence of the Lewis blood group antigen (a glycosyl transferase) to be expressed. Among individuals with a Lewis-negative phenotype (an estimated 5 to 10 percent of the population), CA 19-9 levels are not a useful tumor marker. The specificity of CA 19-9 is also limited. CA 19-9 is frequently elevated in patients with various benign pancreaticobiliary disorders. The degree of elevation of CA 19-9 (both at initial presentation and in the postoperative setting) is associated with long-term prognosis. Furthermore, in patients who appear to have potentially resectable disease, the magnitude of the CA 19-9 level can also help to predict the presence of radiographically occult metastatic disease as well. Serial monitoring of CA 19-9 levels is useful to follow patients after potentially curative surgery and for those who are receiving chemotherapy for advanced disease. Rising CA 19-9 levels usually precede the radiographic appearance of recurrent disease, but confirmation of disease progression should be pursued with imaging studies and/or biopsy. Testing of biomarker levels in combination with CA 19-9 may, for example, improve improve sensitivity, specificity, and/or AUC for detecting pancreatic cancer (or other pancreatic cancer-related uses) as compared to CA 19-9 alone.

[0135] The term "area under the curve" or "AUC" refers to the area under the curve of a

receiver operating characteristic (ROC) curve, both of which are well known in the art. AUC measures are useful for comparing the accuracy of a classifier across the complete data range. Classifiers with a greater AUC have a greater capacity to classify unknowns correctly between two groups of interest (e.g., pancreatic cancer samples and normal or control samples). ROC curves are useful for plotting the performance of a particular feature (e.g., any of the biomarkers described herein and/or any item of additional biomedical information) in distinguishing between two populations (e.g., cases having pancreatic cancer and controls without pancreatic cancer). Typically, the feature data across the entire population (e.g., the cases and controls) are sorted in ascending order based on the value of a single feature. Then, for each value for that feature, the true positive and false positive rates for the data are calculated. The true positive rate is determined by counting the number of cases above the value for that feature and then dividing by the total number of cases. The false positive rate is determined by counting the number of controls above the value for that feature and then dividing by the total number of controls. Although this definition refers to scenarios in which a feature is elevated in cases compared to controls, this definition also applies to scenarios in which a feature is lower in cases compared to the controls (in such a scenario, samples below the value for that feature would be counted). ROC curves can be generated for a single feature as well as for other single outputs, for example, a combination of two or more features can be mathematically combined (e.g., added, subtracted, multiplied, etc.) to provide a single sum value, and this single sum value can be plotted in a ROC curve. Additionally, any combination of multiple features, in which the combination derives a single output value, can be plotted in a ROC curve. These combinations of features may comprise a test. The ROC curve is the plot of the true positive rate (sensitivity) of a test against the false positive rate (1-specificity) of the test.

[0136] As used herein, "detecting" or "determining" with respect to a biomarker value includes the use of both the instrument required to observe and record a signal corresponding to a biomarker value and the material/s required to generate that signal. In various embodiments, the biomarker value is detected using any suitable method, including fluorescence, chemiluminescence, surface plasmon resonance, surface acoustic waves, mass spectrometry, infrared spectroscopy, Raman spectroscopy, atomic force microscopy, scanning tunneling microscopy, electrochemical detection methods, nuclear magnetic resonance, quantum dots, and

the like.

[0137] "Solid support" refers herein to any substrate having a surface to which molecules may be attached, directly or indirectly, through either covalent or non-covalent bonds. A "solid support" can have a variety of physical formats, which can include, for example, a membrane; a chip (e.g., a protein chip); a slide (e.g., a glass slide or coverslip); a column; a hollow, solid, semi-solid, pore- or cavity- containing particle, such as, for example, a bead; a gel; a fiber, including a fiber optic material; a matrix; and a sample receptacle. Exemplary sample receptacles include sample wells, tubes, capillaries, vials, and any other vessel, groove or indentation capable of holding a sample. A sample receptacle can be contained on a multi-sample platform, such as a microtiter plate, slide, microfluidics device, and the like. A support can be composed of a natural or synthetic material, an organic or inorganic material. The composition of the solid support on which capture reagents are attached generally depends on the method of attachment (e.g., covalent attachment). Other exemplary receptacles include microdroplets and microfluidic controlled or bulk oil/aqueous emulsions within which assays and related manipulations can occur. Suitable solid supports include, for example, plastics, resins, polysaccharides, silica or silica-based materials, functionalized glass, modified silicon, carbon, metals, inorganic glasses, membranes, nylon, natural fibers (such as, for example, silk, wool and cotton), polymers, and the like. The material composing the solid support can include reactive groups such as, for example, carboxy, amino, or hydroxyl groups, which are used for attachment of the capture reagents. Polymeric solid supports can include, e.g., polystyrene, polyethylene glycol tetraphthalate, polyvinyl acetate, polyvinyl chloride, polyvinyl pyrrolidone, polyacrylonitrile, polymethyl methacrylate, polytetrafluoroethylene, butyl rubber, styrenebutadiene rubber, natural rubber, polyethylene, polypropylene, (poly)tetrafluoroethylene, (poly)vinylidenefluoride, polycarbonate, and polymethylpentene. Suitable solid support particles that can be used include, e.g., encoded particles, such as Luminex-type encoded particles, magnetic particles, and glass particles.

Exemplary Uses of Biomarkers

[0138] In various exemplary embodiments, methods are provided for diagnosing pancreatic cancer in an individual by detecting one or more biomarker values corresponding to one or more biomarkers that are present in the circulation of an individual, such as in serum or plasma, by

any number of analytical methods, including any of the analytical methods described herein. These biomarkers are, for example, differentially expressed in individuals with pancreatic cancer as compared to individuals without pancreatic cancer. Detection of the differential expression of a biomarker in an individual can be used, for example, to permit the early diagnosis of pancreatic cancer, to distinguish between a benign and malignant mass (such as, for example, a mass observed on a computed tomography (CT) scan, MRI or ultrasound), to monitor pancreatic cancer recurrence, or for differential diagnosis from other clinical conditions such as acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign.

[0139] Any of the biomarkers described herein may be used in a variety of clinical indications for pancreatic cancer, including any of the following: detection of pancreatic cancer (such as in a high-risk individual or population); characterizing pancreatic cancer (e.g., determining pancreatic cancer type, sub-type, or stage), such as by distinguishing between pancreatic cancer (pancreatic cancer) and acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign and/or between adenocarcinoma and other malignant cell types (or otherwise facilitating histopathology); determining whether a pancreatic mass is benign or a malignant pancreatic tumor; determining pancreatic cancer prognosis; monitoring pancreatic cancer progression or remission; monitoring for pancreatic cancer recurrence; monitoring metastasis; treatment selection; monitoring response to a therapeutic agent or other treatment; stratification of individuals for endoscopic ultrasound (EUS) screening (e.g., identifying those individuals at greater risk of pancreatic cancer and thereby most likely to benefit from radiologic screening, thus increasing the positive predictive value of EUS); combining biomarker testing with additional biomedical information, such as smoking or alcohol history, etc., or CA 19-9 level, the presence of a genetic marker(s) indicating a higher risk for pancreatic cancer, etc., or with mass size, morphology, presence of ascites, etc. (such as to provide an assay with increased diagnostic performance compared to CA 19-9 testing or other biomarker testing or with mass size, morphology, etc.); facilitating the diagnosis of an abdominal mass as malignant or benign; facilitating clinical decision making once an abdominal mass is observed on CT, MRI, PET or EUS (e.g., ordering repeat radiologic scans if the abdominal mass is deemed to be low risk, such as if a biomarker-based test is negative, with or without categorization of mass size, or considering biopsy if the mass

is deemed medium to high risk, such as if a biomarker-based test is positive, with or without categorization of mass size or extent of tissue invasion); and facilitating decisions regarding clinical follow-up (e.g., whether to implement repeat radiologic imaging scans, fine needle biopsy, or surgery after observing an abdominal mass on imaging). Biomarker testing may improve positive predictive value (PPV) over EUS screening of high risk individuals alone. In addition to their utilities in conjunction with EUS screening, the biomarkers described herein can also be used in conjunction with any other imaging modalities used for pancreatic cancer, such as CT, MRI or PET scan. Furthermore, the described biomarkers may also be useful in permitting certain of these uses before indications of pancreatic cancer are detected by imaging modalities or other clinical correlates, or before symptoms appear. It further includes distinguishing acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign from pancreatic cancer.

[0140] As an example of the manner in which any of the biomarkers described herein can be used to diagnose pancreatic cancer, differential expression of one or more of the described biomarkers in an individual who is not known to have pancreatic cancer may indicate that the individual has pancreatic cancer, thereby enabling detection of pancreatic cancer at an early stage of the disease when treatment is most effective, perhaps before the pancreatic cancer is detected by other means or before symptoms appear. Over-expression of one or more of the biomarkers during the course of pancreatic cancer may be indicative of pancreatic cancer progression, e.g., a pancreatic tumor is growing and/or metastasizing (and thus indicate a poor prognosis), whereas a decrease in the degree to which one or more of the biomarkers is differentially expressed (i.e., in subsequent biomarker tests, the expression level in the individual is moving toward or approaching a "normal" expression level) may be indicative of pancreatic cancer remission, e.g., a pancreatic tumor is shrinking (and thus indicate a good or better prognosis). Similarly, an increase in the degree to which one or more of the biomarkers is differentially expressed (i.e., in subsequent biomarker tests, the expression level in the individual is moving further away from a "normal" expression level) during the course of pancreatic cancer treatment may indicate that the pancreatic cancer is progressing and therefore indicate that the treatment is ineffective, whereas a decrease in differential expression of one or more of the biomarkers during the course of pancreatic cancer treatment may be indicative of pancreatic cancer remission and therefore indicate that the treatment is working success-

fully. Additionally, an increase or decrease in the differential expression of one or more of the biomarkers after an individual has apparently been cured of pancreatic cancer may be indicative of pancreatic cancer recurrence. In a situation such as this, for example, the individual can be re-started on therapy (or the therapeutic regimen modified such as to increase dosage amount and/or frequency, if the individual has maintained therapy) at an earlier stage than if the recurrence of pancreatic cancer was not detected until later. Furthermore, a differential expression level of one or more of the biomarkers in an individual may be predictive of the individual's response to a particular therapeutic agent. In monitoring for pancreatic cancer recurrence or progression, changes in the biomarker expression levels may indicate the need for repeat imaging (e.g., repeat EUS), such as to determine pancreatic cancer activity or to determine the need for changes in treatment.

[0141] Detection of any of the biomarkers described herein may be particularly useful following, or in conjunction with, pancreatic cancer treatment, such as to evaluate the success of the treatment or to monitor pancreatic cancer remission, recurrence, and/or progression (including metastasis) following treatment. Pancreatic cancer treatment may include, for example, administration of a therapeutic agent to the individual, performance of surgery (e.g., surgical resection of at least a portion of a pancreatic tumor or removal of pancreatic and surrounding tissue), administration of radiation therapy, or any other type of pancreatic cancer treatment used in the art, and any combination of these treatments. For example, any of the biomarkers may be detected at least once after treatment or may be detected multiple times after treatment (such as at periodic intervals), or may be detected both before and after treatment. Differential expression levels of any of the biomarkers in an individual over time may be indicative of pancreatic cancer progression, remission, or recurrence, examples of which include any of the following: an increase or decrease in the expression level of the biomarkers after treatment compared with the expression level of the biomarker before treatment; an increase or decrease in the expression level of the biomarker at a later time point after treatment compared with the expression level of the biomarker at an earlier time point after treatment; and a differential expression level of the biomarker at a single time point after treatment compared with normal levels of the biomarker.

[0142] As a specific example, the biomarker levels for any of the biomarkers described herein can be determined in pre-surgery and post-surgery (e.g., 2-8 weeks after surgery) serum or

plasma samples. An increase in the biomarker expression level(s) in the post-surgery sample compared with the pre-surgery sample can indicate progression of pancreatic cancer (e.g., unsuccessful surgery), whereas a decrease in the biomarker expression level(s) in the post-surgery sample compared with the pre-surgery sample can indicate regression of pancreatic cancer (e.g., the surgery successfully removed the pancreatic tumor). Similar analyses of the biomarker levels can be carried out before and after other forms of treatment, such as before and after radiation therapy or administration of a therapeutic agent or cancer vaccine.

[0143] In addition to testing biomarker levels as a stand-alone diagnostic test, biomarker levels can also be done in conjunction with determination of SNPs or other genetic lesions or variability that are indicative of increased risk of susceptibility of disease. (See, e.g., Amos et al., Nature Genetics 40, 616-622 (2009)).

[0144] In addition to testing biomarker levels as a stand-alone diagnostic test, biomarker levels can also be done in conjunction with radiologic screening. In addition to testing biomarker levels as a stand-alone diagnostic test, biomarker levels can also be done in conjunction with relevant symptoms or genetic testing. Detection of any of the biomarkers described herein may be useful after pancreatic mass has been observed through imaging to aid in the diagnosis of pancreatic cancer and guide appropriate clinical care of the individual, including care by an appropriate surgical specialist or by palliative therapy in the unresectable patient. In addition to testing biomarker levels in conjunction with relevant symptoms or risk factors, information regarding the biomarkers can also be evaluated in conjunction with other types of data, particularly data that indicates an individual's risk for pancreatic cancer (e.g., patient clinical history, symptoms, family history of pancreatic cancer, history of smoking or alcohol use, sudden onset of diabetes mellitus, jaundice, risk factors such as the presence of a genetic marker(s), and/or status of other biomarkers, etc.). These various data can be assessed by automated methods, such as a computer program/software, which can be embodied in a computer or other apparatus/device.

[0145] In addition to testing biomarker levels in conjunction with radiologic screening in high risk individuals (e.g., assessing biomarker levels in conjunction with size or other characteristics of a pancreatic mass observed on an imaging scan), information regarding the biomarkers can also be evaluated in conjunction with other types of data, particularly data that indicates an individual's risk for pancreatic cancer (e.g., patient clinical history, symptoms, family history

of cancer, risk factors such as whether or not the individual is a smoker, heavy alcohol user and/or status of other biomarkers, etc.). These various data can be assessed by automated methods, such as a computer program/software, which can be embodied in a computer or other apparatus/device.

[0146] Any of the described biomarkers may also be used in imaging tests. For example, an imaging agent can be coupled to any of the described biomarkers, which can be used to aid in pancreatic cancer diagnosis, to monitor disease progression/remission or metastasis, to monitor for disease recurrence, or to monitor response to therapy, among other uses.

Detection and Determination of Biomarkers and Biomarker Values

[0147] A biomarker value for the biomarkers described herein can be detected using any of a variety of known analytical methods. In one embodiment, a biomarker value is detected using a capture reagent. As used herein, a "capture agent" or "capture reagent" refers to a molecule that is capable of binding specifically to a biomarker. In various embodiments, the capture reagent can be exposed to the biomarker in solution or can be exposed to the biomarker while the capture reagent is immobilized on a solid support. In other embodiments, the capture reagent contains a feature that is reactive with a secondary feature on a solid support. In these embodiments, the capture reagent can be exposed to the biomarker in solution, and then the feature on the capture reagent can be used in conjunction with the secondary feature on the solid support to immobilize the biomarker on the solid support. The capture reagent is selected based on the type of analysis to be conducted. Capture reagents include but are not limited to aptamers, antibodies, adnectins, ankyrins, other antibody mimetics and other protein scaffolds, autoantibodies, chimeras, small molecules, an $F(ab')_2$ fragment, a single chain antibody fragment, an Fv fragment, a single chain Fv fragment, a nucleic acid, a lectin, a ligand-binding receptor, affybodies, nanobodies, imprinted polymers, avimers, peptidomimetics, a hormone receptor, a cytokine receptor, and synthetic receptors, and modifications and fragments of these.

[0148] In some embodiments, a biomarker value is detected using a biomarker/capture reagent complex.

[0149] In other embodiments, the biomarker value is derived from the biomarker/capture reagent complex and is detected indirectly, such as, for example, as a result of a reaction that

is subsequent to the biomarker/capture reagent interaction, but is dependent on the formation of the biomarker/capture reagent complex.

[0150] In some embodiments, the biomarker value is detected directly from the biomarker in a biological sample.

[0151] In one embodiment, the biomarkers are detected using a multiplexed format that allows for the simultaneous detection of two or more biomarkers in a biological sample. In one embodiment of the multiplexed format, capture reagents are immobilized, directly or indirectly, covalently or non-covalently, in discrete locations on a solid support. In another embodiment, a multiplexed format uses discrete solid supports where each solid support has a unique capture reagent associated with that solid support, such as, for example quantum dots. In another embodiment, an individual device is used for the detection of each one of multiple biomarkers to be detected in a biological sample. Individual devices can be configured to permit each biomarker in the biological sample to be processed simultaneously. For example, a microtiter plate can be used such that each well in the plate is used to uniquely analyze one of multiple biomarkers to be detected in a biological sample.

[0152] In one or more of the foregoing embodiments, a fluorescent tag can be used to label a component of the biomarker/capture complex to enable the detection of the biomarker value. In various embodiments, the fluorescent label can be conjugated to a capture reagent specific to any of the biomarkers described herein using known techniques, and the fluorescent label can then be used to detect the corresponding biomarker value. Suitable fluorescent labels include rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, allophycocyanin, PBXL-3, Qdot 605, Lissamine, phycoerythrin, Texas Red, and other such compounds.

[0153] In one embodiment, the fluorescent label is a fluorescent dye molecule. In some embodiments, the fluorescent dye molecule includes at least one substituted indolium ring system in which the substituent on the 3-carbon of the indolium ring contains a chemically reactive group or a conjugated substance. In some embodiments, the dye molecule includes an AlexFluor molecule, such as, for example, AlexaFluor 488, AlexaFluor 532, AlexaFluor 647, AlexaFluor 680, or AlexaFluor 700. In other embodiments, the dye molecule includes a first type and a second type of dye molecule, such as, e.g., two different AlexaFluor molecules. In other embodiments, the dye molecule includes a first type and a second type of dye molecule,

and the two dye molecules have different emission spectra.

[0154] Fluorescence can be measured with a variety of instrumentation compatible with a wide range of assay formats. For example, spectrofluorimeters have been designed to analyze microtiter plates, microscope slides, printed arrays, cuvettes, etc. See Principles of Fluorescence Spectroscopy, by J.R. Lakowicz, Springer Science + Business Media, Inc., 2004. See Bioluminescence & Chemiluminescence: Progress & Current Applications; Philip E. Stanley and Larry J. Kricka editors, World Scientific Publishing Company, January 2002.

[0155] In one or more of the foregoing embodiments, a chemiluminescence tag can optionally be used to label a component of the biomarker/capture complex to enable the detection of a biomarker value. Suitable chemiluminescent materials include any of oxalyl chloride, Rodamin 6G, Ru(bipy)32+ , TMAE (tetrakis(dimethylamino)ethylene), Pyrogallol (1,2,3-trihydroxibenzene), Lucigenin, peroxyoxalates, Aryl oxalates, Acridinium esters, dioxetanes, and others.

[0156] In yet other embodiments, the detection method includes an enzyme/substrate combination that generates a detectable signal that corresponds to the biomarker value. Generally, the enzyme catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques, including spectrophotometry, fluorescence, and chemiluminescence. Suitable enzymes include, for example, luciferases, luciferin, malate dehydrogenase, urease, horseradish peroxidase (HRPO), alkaline phosphatase, beta-galactosidase, glucoamylase, lysozyme, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, uricase, xanthine oxidase, lactoperoxidase, microperoxidase, and the like.

[0157] In yet other embodiments, the detection method can be a combination of fluorescence, chemiluminescence, radionuclide or enzyme/substrate combinations that generate a measurable signal. Multimodal signaling could have unique and advantageous characteristics in biomarker assay formats.

[0158] More specifically, the biomarker values for the biomarkers described herein can be detected using known analytical methods including, singleplex aptamer assays, multiplexed aptamer assays, singleplex or multiplexed immunoassays, mRNA expression profiling, miRNA expression profiling, mass spectrometric analysis, histological/cytological methods, etc. as detailed below.

Determination of Biomarker Values using Aptamer-Based Assays

[0159] Assays directed to the detection and quantification of physiologically significant molecules in biological samples and other samples are important tools in scientific research and in the health care field. One class of such assays involves the use of a microarray that includes one or more aptamers immobilized on a solid support. The aptamers are each capable of binding to a target molecule in a highly specific manner and with very high affinity. See, e.g., U.S. Patent No. 5,475,096 entitled "Nucleic Acid Ligands"; see also, e.g., U.S. Patent No. 6,242,246, U.S. Patent No. 6,458,543, and U.S. Patent No. 6,503,715, each of which is entitled "Nucleic Acid Ligand Diagnostic Biochip". Once the microarray is contacted with a sample, the aptamers bind to their respective target molecules present in the sample and thereby enable a determination of a biomarker value corresponding to a biomarker.

[0160] As used herein, an "aptamer" refers to a nucleic acid that has a specific binding affinity for a target molecule. It is recognized that affinity interactions are a matter of degree; however, in this context, the "specific binding affinity" of an aptamer for its target means that the aptamer binds to its target generally with a much higher degree of affinity than it binds to other components in a test sample. An "aptamer" is a set of copies of one type or species of nucleic acid molecule that has a particular nucleotide sequence. An aptamer can include any suitable number of nucleotides, including any number of chemically modified nucleotides. "Aptamers" refers to more than one such set of molecules. Different aptamers can have either the same or different numbers of nucleotides. Aptamers can be DNA or RNA or chemically modified nucleic acids and can be single stranded, double stranded, or contain double stranded regions, and can include higher ordered structures. An aptamer can also be a photoaptamer, where a photoreactive or chemically reactive functional group is included in the aptamer to allow it to be covalently linked to its corresponding target. Any of the aptamer methods disclosed herein can include the use of two or more aptamers that specifically bind the same target molecule. As further described below, an aptamer may include a tag. If an aptamer includes a tag, all copies of the aptamer need not have the same tag. Moreover, if different aptamers each include a tag, these different aptamers can have either the same tag or a different tag.

[0161] An aptamer can be identified using any known method, including the SELEX process. Once identified, an aptamer can be prepared or synthesized in accordance with any known

method, including chemical synthetic methods and enzymatic synthetic methods.

[0162] As used herein, a "SOMAmer" or Slow Off-Rate Modified Aptamer refers to an aptamer having improved off-rate characteristics. SOMAmers can be generated using the improved SELEX methods described in U.S. Publication No. 2009/0004667, entitled "Method for Generating Aptamers with Improved Off-Rates."

[0163] The terms "SELEX" and "SELEX process" are used interchangeably herein to refer generally to a combination of (1) the selection of aptamers that interact with a target molecule in a desirable manner, for example binding with high affinity to a protein, with (2) the amplification of those selected nucleic acids. The SELEX process can be used to identify aptamers with high affinity to a specific target or biomarker.

[0164] SELEX generally includes preparing a candidate mixture of nucleic acids, binding of the candidate mixture to the desired target molecule to form an affinity complex, separating the affinity complexes from the unbound candidate nucleic acids, separating and isolating the nucleic acid from the affinity complex, purifying the nucleic acid, and identifying a specific aptamer sequence. The process may include multiple rounds to further refine the affinity of the selected aptamer. The process can include amplification steps at one or more points in the process. See, e.g., U.S. Patent No. 5,475,096, entitled "Nucleic Acid Ligands". The SELEX process can be used to generate an aptamer that covalently binds its target as well as an aptamer that non-covalently binds its target. See, e.g., U.S. Patent No. 5,705,337 entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Chemi-SELEX."

[0165] The SELEX process can be used to identify high-affinity aptamers containing modified nucleotides that confer improved characteristics on the aptamer, such as, for example, improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX process-identified aptamers containing modified nucleotides are described in U.S. Patent No. 5,660,985, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides", which describes oligonucleotides containing nucleotide derivatives chemically modified at the 5'- and 2'-positions of pyrimidines. U.S. Patent No. 5,580,737, see supra, describes highly specific aptamers containing one or more nucleotides modified with 2'-amino (2'-NH2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). See also, U.S. Patent Application Publication 20090098549, entitled "SELEX and PHOTOSELEX", which describes nucleic acid libraries

having expanded physical and chemical properties and their use in SELEX and photoSELEX.

[0166] SELEX can also be used to identify aptamers that have desirable off-rate characteristics. See U.S. Patent Application Publication 20090004667, entitled "Method for Generating Aptamers with Improved Off-Rates", which describes improved SELEX methods for generating aptamers that can bind to target molecules. Methods for producing aptamers and photoaptamers having slower rates of dissociation from their respective target molecules are described. The methods involve contacting the candidate mixture with the target molecule, allowing the formation of nucleic acid-target complexes to occur, and performing a slow off-rate enrichment process wherein nucleic acid-target complexes with fast dissociation rates will dissociate and not reform, while complexes with slow dissociation rates will remain intact. Additionally, the methods include the use of modified nucleotides in the production of candidate nucleic acid mixtures to generate aptamers with improved off-rate performance.

[0167] A variation of this assay employs aptamers that include photoreactive functional groups that enable the aptamers to covalently bind or "photocrosslink" their target molecules. See, e.g., U.S. Patent No. 6,544,776 entitled "Nucleic Acid Ligand Diagnostic Biochip". These photoreactive aptamers are also referred to as photoaptamers. See, e.g., U.S. Patent No. 5,763,177, U.S. Patent No. 6,001,577, and U.S. Patent No. 6,291,184, each of which is entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Photoselection of Nucleic Acid Ligands and Solution SELEX"; see also, e.g., U.S. Patent No. 6,458,539, entitled "Photoselection of Nucleic Acid Ligands". After the microarray is contacted with the sample and the photoaptamers have had an opportunity to bind to their target molecules, the photoaptamers are photoactivated, and the solid support is washed to remove any non-specifically bound molecules. Harsh wash conditions may be used, since target molecules that are bound to the photoaptamers are generally not removed, due to the covalent bonds created by the photoactivated functional group(s) on the photoaptamers. In this manner, the assay enables the detection of a biomarker value corresponding to a biomarker in the test sample.

[0168] In both of these assay formats, the aptamers are immobilized on the solid support prior to being contacted with the sample. Under certain circumstances, however, immobilization of the aptamers prior to contact with the sample may not provide an optimal assay. For example, pre-immobilization of the aptamers may result in inefficient mixing of the aptamers with the target molecules on the surface of the solid support, perhaps leading to lengthy re-

action times and, therefore, extended incubation periods to permit efficient binding of the aptamers to their target molecules. Further, when photoaptamers are employed in the assay and depending upon the material utilized as a solid support, the solid support may tend to scatter or absorb the light used to effect the formation of covalent bonds between the photoaptamers and their target molecules. Moreover, depending upon the method employed, detection of target molecules bound to their aptamers can be subject to imprecision, since the surface of the solid support may also be exposed to and affected by any labeling agents that are used. Finally, immobilization of the aptamers on the solid support generally involves an aptamer-preparation step (i.e., the immobilization) prior to exposure of the aptamers to the sample, and this preparation step may affect the activity or functionality of the aptamers.

[0169] Aptamer assays that permit an aptamer to capture its target in solution and then employ separation steps that are designed to remove specific components of the aptamer-target mixture prior to detection have also been described (see U.S. Patent Application Publication 20090042206, entitled "Multiplexed Analyses of Test Samples"). The described aptamer assay methods enable the detection and quantification of a non-nucleic acid target (e.g., a protein target) in a test sample by detecting and quantifying a nucleic acid (i.e., an aptamer). The described methods create a nucleic acid surrogate (i.e, the aptamer) for detecting and quantifying a non-nucleic acid target, thus allowing the wide variety of nucleic acid technologies, including amplification, to be applied to a broader range of desired targets, including protein targets.

[0170] Aptamers can be constructed to facilitate the separation of the assay components from an aptamer biomarker complex (or photoaptamer biomarker covalent complex) and permit isolation of the aptamer for detection and/or quantification. In one embodiment, these constructs can include a cleavable or releasable element within the aptamer sequence. In other embodiments, additional functionality can be introduced into the aptamer, for example, a labeled or detectable component, a spacer component, or a specific binding tag or immobilization element. For example, the aptamer can include a tag connected to the aptamer via a cleavable moiety, a label, a spacer component separating the label, and the cleavable moiety. In one embodiment, a cleavable element is a photocleavable linker. The photocleavable linker can be attached to a biotin moiety and a spacer section, can include an NHS group for derivatization of amines, and can be used to introduce a biotin group to an aptamer, thereby

allowing for the release of the aptamer later in an assay method.

[0171] Homogenous assays, done with all assay components in solution, do not require separation of sample and reagents prior to the detection of signal. These methods are rapid and easy to use. These methods generate signal based on a molecular capture or binding reagent that reacts with its specific target. For pancreatic cancer, the molecular capture reagents would be an aptamer or an antibody or the like and the specific target would be a pancreatic cancer biomarker of Table 1, Col. 2.

[0172] In one embodiment, a method for signal generation takes advantage of anisotropy signal change due to the interaction of a fluorophore-labeled capture reagent with its specific biomarker target. When the labeled capture reacts with its target, the increased molecular weight causes the rotational motion of the fluorophore attached to the complex to become much slower changing the anisotropy value. By monitoring the anisotropy change, binding events may be used to quantitatively measure the biomarkers in solutions. Other methods include fluorescence polarization assays, molecular beacon methods, time resolved fluorescence quenching, chemiluminescence, fluorescence resonance energy transfer, and the like.

[0173] An exemplary solution-based aptamer assay that can be used to detect a biomarker value corresponding to a biomarker in a biological sample includes the following: (a) preparing a mixture by contacting the biological sample with an aptamer that includes a first tag and has a specific affinity for the biomarker, wherein an aptamer affinity complex is formed when the biomarker is present in the sample; (b) exposing the mixture to a first solid support including a first capture element, and allowing the first tag to associate with the first capture element; (c) removing any components of the mixture not associated with the first solid support; (d) attaching a second tag to the biomarker component of the aptamer affinity complex; (e) releasing the aptamer affinity complex from the first solid support; (f) exposing the released aptamer affinity complex to a second solid support that includes a second capture element and allowing the second tag to associate with the second capture element; (g) removing any non-complexed aptamer from the mixture by partitioning the non-complexed aptamer from the aptamer affinity complex; (h) eluting the aptamer from the solid support; and (i) detecting the biomarker by detecting the aptamer component of the aptamer affinity complex.

[0174] Any means known in the art can be used to detect a biomarker value by detecting the aptamer component of an aptamer affinity complex. A number of different detection

methods can be used to detect the aptamer component of an affinity complex, such as, for example, hybridization assays, mass spectroscopy, or QPCR. In some embodiments, nucleic acid sequencing methods can be used to detect the aptamer component of an aptamer affinity complex and thereby detect a biomarker value. Briefly, a test sample can be subjected to any kind of nucleic acid sequencing method to identify and quantify the sequence or sequences of one or more aptamers present in the test sample. In some embodiments, the sequence includes the entire aptamer molecule or any portion of the molecule that may be used to uniquely identify the molecule. In other embodiments, the identifying sequencing is a specific sequence added to the aptamer; such sequences are often referred to as "tags," "barcodes," or "zipcodes." In some embodiments, the sequencing method includes enzymatic steps to amplify the aptamer sequence or to convert any kind of nucleic acid, including RNA and DNA that contain chemical modifications to any position, to any other kind of nucleic acid appropriate for sequencing.

[0175] In some embodiments, the sequencing method includes one or more cloning steps. In other embodiments the sequencing method includes a direct sequencing method without cloning.

[0176] In some embodiments, the sequencing method includes a directed approach with specific primers that target one or more aptamers in the test sample. In other embodiments, the sequencing method includes a shotgun approach that targets all aptamers in the test sample.

[0177] In some embodiments, the sequencing method includes enzymatic steps to amplify the molecule targeted for sequencing. In other embodiments, the sequencing method directly sequences single molecules. An exemplary nucleic acid sequencing-based method that can be used to detect a biomarker value corresponding to a biomarker in a biological sample includes the following: (a) converting a mixture of aptamers that contain chemically modified nucleotides to unmodified nucleic acids with an enzymatic step; (b) shotgun sequencing the resulting unmodified nucleic acids with a massively parallel sequencing platform such as, for example, the 454 Sequencing System (454 Life Sciences/Roche), the Illumina Sequencing System (Illumina), the ABI SOLiD Sequencing System (Applied Biosystems), the HeliScope Single Molecule Sequencer (Helicos Biosciences), or the Pacific Biosciences Real Time Single-Molecule Sequencing System (Pacific BioSciences) or the Polonator G Sequencing System

(Dover Systems); and (c) identifying and quantifying the aptamers present in the mixture by specific sequence and sequence count.

Determination of Biomarker Values using Immunoassays

[0178] Immunoassay methods are based on the reaction of an antibody to its corresponding target or analyte and can detect the analyte in a sample depending on the specific assay format. To improve specificity and sensitivity of an assay method based on immuno-reactivity, monoclonal antibodies are often used because of their specific epitope recognition. Polyclonal antibodies have also been successfully used in various immunoassays because of their increased affinity for the target as compared to monoclonal antibodies. Immunoassays have been designed for use with a wide range of biological sample matrices. Immunoassay formats have been designed to provide qualitative, semi-quantitative, and quantitative results.

[0179] Quantitative results are generated through the use of a standard curve created with known concentrations of the specific analyte to be detected. The response or signal from an unknown sample is plotted onto the standard curve, and a quantity or value corresponding to the target in the unknown sample is established.

[0180] Numerous immunoassay formats have been designed. ELISA or EIA can be quantitative for the detection of an analyte. This method relies on attachment of a label to either the analyte or the antibody and the label component includes, either directly or indirectly, an enzyme. ELISA tests may be formatted for direct, indirect, competitive, or sandwich detection of the analyte. Other methods rely on labels such as, for example, radioisotopes (I125) or fluorescence. Additional techniques include, for example, agglutination, nephelometry, turbidimetry, Western blot, immunoprecipitation, immunocytochemistry, immunohistochemistry, flow cytometry, Luminex assay, and others (see ImmunoAssay: A Practical Guide, edited by Brian Law, published by Taylor & Francis, Ltd., 2005 edition).

[0181] Exemplary assay formats include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, fluorescent, chemiluminescence, and fluorescence resonance energy transfer (FRET) or time resolved-FRET (TR-FRET) immunoassays. Examples of procedures for detecting biomarkers include biomarker immunoprecipitation followed by quantitative methods that allow size and peptide level discrimination, such as gel electrophoresis, capillary electrophoresis, planar electrochromatography, and the like.

[0182] Methods of detecting and/or quantifying a detectable label or signal generating material depend on the nature of the label. The products of reactions catalyzed by appropriate enzymes (where the detectable label is an enzyme; see above) can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors suitable for detecting such detectable labels include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

[0183] Any of the methods for detection can be performed in any format that allows for any suitable preparation, processing, and analysis of the reactions. This can be, for example, in multi-well assay plates (e.g., 96 wells or 384 wells) or using any suitable array or microarray. Stock solutions for various agents can be made manually or robotically, and all subsequent pipetting, diluting, mixing, distribution, washing, incubating, sample readout, data collection and analysis can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting a detectable label.

Determination of Biomarker Values using Gene Expression Profiling

[0184] Measuring mRNA in a biological sample may be used as a surrogate for detection of the level of the corresponding protein in the biological sample. Thus, any of the biomarkers or biomarker panels described herein can also be detected by detecting the appropriate RNA.

[0185] mRNA expression levels are measured by reverse transcription quantitative polymerase chain reaction (RT-PCR followed with qPCR). RT-PCR is used to create a cDNA from the mRNA. The cDNA may be used in a qPCR assay to produce fluorescence as the DNA amplification process progresses. By comparison to a standard curve, qPCR can produce an absolute measurement such as number of copies of mRNA per cell. Northern blots, microarrays, Invader assays, and RT-PCR combined with capillary electrophoresis have all been used to measure expression levels of mRNA in a sample. See Gene Expression Profiling: Methods and Protocols, Richard A. Shimkets, editor, Humana Press, 2004.

[0186] miRNA molecules are small RNAs that are non-coding but may regulate gene expression. Any of the methods suited to the measurement of mRNA expression levels can also be used for the corresponding miRNA. Recently many laboratories have investigated the use of miRNAs as biomarkers for disease. Many diseases involve wide-spread transcriptional

regulation, and it is not surprising that miRNAs might find a role as biomarkers. The connection between miRNA concentrations and disease is often even less clear than the connections between protein levels and disease, yet the value of miRNA biomarkers might be substantial. Of course, as with any RNA expressed differentially during disease, the problems facing the development of an in vitro diagnostic product will include the requirement that the miRNAs survive in the diseased cell and are easily extracted for analysis, or that the miRNAs are released into blood or other matrices where they must survive long enough to be measured. Protein biomarkers have similar requirements, although many potential protein biomarkers are secreted intentionally at the site of pathology and function, during disease, in a paracrine fashion. Many potential protein biomarkers are designed to function outside the cells within which those proteins are synthesized.

Detection of Biomarkers Using In Vivo Molecular Imaging Technologies

[0187] Any of the described biomarkers (see Table 1, Col. 2) may also be used in molecular imaging tests. For example, an imaging agent can be coupled to any of the described biomarkers, which can be used to aid in pancreatic cancer diagnosis, to monitor disease progression/remission or metastasis, to monitor for disease recurrence, or to monitor response to therapy, among other uses.

[0188] In vivo imaging technologies provide non-invasive methods for determining the state of a particular disease in the body of an individual. For example, entire portions of the body, or even the entire body, may be viewed as a three dimensional image, thereby providing valuable information concerning morphology and structures in the body. Such technologies may be combined with the detection of the biomarkers described herein to provide information concerning the cancer status, in particular the pancreatic cancer status, of an individual.

[0189] The use of in vivo molecular imaging technologies is expanding due to various advances in technology. These advances include the development of new contrast agents or labels, such as radiolabels and/or fluorescent labels, which can provide strong signals within the body; and the development of powerful new imaging technology, which can detect and analyze these signals from outside the body, with sufficient sensitivity and accuracy to provide useful information. The contrast agent can be visualized in an appropriate imaging system,

thereby providing an image of the portion or portions of the body in which the contrast agent is located. The contrast agent may be bound to or associated with a capture reagent, such as an aptamer or an antibody, for example, and/or with a peptide or protein, or an oligonucleotide (for example, for the detection of gene expression), or a complex containing any of these with one or more macromolecules and/or other particulate forms.

[0190] The contrast agent may also feature a radioactive atom that is useful in imaging. Suitable radioactive atoms include technetium-99m or iodine-123 for scintigraphic studies. Other readily detectable moieties include, for example, spin labels for magnetic resonance imaging (MRI) such as, for example, iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Such labels are well known in the art and could easily be selected by one of ordinary skill in the art.

[0191] Standard imaging techniques include but are not limited to magnetic resonance imaging, computed tomography scanning, positron emission tomography (PET), single photon emission computed tomography (SPECT), and the like. For diagnostic in vivo imaging, the type of detection instrument available is a major factor in selecting a given contrast agent, such as a given radionuclide and the particular biomarker that it is used to target (protein, mRNA, and the like). The radionuclide chosen typically has a type of decay that is detectable by a given type of instrument. Also, when selecting a radionuclide for in vivo diagnosis, its half-life should be long enough to enable detection at the time of maximum uptake by the target tissue but short enough that deleterious radiation of the host is minimized.

[0192] Exemplary imaging techniques include but are not limited to PET and SPECT, which are imaging techniques in which a radionuclide is synthetically or locally administered to an individual. The subsequent uptake of the radiotracer is measured over time and used to obtain information about the targeted tissue and the biomarker. Because of the high-energy (gamma-ray) emissions of the specific isotopes employed and the sensitivity and sophistication of the instruments used to detect them, the two-dimensional distribution of radioactivity may be inferred from outside of the body.

[0193] Commonly used positron-emitting nuclides in PET include, for example, carbon-11, nitrogen-13, oxygen-15, and fluorine-18. Isotopes that decay by electron capture and/or gamma-emission are used in SPECT and include, for example iodine-123 and technetium-99m. An exemplary method for labeling amino acids with technetium-99m is the reduction of

pertechnetate ion in the presence of a chelating precursor to form the labile technetium-99m-precursor complex, which, in turn, reacts with the metal binding group of a bifunctionally modified chemotactic peptide to form a technetium-99m-chemotactic peptide conjugate.

[0194] Antibodies are frequently used for such in vivo imaging diagnostic methods. The preparation and use of antibodies for in vivo diagnosis is well known in the art. Labeled antibodies which specifically bind any of the biomarkers in Table 1, Col. 2 can be injected into an individual suspected of having a certain type of cancer (e.g., pancreatic cancer), detectable according to the particular biomarker used, for the purpose of diagnosing or evaluating the disease status of the individual. The label used will be selected in accordance with the imaging modality to be used, as previously described. Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

[0195] Similarly, aptamers may be used for such in vivo imaging diagnostic methods. For example, an aptamer that was used to identify a particular biomarker described in Table 1, Col. 2 (and therefore binds specifically to that particular biomarker) may be appropriately labeled and injected into an individual suspected of having pancreatic cancer, detectable according to the particular biomarker, for the purpose of diagnosing or evaluating the pancreatic cancer status of the individual. The label used will be selected in accordance with the imaging modality to be used, as previously described. Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue. Aptamer-directed imaging agents could have unique and advantageous characteristics relating to tissue penetration, tissue distribution, kinetics, elimination, potency, and selectivity as compared to other imaging agents.

[0196] Such techniques may also optionally be performed with labeled oligonucleotides, for example, for detection of gene expression through imaging with antisense oligonucleotides. These methods are used for in situ hybridization, for example, with fluorescent molecules or radionuclides as the label. Other methods for detection of gene expression include, for example, detection of the activity of a reporter gene.

[0197] Another general type of imaging technology is optical imaging, in which fluorescent signals within the subject are detected by an optical device that is external to the subject.

These signals may be due to actual fluorescence and/or to bioluminescence. Improvements in the sensitivity of optical detection devices have increased the usefulness of optical imaging for in vivo diagnostic assays.

[0198] The use of in vivo molecular biomarker imaging is increasing, including for clinical trials, for example, to more rapidly measure clinical efficacy in trials for new cancer therapies and/or to avoid prolonged treatment with a placebo for those diseases, such as multiple sclerosis, in which such prolonged treatment may be considered to be ethically questionable.

[0199] For a review of other techniques, see N. Blow, Nature Methods, 6, 465-469, 2009.

Determination of Biomarker Values using Histology/Cytology Methods

[0200] For evaluation of pancreatic cancer, a variety of tissue samples may be used in histological or cytological methods. Sample selection depends on the primary tumor location and sites of metastases. For example, tissue samples (forceps biopsy, fine needle aspiration (FNA), and/or brush cytology) collected at the time of endoscopic retrograde cholangiopancreatography (ERCP), or endoscopic ultrasound (EUS)-guided FNA can be used for histology. Ascites or peritoneal washings or pancreatic fluid can be used for cyotology. Any of the biomarkers identified herein that were shown to be up-regulated (ee Table 1, Col. 6) in the individuals with pancreatic can be used to stain a histological specimen as an indication of disease.

[0201] In one embodiment, one or more capture reagent/s specific to the corresponding biomarker/s are used in a cytological evaluation of a pancreatic cell sample and may include one or more of the following: collecting a cell sample, fixing the cell sample, dehydrating, clearing, immobilizing the cell sample on a microscope slide, permeabilizing the cell sample, treating for analyte retrieval, staining, destaining, washing, blocking, and reacting with one or more capture reagent/s in a buffered solution. In another embodiment, the cell sample is produced from a cell block.

[0202] In another embodiment, one or more capture reagent/s specific to the corresponding biomarkers are used in a histological evaluation of a pancreatic tissue sample and may include one or more of the following: collecting a tissue specimen, fixing the tissue sample, dehydrating, clearing, immobilizing the tissue sample on a microscope slide, permeabilizing the tissue sample, treating for analyte retrieval, staining, destaining, washing, blocking, rehydrating,

and reacting with capture reagent/s in a buffered solution. In another embodiment, fixing and dehydrating are replaced with freezing.

[0203] In another embodiment, the one or more aptamer/s specific to the corresponding biomarker/s are reacted with the histological or cytological sample and can serve as the nucleic acid target in a nucleic acid amplification method. Suitable nucleic acid amplification methods include, for example, PCR, q-beta replicase, rolling circle amplification, strand displacement, helicase dependent amplification, loop mediated isothermal amplification, ligase chain reaction, and restriction and circularization aided rolling circle amplification.

[0204] In one embodiment, the one or more capture reagent/s specific to the corresponding biomarkers for use in the histological or cytological evaluation are mixed in a buffered solution that can include any of the following: blocking materials, competitors, detergents, stabilizers, carrier nucleic acid, polyanionic materials, etc.

[0205] A "cytology protocol" generally includes sample collection, sample fixation, sample immobilization, and staining. "Cell preparation" can include several processing steps after sample collection, including the use of one or more slow off-rate aptamers for the staining of the prepared cells.

[0206] Sample collection can include directly placing the sample in an untreated transport container, placing the sample in a transport container containing some type of media, or placing the sample directly onto a slide (immobilization) without any treatment or fixation.

[0207] Sample immobilization can be improved by applying a portion of the collected specimen to a glass slide that is treated with polylysine, gelatin, or a silane. Slides can be prepared by smearing a thin and even layer of cells across the slide. Care is generally taken to minimize mechanical distortion and drying artifacts. Liquid specimens can be processed in a cell block method. Or, alternatively, liquid specimens can be mixed 1:1 with the fixative solution for about 10 minutes at room temperature.

[0208] Cell blocks can be prepared from residual effusions, sputum, urine sediments, gastrointestinal fluids, cell scraping, or fine needle aspirates. Cells are concentrated or packed by centrifugation or membrane filtration. A number of methods for cell block preparation have been developed. Representative procedures include the fixed sediment, bacterial agar, or membrane filtration methods. In the fixed sediment method, the cell sediment is mixed with a fixative like Bouins, picric acid, or buffered formalin and then the mixture is centrifuged

to pellet the fixed cells. The supernatant is removed, drying the cell pellet as completely as possible. The pellet is collected and wrapped in lens paper and then placed in a tissue cassette. The tissue cassette is placed in a jar with additional fixative and processed as a tissue sample. Agar method is very similar but the pellet is removed and dried on paper towel and then cut in half. The cut side is placed in a drop of melted agar on a glass slide and then the pellet is covered with agar making sure that no bubbles form in the agar. The agar is allowed to harden and then any excess agar is trimmed away. This is placed in a tissue cassette and the tissue process completed. Alternatively, the pellet may be directly suspended in 2% liquid agar at 65°C and the sample centrifuged. The agar cell pellet is allowed to solidify for an hour at 4°C. The solid agar may be removed from the centrifuge tube and sliced in half. The agar is wrapped in filter paper and then the tissue cassette. Processing from this point forward is as described above. Centrifugation can be replaced in any these procedures with membrane filtration. Any of these processes may be used to generate a "cell block sample".

[0209] Cell blocks can be prepared using specialized resin including Lowicryl resins, LR White, LR Gold, Unicryl, and MonoStep. These resins have low viscosity and can be polymerized at low temperatures and with ultra violet (UV) light. The embedding process relies on progressively cooling the sample during dehydration, transferring the sample to the resin, and polymerizing a block at the final low temperature at the appropriate UV wavelength.

[0210] Cell block sections can be stained with hematoxylin-eosin for cytomorphological examination while additional sections are used for examination for specific markers.

[0211] Whether the process is cytologoical or histological, the sample may be fixed prior to additional processing to prevent sample degradation. This process is called "fixation" and describes a wide range of materials and procedures that may be used interchangeably. The sample fixation protocol and reagents are best selected empirically based on the targets to be detected and the specific cell/tissue type to be analyzed. Sample fixation relies on reagents such as ethanol, polyethylene glycol, methanol, formalin, or isopropanol. The samples should be fixed as soon after collection and affixation to the slide as possible. However, the fixative selected can introduce structural changes into various molecular targets making their subsequent detection more difficult. The fixation and immobilization processes and their sequence can modify the appearance of the cell and these changes must be anticipated and recognized by the cytotechnologist. Fixatives can cause shrinkage of certain cell types and cause the cytoplasm

to appear granular or reticular. Many fixatives function by crosslinking cellular components. This can damage or modify specific epitopes, generate new epitopes, cause molecular associations, and reduce membrane permeability. Formalin fixation is one of the most common cytological/histological approaches. Formalin forms methyl bridges between neighboring proteins or within proteins. Precipitation or coagulation is also used for fixation and ethanol is frequently used in this type of fixation. A combination of crosslinking and precipitation can also be used for fixation. A strong fixation process is best at preserving morphological information while a weaker fixation process is best for the preservation of molecular targets.

[0212] A representative fixative is 50% absolute ethanol, 2 mM polyethylene glycol (PEG), 1.85% formaldehyde. Variations on this formulation include ethanol (50% to 95%), methanol (20% - 50%), and formalin (formaldehyde) only. Another common fixative is 2% PEG 1500, 50% ethanol, and 3% methanol. Slides are place in the fixative for about 10 to 15 minutes at room temperature and then removed and allowed to dry. Once slides are fixed they can be rinsed with a buffered solution like PBS.

[0213] A wide range of dyes can be used to differentially highlight and contrast or "stain" cellular, sub-cellular, and tissue features or morphological structures. Hematoylin is used to stain nuclei a blue or black color. Orange G-6 and Eosin Azure both stain the cell's cytoplasm. Orange G stains keratin and glycogen containing cells yellow. Eosin Y is used to stain nucleoli, cilia, red blood cells, and superficial epithelial squamous cells. Romanowsky stains are used for air dried slides and are useful in enhancing pleomorphism and distinguishing extracellular from intracytoplasmic material.

[0214] The staining process can include a treatment to increase the permeability of the cells to the stain. Treatment of the cells with a detergent can be used to increase permeability. To increase cell and tissue permeability, fixed samples can be further treated with solvents, saponins, or non-ionic detergents. Enzymatic digestion can also improve the accessibility of specific targets in a tissue sample.

[0215] After staining, the sample is dehydrated using a succession of alcohol rinses with increasing alcohol concentration. The final wash is done with xylene or a xylene substitute, such as a citrus terpene, that has a refractive index close to that of the coverslip to be applied to the slide. This final step is referred to as clearing. Once the sample is dehydrated and cleared, a mounting medium is applied. The mounting medium is selected to have a refractive

index close to the glass and is capable of bonding the coverslip to the slide. It will also inhibit the additional drying, shrinking, or fading of the cell sample.

[0216] Regardless of the stains or processing used, the final evaluation of the pancreatic cytological specimen is made by some type of microscopy to permit a visual inspection of the morphology and a determination of the marker's presence or absence. Exemplary microscopic methods include brightfield, phase contrast, fluorescence, and differential interference contrast.

[0217] If secondary tests are required on the sample after examination, the coverslip may be removed and the slide destained. Destaining involves using the original solvent systems used in staining the slide originally without the added dye and in a reverse order to the original staining procedure. Destaining may also be completed by soaking the slide in an acid alcohol until the cells are colorless. Once colorless the slides are rinsed well in a water bath and the second staining procedure applied.

[0218] In addition, specific molecular differentiation may be possible in conjunction with the cellular morphological analysis through the use of specific molecular reagents such as antibodies or nucleic acid probes or aptamers. This improves the accuracy of diagnostic cytology. Micro-dissection can be used to isolate a subset of cells for additional evaluation, in particular, for genetic evaluation of abnormal chromosomes, gene expression, or mutations.

[0219] Preparation of a tissue sample for histological evaluation involves fixation, dehydration, infiltration, embedding, and sectioning. The fixation reagents used in histology are very similar or identical to those used in cytology and have the same issues of preserving morphological features at the expense of molecular ones such as individual proteins. Time can be saved if the tissue sample is not fixed and dehydrated but instead is frozen and then sectioned while frozen. This is a more gentle processing procedure and can preserve more individual markers. However, freezing is not acceptable for long term storage of a tissue sample as subcellular information is lost due to the introduction of ice crystals. Ice in the frozen tissue sample also prevents the sectioning process from producing a very thin slice and thus some microscopic resolution and imaging of subcellular structures can be lost. In addition to formalin fixation, osmium tetroxide is used to fix and stain phospholipids (membranes).

[0220] Dehydration of tissues is accomplished with successive washes of increasing alcohol concentration. Clearing employs a material that is miscible with alcohol and the embedding material and involves a stepwise process starting at 50:50 alcohol:clearing reagent and then

100% clearing agent (xylene or xylene substitute). Infiltration involves incubating the tissue with a liquid form of the embedding agent (warm wax, nitrocellulose solution) first at 50:50 embedding agent: clearing agent and the 100% embedding agent. Embedding is completed by placing the tissue in a mold or cassette and filling with melted embedding agent such as wax, agar, or gelatin. The embedding agent is allowed to harden. The hardened tissue sample may then be sliced into thin section for staining and subsequent examination.

[0221] Prior to staining, the tissue section is dewaxed and rehydrated. Xylene is used to dewax the section, one or more changes of xylene may be used, and the tissue is rehydrated by successive washes in alcohol of decreasing concentration. Prior to dewax, the tissue section may be heat immobilized to a glass slide at about 80°C for about 20 minutes.

[0222] Laser capture micro-dissection allows the isolation of a subset of cells for further analysis from a tissue section.

[0223] As in cytology, to enhance the visualization of the microscopic features, the tissue section or slice can be stained with a variety of stains. A large menu of commercially available stains can be used to enhance or identify specific features.

[0224] To further increase the interaction of molecular reagents with cytological/histological samples, a number of techniques for "analyte retrieval" have been developed. The first such technique uses high temperature heating of a fixed sample. This method is also referred to as heat-induced epitope retrieval or HIER. A variety of heating techniques have been used, including steam heating, microwaving, autoclaving, water baths, and pressure cooking or a combination of these methods of heating. Analyte retrieval solutions include, for example, water, citrate, and normal saline buffers. The key to analyte retrieval is the time at high temperature but lower temperatures for longer times have also been successfully used. Another key to analyte retrieval is the pH of the heating solution. Low pH has been found to provide the best immunostaining but also gives rise to backgrounds that frequently require the use of a second tissue section as a negative control. The most consistent benefit (increased immunostaining without increase in background) is generally obtained with a high pH solution regardless of the buffer composition. The analyte retrieval process for a specific target is empirically optimized for the target using heat, time, pH, and buffer composition as variables for process optimization. Using the microwave analyte retrieval method allows for sequential staining of different targets with antibody reagents. But the time required to achieve antibody

and enzyme complexes between staining steps has also been shown to degrade cell membrane analytes. Microwave heating methods have improved in situ hybridization methods as well.

[0225] To initiate the analyte retrieval process, the section is first dewaxed and hydrated. The slide is then placed in 10mM sodium citrate buffer pH 6.0 in a dish or jar. A representative procedure uses an 1100W microwave and microwaves the slide at 100% power for 2 minutes followed by microwaving the slides using 20% power for 18 minutes after checking to be sure the slide remains covered in liquid. The slide is then allowed to cool in the uncovered container and then rinsed with distilled water. HIER may be used in combination with an enzymatic digestion to improve the reactivity of the target to immunochemical reagents.

[0226] One such enzymatic digestion protocol uses proteinase K. A 20 g/ml concentration of proteinase K is prepared in 50 mM Tris Base, 1mM EDTA, 0.5% Triton X-100, pH 8.0 buffer. The process first involves dewaxing sections in 2 changes of xylene, 5 minutes each. Then the sample is hydrated in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each, and then rinsed in distilled water. Sections are covered with Proteinase K working solution and incubated 10-20 minutes at 37C in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation). The sections are cooled at room temperature for 10 minutes and then rinsed in PBS Tween 20 for 2x2 min. If desired, sections can be blocked to eliminate potential interference from endogenous compounds and enzymes. The section is then incubated with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight at 4C. The section is then rinsed with PBS Tween 20 for 2x2 min. Additional blocking can be performed, if required for the specific application, followed by additional rinsing with PBS Tween 20 for 3x2 min and then finally the immunostaining protocol completed.

[0227] A simple treatment with 1% SDS at room temperature has also been demonstrated to improve immunohistochemical staining. Analyte retrieval methods have been applied to slide mounted sections as well as free floating sections. Another treatment option is to place the slide in a jar containing citric acid and 0.1 Nonident P40 at pH 6.0 and heating to 95°C. The slide is then washed with a buffer solution like PBS.

[0228] For immunological staining of tissues it may be useful to block non-specific association of the antibody with tissue proteins by soaking the section in a protein solution like serum or non-fat dry milk.

[0229] Blocking reactions may include the need to reduce the level of endogenous biotin; eliminate endogenous charge effects; inactivate endogenous nucleases; and/ or inactivate endogenous enzymes like peroxidase and alkaline phosphatase. Endogenous nucleases may be inactivated by degradation with proteinase K, by heat treatment, use of a chelating agent such as EDTA or EGTA, the introduction of carrier DNA or RNA, treatment with a chaotrope such as urea, thiourea, guanidine hydrochloride, guanidine thiocyanate, lithium perchlorate, etc, or diethyl pyrocarbonate. Alkaline phosphatase may be inactivated by treated with 0.1N HCl for 5 minutes at room temperature or treatment with 1 mM levamisole. Peroxidase activity may be eliminated by treatment with 0.03% hydrogen peroxide. Endogenous biotin may be blocked by soaking the slide or section in an avidin (streptavidin, neutravidin may be substituted) solution for at least 15 minutes at room temperature. The slide or section is then washed for at least 10 minutes in buffer. This may be repeated at least three times. Then the slide or section is soaked in a biotin solution for 10 minutes. This may be repeated at least three times with a fresh biotin solution each time. The buffer wash procedure is repeated. Blocking protocols should be minimized to prevent damaging either the cell or tissue structure or the target or targets of interest but one or more of these protocols could be combined to "block" a slide or section prior to reaction with one or more slow off-rate aptamers. See Basic Medical Histology: the Biology of Cells, Tissues and Organs, authored by Richard G. Kessel, Oxford University Press, 1998.

Determination of Biomarker Values using Mass Spectrometry Methods

[0230] A variety of configurations of mass spectrometers can be used to detect biomarker values. Several types of mass spectrometers are available or can be produced with various configurations. In general, a mass spectrometer has the following major components: a sample inlet, an ion source, a mass analyzer, a detector, a vacuum system, and instrument-control system, and a data system. Difference in the sample inlet, ion source, and mass analyzer generally define the type of instrument and its capabilities. For example, an inlet can be a capillary-column liquid chromatography source or can be a direct probe or stage such as used in matrix-assisted laser desorption. Common ion sources are, for example, electrospray, including nanospray and microspray or matrix-assisted laser desorption. Common mass analyzers

include a quadrupole mass filter, ion trap mass analyzer and time-of-flight mass analyzer. Additional mass spectrometry methods are well known in the art (see Burlingame et al. Anal. Chem. 70:647 R-716R (1998); Kinter and Sherman, New York (2000)).

[0231] Protein biomarkers and biomarker values can be detected and measured by any of the following: electrospray ionization mass spectrometry (ESI-MS), ESI-MS/MS, ESI-MS/(MS)n, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), desorption/ionization on silicon (DIOS), secondary ion mass spectrometry (SIMS), quadrupole time-of-flight (Q-TOF), tandem time-of-flight (TOF/TOF) technology, called ultraflex III TOF/TOF, atmospheric pressure chemical ionization mass spectrometry (APCI-MS), APCI-MS/MS, APCI-(MS)N, atmospheric pressure photoionization mass spectrometry (APPI-MS), APPI-MS/MS, and APPI-(MS)N, quadrupole mass spectrometry, Fourier transform mass spectrometry (FTMS), quantitative mass spectrometry, and ion trap mass spectrometry.

[0232] Sample preparation strategies are used to label and enrich samples before mass spectroscopic characterization of protein biomarkers and determination biomarker values. Labeling methods include but are not limited to isobaric tag for relative and absolute quantitation (iTRAQ) and stable isotope labeling with amino acids in cell culture (SILAC). Capture reagents used to selectively enrich samples for candidate biomarker proteins prior to mass spectroscopic analysis include but are not limited to aptamers, antibodies, nucleic acid probes, chimeras, small molecules, an F(ab')2 fragment, a single chain antibody fragment, an Fv fragment, a single chain Fv fragment, a nucleic acid, a lectin, a ligand-binding receptor, affybodies, nanobodies, ankyrins, domain antibodies, alternative antibody scaffolds (e.g. diabodies etc) imprinted polymers, avimers, peptidomimetics, peptoids, peptide nucleic acids, threose nucleic acid, a hormone receptor, a cytokine receptor, and synthetic receptors, and modifications and fragments of these.

Determination of Biomarker Values using a Proximity Ligation Assay

[0233] A proximity ligation assay can be used to determine biomarker values. Briefly, a test sample is contacted with a pair of affinity probes that may be a pair of antibodies or a pair

of aptamers, with each member of the pair extended with an oligonucleotide. The targets for the pair of affinity probes may be two distinct determinates on one protein or one determinate on each of two different proteins, which may exist as homo- or hetero-multimeric complexes. When probes bind to the target determinates, the free ends of the oligonucleotide extensions are brought into sufficiently close proximity to hybridize together. The hybridization of the oligonucleotide extensions is facilitated by a common connector oligonucleotide which serves to bridge together the oligonucleotide extensions when they are positioned in sufficient proximity. Once the oligonucleotide extensions of the probes are hybridized, the ends of the extensions are joined together by enzymatic DNA ligation.

[0234] Each oligonucleotide extension comprises a primer site for PCR amplification. Once the oligonucleotide extensions are ligated together, the oligonucleotides form a continuous DNA sequence which, through PCR amplification, reveals information regarding the identity and amount of the target protein, as well as, information regarding protein-protein interactions where the target determinates are on two different proteins. Proximity ligation can provide a highly sensitive and specific assay for real-time protein concentration and interaction information through use of real-time PCR. Probes that do not bind the determinates of interest do not have the corresponding oligonucleotide extensions brought into proximity and no ligation or PCR amplification can proceed, resulting in no signal being produced.

[0235] The foregoing assays enable the detection of biomarker values that are useful in methods for diagnosing pancreatic cancer, where the methods comprise detecting, in a biological sample from an individual, at least N biomarker values that each correspond to a biomarker selected from the group consisting of the biomarkers provided in Table 1, Col. 2, wherein a classification, as described in detail below, using the biomarker values indicates whether the individual has pancreatic cancer. While certain of the described pancreatic cancer biomarkers are useful alone for detecting and diagnosing pancreatic cancer, methods are also described herein for the grouping of multiple subsets of the pancreatic cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. In other embodiments, N is selected to be any number from 2-65 biomarkers. It will be appreciated that N can be selected to be any number from any of the above described ranges, as well as similar, but higher order, ranges. In accordance with any of the methods described

herein, biomarker values can be detected and classified individually or they can be detected and classified collectively, as for example in a multiplex assay format.

[0236] In another aspect, methods are provided for detecting an absence of pancreatic cancer, the methods comprising detecting, in a biological sample from an individual, at least N biomarker values that each correspond to a biomarker selected from the group consisting of the biomarkers provided in Table 1, Col. 2, wherein a classification, as described in detail below, of the biomarker values indicates an absence of pancreatic cancer in the individual. While certain of the described pancreatic cancer biomarkers are useful alone for detecting and diagnosing the absence of pancreatic cancer, methods are also described herein for the grouping of multiple subsets of the pancreatic cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. In other embodiments, N is selected to be any number from 2-65 biomarkers. It will be appreciated that N can be selected to be any number from any of the above described ranges, as well as similar, but higher order, ranges. In accordance with any of the methods described herein, biomarker values can be detected and classified individually or they can be detected and classified collectively, as for example in a multiplex assay format.

Classification of Biomarkers and Calculation of Disease Scores

[0237] A biomarker "signature" for a given diagnostic test contains a set of markers, each marker having different levels in the populations of interest. Different levels, in this context, may refer to different means of the marker levels for the individuals in two or more groups, or different variances in the two or more groups, or a combination of both. For the simplest form of a diagnostic test, these markers can be used to assign an unknown sample from an individual into one of two groups, either diseased or not diseased. The assignment of a sample into one of two or more groups is known as classification, and the procedure used to accomplish this assignment is known as a classifier or a classification method. Classification methods may also be referred to as scoring methods. There are many classification methods that can be used to construct a diagnostic classifier from a set of biomarker values. In general, classification methods are most easily performed using supervised learning techniques where a data set is collected using samples obtained from individuals within two (or more, for multiple classifica-

tion states) distinct groups one wishes to distinguish. Since the class (group or population) to which each sample belongs is known in advance for each sample, the classification method can be trained to give the desired classification response. It is also possible to use unsupervised learning techniques to produce a diagnostic classifier.

[0238] Common approaches for developing diagnostic classifiers include decision trees; bagging, boosting, forests and random forests; rule inference based learning; Parzen Windows; linear models; logistic; neural network methods; unsupervised clustering; K-means; hierarchical ascending/ descending; semi-supervised learning; prototype methods; nearest neighbor; kernel density estimation; support vector machines; hidden Markov models; Boltzmann Learning; and classifiers may be combined either simply or in ways which minimize particular objective functions. For a review, see, e.g., Pattern Classification, R.O. Duda, et al., editors, John Wiley & Sons, 2nd edition, 2001; see also, The Elements of Statistical Learning - Data Mining, Inference, and Prediction, T. Hastie, et al., editors, Springer Science+Business Media, LLC, 2nd edition, 2009.

[0239] To produce a classifier using supervised learning techniques, a set of samples called training data are obtained. In the context of diagnostic tests, training data includes samples from the distinct groups (classes) to which unknown samples will later be assigned. For example, samples collected from individuals in a control population and individuals in a particular disease population can constitute training data to develop a classifier that can classify unknown samples (or, more particularly, the individuals from whom the samples were obtained) as either having the disease or being free from the disease. The development of the classifier from the training data is known as training the classifier. Specific details on classifier training depend on the nature of the supervised learning technique. For purposes of illustration, an example of training a naïve Bayesian classifier will be described below (see, e.g., Pattern Classification, R.O. Duda, et al., editors, John Wiley & Sons, 2nd edition, 2001; see also, The Elements of Statistical Learning - Data Mining, Inference, and Prediction, T. Hastie, et al., editors, Springer Science+Business Media, LLC, 2nd edition, 2009).

[0240] Since typically there are many more potential biomarker values than samples in a training set, care must be used to avoid over-fitting. Over-fitting occurs when a statistical model describes random error or noise instead of the underlying relationship. Over-fitting can be avoided in a variety of way, including, for example, by limiting the number of markers

used in developing the classifier, by assuming that the marker responses are independent of one another, by limiting the complexity of the underlying statistical model employed, and by ensuring that the underlying statistical model conforms to the data.

[0241] An illustrative example of the development of a diagnostic test using a set of biomarkers includes the application of a naïve Bayes classifier, a simple probabilistic classifier based on Bayes theorem with strict independent treatment of the biomarkers. Each biomarker is described by a class-dependent probability density function (pdf) for the measured RFU values or log RFU (relative fluorescence units) values in each class. The joint pdfs for the set of markers in one class is assumed to be the product of the individual class-dependent pdfs for each biomarker. Training a naïve Bayes classifier in this context amounts to assigning parameters ("parameterization") to characterize the class dependent pdfs. Any underlying model for the class-dependent pdfs may be used, but the model should generally conform to the data observed in the training set.

[0242] Specifically, the class-dependent probability of measuring a value x_i for biomarker i in the disease class is written as $p(x_i|d)$ and the overall naïve Bayes probability of observing n markers with values $\tilde{x} = (x_1, x_2, ...x_n)$ is written as $p(\tilde{x}|d) = \prod_{i=1}^{n} p(x_i|d)$ where the individual x_is are the measured biomarker levels in RFU or log RFU. The classification assignment for an unknown is facilitated by calculating the probability of being diseased $p(d|\tilde{x})$ having measured $\tilde{x}$ compared to the probability of being disease free (control) $p(c|\tilde{x})$ for the same measured values. The ratio of these probabilities is computed from the class-dependent pdfs by application of Bayes theorem, i.e., $\frac{p(d|\tilde{x})}{p(c|\tilde{x})} = \frac{p(\tilde{x}|d)p(d)}{p(\tilde{x}|c)(1-p(d))}$ where $p(d)$ is the prevalence of the disease in the population appropriate to the test. Taking the logarithm of both sides of this ratio and substituting the naïve Bayes class-dependent probabilities from above gives $ln\left(\frac{p(d|\tilde{x})}{p(c|\tilde{x})}\right) = \sum_{i=1}^{n} ln\left(\frac{p(x_i|d)}{p(x_i|c)}\right) + ln\left(\frac{p(d)}{1-p(d)}\right)$. This form is known as the log likelihood ratio and simply states that the log likelihood of being free of the particular disease versus having the disease and is primarily composed of the sum of individual log likelihood ratios of the n individual biomarkers. In its simplest form, an unknown sample (or, more particularly, the individual from whom the sample was obtained) is classified as being free of the disease if the above ratio is greater than zero and having the disease if the ratio is less than zero.

[0243] In one exemplary embodiment, the class-dependent biomarker pdfs $p(x_i|c)$ and $p(x_i|d)$ are assumed to be normal or log-normal distributions in the measured RFU values x_i, i.e.

$p(x_i|c) - \frac{1}{\sqrt{2\pi}\sigma_{c,i}} exp\left(-\frac{(x_i-\mu_{c,i})^2}{2\sigma_{c,i}^2}\right)$, with a similar expression for with and. Parameterization of the model requires estimation of two parameters for each class-dependent pdf, a mean μ and a variance σ^2, from the training data. This may be accomplished in a number of ways, including, for example, by maximum likelihood estimates, by least-squares, and by any other methods known to one skilled in the art. Substituting the normal distributions for and into the log-likelihood ratio defined above gives the following expression:

[0244] $$ln\left(\frac{p(d|\tilde{x})}{p(c|\tilde{x})}\right) = \sum_{i=1}^{n} ln\left(\frac{\sigma_{c,i}}{\sigma_{d_i}}\right) - \frac{1}{2}\sum_{i=1}^{n}\left[(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}})^2 - (\frac{x_i-\mu_{c,i}}{\sigma_{c,i}})^2\right] + ln\left(\frac{p(d)}{1-p(d)}\right)$$

[0245] Once a set of μs and σ^2s have been defined for each pdf in each class from the training data and the disease prevalence in the population is specified, the Bayes classifier is fully determined and may be used to classify unknown samples with measured values $\tilde{x}$.

[0246] The performance of the naïve Bayes classifier is dependent upon the number and quality of the biomarkers used to construct and train the classifier. A single biomarker will perform in accordance with its KS-distance (Kolmogorov-Smirnov), as defined in Example 3, below. If a classifier performance metric is defined as the area under the receiver operator characteristic curve (AUC), a perfect classifier will have a score of 1 and a random classifier, on average, will have a score of 0.5. The definition of the KS-distance between two sets A and B of sizes n and m is the value, $D_{n,m} = sup_x|F_{A,n}(x) - F_{B,m}(x)|$, which is the largest difference between two empirical cumulative distribution functions (cdf). The empirical cdf for a set A of n observations X_i is defined as, $F_{A,n}(x) = \frac{1}{n}\sum_{i-1}^{n} I_{X_i \leq x}$, where $I_{X_i \leq x}$ is the indicator function which is equal to 1 if $X_i < x$ and is otherwise equal to 0. By definition, this value is bounded between 0 and 1, where a KS-distance of 1 indicates that the emperical distributions do not overlap.

[0247] The addition of subsequent markers with good KS distances (>0.3, for example) will, in general, improve the classification performance if the subsequently added markers are independent of the first marker. Using the sensitivity plus specificity as a classifier score, it is straightforward to generate many high scoring classifiers with a variation of a greedy algorithm. (A greedy algorithm is any algorithm that follows the problem solving metaheuristic of making the locally optimal choice at each stage with the hope of finding the global optimum.)

[0248] The algorithm approach used here is described in detail in Example 4. Briefly, all single analyte classifiers are generated from a table of potential biomarkers and added to a list. Next, all possible additions of a second analyte to each of the stored single analyte classifiers

is then performed, saving a predetermined number of the best scoring pairs, say, for example, a thousand, on a new list. All possible three marker classifiers are explored using this new list of the best two-marker classifiers, again saving the best thousand of these. This process continues until the score either plateaus or begins to deteriorate as additional markers are added. Those high scoring classifiers that remain after convergence can be evaluated for the desired performance for an intended use. For example, in one diagnostic application, classifiers with a high sensitivity and modest specificity may be more desirable than modest sensitivity and high specificity. In another diagnostic application, classifiers with a high specificity and a modest sensitivity may be more desirable. The desired level of performance is generally selected based upon a trade-off that must be made between the number of false positives and false negatives that can each be tolerated for the particular diagnostic application. Such trade-offs generally depend on the medical consequences of an error, either false positive or false negative.

[0249] Various other techniques are known in the art and may be employed to generate many potential classifiers from a list of biomarkers using a naïve Bayes classifier. In one embodiment, what is referred to as a genetic algorithm can be used to combine different markers using the fitness score as defined above. Genetic algorithms are particularly well suited to exploring a large diverse population of potential classifiers. In another embodiment, so-called ant colony optimization can be used to generate sets of classifiers. Other strategies that are known in the art can also be employed, including, for example, other evolutionary strategies as well as simulated annealing and other stochastic search methods. Metaheuristic methods, such as, for example, harmony search may also be employed.

[0250] Exemplary embodiments use any number of the pancreatic cancer biomarkers listed in Table 1, Col. 2 in various combinations to produce diagnostic tests for detecting pancreatic cancer (see Example 2 for a detailed description of how these biomarkers were identified). In one embodiment, a method for diagnosing pancreatic cancer uses a naïve Bayes classification method in conjunction with any number of the pancreatic cancer biomarkers listed in Table 1, Col. 2. In an illustrative example (Example 3), the simplest test for detecting pancreatic cancer from a population of GI and normal controls can be constructed using a single biomarker, for example, CTSB which is differentially expressed in pancreatic cancer with a KS-distance of 0.52. Using the parameters, $\mu_{c,i}, \sigma_{c,i}, \mu_{d,i}$, and, $\sigma_{d,i}$ for CTSB from Table 16 and the equation

for the log-likelihood described above, a diagnostic test with an AUC of 0.79 can be derived, see Table 15. The ROC curve for this test is displayed in Figure 2.

[0251] Addition of biomarker C5a, for example, with a KS-distance of 0.40, significantly improves the classifier performance to an AUC of 0.85. Note that the score for a classifier constructed of two biomarkers is not a simple sum of the KS-distances; KS-distances are not additive when combining biomarkers and it takes many more weak markers to achieve the same level of performance as a strong marker. Adding a third marker, C5, for example, boosts the classifier performance to an AUC of 0.88. Adding additional biomarkers, such as, for example, CCL18, CSF1R, KLK7, ETHE1, C5-C6, KLK8, and VEGFA, produces a series of pancreatic cancer tests summarized in Table 15 and displayed as a series of ROC curves in Figure 3. The score of the classifiers as a function of the number of analytes used in classifier construction is displayed in Figure 4. The AUC of this exemplary ten-marker classifier is 0.91.

[0252] The markers listed in Table 1, Col. 2 can be combined in many ways to produce classifiers for diagnosing pancreatic cancer. In some embodiments, panels of biomarkers are comprised of different numbers of analytes depending on a specific diagnostic performance criterion that is selected. For example, certain combinations of biomarkers will produce tests that are more sensitive (or more specific) than other combinations.

[0253] Once a panel is defined to include a particular set of biomarkers from Table 1, Col. 2 and a classifier is constructed from a set of training data, the definition of the diagnostic test is complete. In one embodiment, the procedure used to classify an unknown sample is outlined in Figure 1A. In another embodiment the procedure used to classify an unknown sample is outlined in Figure1B. The biological sample is appropriately diluted and then run in one or more assays to produce the relevant quantitative biomarker levels used for classification. The measured biomarker levels are used as input for the classification method that outputs a classification and an optional score for the sample that reflects the confidence of the class assignment.

[0254] Table 1 identifies 65 biomarkers that are useful for diagnosing pancreatic cancer. This is a surprisingly larger number than expected when compared to what is typically found during biomarker discovery efforts and may be attributable to the scale of the described study, which encompassed over 800 proteins measured in hundreds of individual samples, in some cases at concentrations in the low femtomolar range. Presumably, the large number of discovered

biomarkers reflects the diverse biochemical pathways implicated in both tumor biology and the body's response to the tumor's presence; each pathway and process involves many proteins. The results show that no single protein of a small group of proteins is uniquely informative about such complex processes; rather, that multiple proteins are involved in relevant processes, such as apoptosis or extracellular matrix repair, for example.

[0255] Given the numerous biomarkers identified during the described study, one would expect to be able to derive large numbers of high-performing classifiers that can be used in various diagnostic methods. To test this notion, tens of thousands of classifiers were evaluated using the biomarkers in Table 1. As described in Example 4, many subsets of the biomarkers presented in Table 1 can be combined to generate useful classifiers. By way of example, descriptions are provided for classifiers containing 1, 2, and 3 biomarkers for detection of pancreatic cancer. As described in Example 4, all classifiers that were built using the biomarkers in Table 1 perform distinctly better than classifiers that were built using "non-markers".

[0256] The performance of classifiers obtained by randomly excluding some of the markers in Table 1, which resulted in smaller subsets from which to build the classifiers, was also tested. As described in Example 4, Part 3, the classifiers that were built from random subsets of the markers in Table 1 performed similarly to optimal classifiers that were built using the full list of markers in Table 1.

[0257] The performance of ten-marker classifiers obtained by excluding the "best" individual markers from the ten-marker aggregation was also tested. As described in Example 4, Part 3, classifiers constructed without the "best" markers in Table 1 also performed well. Many subsets of the biomarkers listed in Table 1 performed close to optimally, even after removing the top 15 of the markers listed in the Table. This implies that the performance characteristics of any particular classifier are likely not due to some small core group of biomarkers and that the disease process likely impacts numerous biochemical pathways, which alters the expression level of many proteins.

[0258] The results from Example 4 suggest certain possible conclusions: First, the identification of a large number of biomarkers enables their aggregation into a vast number of classifiers that offer similarly high performance. Second, classifiers can be constructed such that particular biomarkers may be substituted for other biomarkers in a manner that reflects the redundancies that undoubtedly pervade the complexities of the underlying disease

processes. That is to say, the information about the disease contributed by any individual biomarker identified in Table 1 overlaps with the information contributed by other biomarkers, such that it may be that no particular biomarker or small group of biomarkers in Table 1 must be included in any classifier.

[0259] Exemplary embodiments use naïve Bayes classifiers constructed from the data in Table 16 to classify an unknown sample. The procedure is outlined in Figures 1A and 1B. In one embodiment, the biological sample is optionally diluted and run in a multiplexed aptamer assay. The data from the assay are normalized and calibrated as outlined in Example 3, and the resulting biomarker levels are used as input to a Bayes classification scheme. The log-likelihood ratio is computed for each measured biomarker individually and then summed to produce a final classification score, which is also referred to as a diagnostic score. The resulting assignment as well as the overall classification score can be reported. Optionally, the individual log-likelihood risk factors computed for each biomarker level can be reported as well. The details of the classification score calculation are presented in Example 3.

Kits

[0260] Any combination of the biomarkers of Table 1, Col. 2 (as well as additional biomedical information) can be detected using a suitable kit, such as for use in performing the methods disclosed herein. Furthermore, any kit can contain one or more detectable labels as described herein, such as a fluorescent moiety, etc.

[0261] In one embodiment, a kit includes (a) one or more capture reagents (such as, for example, at least one aptamer or antibody) for detecting one or more biomarkers in a biological sample, wherein the biomarkers include any of the biomarkers set forth in Table 1, Col. 2, and optionally (b) one or more software or computer program products for classifying the individual from whom the biological sample was obtained as either having or not having pancreatic cancer or for determining the likelihood that the individual has pancreatic cancer, as further described herein. Alternatively, rather than one or more computer program products, one or more instructions for manually performing the above steps by a human can be provided.

[0262] The combination of a solid support with a corresponding capture reagent and a signal generating material is referred to herein as a "detection device" or "kit". The kit can also include instructions for using the devices and reagents, handling the sample, and analyzing

the data. Further the kit may be used with a computer system or software to analyze and report the result of the analysis of the biological sample.

[0263] The kits can also contain one or more reagents (e.g., solubilization buffers, detergents, washes, or buffers) for processing a biological sample. Any of the kits described herein can also include, e.g., buffers, blocking agents, mass spectrometry matrix materials, antibody capture agents, positive control samples, negative control samples, software and information such as protocols, guidance and reference data.

[0264] In one aspect, the invention provides kits for the analysis of pancreatic cancer status. The kits include PCR primers for one or more biomarkers selected from Table 1, Col. 2. The kit may further include instructions for use and correlation of the biomarkers with pancreatic cancer. The kit may also include a DNA array containing the complement of one or more of the biomarkers selected from Table 1, Col. 2, reagents, and/or enzymes for amplifying or isolating sample DNA. The kits may include reagents for real-time PCR, for example, TaqMan probes and/or primers, and enzymes.

[0265] For example, a kit can comprise (a) reagents comprising at least capture reagent for quantifying one or more biomarkers in a test sample, wherein said biomarkers comprise the biomarkers set forth in Table 1, Col. 2, or any other biomarkers or biomarkers panels described herein, and optionally (b) one or more algorithms or computer programs for performing the steps of comparing the amount of each biomarker quantified in the test sample to one or more predetermined cutoffs and assigning a score for each biomarker quantified based on said comparison, combining the assigned scores for each biomarker quantified to obtain a total score, comparing the total score with a predetermined score, and using said comparison to determine whether an individual has pancreatic cancer. Alternatively, rather than one or more algorithms or computer programs, one or more instructions for manually performing the above steps by a human can be provided.

Computer Methods and Software

[0266] Once a biomarker or biomarker panel is selected, a method for diagnosing an individual can comprise the following: 1) collect or otherwise obtain a biological sample; 2) perform an analytical method to detect and measure the biomarker or biomarkers in the panel in the biological sample; 3) perform any data normalization or standardization required for the

method used to collect biomarker values; 4) calculate the marker score; 5) combine the marker scores to obtain a total diagnostic score; and 6) report the individual's diagnostic score. In this approach, the diagnostic score may be a single number determined from the sum of all the marker calculations that is compared to a preset threshold value that is an indication of the presence or absence of disease. Or the diagnostic score may be a series of bars that each represent a biomarker value and the pattern of the responses may be compared to a pre-set pattern for determination of the presence or absence of disease.

[0267] At least some embodiments of the methods described herein can be implemented with the use of a computer. An example of a computer system 100 is shown in Figure 6. With reference to Figure 6, system 100 is shown comprised of hardware elements that are electrically coupled via bus 108, including a processor 101, input device 102, output device 103, storage device 104, computer-readable storage media reader 105a, communications system 106 processing acceleration (e.g., DSP or special-purpose processors) 107 and memory 109. Computer-readable storage media reader 105a is further coupled to computer-readable storage media 105b, the combination comprehensively representing remote, local, fixed and/or removable storage devices plus storage media, memory, etc. for temporarily and/or more permanently containing computer-readable information, which can include storage device 104, memory 109 and/or any other such accessible system 100 resource. System 100 also comprises software elements (shown as being currently located within working memory 191) including an operating system 192 and other code 193, such as programs, data and the like.

[0268] With respect to Figure 6, system 100 has extensive flexibility and configurability. Thus, for example, a single architecture might be utilized to implement one or more servers that can be further configured in accordance with currently desirable protocols, protocol variations, extensions, etc. However, it will be apparent to those skilled in the art that embodiments may well be utilized in accordance with more specific application requirements. For example, one or more system elements might be implemented as sub-elements within a system 100 component (e.g., within communications system 106). Customized hardware might also be utilized and/or particular elements might be implemented in hardware, software or both. Further, while connection to other computing devices such as network input/output devices (not shown) may be employed, it is to be understood that wired, wireless, modem, and/or other connection or connections to other computing devices might also be utilized.

[0269] In one aspect, the system can comprise a database containing features of biomarkers characteristic of pancreatic cancer. The biomarker data (or biomarker information) can be utilized as an input to the computer for use as part of a computer implemented method. The biomarker data can include the data as described herein.

[0270] In one aspect, the system further comprises one or more devices for providing input data to the one or more processors.

[0271] The system further comprises a memory for storing a data set of ranked data elements.

[0272] In another aspect, the device for providing input data comprises a detector for detecting the characteristic of the data element, e.g., such as a mass spectrometer or gene chip reader.

[0273] The system additionally may comprise a database management system. User requests or queries can be formatted in an appropriate language understood by the database management system that processes the query to extract the relevant information from the database of training sets.

[0274] The system may be connectable to a network to which a network server and one or more clients are connected. The network may be a local area network (LAN) or a wide area network (WAN), as is known in the art. Preferably, the server includes the hardware necessary for running computer program products (e.g., software) to access database data for processing user requests.

[0275] The system may include an operating system (e.g., UNIX or Linux) for executing instructions from a database management system. In one aspect, the operating system can operate on a global communications network, such as the internet, and utilize a global communications network server to connect to such a network.

[0276] The system may include one or more devices that comprise a graphical display interface comprising interface elements such as buttons, pull down menus, scroll bars, fields for entering text, and the like as are routinely found in graphical user interfaces known in the art. Requests entered on a user interface can be transmitted to an application program in the system for formatting to search for relevant information in one or more of the system databases. Requests or queries entered by a user may be constructed in any suitable database language.

[0277] The graphical user interface may be generated by a graphical user interface code as part of the operating system and can be used to input data and/or to display inputted data. The result of processed data can be displayed in the interface, printed on a printer in communication with the system, saved in a memory device, and/or transmitted over the network or can be provided in the form of the computer readable medium.

[0278] The system can be in communication with an input device for providing data regarding data elements to the system (e.g., expression values). In one aspect, the input device can include a gene expression profiling system including, e.g., a mass spectrometer, gene chip or array reader, and the like.

[0279] The methods and apparatus for analyzing pancreatic cancer biomarker information according to various embodiments may be implemented in any suitable manner, for example, using a computer program operating on a computer system. A conventional computer system comprising a processor and a random access memory, such as a remotely-accessible application server, network server, personal computer or workstation may be used. Additional computer system components may include memory devices or information storage systems, such as a mass storage system and a user interface, for example a conventional monitor, keyboard and tracking device. The computer system may be a stand-alone system or part of a network of computers including a server and one or more databases.

[0280] The pancreatic cancer biomarker analysis system can provide functions and operations to complete data analysis, such as data gathering, processing, analysis, reporting and/or diagnosis. For example, in one embodiment, the computer system can execute the computer program that may receive, store, search, analyze, and report information relating to the pancreatic cancer biomarkers. The computer program may comprise multiple modules performing various functions or operations, such as a processing module for processing raw data and generating supplemental data and an analysis module for analyzing raw data and supplemental data to generate a pancreatic cancer status and/or diagnosis. Diagnosing pancreatic cancer status may comprise generating or collecting any other information, including additional biomedical information, regarding the condition of the individual relative to the disease, identifying whether further tests may be desirable, or otherwise evaluating the health status of the individual.

[0281] Referring now to Figure 7, an example of a method of utilizing a computer in ac-

cordance with principles of a disclosed embodiment can be seen. In Figure 7, a flowchart 3000 is shown. In block 3004, biomarker information can be retrieved for an individual. The biomarker information can be retrieved from a computer database, for example, after testing of the individual's biological sample is performed. The biomarker information can comprise biomarker values that each correspond to one of at least N biomarkers selected from a group consisting of the biomarkers provided in Table 1, Col. 2, wherein N = 2-65. In block 3008, a computer can be utilized to classify each of the biomarker values. And, in block 3012, a determination can be made as to the likelihood that an individual has pancreatic cancer based upon a plurality of classifications. The indication can be output to a display or other indicating device so that it is viewable by a person. Thus, for example, it can be displayed on a display screen of a computer or other output device.

[0282] Referring now to Figure 8, an alternative method of utilizing a computer in accordance with another embodiment can be illustrated via flowchart 3200. In block 3204, a computer can be utilized to retrieve biomarker information for an individual. The biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers provided in Table 1, Col. 2. In block 3208, a classification of the biomarker value can be performed with the computer. And, in block 3212, an indication can be made as to the likelihood that the individual has pancreatic cancer based upon the classification. The indication can be output to a display or other indicating device so that it is viewable by a person. Thus, for example, it can be displayed on a display screen of a computer or other output device.

[0283] Some embodiments described herein can be implemented so as to include a computer program product. A computer program product may include a computer readable medium having computer readable program code embodied in the medium for causing an application program to execute on a computer with a database.

[0284] As used herein, a "computer program product" refers to an organized set of instructions in the form of natural or programming language statements that are contained on a physical media of any nature (e.g., written, electronic, magnetic, optical or otherwise) and that may be used with a computer or other automated data processing system. Such programming language statements, when executed by a computer or data processing system, cause the computer or data processing system to act in accordance with the particular content of

the statements. Computer program products include without limitation: programs in source and object code and/or test or data libraries embedded in a computer readable medium. Furthermore, the computer program product that enables a computer system or data processing equipment device to act in pre-selected ways may be provided in a number of forms, including, but not limited to, original source code, assembly code, object code, machine language, encrypted or compressed versions of the foregoing and any and all equivalents.

[0285] In one aspect, a computer program product is provided for indicating a likelihood of pancreatic cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers in the biological sample selected from the group of biomarkers provided in Table 1, Col. 2, wherein N = 2-65; and code that executes a classification method that indicates a pancreatic cancer status of the individual as a function of the biomarker values.

[0286] In still another aspect, a computer program product is provided for indicating a likelihood of pancreatic cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers provided in Table 1, Col. 2; and code that executes a classification method that indicates a pancreatic cancer status of the individual as a function of the biomarker value.

[0287] While various embodiments have been described as methods or apparatuses, it should be understood that embodiments can be implemented through code coupled with a computer, e.g., code resident on a computer or accessible by the computer. For example, software and databases could be utilized to implement many of the methods discussed above. Thus, in addition to embodiments accomplished by hardware, it is also noted that these embodiments can be accomplished through the use of an article of manufacture comprised of a computer usable medium having a computer readable program code embodied therein, which causes the enablement of the functions disclosed in this description. Therefore, it is desired that embodiments also be considered protected by this patent in their program code means as

well. Furthermore, the embodiments may be embodied as code stored in a computer-readable memory of virtually any kind including, without limitation, RAM, ROM, magnetic media, optical media, or magneto-optical media. Even more generally, the embodiments could be implemented in software, or in hardware, or any combination thereof including, but not limited to, software running on a general purpose processor, microcode, PLAs, or ASICs.

[0288] It is also envisioned that embodiments could be accomplished as computer signals embodied in a carrier wave, as well as signals (e.g., electrical and optical) propagated through a transmission medium. Thus, the various types of information discussed above could be formatted in a structure, such as a data structure, and transmitted as an electrical signal through a transmission medium or stored on a computer readable medium.

[0289] It is also noted that many of the structures, materials, and acts recited herein can be recited as means for performing a function or step for performing a function. Therefore, it should be understood that such language is entitled to cover all such structures, materials, or acts disclosed within this specification and their equivalents.

[0290] The biomarker identification process, the utilization of the biomarkers disclosed herein, and the various methods for determining biomarker values are described in detail above with respect to pancreatic cancer. However, the application of the process, the use of identified biomarkers, and the methods for determining biomarker values are fully applicable to other specific types of cancer, to cancer generally, to any other disease or medical condition, or to the identification of individuals who may or may not be benefited by an ancillary medical treatment. Except when referring to specific results related to pancreatic cancer, as is clear from the context, references herein to pancreatic cancer may be understood to include other types of cancer, cancer generally, or any other disease or medical condition.

EXAMPLES

[0291] The following examples are provided for illustrative purposes only and are not intended to limit the scope of the application as defined by the appended claims. All examples described herein were carried out using standard techniques, which are well known and routine to those of skill in the art. Routine molecular biology techniques described in the following

examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (2001).

Example 1. Multiplexed Aptamer Analysis of Samples

[0292] This example describes the multiplex aptamer assay used to analyze the samples and controls for the identification of the biomarkers set forth in Table 1, Col. 2 (see Figure 9) and the identification of the cancer biomarkers set forth in Table 19. For the pancreatic cancer, lung cancer, and mesothelioma studies, the multiplexed analysis utilized 823 aptamers, each unique to a specific target.

[0293] In this method, pipette tips were changed for each solution addition.

[0294] Also, unless otherwise indicated, most solution transfers and wash additions used the 96-well head of a Beckman Biomek FxP. Method steps manually pipetted used a twelve channel P200 Pipetteman (Rainin Instruments, LLC, Oakland, CA), unless otherwise indicated. A custom buffer referred to as SB17 was prepared in-house, comprising 40 mM HEPES, 100 mM NaCl, 5 mM KCl, 5 mM MgCl2, 1 mM EDTA at pH 7.5. A custom buffer referred to as SB18 was prepared in-house, comprising 40 mM HEPES, 100 mM NaCl, 5 mM KCl, 5 mM MgCl2 at pH 7.5. All steps were performed at room temperature unless otherwise indicated.

[0295] 1. Preparation of Aptamer Stock Solution

[0296] For aptamers without a photo-cleavable biotin linker, custom stock aptamer solutions for 10%, 1% and 0.03% plasma were prepared at 8x concentration in 1x SB17, 0.05% Tween-20 with appropriate photo-cleavable, biotinylated primers, where the resultant primer concentration was 3 times the relevant aptamer concentration. The primers hybridized to all or part of the corresponding aptamer.

[0297] Each of the 3, 8x aptamer solutions were diluted separately 1:4 into 1xSB17, 0.05% Tween-20 (1500 μL of 8x stock into 4500 μL of 1xSB17, 0.05% Tween-20) to achieve a 2x concentration. Each diluted aptamer master mix was then split, 1500 μL each, into 4 2 mL screw cap tubes and brought to 95°C for 5 minutes, followed by a 37°C incubation for 15 minutes. After incubation the 4 2 mL tubes corresponding to a particular aptamer master mix were combined into a reagent trough, and 55 μL of a 2x aptamer mix (for all three mixes) was manually pipetted into a 96-well Hybaid plate and the plate foil sealed. The final result

was 3, 96-well, foil-sealed Hybaid plates. The individual aptamer concentration was 0.5 nM.

[0298] 2. Assay Sample Preparation

[0299] Frozen aliquots of 100% plasma, stored at -80°C, were placed in 25°C water bath for 10 minutes. Thawed samples were placed on ice, gently vortexed (set on 4) for 8 seconds and then replaced on ice.

[0300] A 20% sample solution was prepared by transferring 16 μL of sample using a 50 μL 8-channel spanning pipettor into 96-well Hybaid plates, each well containing 64 μL of the appropriate sample diluent at 4°C (0.8x SB18, 0.05% Tween-20, 2 μM Z-block_2, 0.6 mM MgCl2 for plasma). This plate was stored on ice until the next sample dilution steps were initiated.

[0301] To commence sample and aptamer equilibration, the 20% sample plate was briefly centrifuged and placed on the Beckman FX where it was mixed by pipetting up and down with the 96-well pipettor. A 2% sample was then prepared by diluting 10 μL of the 20% sample into 90 μL of 1xSB17, 0.05% Tween-20. Next, dilution of 6 μL of the resultant 2% sample into 194 μL of 1xSB17, 0.05% Tween-20 made a 0.06% sample plate. Dilutions were done on the Beckman Biomek FxP. After each transfer, the solutions were mixed by pipetting up and down. The 3 sample dilution plates were then transferred to their respective aptamer solutions by adding 55 μL of the sample to 55 μL of the appropriate 2x aptamer mix. The sample and aptamer solutions were mixed on the robot by pipetting up and down.

[0302] 3. Sample Equilibration binding

[0303] The sample/aptamer plates were foil sealed and placed into a 37°C incubator for 3.5 hours before proceeding to the Catch 1 step.

[0304] 4. Preparation of Catch 2 bead plate

[0305] An 5.5 mL aliquot of MyOne (Invitrogen Corp., Carlsbad, CA) Streptavidin C1 beads (10 mg/mL) was washed 2 times with equal volumes of 20 mM NaOH (5 minute incubation for each wash), 3 times with equal volumes of 1x SB17, 0.05% Tween-20 and resuspended in 5.5 mL 1x SB17, 0.05% Tween-20. Using a 12-span multichannel pipettor, 50 μL of this solution was manually pipetted into each well of a 96-well Hybaid plate. The plate was then covered with foil and stored at 4°C for use in the assay.

[0306] 5. Preparation of Catch 1 bead plates

[0307] Three 0.45 μm Millipore HV plates (Durapore membrane, Cat# MAHVN4550) were

equilibrated with 100 μL of 1x SB17, 0.05% Tween-20 for at least 10 minutes. The equilibration buffer was then filtered through the plate and 133.3 μL of a 7.5% streptavidin-agarose bead slurry (in 1x SB17, 0.05% Tween-20) was added into each well. To keep the streptavidin-agarose beads suspended while transferring them into the filter plate, the bead solution was manually mixed with a 200 μL, 12-channel pipettor, 15 times. After the beads were distributed across the 3 filter plates, a vacuum was applied to remove the bead supernatant. Finally, the beads were washed in the filter plates with 200 μL 1x SB17, 0.05% Tween-20 and then resuspended in 200 μL 1x SB17, 0.05% Tween-20. The bottoms of the filter plates were blotted and the plates stored for use in the assay.

[0308] 6. Loading the Cytomat

[0309] The cytomat was loaded with all tips, plates, all reagents in troughs (except NHS-biotin reagent which was prepared fresh right before addition to the plates), 3 prepared Catch 1 filter plates and 1 prepared MyOne plate.

[0310] 7. Catch 1

[0311] After a 3.5 hour equilibration time, the sample/aptamer plates were removed from the incubator, centrifuged for about 1 minute, foil removed, and placed on the deck of the Beckman Biomek FxP. The Beckman Biomek FxP program was initiated. All subsequent steps in Catch 1 were performed by the Beckman Biomek FxP robot unless otherwise noted. Within the program, the vacuum was applied to the Catch 1 filter plates to remove the bead supernatant. One hundred microlitres of each of the 10%, 1% and 0.03% equilibration binding reactions were added to their respective Catch 1 filtration plates, and each plate was mixed using an on-deck orbital shaker at 800 rpm for 10 minutes.

[0312] Unbound solution was removed via vacuum filtration. The Catch 1 beads were washed with 190 μL of 100 μM biotin in 1x SB17, 0.05% Tween-20 followed by 190 μL of 1x SB17, 0.05% Tween-20 by dispensing the solution and immediately drawing a vacuum to filter the solution through the plate.

[0313] Next, 190 μL 1x SB17, 0.05% Tween-20 was added to the Catch 1 plates. Plates were blotted to remove droplets using an on-deck blot station and then incubated with orbital shakers at 800 rpm for 10 minutes at 25°C.

[0314] The robot removed this wash via vacuum filtration and blotted the bottom of the filter plate to remove droplets using the on-deck blot station.

[0315] 8. Tagging

[0316] A NHS-PEO4-biotin aliquot was thawed at 37°C for 6 minutes and then diluted 1:100 with tagging buffer (SB17 at pH=7.25 0.05% Tween-20). The NHS-PEO4-biotin reagent was dissolved at 100 mM concentration in anhydrous DMSO and had been stored frozen at -20°C. Upon a robot prompt, the diluted NHS-PEO4-biotin reagent was manually added to an on-deck trough and the robot program was manually re-initiated to dispense 100 μL of the NHS-PEO4-biotin into each well of each Catch 1 filter plate. This solution was allowed to incubate with Catch 1 beads shaking at 800 rpm for 5 minutes on the orbital shakers.

[0317] 9. Kinetic Challenge and Photo-cleavage

[0318] The tagging reaction was quenched by the addition of 150 μL of 20 mM glycine in 1x SB17, 0.05% Tween-20 to the Catch 1 plates while still containing the NHS tag. The plates were then incubated for 1 minute on orbital shakers at 800 rpm. The NHS-tag/glycine solution was removed via vacuum filtration. Next, 190 μL 20 mM glycine (1x SB17, 0.05% Tween-20) was added to each plate and incubated for 1 minute on orbital shakers at 800 rpm before removal by vacuum filtration.

[0319] 190 μL of 1x SB17, 0.05% Tween-20 was added to each plate and removed by vacuum filtration.

[0320] The wells of the Catch 1 plates were subsequently washed three times by adding 190 μL 1x SB17, 0.05% Tween-20, placing the plates on orbital shakers for 1 minute at 800 rpm followed by vacuum filtration. After the last wash the plates were placed on top of a 1 mL deep-well plate and removed from the deck. The Catch 1 plates were centrifuged at 1000 rpm for 1 minute to remove as much extraneous volume from the agarose beads before elution as possible.

[0321] The plates were placed back onto the Beckman Biomek FxP and 85 μL of 10 mM DxSO4 in 1x SB17, 0.05% Tween-20 was added to each well of the filter plates.

[0322] The filter plates were removed from the deck, placed onto a Variomag Thermoshaker (Thermo Fisher Scientific, Inc., Waltham, MA) under the BlackRay (Ted Pella, Inc., Redding, CA) light sources, and irradiated for 10 minutes while shaking at 800 rpm.

[0323] The photocleaved solutions were sequentially eluted from each Catch 1 plate into a common deep well plate by first placing the 10% Catch 1 filter plate on top of a 1 mL deep-well plate and centrifuging at 1000 rpm for 1 minute. The 1% and 0.03% Catch 1 plates were then

sequentially centrifuged into the same deep well plate.

[0324] 10. Catch 2 bead capture

[0325] The 1 mL deep well block containing the combined eluates of Catch 1 was placed on the deck of the Beckman Biomek FxP for Catch 2.

[0326] The robot transferred all of the photo-cleaved eluate from the 1 mL deep-well plate onto the Hybaid plate containing the previously prepared Catch 2 MyOne magnetic beads (after removal of the MyOne buffer via magnetic separation).

[0327] The solution was incubated while shaking at 1350 rpm for 5 minutes at 25°C on a Variomag Thermoshaker (Thermo Fisher Scientific, Inc., Waltham, MA).

[0328] The robot transferred the plate to the on deck magnetic separator station. The plate was incubated on the magnet for 90 seconds before removal and discarding of the supernatant.

[0329] 11. 37°C 30% glycerol washes

[0330] The Catch 2 plate was moved to the on-deck thermal shaker and 75 μL of 1x SB17, 0.05% Tween-20 was transferred to each well. The plate was mixed for 1 minute at 1350 rpm and 37°C to resuspend and warm the beads. To each well of the Catch 2 plate, 75 μL of 60% glycerol at 37°C was transferred and the plate continued to mix for another minute at 1350 rpm and 37°C. The robot transferred the plate to the 37°C magnetic separator where it was incubated on the magnet for 2 minutes and then the robot removed and discarded the supernatant. These washes were repeated two more times.

[0331] After removal of the third 30% glycerol wash from the Catch 2 beads, 150 μL of 1x SB17, 0.05% Tween-20 was added to each well and incubated at 37°C, shaking at 1350 rpm for 1 minute, before removal by magnetic separation on the 37°C magnet.

[0332] The Catch 2 beads were washed a final time using 150 μL 1x SB19, 0.05% Tween-20 with incubation for 1 minute while shaking at 1350 rpm at 25°C prior to magnetic separation.

[0333] 12. Catch 2 Bead Elution and Neutralization

[0334] The aptamers were eluted from Catch 2 beads by adding 105 μL of 100 mM CAPSO with 1 M NaCl, 0.05% Tween-20 to each well. The beads were incubated with this solution with shaking at 1350 rpm for 5 minutes.

[0335] The Catch 2 plate was then placed onto the magnetic separator for 90 seconds prior to transferring 90 μL of the eluate to a new 96-well plate containing 10 μL of 500 mM HCl, 500 mM HEPES, 0.05% Tween-20 in each well. After transfer, the solution was mixed robotically

by pipetting 90 μL up and down five times.

[0336] 13. Hybridization

[0337] The Beckman Biomek FxP transferred 20 μL of the neutralized Catch 2 eluate to a fresh Hybaid plate, and 5 μL of 10x Agilent Block, containing a 10x spike of hybridization controls, was added to each well. Next, 25 μL of 2x Agilent Hybridization buffer was manually pipetted to the each well of the plate containing the neutralized samples and blocking buffer and the solution was mixed by manually pipetting 25 μL up and down 15 times slowly to avoid extensive bubble formation. The plate was spun at 1000 rpm for 1 minute.

[0338] A gasket slide was placed into an Agilent hybridization chamber and 40 μL of each of the samples containing hybridization and blocking solution was manually pipetted into each gasket. An 8-channel variable spanning pipettor was used in a manner intended to minimize bubble formation. Custom Agilent microarray slides (Agilent Technologies, Inc., Santa Clara, CA),with their Number Barcode facing up, were then slowly lowered onto the gasket slides (see Agilent manual for detailed description).

[0339] The top of the hybridization chambers were placed onto the slide/backing sandwich and clamping brackets slid over the whole assembly. These assemblies were tightly clamped by turning the screws securely.

[0340] Each slide/backing slide sandwich was visually inspected to assure the solution bubble could move freely within the sample. If the bubble did not move freely the hybridization chamber assembly was gently tapped to disengage bubbles lodged near the gasket.

[0341] The assembled hybridization chambers were incubated in an Agilent hybridization oven for 19 hours at 60°C rotating at 20 rpm.

[0342] 14. Post Hybridization Washing

[0343] Approximately 400 mL Agilent Wash Buffer 1 was placed into each of two separate glass staining dishes. One of the staining dishes was placed on a magnetic stir plate and a slide rack and stir bar were placed into the buffer.

[0344] A staining dish for Agilent Wash 2 was prepared by placing a stir bar into an empty glass staining dish.

[0345] A fourth glass staining dish was set aside for the final acetonitrile wash.

[0346] Each of six hybridization chambers was disassembled. One-by-one, the slide/backing sandwich was removed from its hybridization chamber and submerged into the staining dish

containing Wash 1. The slide/backing sandwich was pried apart using a pair of tweezers, while still submerging the microarray slide. The slide was quickly transferred into the slide rack in the Wash 1 staining dish on the magnetic stir plate.

[0347] The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0348] When one minute was remaining for Wash 1, Wash Buffer 2 pre-warmed to 37°C in an incubator was added to the second prepared staining dish. The slide rack was quickly transferred to Wash Buffer 2 and any excess buffer on the bottom of the rack was removed by scraping it on the top of the stain dish. The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0349] The slide rack was slowly pulled out of Wash 2, taking approximately 15 seconds to remove the slides from the solution.

[0350] With one minute remaining in Wash 2 acetonitrile (ACN) was added to the fourth staining dish. The slide rack was transferred to the acetonitrile stain dish. The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0351] The slide rack was slowly pulled out of the ACN stain dish and placed on an absorbent towel. The bottom edges of the slides were quickly dried and the slide was placed into a clean slide box.

[0352] 15. Microarray Imaging

[0353] The microarray slides were placed into Agilent scanner slide holders and loaded into the Agilent Microarray scanner according to the manufacturers instructions.

[0354] The slides were imaged in the Cy3-channel at 5 μm resolution at the100% PMT setting and the XRD option enabled at 0.05. The resulting tiff images were processed using Agilent feature extraction software version 10.5.

Example 2. Biomarker Identification

[0355] The identification of potential pancreatic cancer biomarkers was performed for diagnosis of pancreatic cancer in asymptomatic individuals and symptomatic individuals with acute or chronic pancreatitis (or both), pancreatic obstruction, GERD, gallstones, or abnormal imaging later found to be benign, collectively the GI and normal controls. Enrollment

criteria for this study were age 18 or older, able to give informed consent, and plasma sample and documented diagnosis of pancreatic cancer or benign findings. For cases, blood samples collected prior to treatment or surgery and subsequently diagnosed with pancreatic cancer. Exclusion criteria included prior diagnosis or treatment of cancer (excluding squamous cell carcinoma of the skin) within 5 years of the blood draw. Plasma samples were collected from 2 different sites and included 143 pancreatic cancer samples and 115 control group samples. The multiplexed aptamer affinity assay as described in Example 1 was used to measure and report the RFU value for 823 analytes in each of these 258 samples. Since the plasma samples were obtained from 2 independent studies and sites under similar protocols, an examination of site differences prior to the analysis for biomarkers discovery was performed.

[0356] Each of the case and control populations were separately compared by generating class-dependent cumulative distribution functions (cdfs) for each of the 823 analytes. The KS-distance (Kolmogorov-Smirnov statistic) between values from two sets of samples is a non parametric measurement of the extent to which the empirical distribution of the values from one set (Set A) differs from the distribution of values from the other set (Set B). For any value of a threshold T some proportion of the values from Set A will be less than T, and some proportion of the values from Set B will be less than T. The KS-distance measures the maximum (unsigned) difference between the proportion of the values from the two sets for any choice of T.

[0357] This set of potential biomarkers can be used to build classifiers that assign samples to either a control or disease group. In fact, many such classifiers were produced from these sets of biomarkers and the frequency with which any biomarker was used in good scoring classifiers determined. Those biomarkers that occurred most frequently among the top scoring classifiers were the most useful for creating a diagnostic test. In this example, Bayesian classifiers were used to explore the classification space but many other supervised learning techniques may be employed for this purpose. The scoring fitness of any individual classifier was gauged by the area under the receiver operating characterisic curve (AUC of the ROC) of the classifier at the Bayesian surface assuming a disease prevalence of 0.5. This scoring metric varies from zero to one, with one being an error-free classifier. The details of constructing a Baycsian classifier from biomarker population measurements are described in Example 3.

[0358] Using the 65 analytes in Table 1, a total of 973 10-analyte classifiers were found

with an AUC of 0.90 for diagnosing pancreatic cancer from the control group. From this set of classifiers, a total of 11 biomarkers were found to be present in 30% or more of the high scoring classifiers. Table 13 provides a list of these potential biomarkers and Figure 10 is a frequency plot for the identified biomarkers.

Example 3. Naïve Bayesian Classification for Pancreatic Cancer

[0359] From the list of biomarkers identified as useful for discriminating between pancreatic cancer and controls, a panel of ten biomarkers was selected and a naïve Bayes classifier was constructed, see Table 16. The class-dependent probability density functions (pdfs), $p(x_i|c)$ and $p(x_i|d)$, where x_i is the log of the measured RFU value for biomarker i, and c and d refer to the control and disease populations, were modeled as log-normal distribution functions characterized by a mean μ and variance σ^2. The parameters for pdfs of the ten biomarkers are listed in Table 16 and an example of the raw data along with the model fit to a normal pdf is displayed in Figure 5. The underlying assumption appears to fit the data quite well as evidenced by Figure 5.

[0360] The naïve Bayes classification for such a model is given by the following equation, where $p(d)$ is the prevalence of the disease in the population,
$ln\left(\frac{p(d|\tilde{x})}{p(c|\tilde{x})}\right) = \sum_{i=1}^{n} ln\left(\frac{\sigma_{c,i}}{\sigma_{d_i}}\right) - \frac{1}{2}\sum_{i=1}^{n}\left[(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}})^2 - (\frac{x_i-\mu_{c,i}}{\sigma_{c,i}})^2\right] + ln\left(\frac{p(d)}{1-p(d)}\right)$, appropriate to the test and $n = 10$. Each of the terms in the summation is a log-likelihood ratio for an individual marker and the total log-likelihood ratio of a sample $\tilde{x}$ being free from the disease of interest (i.e. in this case, pancreatic cancer) versus having the disease is simply the sum of these individual terms plus a term that accounts for the prevalence of the disease. For simplicity, we assume $p(d) = 0.5$ so that $ln\left(\frac{p(d)}{1-p(d)}\right) = 0$.

[0361] Given an unknown sample measurement in log(RFU) for each of the ten biomarkers of 6.3, 9.3, 8.7, 10.8, 7.4, 11.4, 11.7, 9.0, 8.0, 7.3, the calculation of the classification is detailed in Table 16. The individual components comprising the log likelihood ratio for disease versus control class are tabulated and can be computed from the parameters in Table 16 and the values of $\tilde{x}$. The sum of the individual log likelihood ratios is -3.044, or a likelihood of being free from the disease versus having the disease of 21, where likelihood $e^{3.044} = 21$. The first 3 biomarker values have likelihoods more consistent with the disease group (log likelihood > 0) but the remaining 7 biomarkers are all consistently found to favor the control group.

Multiplying the likelihoods together gives the same results as that shown above; a likelihood of 21 that the unknown sample is free from the disease. In fact, this sample came from the control population in the training set.

Example 4. Greedy Algorithm for Selecting Biomarker Panels for Classifiers.

[0362] This example describes the selection of biomarkers from Table 1 to form panels that can be used as classifiers in any of the methods described herein. Subsets of the biomarkers in Table 1 were selected to construct classifiers with good performance. This method was also used to determine which potential markers were included as biomarkers in Example 2.

[0363] The measure of classifier performance used here is the AUC; a performance of 0.5 is the baseline expectation for a random (coin toss) classifier, a classifier worse than random would score between 0.0 and 0.5, a classifier with better than random performance would score between 0.5 and 1.0. A perfect classifier with no errors would have a sensitivity of 1.0 and a specificity of 1.0. One can apply the methods described in Example 4 to other common measures of performance such as the F-measure, the sum of sensitivity and specificity, or the product of sensitivity and specificity. Specifically one might want to treat sensitivity and specificity with differing weight, so as to select those classifiers which perform with higher specificity at the expense of some sensitivity, or to select those classifiers which perform with higher sensitivity at the expense of some specificity. Since the method described here only involves a measure of "performance", any weighting scheme which results in a single performance measure can be used. Different applications will have different benefits for true positive and true negative findings, and also different costs associated with false positive findings from false negative findings. For example, screening asymptomatic high risk individuals and the differential diagnosis of pancreatic cancer from benign GI symptoms will not in general have the same optimal trade-off between specificity and sensitivity. The different demands of the two tests will in general require setting different weighting to positive and negative misclassifications, reflected in the performance measure. Changing the performance measure will in general change the exact subset of markers selected from Table 1, Col. 2 for a given set of data.

[0364] For the Bayesian approach to the discrimination of pancreatic cancer samples from control samples described in Example 3, the classifier was completely parameterized by the distributions of biomarkers in the disease and benign training samples, and the list of biomarkers was chosen from Table 1; that is to say, the subset of markers chosen forinclusion determined a classifier in a one-to-one manner given a set of training data.

[0365] The greedy method employed here was used to search for the optimal subset of markers from Table 1. For small numbers of markers or classifiers with relatively few markers, every possible subset of markers was enumerated and evaluated in terms of the performance of the classifier constructed with that particular set of markers (see Example 4, Part 2). (This approach is well known in the field of statistics as "best subset selection"; see, e.g., Hastie et al). However, for the classifiers described herein, the number of combinations of multiple markers can be very large, and it was not feasible to evaluate every possible set of 10 markers, as there are 30,045,015 possible combinations that can be generated from a list of only 30 total analytes. Because of the impracticality of searching through every subset of markers, the single optimal subset may not be found; however, by using this approach, many excellent subsets were found, and, in many cases, any of these subsets may represent an optimal one.

[0366] Instead of evaluating every possible set of markers, a "greedy" forward stepwise approach may be followed (see, e.g., Dabney AR, Storey JD (2007) Optimality Driven Nearest Centroid Classification from Genomic Data.

PLoS ONE 2(10): e1002. doi:10.1371/journal.pone.0001002). Using this method, a classifier is started with the best single marker (based on KS-distance for the individual markers) and is grown at each step by trying, in turn, each member of a marker list that is not currently a member of the set of markers in the classifier. The one marker which scores best in combination with the existing classifier is added to the classifier. This is repeated until no further improvement in performance is achieved. Unfortunately, this approach may miss valuable combinations of markers for which some of the individual markers are not all chosen before the process stops.

[0367] The greedy procedure used here was an elaboration of the preceding forward stepwise approach, in that, to broaden the search, rather than keeping just a single candidate classifier (marker subset) at each step, a list of candidate classifiers was kept. The list was seeded with every single marker subset (using every marker in the table on its own). The list was

expanded in steps by deriving new classifiers (marker subsets) from the ones currently on the list and adding them to the list. Each marker subset currently on the list was extended by adding any marker from Table 1 not already part of that classifier, and which would not, on its addition to the subset, duplicate an existing subset (these are termed "permissible markers"). Every existing marker subset was extended by every permissible marker from the list. Clearly, such a process would eventually generate every possible subset, and the list would run out of space. Therefore, all the generated classifiers were kept only while the list was less than some predetermined size (often enough to hold all three marker subsets). Once the list reached the predetermined size limit, it became elitist; that is, only those classifiers which showed a certain level of performance were kept on the list, and the others fell off the end of the list and were lost. This was achieved by keeping the list sorted in order of classifier performance; new classifiers which were at least as good as the worst classifier currently on the list were inserted, forcing the expulsion of the current bottom underachiever. One further implementation detail is that the list was completely replaced on each generational step; therefore, every classifier on the list had the same number of markers, and at each step the number of markers per classifier grew by one.

[**0368**] Since this method produced a list of candidate classifiers using different combinations of markers, one may ask if the classifiers can be combined in order to avoid errors which might be made by the best single classifier, or by minority groups of the best classifiers. Such "ensemble" and "committee of experts" methods are well known in the fields of statistical and machine learning and include, for example, "Averaging", "Voting", "Stacking", "Bagging" and "Boosting" (see, e.g., Hastie et al.). These combinations of simple classifiers provide a method for reducing the variance in the classifications due to noise in any particular set of markers by including several different classifiers and therefore information from a larger set of the markers from the biomarker table, effectively averaging between the classifiers. An example of the usefulness of this approach is that it can prevent outliers in a single marker from adversely affecting the classification of a single sample. The requirement to measure a larger number of signals may be impractical in conventional "one marker at a time" antibody assays but has no downside for a fully multiplexed aptamer assay. Techniques such as these benefit from a more extensive table of biomarkers and use the multiple sources of information concerning the disease processes to provide a more robust classification.

[0369] The biomarkers selected in Table 1 gave rise to classifiers which perform better than classifiers built with "non-markers" (i.e., proteins having signals that did not meet the criteria for inclusion in Table 1 (as described in Example 2)).

[0370] For classifiers containing only one, two, and three markers, all possible classifiers obtained using the biomarkers in Table 1 were enumerated and examined for the distribution of performance compared to classifiers built from a similar table of randomly selected non-markers signals.

[0371] In Figure 11, the AUC was used as the measure of performance; a performance of 0.5 is the baseline expectation for a random (coin toss) classifier. The histogram of classifier performance was compared with the histogram of performance from a similar exhaustive enumeration of classifiers built from a "non-marker" table of 65 non-marker signals; the 65 signals were randomly chosen from aptamers that did not demonstrate differential signaling between control and disease populations.

[0372] Figure 11 shows histograms of the performance of all possible one, two, and three-marker classifiers built from the biomarker parameters in Table 14 for biomarkers that can discriminate between the control group and pancreatic cancer and compares these classifiers with all possible one, two, and three-marker classifiers built using the 65 "non-marker" aptamer RFU signals. Figure 11A shows the histograms of single marker classifier performance, Figure 11B shows the histogram of two marker classifier performance, and Figure 11C shows the histogram of three marker classifier performance.

[0373] In Figure 11, the solid lines represent the histograms of the classifier performance of all one, two, and three-marker classifiers using the biomarker data for GI and normal controls and pancreatic cancer in Table 14. The dotted lines are the histograms of the classifier performance of all one, two, and three-marker classifiers using the data for controls and pancreatic cancer but using the set of random non-marker signals.

[0374] The classifiers built from the markers listed in Table 1 form a distinct histogram, well separated from the classifiers built with signals from the "non-markers" for all one-marker, two-marker, and three-marker comparisons. The performance and AUC score of the classifiers built from the biomarkers in Table 1 also increase faster with the number of markers than do the classifiers built from the non-markers, the separation increases between the marker and non-marker classifiers as the number of markers per classifier increases. All classifiers built

using the biomarkers listed in Table 14 perform distinctly better than classifiers built using the "non-markers".

[0375] The distributions of classifier performance show that there are many possible multiple-marker classifiers that can be derived from the set of analytes in Table 1. Although some biomarkers are better than others on their own, as evidenced by the distribution of classifier scores and AUCs for single analytes, it was desirable to determine whether such biomarkers are required to construct high performing classifiers. To make this determination, the behavior of classifier performance was examined by leaving out some number of the best biomarkers. Figure 12 compares the performance of classifiers built with the full list of biomarkers in Table 1 with the performance of classifiers built with subsets of biomarkers from Table 1 that excluded top-ranked markers.

[0376] Figure 12 demonstrates that classifiers constructed without the best markers perform well, implying that the performance of the classifiers was not due to some small core group of markers and that the changes in the underlying processes associated with disease are reflected in the activities of many proteins. Many subsets of the biomarkers in Table 1 performed close to optimally, even after removing the top 15 of the 65 markers from Table 1. After dropping the 15 top-ranked markers (ranked by KS-distance) from Table 1, the classifier performance increased with the number of markers selected from the table to reach an AUC of almost 0.87, close to the performance of the optimal classifier score of 0.91 selected from the full list of biomarkers.

[0377] Finally, Figure 16 shows how the ROC performance of typical classifiers constructed from the list of parameters in Table 14 according to Example 3. A five analyte classifier was constructed with CTSB, C5a, C5, CCL18, and CSF1R. Figure 16A shows the performance of the model, assuming independence of these markers, as in Example 3, and Figure 16B shows the empirical ROC curves generated from the study data set used to define the parameters in Table 14. It can be seen that the performance for a given number of selected markers was qualitatively in agreement, and that quantitative agreement was generally quite good, as evidenced by the AUCs, although the model calculation tends to overestimate classifier performance. This is consistent with the notion that the information contributed by any particular biomarker concerning the disease processes is redundant with the information contributed by other biomarkers provided in Table 1 while the model calculation assumes complete indepen-

dence. Figure 16 thus demonstrates that Table 1 in combination with the methods described in Example 3 enable the construction and evaluation of a great many classifiers useful for the discrimination of pancreatic cancer from the control group.

Example 5. Incorporating CA19-9

[0378] Cancer associated antigen 19-9 (CA 19-9) is a known serum marker for pancreatic cancer. The reported sensitivity and specificity of CA 19-9 for pancreatic cancer are 80 to 90 percent, respectively. However, the accuracy of CA 19-9 to identify patients with small surgically resectable cancers is limited. The specificity of CA 19-9 is also limited; CA 19-9 is frequently elevated in patients with various benign pancreaticobiliary disorders.

[0379] The degree of elevation of CA 19-9 in pancreatic cancer is associated with long-term prognosis. Furthermore, in patients who appear to have potentially resectable disease, the magnitude of the CA 19-9 level can also help to predict the presence of radiographically occult metastatic disease. Serial monitoring of CA 19-9 levels is useful to follow patients after potentially curative surgery and for those who are receiving chemotherapy for advanced disease. Rising CA 19-9 levels usually precede the radiographic appearance of recurrent disease, but confirmation of disease progression should be pursued with imaging studies and/or biopsy. Testing of biomarker levels in combination with CA 19-9 may improve sensitivity, specificity, and/or AUC for detecting pancreatic cancer (or other pancreatic cancer-related uses) as compared to CA 19-9 alone.

[0380] An elevated level of CA19-9 is considered to be 35-40 U/ml in serum.

[0381] We received clinical CA19-9 measurements for a subset of the training samples. Of the original 100 cases and 69 controls, we had CA19-9 measurements for 99 cases and 52 controls. Therefore, we trained a new set of random forest models on this subset of samples using subsets of the SOMAmers in Table 1. We also trained new classifiers which incorporated the CA19-9 measurement with our SOMAmer panel (combined panel).

[0382] The classifier performance of the three different approaches (SOMAmer, CA19-9, and a combined panel) is shown in Figure 13. The SOMAmer panel and CA19-9 perform similarly, however when the two are combined into a single classifier the performance improves dramatically. For a specificity of 100%, the SOMAmer panel and CA19-9 have a sensitivity

just under 50%, whereas the combined classifier has a sensitivity of around 75%.

[0383] Further analysis revealed that when CA19-9 is included in the classifier, the number of SOMAmers required for the same relative performance is reduced. Figure 14 shows the performance of random forest classifiers that use CA19-9 and either one or two additional SOMAmers. The left panel shows the performance of a model trained using CA19-9 and HAMP and the right panel shows the performance of CA19-9, HAMP, and CTSB.

Example 6. Clinical Biomarker Panel

[0384] A random forest classifier was built from a panel of biomarkers selected that may be the most appropriate for use in a clinical diagnostic test. Unlike the models selected by the naïve Bayes greedy forward algorithm, the random forest classifier does not assume that the biomarker measurements are randomly distributed. Therefore this model can utilize biomarkers from Table 1 that are not effective in the naïve Bayes classifier.

[0385] The panel was selected using a backward elimination procedure that utilized the gini importance measure provided by the random forest classifier. The gini importance is a measure of the effectiveness of a biomarker at correctly classifying samples in the training set. This measure of biomarker importance can be used to eliminate markers that are less vital to the performance of the classifier. The backward elimination procedure was initiated by building a random forest classifier that included all 65 in Table 1. The least important biomarker was then eliminated and a new model was built with the remaining biomakers. This procedure continued until only single biomarker remained.

[0386] The final panel that was selected provided the best balance between the greatest AUC and the lowest number of markers in the model. The panel of ten biomarkers that satisfied these criteria is composed of the following analytes, APOA1, CTSB, C2, MMP7, HAMP, TFPI, C5, c5a, SFRP1, and ETHE1. A plot of the ROC curve for this biomarker panel is shown in Figure 15. The figure indicates two possible decision cutoffs illustrated by arrows: a symptomatic cutoff where a sensitivity of 84% or more can be obtained with at least 80% specificity; and an asymptomatic cutoff where a specificity of 97.5% can be obtained with at least 60% sensitivity.

Example 7. Biomarkers for the Diagnosis of Cancer

[0387] The identification of potential biomarkers for the general diagnosis of cancer was performed. Both case and control samples were evaluated from 3 different types of cancer (pancreatic cancer, lung cancer, and mesothelioma). Across the collection sites, inclusion criteria were at least 18 years old with signed informed consent. Both cases and controls were excluded for known malignancy other than the cancer in question.

[0388] **Pancreatic Cancer.** Case and control samples were obtained as described in Example 2.

[0389] **Lung Cancer.** Case and control samples were obtained from three academic cancer center biorepositories and one commercial biorepository to identify potential markers for the differential diagnosis of non-small cell lung cancer (NSCLC) from a control group of high risk smokers and individuals with benign pulmonary nodules. The study was composed of 978 samples collected from smokers and patients with benign nodules as well as 320 individuals diagnosed with NSCLC.

[0390] **Pleural Mesothelioma.** Case and control samples were obtained from an academic cancer center biorepository to identify potential markers for the differential diagnosis of malignant pleural mesothelioma from individuals with a history of asbestos exposure or benign lung disease, including suspicious radiology findings that were later diagnosed as non-malignant. The study was composed of 30 samples collected from asbestos exposed individuals and 41 samples collected from mesothelioma patients.

[0391] A final list of cancer biomarkers was identified by combining the sets of biomarkers considered for each of the 3 different cancer studies. Bayesian classifiers that used biomarker sets of increasing size were successively constructed using a greedy algorithm (as described in greater detail in Section 7.2 of this Example). The sets (or panels) of biomarkers that were useful for diagnosing cancer in general among the types of cancer were compiled as a function of set (or panel) size and analyzed for their performance. This analysis resulted in the list of 10 cancer biomarkers shown in Table 19, each of which was present in at least one of these successive marker sets, which ranged in size from three to ten markers. As an illustrative example, we describe the generation of a specific panel composed of ten cancer biomarkers, which is shown in Table 32.

7.1 Naïve Bayesian Classification for Cancer

[0392] From the list of biomarkers in Table 1, a panel of ten potential cancer biomarkers was selected using a greedy algorithm for biomarker selection, as outlined in Section 7.2 of this Example. A distinct naïve Bayes classifier was constructed for each of the 3. The class-dependent probability density functions (pdfs), $p(x_i|c)$ and $p(x_i|d)$, where x_i is the log of the measured RFU value for biomarker i, and c and d refer to the control and disease populations, were modeled as log-normal distribution functions characterized by a mean μ and variance σ^2. The parameters for pdfs of the 3 models composed of the ten potential biomarkers are listed in Table 31.

[0393] The naïve Bayes classification for such a model is given by the following equation, where $p(d)$ is the prevalence of the disease in the population, $ln\left(\frac{p(d|\tilde{x})}{p(c|\tilde{x})}\right) = \sum_{i=1}^{n} ln\left(\frac{\sigma_{c,i}}{\sigma_{d_i}}\right) - \frac{1}{2}\sum_{i=1}^{n}\left[(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}})^2 - (\frac{x_i-\mu_{c,i}}{\sigma_{c,i}})^2\right] + ln\left(\frac{p(d)}{1-p(d)}\right)$, appropriate to the test and $n = 10$. Each of the terms in the summation is a log-likelihood ratio for an individual marker and the total log-likelihood ratio of a sample $\tilde{x}$ being free from the disease interest (i.e., in this case, each particular disease from the 3 different cancer types) versus having the disease is simply the sum of these individual terms plus a term that accounts for the prevalence of the disease. For simplicity, we assume $p(d) = 0.5$ so that $ln\left(\frac{p(d)}{1-p(d)}\right) = 0$.

[0394] Given an unknown sample measurement in log(RFU) for each of the ten biomarkers of 10.1, 8.9, 8.8, 8.8, 9.1, 7.3, 8.2, 9.5, 6.7, 7.7, the calculation of the classification is detailed in Table 32. The individual components comprising the log likelihood ratio for disease versus control class are tabulated and can be computed from the parameters in Table 31 and the values of $\tilde{x}$. The sum of the individual log likelihood ratios is -4.568, or a likelihood of being free from the disease versus having the disease of 96, where likelihood $e^{4.568} = 96$. Only 1 of the biomarker values have likelihoods more consistent with the disease group (log likelihood > 0) but the remaining 9 biomarkers are all consistently found to favor the control group. Multiplying the likelihoods together gives the same results as that shown above; a likelihood of 96 that the unknown sample is free from the disease. In fact, this sample came from the control population in the NSCLC training set.

7.2 Greedy Algorithm for Selecting Cancer Biomarker Panels for Classifiers

Part 1

[0395] Subsets of the biomarkers in Table 1 were selected to construct potential classifiers that could be used to determine which of the markers could be used as general cancer biomarkers to detect cancer.

[0396] Given a set of markers, a distinct model was trained for each of the 3 cancer studies, so a global measure of performance was required to select a set of biomarkers that was able to classify simultaneously many different types of cancer. The measure of classifier performance used here was the mean of the area under ROC curve across all naïve Bayes classifiers. The ROC curve is a plot of a single classifier true positive rate (sensitivity) versus the false positive rate (1-specificity). The area under the ROC curve (AUC) ranges from 0 to 1.0, where an AUC of 1.0 corresponds to perfect classification and an AUC of 0.5 corresponds to random (coin toss) classifier. One can apply other common measures of performance such as the F-measure or the sum or product of sensitivity and specificity. Specifically, one might want to treat sensitivity and specificity with differing weight, in order to select those classifiers that perform with higher specificity at the expense of some sensitivity, or to select those classifiers which perform with higher sensitivity at the expense of specificity. We chose to use the AUC because it encompasses all combinations of sensitivity and specificity in a single measure. Different applications will have different benefits for true positive and true negative findings, and will have different costs associated with false positive findings from false negative findings. Changing the performance measure may change the exact subset of markers selected for a given set of data.

[0397] For the Bayesian approach to the discrimination of cancer samples from control samples described in Section 7.1 of this Example, the classifier was completely parameterized by the distributions of biomarkers in each of the 3 cancer studies, and the list of biomarkers was chosen from Table 19. That is to say, the subset of markers chosen for inclusion determined a classifier in a one-to-one manner given a set of training data.

[0398] The greedy method employed here was used to search for the optimal subset of

markers from Table 1. For small numbers of markers or classifiers with relatively few markers, every possible subset of markers was enumerated and evaluated in terms of the performance of the classifier constructed with that particular set of markers (see Example 4, Part 2). (This approach is well known in the field of statistics as "best subset selection"; see, e.g., Hastie et al). However, for the classifiers described herein, the number of combinations of multiple markers can be very large, and it was not feasible to evaluate every possible set of 10 markers, as there are 30,045,015 possible combinations that can be generated from a list of only 30 total analytes. Because of the impracticality of searching through every subset of markers, the single optimal subset may not be found; however, by using this approach, many excellent subsets were found, and, in many cases, any of these subsets may represent an optimal one.

[0399] Instead of evaluating every possible set of markers, a "greedy" forward stepwise approach may be followed (see, e.g., Dabney AR, Storey JD (2007) Optimality Driven Nearest Centroid Classification from Genomic Data.

PLoS ONE 2(10): e1002. doi:10.1371/journal.pone.0001002). Using this method, a classifier is started with the best single marker (based on KS-distance for the individual markers) and is grown at each step by trying, in turn, each member of a marker list that is not currently a member of the set of markers in the classifier. The one marker that scores the best in combination with the existing classifier is added to the classifier. This is repeated until no further improvement in performance is achieved. Unfortunately, this approach may miss valuable combinations of markers for which some of the individual markers are not all chosen before the process stops.

[0400] The greedy procedure used here was an elaboration of the preceding forward stepwise approach, in that, to broaden the search, rather than keeping just a single marker subset at each step, a list of candidate marker sets was kept. The list was seeded with a list of single markers. The list was expanded in steps by deriving new marker subsets from the ones currently on the list and adding them to the list. Each marker subset currently on the list was extended by adding any marker from Table 1 not already part of that classifier, and which would not, on its addition to the subset, duplicate an existing subset (these are termed "permissible markers"). Each time a new set of markers was defined, a set of classifiers composed of one for each cancer study was trained using these markers, and the global performance was measured via the mean AUC across all 3 studies. To avoid potential over fitting, the AUC

for each cancer study model was calculated via a ten-fold cross validation procedure. Every existing marker subset was extended by every permissible marker from the list. Clearly, such a process would eventually generate every possible subset, and the list would run out of space. Therefore, all the generated marker sets were kept only while the list was less than some predetermined size. Once the list reached the predetermined size limit, it became elitist; that is, only those classifier sets which showed a certain level of performance were kept on the list, and the others fell off the end of the list and were lost. This was achieved by keeping the list sorted in order of classifier set performance; new marker sets whose classifiers were globally at least as good as the worst set of classifiers currently on the list were inserted, forcing the expulsion of the current bottom underachieving classifier sets. One further implementation detail is that the list was completely replaced on each generational step; therefore, every marker set on the list had the same number of markers, and at each step the number of markers per classifier grew by one.

[0401] In one embodiment, the set (or panel) of biomarkers useful for constructing classifiers for diagnosing general cancer from non-cancer is based on the mean AUC for the particular combination of biomarkers used in the classification scheme. We identified many combinations of biomarkers derived from the markers in Table 19 that were able to effectively classify different cancer samples from controls. Representative panels are set forth in Tables 22-29, which set forth a series of 100 different panels of 3-10 biomarkers, which have the indicated mean cross validation (CV) AUC for each panel. The total number of occurrences of each marker in each of these panels is indicated at the bottom of each table.

[0402] The biomarkers selected in Table 19 gave rise to classifiers that perform better than classifiers built with "non-markers." In Figure 17, we display the performance of our ten biomarker classifiers compared to the performance of other possible classifiers.

[0403] Figure 17A shows the distribution of mean AUCs for classifiers built from randomly sampled sets of ten "non-markers" taken from the entire set of 10 present in all 3 studies, excluding the ten markers in Table 19. The performance of the ten potential cancer biomarkers is displayed as a vertical dashed line. This plot clearly shows that the performance of the ten potential biomarkers is well beyond the distribution of other marker combinations.

[0404] Figure 17B displays a similar distribution as Figure 17A, however the randomly sampled sets were restricted to the 55 biomarkers from Table 1 that were not selected by

the greedy biomarker selection procedure for ten analyte classifiers. This plot demonstrates that the ten markers chosen by the greedy algorithm represent a subset of biomarkers that generalize to other types of cancer far better than classifiers built with the remaining 55 biomarkers.

[0405] Finally, Figure 18 shows the classifier ROC curve for each of the 3 cancer studies classifiers. The foregoing embodiments and examples are intended only as examples. No particular embodiment, example, or element of a particular embodiment or example is to be construed as a critical, required, or essential element or feature of any of the claims. Further, no element described herein is required for the practice of the appended claims unless expressly described as "essential" or "critical." Various alterations, modifications, substitutions, and other variations can be made to the disclosed embodiments without departing from the scope of the present application, which is defined by the appended claims. The specification, including the figures and examples, is to be regarded in an illustrative manner, rather than a restrictive one, and all such modifications and substitutions are intended to be included within the scope of the application. Accordingly, the scope of the application should be determined by the appended claims and their legal equivalents, rather than by the examples given above. For example, steps recited in any of the method or process claims may be executed in any feasible order and are not limited to an order presented in any of the embodiments, the examples, or the claims. Further, in any of the aforementioned methods, one or more biomarkers of Table 1 or Table 19 can be specifically excluded either as an individual biomarker or as a biomarker from any panel.

Table 1: Cancer Biomarkers

Column #1	Column #2	Column #3	Column #4	Column #5	Column #6
Biomarker #	Biomarker Designation Entrez Gene Symbol(s)	Entrez Gene ID	SwissProt ID	Public Name	Direction
1	ACP5	54	P13686	TrATPase	Up
2	ACY1	95	Q03154	Aminoacylase-1	Up
3	AHSG	197	P02765	α2-HS-Glycoprotein	Down
4	ALPL	249	P05186	Alkaline phosphatase, bone	Down
5	APOA1	335	P02647	Apo A-I	Down
6	APOE	348	P02649	Apo E2	Up
7	BMP6	654	P22004	BMP-6	Up
8	C2	717	P06681	C2	Up
9	C5	727	P01031	C5	Up
10	C5	727	P01031	C5a	Up
11	C5-C6	727; 729	P01031; P13671	C5b,6 Complex	Up
12	C9	735	P02748	C9	Up
13	CCL18	6362	P55774	MIP-4	Up
14	CCL23	6368	P55773	MPIF-1	Up
15	CCL23	6368	P55773	Ck-β-8-1	Up
16	CDK5-CDK5R1	1020; 1775	Q00535; Q15078	CDK5/p35	Up
17	CKB-CKM-	1152; 1158	P12277; P06732	CK-MB	Down
18	CKM	1158	P06732	CK-MM	Down
19	CRP	1401	P02741	CRP	Up
20	CSF1R	1436	P07333	M-CSF R	Up
21	CTSB	1508	P07858	Cathepsin B	Up
22	ENTPD1	953	P49961	CD39	Up
23	ESM1	11082	Q9NQ30	Endocan	Up
24	ETHE1	23474	O95571	ETHE1	Up
25	FCGR3B	2215	O75015	FCγ3B	Up
26	FGFR3	2261	P22607	FGFR-3	Up
27	FSTL3	10272	O95633	FSTL3	Up
28	GDF11	10220	O95390	GDF-11	Down
29	GFRA1	2674	P56159	GFR α-1	Up
30	HAMP	57817	P81172	Hepcidin-25	Up
31	HINT1	3094	P49773	HINT1	Down
32	IDUA	3425	P35475	IDUA	Up
33	IL11RA	3590	Q14626	IL-11 Rα	Down
34	IL12A-IL12B	3592; 3593	P29459; P29460	IL-12	Down
35	IL18R1	8809	Q13478	IL-18 Rα	Up
36	IL1RL1	9173	Q01638	IL-1 R4	Up
37	INSR	3643	P06213	IR	Up
38	KIT	3815	P10721	SCF sR	Down
39	KLK3-SERPINA3	354; 12	P07288; P01011	PSA-ACT	Up
40	KLK7	5650	P49862	Kallikrein 7	Down
41	KLK8	11202	O60259	Kallikrein 8	Up
42	KLKB1	3818	P03952	Prekallikrein	Down
43	LBP	3929	P18428	LBP	Up
44	LTF	4057	P02788	Lactoferrin	Down
45	MCM2	4171	P49736	MCM2	Up
46	MDK	4192	P21741	Midkine	Up
47	MMP7	4316	P09237	MMP-7	Up
48	MRC1	4360	P22897	Macrophage mannose receptor	Up
49	NID1	4811	P14543	Nidogen	Up
50	NID2	22795	Q14112	Nidogen-2	Up
51	NRP1	8829	O14786	NRP1	Up
52	PLAT	5327	P00750	tPA	Up
53	SERPINA5	5104	P05154	Protein C Inhibitor	Down
54	SERPINF2	5345	P08697	α2-Antiplasmin	Down
55	SFRP1	6422	Q8N474	FRP-1, soluble	Up
56	SCTA	6449	O43765	SCTα	Down
57	TFPI	7035	P10646	TFPI	Up
58	THBS2	7058	P35442	Thrombospondin-2	Up
59	THBS4	7060	P35443	Thrombospondin-4	Down
60	TIMP1	7076	P01033	TIMP-1	Up
61	TNFRSF18	8784	Q9Y5U5	GITR/TNFRSF18	Down

Table 1 – continued from previous page

Column #1	Column #2	Column #3	Column #4	Column #5	Column #6
Biomarker #	Biomarker Designation Entrez Gene Symbol(s)	Entrez Gene ID	SwissProt ID	Public Name	Direction
62	TNFRSF1B	7133	P20333	TNF sR-II	Up
63	TOP1	7150	P11387	Topoisomerase I	Down
64	VEGFA	7422	P15692	VEGF	Down
65	VEGFC	7424	P49767	VEGF-C	Up

Table 2: Panels of 1 Biomarker

Markers		CV AUC
1	CTSB	0.780
2	C2	0.771
3	APOA1	0.754
4	C5	0.745
5	TFPI	0.739
6	C5a	0.724
7	TIMP1	0.720
8	FCGR3B	0.719
9	HAMP	0.718
10	CRP	0.717
11	NRP1	0.716
12	THBS2	0.715
13	MMP7	0.711
14	CCL18	0.709
15	CSF1R	0.705
16	ACP5	0.704
17	LBP	0.703
18	MRC1	0.699
19	PLAT	0.699
20	GFRA1	0.698
21	CCL23	0.696
22	KLK7	0.696
23	MDK	0.694
24	CKB-CKM	0.694
25	KLK3-SERPINA3	0.693
26	CKM	0.693
27	GDF11	0.692
28	IL11RA	0.690
29	IL1RL1	0.690
30	ETHE1	0.684
31	FSTL3	0.681
32	KIT	0.680
33	FGFR3	0.677
34	KLKB1	0.677
35	THBS4	0.669
36	ACY1	0.666
37	C5-C6	0.664
38	INSR	0.663
39	IL18R1	0.663
40	BMP6	0.663
41	TNFRSF1B	0.660

Table 2 – continued from previous page

Markers		CV AUC
42	C9	0.657
43	SERPINA5	0.655
44	IL12A-IL12B	0.655
45	NID2	0.649
46	TOP1	0.647
47	NID1	0.642
48	CCL23	0.641
49	MCM2	0.641
50	AHSG	0.638
51	VEGFC	0.637
52	ENTPD1	0.637
53	HINT1	0.637
54	ALPL	0.635
55	LTF	0.632
56	ESM1	0.625
57	SERPINF2	0.624
58	CDK5-CDK5R1	0.623
59	SGTA	0.603
60	KLK8	0.597
61	IDUA	0.594
62	SFRP1	0.586
63	VEGFA	0.585
64	APOE	0.574
65	TNFRSF18	0.527

Table 3: Panels of 2 Biomarkers

Markers			CV AUC
1	C5	CTSB	0.848
2	C5a	CTSB	0.841
3	CTSB	ETHE1	0.833
4	CTSB	HAMP	0.830
5	CTSB	THBS4	0.830
6	KIT	CTSB	0.829
7	C9	CTSB	0.828
8	CTSB	KLK7	0.826
9	CTSB	C2	0.821
10	C5	APOA1	0.820
11	CTSB	CRP	0.818
12	CCL23	CTSB	0.817
13	C5-C6	CTSB	0.814
14	CTSB	IL11RA	0.812
15	CCL18	CTSB	0.811
16	APOA1	CTSB	0.809
17	C5	CSF1R	0.808
18	GDF11	CTSB	0.807
19	C5	C2	0.806
20	C2	TFPI	0.806
21	C5	CCL18	0.804
22	C2	IL11RA	0.803

Table 3 – continued from previous page

Markers			CV AUC
23	CCL23	CTSB	0.802
24	TIMP1	C5	0.801
25	CTSB	LBP	0.799
26	CTSB	TFPI	0.799
27	PLAT	C5	0.799
28	CTSB	AHSG	0.799
29	CCL18	C2	0.799
30	C5	MRC1	0.799
31	APOA1	C2	0.798
32	C5	FCGR3B	0.797
33	C5	TFPI	0.797
34	ALPL	CTSB	0.796
35	C5	MMP7	0.796
36	CTSB	KLK3-SERPINA3	0.795
37	MMP7	CTSB	0.795
38	MMP7	C2	0.794
39	TFPI	HAMP	0.793
40	CCL18	ETHE1	0.793
41	C2	HAMP	0.792
42	PLAT	C2	0.792
43	CTSB	NRP1	0.792
44	LTF	CTSB	0.791
45	C5	ACP5	0.790
46	APOA1	TFPI	0.790
47	C5a	TNFRSF1B	0.789
48	C5a	CCL18	0.789
49	CKM	CTSB	0.789
50	C5	THBS2	0.789
51	C2	CRP	0.788
52	C5a	KLK7	0.788
53	C2	THBS4	0.788
54	CSF1R	THBS4	0.788
55	C5a	FSTL3	0.788
56	C5	NRP1	0.788
57	C5a	C2	0.788
58	C5a	TFPI	0.787
59	CTSB	CKB-CKM	0.787
60	C5a	NRP1	0.787
61	CSF1R	APOA1	0.787
62	TFPI	CRP	0.787
63	MMP7	KLK7	0.787
64	C5a	FCGR3B	0.787
65	C2	ETHE1	0.786
66	CCL23	C2	0.786
67	PLAT	CTSB	0.786
68	CCL18	TFPI	0.786
69	ACP5	CRP	0.785
70	C2	KLK7	0.785
71	C5	CCL23	0.784
72	MMP7	C5a	0.784
73	APOA1	KLK7	0.784
74	C5	GFRA1	0.784
75	C5	HAMP	0.784

Table 3 – continued from previous page

Markers			CV AUC
76	C5	C5a	0.784
77	NRP1	CRP	0.783
78	KIT	C2	0.783
79	C5	IL1RL1	0.783
80	APOA1	ETHE1	0.783
81	CTSB	CDK5-CDK5R1	0.782
82	CSF1R	CRP	0.782
83	TIMP1	CTSB	0.782
84	IL1RL1	CTSB	0.782
85	CSF1R	C5a	0.782
86	TIMP1	C5a	0.781
87	TFPI	KLK7	0.781
88	C5	KLKB1	0.781
89	CTSB	FCGR3B	0.781
90	APOA1	MMP7	0.781
91	IL12A-IL12B	CTSB	0.781
92	C5	MDK	0.780
93	MDK	CTSB	0.780
94	C5	TNFRSF1B	0.780
95	C2	ACP5	0.780
96	IL12A-IL12B	C2	0.780
97	NRP1	TFPI	0.780
98	C5	KIT	0.779
99	FCGR3B	ETHE1	0.779
100	C5-C6	C2	0.779

Table 4: Panels of 3 Biomarkers

Markers				CV AUC
1	C5	C5a	CTSB	0.871
2	C5	CTSB	ETHE1	0.870
3	C5	CTSB	HAMP	0.866
4	C5	CCL18	CTSB	0.865
5	C5	KIT	CTSB	0.862
6	C5	CTSB	THBS4	0.861
7	KIT	C5a	CTSB	0.861
8	C5a	CCL18	CTSB	0.859
9	CTSB	HAMP	ETHE1	0.859
10	CTSB	KLK7	HAMP	0.859
11	C5a	CTSB	KLK7	0.859
12	C9	C5	CTSB	0.859
13	C5-C6	C5a	CTSB	0.858
14	C5a	CTSB	ETHE1	0.858
15	C5	ALPL	CTSB	0.857
16	C5a	CTSB	HAMP	0.856
17	KIT	CTSB	HAMP	0.856
18	KIT	CTSB	ETHE1	0.854
19	C5	LTF	CTSB	0.854
20	C5a	CTSB	THBS4	0.854
21	C5	CCL23	CTSB	0.854

Table 4 – continued from previous page

Markers				CV AUC
22	C5	APOA1	CTSB	0.854
23	CCL18	CTSB	ETHE1	0.853
24	C5	CTSB	IL11RA	0.853
25	C5	CTSB	C2	0.852
26	C5a	CTSB	C2	0.852
27	CTSB	THBS4	ETHE1	0.852
28	CTSB	KLK7	ETHE1	0.851
29	C5-C6	CTSB	HAMP	0.851
30	C5a	CCL23	CTSB	0.850
31	C9	CTSB	ETHE1	0.850
32	C5	CTSB	KLK7	0.849
33	C5-C6	CTSB	ETHE1	0.849
34	GDF11	C5a	CTSB	0.849
35	CTSB	THBS4	HAMP	0.848
36	CCL23	CTSB	ETHE1	0.848
37	PLAT	C5	CTSB	0.848
38	C5a	CTSB	IL11RA	0.848
39	PLAT	C5a	CTSB	0.848
40	C9	KIT	CTSB	0.847
41	C5	VEGFA	CTSB	0.847
42	CTSB	C2	HAMP	0.847
43	C5	CCL23	CTSB	0.847
44	CTSB	C2	ETHE1	0.847
45	C5	CSF1R	APOA1	0.846
46	CCL18	CTSB	HAMP	0.846
47	CTSB	C2	IL11RA	0.846
48	C5a	CCL23	CTSB	0.846
49	GDF11	CTSB	HAMP	0.846
50	C9	CTSB	THBS4	0.845
51	C5a	CTSB	CDK5-CDK5R1	0.845
52	APOA1	CTSB	ETHE1	0.845
53	C9	C5a	CTSB	0.845
54	C9	CCL18	CTSB	0.844
55	C5	CTSB	TFPI	0.844
56	CCL18	CTSB	THBS4	0.844
57	KIT	CTSB	THBS4	0.844
58	C9	CTSB	KLK7	0.844
59	CTSB	C2	THBS4	0.844
60	C5-C6	CCL18	CTSB	0.843
61	C5	CSF1R	CTSB	0.843
62	C9	CTSB	HAMP	0.843
63	C5	CTSB	ACP5	0.843
64	C5	CSF1R	CCL18	0.842
65	C5-C6	KIT	CTSB	0.842
66	C5	MMP7	CTSB	0.842
67	PLAT	C9	CTSB	0.842
68	C5	CTSB	NRP1	0.842
69	MMP7	C5a	CTSB	0.842
70	C5	CTSB	LBP	0.841
71	CSF1R	C5a	CTSB	0.841
72	C5-C6	C9	CTSB	0.841
73	C5	GDF11	CTSB	0.841
74	KIT	CCL18	CTSB	0.840

Table 4 – continued from previous page

Markers				CV AUC
75	CTSB	THBS4	CRP	0.840
76	C5	CTSB	AHSG	0.840
77	C9	CTSB	C2	0.840
78	LTF	C5a	CTSB	0.840
79	C5a	CTSB	TFPI	0.840
80	C5a	CTSB	TNFRSF1B	0.839
81	ALPL	C5a	CTSB	0.839
82	C5a	CTSB	NRP1	0.839
83	APOA1	C5a	CTSB	0.839
84	CCL23	CTSB	ETHE1	0.839
85	C5a	CTSB	FCGR3B	0.838
86	CTSB	TFPI	ETHE1	0.838
87	C5	KLK8	CTSB	0.838
88	C5-C6	C5	CTSB	0.838
89	C5a	CTSB	KLK3-SERPINA3	0.838
90	CTSB	LBP	ETHE1	0.838
91	CTSB	IL11RA	ETHE1	0.838
92	CTSB	HAMP	IL11RA	0.838
93	ALPL	CTSB	KLK7	0.838
94	CCL18	CTSB	KLK7	0.838
95	C5a	CTSB	INSR	0.838
96	CTSB	C2	KLK7	0.838
97	CTSB	AHSG	ETHE1	0.838
98	C5-C6	CTSB	THBS4	0.838
99	C5	CTSB	KLKB1	0.837
100	C5a	CTSB	LBP	0.837

Table 5: Panels of 4 Biomarkers

Markers					CV AUC
1	C5	CCL18	CTSB	ETHE1	0.882
2	C5	C5a	CCL18	CTSB	0.880
3	C5	CTSB	HAMP	ETHE1	0.880
4	C5	KIT	C5a	CTSB	0.880
5	C5	C5a	CTSB	ETHE1	0.880
6	C5	CTSB	THBS4	ETHE1	0.878
7	C5	LTF	CTSB	ETHE1	0.877
8	C5	CSF1R	C5a	CTSB	0.877
9	PLAT	C5	C5a	CTSB	0.876
10	C5	ALPL	CTSB	ETHE1	0.876
11	C5	KIT	CTSB	ETHE1	0.875
12	C5a	CTSB	KLK7	HAMP	0.875
13	C5	KIT	CTSB	HAMP	0.875
14	C5	CCL23	CTSB	ETHE1	0.875
15	C5a	CTSB	KLK7	ETHE1	0.875
16	PLAT	C5	CTSB	ETHE1	0.875
17	C5	C5a	CTSB	HAMP	0.874
18	CTSB	KLK7	HAMP	ETHE1	0.874
19	C5	KIT	CCL18	CTSB	0.874
20	C9	C5	CTSB	ETHE1	0.874

Table 5 – continued from previous page

Markers					CV AUC
21	C5	CCL18	CTSB	THBS4	0.874
22	C5-C6	CCL18	CTSB	ETHE1	0.874
23	C5	LTF	C5a	CTSB	0.874
24	C5	ALPL	C5a	CTSB	0.873
25	KIT	CTSB	HAMP	ETHE1	0.873
26	C5	C5a	CTSB	KLK7	0.873
27	C5	C5a	CTSB	THBS4	0.872
28	C5-C6	C5a	CCL18	CTSB	0.872
29	C5	CTSB	KLK7	HAMP	0.872
30	C5	C5a	CTSB	ACP5	0.872
31	C5	C5a	CTSB	IL11RA	0.872
32	C5	C5a	CCL23	CTSB	0.872
33	C5	CCL18	CTSB	HAMP	0.872
34	C5a	CCL18	CTSB	KLK7	0.872
35	C5	KIT	VEGFA	CTSB	0.871
36	KIT	C5a	CCL18	CTSB	0.871
37	C5	APOA1	CTSB	ETHE1	0.871
38	C5a	CCL18	CTSB	ETHE1	0.871
39	KIT	C5a	CTSB	ETHE1	0.871
40	PLAT	C9	C5	CTSB	0.871
41	C5	ALPL	CTSB	THBS4	0.870
42	C5	ALPL	CCL18	CTSB	0.870
43	C5	CSF1R	CTSB	ETHE1	0.870
44	C5	CTSB	THBS4	HAMP	0.870
45	C5a	CCL18	CTSB	THBS4	0.870
46	C5	CTSB	KLK7	ETHE1	0.869
47	ALPL	C5a	CTSB	KLK7	0.869
48	MMP7	C5a	CTSB	KLK7	0.869
49	C5	LTF	CCL18	CTSB	0.869
50	C9	C5	KIT	CTSB	0.869
51	C5-C6	CTSB	HAMP	ETHE1	0.869
52	C5	ALPL	CTSB	HAMP	0.869
53	LTF	C5a	CTSB	KLK7	0.869
54	C5-C6	KIT	C5a	CTSB	0.869
55	C5	ALPL	CTSB	IL11RA	0.868
56	KIT	C5a	CTSB	HAMP	0.868
57	C9	C5	CCL18	CTSB	0.868
58	C5	LTF	CTSB	THBS4	0.868
59	C5	CTSB	ACP5	ETHE1	0.868
60	C5	CCL18	CTSB	IL11RA	0.868
61	CCL18	CTSB	HAMP	ETHE1	0.868
62	PLAT	KIT	C5a	CTSB	0.868
63	C5-C6	C5a	CTSB	ETHE1	0.868
64	C5	C5a	CTSB	C2	0.868
65	C9	C5	ALPL	CTSB	0.868
66	C5	CTSB	IL11RA	ETHE1	0.868
67	CCL18	CTSB	KLK7	ETHE1	0.868
68	C5	CTSB	C2	ETHE1	0.868
69	C5	KIT	CTSB	THBS4	0.867
70	CCL18	CTSB	THBS4	ETHE1	0.867
71	CCL18	CTSB	KLK7	HAMP	0.867
72	C5	CSF1R	CCL18	ETHE1	0.867
73	KIT	CCL18	CTSB	ETHE1	0.867

Table 5 – continued from previous page

Markers					CV AUC
74	C5	C5a	CTSB	CDK5-CDK5R1	0.867
75	C5	C5a	CCL23	CTSB	0.867
76	C5	KIT	ALPL	CTSB	0.867
77	KIT	CSF1R	C5a	CTSB	0.867
78	C5	KIT	LTF	CTSB	0.867
79	C5	LTF	CTSB	IL11RA	0.867
80	C9	C5	CSF1R	CTSB	0.866
81	C5-C6	C5a	CTSB	KLK7	0.866
82	C5	C5a	CTSB	INSR	0.866
83	C5a	CCL23	CTSB	KLK7	0.866
84	C5	GDF11	CTSB	HAMP	0.866
85	C5	GDF11	C5a	CTSB	0.866
86	C5	CSF1R	CTSB	HAMP	0.866
87	C5	C5a	CTSB	TNFRSF1B	0.866
88	C5	CCL23	CTSB	ETHE1	0.866
89	C9	C5	LTF	CTSB	0.866
90	C9	C5	CTSB	HAMP	0.866
91	C9	C5	CTSB	THBS4	0.866
92	C5	LTF	CTSB	HAMP	0.866
93	C5-C6	C5	C5a	CTSB	0.865
94	C5	KLK8	C5a	CTSB	0.865
95	C5	VEGFA	CTSB	ETHE1	0.865
96	C5a	CTSB	HAMP	ETHE1	0.865
97	C5	MMP7	C5a	CTSB	0.865
98	C5	C5a	CTSB	ESM1	0.865
99	C5a	CCL18	CTSB	IL11RA	0.865
100	C5a	CTSB	C2	ETHE1	0.865

Table 6: Panels of 5 Biomarkers

Markers						CV AUC
1	C5	CSF1R	C5a	CTSB	ETHE1	0.892
2	C5	C5a	CCL18	CTSB	ETHE1	0.889
3	C5	CCL18	CTSB	THBS4	ETHE1	0.888
4	C5	CSF1R	CCL18	CTSB	ETHE1	0.887
5	PLAT	C5	C5a	CTSB	ETHE1	0.886
6	C5	KIT	CSF1R	C5a	CTSB	0.886
7	C5	KIT	CCL18	CTSB	ETHE1	0.886
8	C5	KIT	C5a	CCL18	CTSB	0.886
9	C5	LTF	CCL18	CTSB	ETHE1	0.886
10	C5	KIT	C5a	CTSB	ETHE1	0.885
11	C5a	CCL18	CTSB	KLK7	ETHE1	0.885
12	C5	CSF1R	C5a	CCL18	CTSB	0.885
13	C5	C5a	CCL18	CTSB	THBS4	0.885
14	C5	ALPL	CCL18	CTSB	ETHE1	0.884
15	C5	C5a	CTSB	KLK7	ETHE1	0.884
16	C5	ALPL	CTSB	THBS4	ETHE1	0.884
17	C5a	CTSB	KLK7	HAMP	ETHE1	0.884
18	C5	CTSB	KLK7	HAMP	ETHE1	0.884
19	C5	CCL18	CTSB	HAMP	ETHE1	0.884

Table 6 – continued from previous page

Markers						CV AUC
20	C5	KIT	CTSB	HAMP	ETHE1	0.884
21	C5	CSF1R	C5a	CTSB	TIIBS4	0.884
22	C5	LTF	CTSB	TIIBS4	ETIIE1	0.884
23	C5	ALPL	C5a	CTSB	ETIIE1	0.883
24	CCL18	CTSB	KLK7	HAMP	ETHE1	0.883
25	C5	KIT	LTF	C5a	CTSB	0.883
26	PLAT	C5	KIT	C5a	CTSB	0.883
27	PLAT	C9	C5	CTSB	ETHE1	0.883
28	C5	LTF	C5a	CTSB	ETHE1	0.883
29	C5	CSF1R	CTSB	HAMP	ETHE1	0.883
30	C9	C5	CSF1R	CTSB	ETHE1	0.882
31	PLAT	C5	CTSB	THBS4	ETHE1	0.882
32	C5	C5a	CCL23	CTSB	ETHE1	0.882
33	C5	C5a	CCL18	CTSB	KLK7	0.882
34	C5-C6	C5a	CCL18	CTSB	ETHE1	0.882
35	C5	CSF1R	CTSB	THBS4	ETHE1	0.882
36	C5	C5a	CTSB	KLK7	HAMP	0.882
37	C5	LTF	C5a	CCL18	CTSB	0.882
38	C5	KIT	ALPL	C5a	CTSB	0.882
39	C5	CCL23	CCL18	CTSB	ETHE1	0.881
40	PLAT	C5	CCL18	CTSB	ETHE1	0.881
41	C5	C5a	CTSB	THBS4	ETHE1	0.881
42	C5	KLK8	CCL18	CTSB	ETHE1	0.881
43	C5	C5a	CCL18	CTSB	IL11RA	0.881
44	C5	KIT	ALPL	CCL18	CTSB	0.881
45	PLAT	C5	C5a	CCL18	CTSB	0.881
46	C5	CCL18	CTSB	IL11RA	ETHE1	0.881
47	C5	C5a	CTSB	INSR	ETHE1	0.881
48	C5	KIT	C5a	CTSB	HAMP	0.881
49	C5	C5a	CTSB	ACP5	ETIIE1	0.881
50	C5-C6	CCL18	CTSB	TIIBS4	ETIIE1	0.880
51	C5	LTF	C5a	CTSB	KLK7	0.880
52	C5	C5a	CTSB	HAMP	ETHE1	0.880
53	C5	KIT	ALPL	CTSB	ETHE1	0.880
54	C5	KIT	LTF	CTSB	ETHE1	0.880
55	C5	ALPL	C5a	CTSB	KLK7	0.880
56	C5-C6	KIT	C5a	CCL18	CTSB	0.880
57	C5	CTSB	THBS4	HAMP	ETHE1	0.880
58	C5a	CCL18	CTSB	KLK7	HAMP	0.880
59	C5	CCL18	CTSB	KLK7	ETHE1	0.880
60	C5	CCL18	CTSB	ACP5	ETHE1	0.880
61	C5	KIT	LTF	CCL18	CTSB	0.880
62	MMP7	C5a	CTSB	KLK7	HAMP	0.880
63	C5	KIT	C5a	CCL23	CTSB	0.880
64	C9	C5	CCL18	CTSB	ETHE1	0.880
65	C5	CSF1R	C5a	CTSB	HAMP	0.880
66	C5	ALPL	C5a	CCL18	CTSB	0.880
67	C5	C5a	CCL18	CTSB	HAMP	0.880
68	C5	CSF1R	C5a	CTSB	IL11RA	0.880
69	C5a	CCL23	CTSB	KLK7	ETHE1	0.880
70	C5-C6	KIT	CCL18	CTSB	ETHE1	0.879
71	C5	CSF1R	CCL23	CTSB	ETHE1	0.879
72	C5	LTF	CCL23	CTSB	ETHE1	0.879

Table 6 – continued from previous page

Markers						CV AUC
73	C5	KLK8	C5a	CCL18	CTSB	0.879
74	LTF	C5a	CCL18	CTSB	KLK7	0.879
75	PLAT	C5	CCL23	CTSB	ETHE1	0.879
76	C5	KIT	CSF1R	CTSB	ETHE1	0.879
77	ALPL	C5a	CCL18	CTSB	KLK7	0.879
78	KIT	C5a	CCL18	CTSB	ETHE1	0.879
79	CSF1R	C5a	CCL18	CTSB	ETHE1	0.879
80	C5	KIT	C5a	CTSB	THBS4	0.879
81	C5	VEGFA	CCL18	CTSB	ETHE1	0.879
82	C5	CSF1R	C5a	CTSB	KLK7	0.879
83	CSF1R	C5a	CTSB	KLK7	ETHE1	0.879
84	C5-C6	C5a	CTSB	KLK7	ETHE1	0.879
85	MMP7	C5a	CCL18	CTSB	KLK7	0.879
86	C5-C6	C5	CCL18	CTSB	ETHE1	0.879
87	C5	ALPL	CTSB	HAMP	ETHE1	0.879
88	C5	KIT	VEGFA	CTSB	ETHE1	0.879
89	C5	CCL18	CTSB	C2	ETHE1	0.879
90	C5	KIT	CCL18	CTSB	HAMP	0.879
91	C5	CCL23	CTSB	THBS4	ETHE1	0.879
92	C5	ALPL	C5a	CTSB	THBS4	0.879
93	C5	VEGFA	CTSB	THBS4	ETHE1	0.879
94	C5	LTF	CCL18	CTSB	THBS4	0.879
95	C5	LTF	CTSB	IL11RA	ETHE1	0.878
96	MMP7	C5a	CTSB	KLK7	ETHE1	0.878
97	C5	KIT	VEGFA	CCL18	CTSB	0.878
98	C5	ALPL	CCL18	CTSB	THBS4	0.878
99	C5-C6	C5a	CCL18	CTSB	KLK7	0.878
100	C5	APOA1	CCL18	CTSB	ETHE1	0.878

Table 7: Panels of 6 Biomarkers

Markers						CV AUC
1	C5 ETHE1	CSF1R	C5a	CCL18	CTSB	0.898
2	C5 ETHE1	CSF1R	C5a	CTSB	THBS4	0.896
3	C5 ETHE1	KIT	CSF1R	C5a	CTSB	0.895
4	C5 ETHE1	KIT	C5a	CCL18	CTSB	0.894
5	PLAT ETHE1	C5	CSF1R	C5a	CTSB	0.893
6	C5 ETHE1	ALPL	CCL18	CTSB	THBS4	0.893
7	C5 ETHE1	CSF1R	CCL18	CTSB	THBS4	0.892
8	C5 ETHE1	CSF1R	C5a	CTSB	KLK7	0.892
9	C5 ETHE1	LTF	CCL18	CTSB	THBS4	0.892

Table 7 – continued from previous page

Markers						CV AUC
10	C5 ETHE1	C5a	CCL18	CTSB	KLK7	0.892
11	C5 ETHE1	CCL18	CTSB	KLK7	HAMP	0.892
12	C5 ETHE1	LTF	C5a	CCL18	CTSB	0.892
13	PLAT ETHE1	C5	C5a	CCL18	CTSB	0.891
14	C5 CTSB	KIT	CSF1R	C5a	CCL18	0.891
15	C5 THBS4	CSF1R	C5a	CCL18	CTSB	0.891
16	PLAT ETHE1	C5	KIT	C5a	CTSB	0.891
17	C5 ETHE1	KIT	LTF	CCL18	CTSB	0.891
18	C5-C6 ETHE1	C5a	CCL18	CTSB	KLK7	0.890
19	C5 ETHE1	CSF1R	C5a	CCL23	CTSB	0.890
20	C5 ETHE1	ALPL	C5a	CCL18	CTSB	0.890
21	C5 ETHE1	LTF	CCL18	CTSB	KLK7	0.890
22	C5 ETHE1	KIT	CCL18	CTSB	THBS4	0.890
23	C5 ETHE1	C5a	CCL18	CTSB	THBS4	0.890
24	C5 ETHE1	KIT	CCL18	CTSB	HAMP	0.890
25	C5 ETHE1	CSF1R	C5a	CTSB	IL11RA	0.890
26	C5 KLK7	LTF	C5a	CCL18	CTSB	0.890
27	C5 ETHE1	KIT	CSF1R	CCL18	CTSB	0.890
28	C5 ETHE1	KIT	VEGFA	CCL18	CTSB	0.889
29	C5 ETHE1	KIT	ALPL	CCL18	CTSB	0.889
30	CSF1R ETHE1	C5a	CTSB	KLK7	HAMP	0.889
31	PLAT ETHE1	C5	C5a	CTSB	KLK7	0.889
32	C5 CTSB	KIT	LTF	C5a	CCL18	0.889
33	C5 ETHE1	C5a	CTSB	KLK7	HAMP	0.889
34	PLAT ETHE1	C9	C5	CSF1R	CTSB	0.889
35	C5 ETHE1	KIT	LTF	C5a	CTSB	0.889

Table 7 – continued from previous page

Markers						CV AUC
36	C5 ETHE1	LTF	CCL18	CTSB	IL11RA	0.889
37	LTF ETHE1	C5a	CCL18	CTSB	KLK7	0.889
38	C5 ETHE1	CSF1R	CTSB	KLK7	HAMP	0.889
39	C5-C6 ETHE1	CSF1R	C5a	CCL18	CTSB	0.888
40	C5 THBS4	KIT	CSF1R	C5a	CTSB	0.888
41	C5 THBS4	KIT	C5a	CCL18	CTSB	0.888
42	C5 ETHE1	KIT	ALPL	C5a	CTSB	0.888
43	C5 ETHE1	CSF1R	C5a	CCL23	CTSB	0.888
44	C5a ETHE1	CCL18	CTSB	KLK7	HAMP	0.888
45	C5 ETHE1	ALPL	CTSB	KLK7	HAMP	0.888
46	C5 KLK7	ALPL	C5a	CCL18	CTSB	0.888
47	C5 ETHE1	ALPL	CCL18	CTSB	KLK7	0.888
48	C5 ETHE1	ALPL	C5a	CTSB	KLK7	0.888
49	C5 ETHE1	ALPL	CCL18	CTSB	IL11RA	0.888
50	C5 ETHE1	KIT	CSF1R	CTSB	HAMP	0.888
51	C5 ETHE1	CSF1R	CCL23	CCL18	CTSB	0.888
52	CSF1R ETHE1	C5a	CCL18	CTSB	THBS4	0.888
53	C5 IL11RA	CSF1R	C5a	CCL18	CTSB	0.888
54	C5 ETHE1	C5a	CCL18	CTSB	ACP5	0.888
55	CSF1R ETHE1	CCL18	CTSB	KLK7	HAMP	0.888
56	C9 ETHE1	C5	CSF1R	CCL18	CTSB	0.888
57	PLAT ETHE1	C5	C5a	CCL23	CTSB	0.888
58	PLAT CTSB	C5	KIT	CSF1R	C5a	0.888
59	C5 ETHE1	LTF	C5a	CTSB	KLK7	0.888
60	C5-C6 ETHE1	CCL18	CTSB	KLK7	HAMP	0.888
61	C5 ETHE1	CSF1R	C5a	CTSB	ACP5	0.888

Table 7 – continued from previous page

	Markers					CV AUC
62	C5 HAMP	CSF1R	C5a	CTSB	KLK7	0.888
63	C5 IL11RA	LTF	C5a	CCL18	CTSB	0.888
64	C5 ETHE1	CSF1R	ALPL	C5a	CTSB	0.888
65	C5 ETHE1	CSF1R	C5a	CTSB	HAMP	0.888
66	C5 ETHE1	C5a	CCL23	CTSB	KLK7	0.888
67	CSF1R ETHE1	C5a	CCL18	CTSB	KLK7	0.888
68	C5 ETHE1	KIT	KLK8	CCL18	CTSB	0.888
69	C5 ETHE1	C5a	CCL18	CTSB	INSR	0.887
70	C5 ETHE1	LTF	CTSB	KLK7	HAMP	0.887
71	C5 ETHE1	C5a	CCL18	CTSB	HAMP	0.887
72	C5-C6 ETHE1	KIT	CSF1R	C5a	CTSB	0.887
73	C5-C6 ETHE1	KIT	C5a	CCL18	CTSB	0.887
74	ALPL ETHE1	C5a	CCL18	CTSB	KLK7	0.887
75	C5 ETHE1	CSF1R	CCL18	CTSB	IL11RA	0.887
76	C5 ETHE1	C5a	CCL18	CTSB	IL11RA	0.887
77	C5-C6 ETHE1	C5a	CCL18	CTSB	THBS4	0.887
78	C5a ETHE1	CCL18	CTSB	KLK7	INSR	0.887
79	C9 ETHE1	C5	CSF1R	CTSB	THBS4	0.887
80	C5 ETHE1	CSF1R	LTF	C5a	CTSB	0.887
81	C5 THBS4	ALPL	C5a	CCL18	CTSB	0.887
82	C5 ETHE1	KIT	C5a	CCL23	CTSB	0.887
83	C5 HAMP	C5a	CCL18	CTSB	KLK7	0.887
84	MMP7 ETHE1	C5a	CTSB	KLK7	HAMP	0.887
85	C5 ETHE1	C5a	CTSB	ACP5	KLK7	0.887
86	C5 ETHE1	MMP7	C5a	CTSB	KLK7	0.887
87	C5 ETHE1	CCL18	CTSB	THBS4	HAMP	0.887

Table 7 – continued from previous page

	Markers					CV AUC
88	C5-C6 KLK7	LTF	C5a	CCL18	CTSB	0.887
89	C5 ETHE1	CSF1R	ALPL	CTSB	THBS4	0.887
90	C5 ETHE1	CSF1R	CCL18	CTSB	HAMP	0.887
91	C5 KLK7	CSF1R	C5a	CCL18	CTSB	0.887
92	C5-C6 ETHE1	KIT	CCL18	CTSB	HAMP	0.887
93	C5 ETHE1	KIT	C5a	CTSB	ACP5	0.887
94	PLAT ETHE1	C5	C5a	CTSB	THBS4	0.887
95	C5 THBS4	LTF	C5a	CCL18	CTSB	0.887
96	C5 CTSB	KIT	ALPL	C5a	CCL18	0.886
97	KLK8 ETHE1	C5a	CCL18	CTSB	KLK7	0.886
98	C5 ETHE1	VEGFA	CCL18	CTSB	THBS4	0.886
99	C5 ETHE1	KIT	C5a	CTSB	HAMP	0.886
100	C5 ETHE1	LTF	C5a	CCL23	CTSB	0.886

Table 8: Panels of 7 Biomarkers

	Markers					CV AUC
1	C5 THBS4	CSF1R ETHE1	C5a	CCL18	CTSB	0.900
2	C5 CTSB	KIT ETHE1	CSF1R	C5a	CCL18	0.900
3	PLAT CTSB	C5 ETHE1	KIT	CSF1R	C5a	0.899
4	C5 IL11RA	CSF1R ETHE1	C5a	CCL18	CTSB	0.898
5	C5 KLK7	CSF1R ETHE1	C5a	CCL18	CTSB	0.898
6	C5 KLK7	LTF ETHE1	C5a	CCL18	CTSB	0.897
7	C5-C6 THBS4	CSF1R ETHE1	C5a	CCL18	CTSB	0.897
8	C5 HAMP	CSF1R ETHE1	C5a	CTSB	KLK7	0.896
9	C5 THBS4	KIT ETHE1	CSF1R	C5a	CTSB	0.896
10	C5 KLK7	ALPL ETHE1	C5a	CCL18	CTSB	0.896

Table 8 – continued from previous page

Markers						CV AUC
11	C5 CTSB	KIT ETHE1	VEGFA	CSF1R	CCL18	0.896
12	PLAT THBS4	C5 ETHE1	CSF1R	C5a	CTSB	0.896
13	C5 THBS4	CSF1R ETHE1	ALPL	CCL18	CTSB	0.895
14	PLAT CTSB	C5 ETHE1	CSF1R	C5a	CCL18	0.895
15	C5 CTSB	KIT THBS4	CSF1R	C5a	CCL18	0.895
16	C5 THBS4	CSF1R ETHE1	C5a	CCL23	CTSB	0.895
17	C5 CTSB	KIT ETHE1	VEGFA	CSF1R	C5a	0.895
18	C5 THBS4	VEGFA ETHE1	CSF1R	CCL18	CTSB	0.895
19	C5 KLK7	LTF ETHE1	C5a	CCL23	CTSB	0.895
20	C5 CTSB	CSF1R ETHE1	KLK8	C5a	CCL18	0.894
21	C5-C6 CTSB	KIT ETHE1	CSF1R	C5a	CCL18	0.894
22	C5-C6 CTSB	C5 ETHE1	CSF1R	C5a	CCL18	0.894
23	C5 CTSB	KIT ETHE1	LTF	C5a	CCL18	0.894
24	C5-C6 KLK7	CSF1R ETHE1	C5a	CCL18	CTSB	0.894
25	C5 CTSB	KIT ETHE1	CSF1R	LTF	C5a	0.894
26	PLAT CTSB	C5 ETHE1	KIT	C5a	CCL18	0.894
27	C5 CTSB	KIT ETHE1	CSF1R	ALPL	C5a	0.894
28	C5 KLK7	KLK8 ETHE1	C5a	CCL18	CTSB	0.894
29	C5 CTSB	CSF1R ETHE1	ALPL	C5a	CCL18	0.894
30	C5 CTSB	CSF1R ETHE1	LTF	C5a	CCL18	0.894
31	C5-C6 KLK7	LTF ETHE1	C5a	CCL18	CTSB	0.894
32	C5 CDK5-CDK5R1	CSF1R ETHE1	C5a	CCL18	CTSB	0.894
33	PLAT CTSB	C5 ETHE1	CSF1R	C5a	CCL23	0.894
34	C5 HAMP	C5a ETHE1	CCL18	CTSB	KLK7	0.894
35	C5 HAMP	CSF1R ETHE1	CCL18	CTSB	KLK7	0.894
36	C5 CTSB	KIT ETHE1	KLK8	C5a	CCL18	0.894

Table 8 – continued from previous page

	Markers					CV AUC
37	C5 CTSB	KIT ETHE1	CSF1R	C5a	CCL23	0.894
38	C5 THBS4	ALPL ETHE1	C5a	CCL18	CTSB	0.894
39	C5 KLK7	C5a ETHE1	CCL23	CCL18	CTSB	0.894
40	C5 HAMP	KIT ETHE1	CSF1R	CCL18	CTSB	0.894
41	PLAT KLK7	C5 ETHE1	CSF1R	C5a	CTSB	0.894
42	C5 THBS4	KIT ETHE1	CSF1R	CCL18	CTSB	0.894
43	C5 THBS4	KIT ETHE1	C5a	CCL18	CTSB	0.894
44	C5 THBS4	KIT ETHE1	LTF	CCL18	CTSB	0.894
45	C5 KLK7	CSF1R ETHE1	MMP7	C5a	CTSB	0.894
46	C5 THBS4	CSF1R ETHE1	LTF	CCL18	CTSB	0.894
47	C5 ACP5	KIT ETHE1	CSF1R	C5a	CTSB	0.894
48	C5 CTSB	KIT ETHE1	ALPL	C5a	CCL18	0.894
49	C5 KLK7	C5a ETHE1	CCL18	CTSB	ACP5	0.893
50	C5 KLK7	CSF1R ETHE1	ALPL	C5a	CTSB	0.893
51	C5 THBS4	KIT ETHE1	ALPL	CCL18	CTSB	0.893
52	C5-C6 HAMP	CSF1R ETHE1	C5a	CTSB	KLK7	0.893
53	C5 KLK7	CSF1R ETHE1	LTF	C5a	CTSB	0.893
54	C5 THBS4	CSF1R ETHE1	ALPL	C5a	CTSB	0.893
55	C5 ACP5	CSF1R ETHE1	C5a	CCL18	CTSB	0.893
56	C5 TFPI	CSF1R ETHE1	C5a	CCL18	CTSB	0.893
57	C5 HAMP	ALPL ETHE1	CCL18	CTSB	KLK7	0.893
58	C5 KLK7	CSF1R ETHE1	C5a	CTSB	ACP5	0.893
59	PLAT THBS4	C9 ETHE1	C5	CSF1R	CTSB	0.893
60	C5 CTSB	KIT ETHE1	LTF	C5a	CCL23	0.893
61	C5 FGFR3	CSF1R ETHE1	C5a	CCL18	CTSB	0.893
62	PLAT KLK7	C5 ETHE1	C5a	CCL18	CTSB	0.893

Table 8 – continued from previous page

Markers						CV AUC
63	PLAT IL11RA	C5 ETHE1	CSF1R	C5a	CTSB	0.893
64	C5 CTSB	CSF1R ETHE1	C5a	CCL23	CCL18	0.893
65	C5 HAMP	KIT ETHE1	CSF1R	C5a	CTSB	0.893
66	C5 CTSB	CSF1R KLK7	ALPL	C5a	CCL18	0.893
67	C5 HAMP	LTF ETHE1	CCL18	CTSB	KLK7	0.893
68	C5 CTSB	KIT ETHE1	CSF1R	LTF	CCL18	0.893
69	PLAT CTSB	C5 ETHE1	CSF1R	C5a	CCL23	0.893
70	C5 CCL18	KIT CTSB	CSF1R	ALPL	C5a	0.893
71	CSF1R HAMP	MMP7 ETHE1	C5a	CTSB	KLK7	0.893
72	C5 KLK7	CSF1R ETHE1	C5a	CCL23	CTSB	0.892
73	C5 IL11RA	CSF1R ETHE1	ALPL	CCL18	CTSB	0.892
74	C5 KLK7	MMP7 ETHE1	C5a	CCL18	CTSB	0.892
75	PLAT CTSB	C9 ETHE1	C5	CSF1R	C5a	0.892
76	C5 CCL18	KIT CTSB	CSF1R	LTF	C5a	0.892
77	C5 CTSB	KIT ETHE1	CSF1R	MMP7	C5a	0.892
78	C5 CTSB	CSF1R KLK7	LTF	C5a	CCL18	0.892
79	C9 THBS4	C5 ETHE1	CSF1R	CCL18	CTSB	0.892
80	C5 CTSB	CSF1R ETHE1	GDF11	C5a	CCL18	0.892
81	C5 THBS4	LTF ETHE1	C5a	CCL18	CTSB	0.892
82	PLAT CTSB	C9 ETHE1	C5	CSF1R	CCL18	0.892
83	C5 CTSB	KIT ETHE1	VEGFA	KLK8	CCL18	0.892
84	C5 THBS4	CSF1R ETHE1	LTF	C5a	CTSB	0.892
85	CSF1R HAMP	C5a ETHE1	CCL18	CTSB	KLK7	0.892
86	C5 CTSB	CSF1R ETHE1	LTF	C5a	CCL23	0.892
87	C5-C6 KLK7	ALPL ETHE1	C5a	CCL18	CTSB	0.892
88	KIT THBS4	CSF1R ETHE1	C5a	CCL18	CTSB	0.892

Table 8 – continued from previous page

	Markers					CV AUC
89	C5 CTSB	CSF1R THBS4	ALPL	C5a	CCL18	0.892
90	C5 INSR	C5a ETHE1	CCL18	CTSB	KLK7	0.892
91	C5 CTSB	CSF1R IL11RA	LTF	C5a	CCL18	0.892
92	C5 ESM1	CSF1R ETHE1	C5a	CCL18	CTSB	0.892
93	C5-C6 HAMP	C5a ETHE1	CCL18	CTSB	KLK7	0.892
94	PLAT THBS4	C5 ETHE1	C5a	CCL18	CTSB	0.892
95	C5 HAMP	ALPL ETHE1	C5a	CTSB	KLK7	0.892
96	C5 IL11RA	LTF ETHE1	C5a	CCL18	CTSB	0.892
97	C5 CTSB	VEGFA ETHE1	CSF1R	C5a	CCL18	0.892
98	C5 HAMP	LTF ETHE1	C5a	CTSB	KLK7	0.892
99	C5 KLK7	ALPL ETHE1	C5a	CCL23	CTSB	0.892
100	C5 CTSB	KIT THBS4	LTF	C5a	CCL18	0.892

Table 9: Panels of 8 Biomarkers

	Markers					CV AUC
1	C5 CTSB	KIT THBS4	CSF1R ETHE1	C5a	CCL18	0.902
2	C5 CTSB	CSF1R KLK7	LTF ETHE1	C5a	CCL18	0.902
3	PLAT CCL18	C5 CTSB	KIT ETHE1	CSF1R	C5a	0.901
4	C5 CCL18	KIT CTSB	CSF1R ETHE1	LTF	C5a	0.901
5	C5 CTSB	CSF1R KLK7	ALPL ETHE1	C5a	CCL18	0.901
6	C5-C6 CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	CCL18	0.900
7	C5-C6 CTSB	KIT THBS4	CSF1R ETHE1	C5a	CCL18	0.900
8	C5 CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	CCL18	0.899
9	C5 CCL18	KIT CTSB	CSF1R ETHE1	ALPL	C5a	0.899
10	C5 KLK7	CSF1R HAMP	C5a ETHE1	CCL18	CTSB	0.899
11	C5 CTSB	KIT THBS4	VEGFA ETHE1	CSF1R	CCL18	0.899

Table 9 – continued from previous page

Markers						CV AUC
12	C5 CCL18	KIT CTSB	CSF1R ETHE1	KLK8	C5a	0.899
13	C5 CTSB	CSF1R THBS4	LTF ETHE1	C5a	CCL18	0.899
14	C5 CCL18	KIT CTSB	VEGFA ETHE1	CSF1R	C5a	0.899
15	C5 CTSB	CSF1R THBS4	ALPL ETHE1	C5a	CCL18	0.899
16	C5-C6 CTSB	C5 THBS4	CSF1R ETHE1	C5a	CCL18	0.899
17	C5-C6 CTSB	CSF1R KLK7	LTF ETHE1	C5a	CCL18	0.899
18	C5 CCL18	KIT CTSB	VEGFA ETHE1	CSF1R	KLK8	0.898
19	PLAT CTSB	C5 THBS4	CSF1R ETHE1	C5a	CCL18	0.898
20	C5 CTSB	CSF1R IL11RA	ALPL ETHE1	C5a	CCL18	0.898
21	C5 CTSB	LTF KLK7	C5a ETHE1	CCL23	CCL18	0.898
22	C5-C6 CCL18	C5 CTSB	KIT ETHE1	CSF1R	C5a	0.898
23	C5 CTSB	CSF1R IL11RA	LTF ETHE1	C5a	CCL18	0.898
24	C5 CTSB	CSF1R KLK7	MMP7 ETHE1	C5a	CCL18	0.898
25	C5 CCL23	KIT CTSB	CSF1R ETHE1	LTF	C5a	0.898
26	C5 CTSB	ALPL KLK7	KLK8 ETHE1	C5a	CCL18	0.898
27	C5 CTSB	LTF KLK7	KLK8 ETHE1	C5a	CCL18	0.898
28	PLAT CCL23	C5 CTSB	KIT ETHE1	CSF1R	C5a	0.898
29	C5 CTSB	KIT ACP5	CSF1R ETHE1	C5a	CCL18	0.898
30	C5 CCL18	KIT CTSB	CSF1R ETHE1	C5a	CCL23	0.898
31	C5-C6 KLK7	CSF1R HAMP	C5a ETHE1	CCL18	CTSB	0.898
32	C5 CTSB	CSF1R KLK7	KLK8 ETHE1	C5a	CCL18	0.898
33	PLAT CTSB	C5 THBS4	KIT ETHE1	CSF1R	C5a	0.898
34	C5 CTSB	KIT THBS4	CSF1R ETHE1	ALPL	CCL18	0.898
35	C5 CTSB	KIT THBS4	CSF1R ETHE1	LTF	CCL18	0.898
36	C5 CTSB	CSF1R THBS4	C5a ETHE1	CCL23	CCL18	0.898
37	C5 CTSB	CSF1R KLK7	LTF ETHE1	C5a	CCL23	0.898

Table 9 – continued from previous page

	Markers					CV AUC
38	C5 THBS4	CSF1R KLK7	C5a ETHE1	CCL18	CTSB	0.898
39	C5 CTSB	CSF1R THBS4	KLK8 ETHE1	C5a	CCL18	0.898
40	C5 CTSB	VEGFA THBS4	CSF1R ETHE1	C5a	CCL18	0.898
41	C5-C6 CTSB	CSF1R KLK7	MMP7 ETHE1	C5a	CCL18	0.897
42	PLAT CTSB	C5 KLK7	CSF1R ETHE1	C5a	CCL18	0.897
43	C5 CTSB	KIT TFPI	CSF1R ETHE1	C5a	CCL18	0.897
44	C5 CCL18	CSF1R CTSB	GDF11 ETHE1	LTF	C5a	0.897
45	C5 THBS4	LTF KLK7	C5a ETHE1	CCL18	CTSB	0.897
46	C5 KLK7	KIT HAMP	CSF1R ETHE1	C5a	CTSB	0.897
47	C5 CTSB	KIT THBS4	CSF1R ETHE1	LTF	C5a	0.897
48	PLAT CTSB	C5 IL11RA	CSF1R ETHE1	C5a	CCL18	0.897
49	C5 CTSB	LTF KLK7	MMP7 ETHE1	C5a	CCL18	0.897
50	C5-C6 CCL18	KIT CTSB	VEGFA ETHE1	CSF1R	C5a	0.897
51	C5 CTSB	KIT THBS4	CSF1R ETHE1	ALPL	C5a	0.897
52	C5 CTSB	IL12A-IL12B THBS4	CSF1R ETHE1	C5a	CCL18	0.896
53	C5 CTSB	CSF1R KLK7	C5a ETHE1	CCL23	CCL18	0.896
54	PLAT CTSB	C5 KLK7	CSF1R ETHE1	MMP7	C5a	0.896
55	C5 KLK7	CSF1R HAMP	MMP7 ETHE1	C5a	CTSB	0.896
56	C5 CTSB	KIT KLK7	LTF ETHE1	C5a	CCL18	0.896
57	C5 CTSB	KIT IL11RA	CSF1R ETHE1	C5a	CCL18	0.896
58	C5 CTSB	VEGFA KLK7	CSF1R ETHE1	MMP7	C5a	0.896
59	C5 CTSB	CSF1R THBS4	LTF KLK7	C5a	CCL18	0.896
60	C5 THBS4	CSF1R INSR	C5a ETHE1	CCL18	CTSB	0.896
61	C5-C6 CCL18	KIT CTSB	CSF1R ETHE1	C5a	CCL23	0.896
62	C5 KLK7	KIT HAMP	C5a ETHE1	CCL18	CTSB	0.896
63	C5 KLK7	LTF INSR	C5a ETHE1	CCL18	CTSB	0.896

Table 9 – continued from previous page

Markers						CV AUC
64	C5 CCL18	KIT CTSB	CSF1R THBS4	LTF	C5a	0.896
65	C5 CTSB	CSF1R THBS4	LTF ETHE1	C5a	CCL23	0.896
66	C5-C6 CTSB	CSF1R KLK7	ALPL ETHE1	C5a	CCL18	0.896
67	C5 CTSB	VEGFA IL11RA	CSF1R ETHE1	C5a	CCL18	0.896
68	C5 ACP5	CSF1R KLK7	C5a ETHE1	CCL18	CTSB	0.896
69	PLAT CTSB	C9 THBS4	C5 ETHE1	CSF1R	CCL18	0.896
70	PLAT C5a	C5 CTSB	KIT ETHE1	CSF1R	ALPL	0.896
71	C5 CTSB	KIT THBS4	LTF ETHE1	C5a	CCL18	0.896
72	C5-C6 CTSB	LTF KLK7	C5a ETHE1	CCL23	CCL18	0.896
73	C5 THBS4	KIT HAMP	CSF1R ETHE1	CCL18	CTSB	0.896
74	PLAT KLK7	C5 HAMP	CSF1R ETHE1	C5a	CTSB	0.896
75	C5 KLK7	LTF HAMP	C5a ETHE1	CCL18	CTSB	0.896
76	C5 CCL18	KIT CTSB	CSF1R IL11RA	LTF	C5a	0.896
77	PLAT C5a	C5 CTSB	KIT ETHE1	VEGFA	CSF1R	0.896
78	C5 CTSB	KIT THBS4	ALPL ETHE1	C5a	CCL18	0.896
79	KIT KLK7	CSF1R HAMP	C5a ETHE1	CCL18	CTSB	0.896
80	C5 CTSB	ALPL KLK7	C5a ETHE1	CCL23	CCL18	0.896
81	C5 THBS4	CSF1R ESM1	C5a ETHE1	CCL18	CTSB	0.896
82	C5 TFPI	CSF1R THBS4	C5a ETHE1	CCL18	CTSB	0.896
83	C5 CTSB	KIT THBS4	KLK8 ETHE1	C5a	CCL18	0.896
84	C5-C6 THBS4	CSF1R KLK7	C5a ETHE1	CCL18	CTSB	0.896
85	PLAT CTSB	C5 KLK7	CSF1R ETHE1	C5a	CCL23	0.896
86	PLAT C5a	C5 CTSB	KIT ETHE1	CSF1R	KLK8	0.896
87	C5 TFPI	CSF1R KLK7	C5a ETHE1	CCL18	CTSB	0.896
88	C5 CTSB	KIT THBS4	CSF1R ETHE1	C5a	CCL23	0.896
89	C5 CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	CCL23	0.896

Table 9 – continued from previous page

	Markers					CV AUC
90	PLAT CTSB	C5 KLK7	ALPL ETHE1	C5a	CCL18	0.896
91	C5 CCL18	KIT CTSB	CSF1R THBS4	ALPL	C5a	0.896
92	PLAT CTSB	C5 KLK7	LTF ETHE1	C5a	CCL18	0.896
93	PLAT CCL23	C5 CTSB	KIT ETHE1	CSF1R	C5a	0.896
94	PLAT CCL18	C9 CTSB	C5 ETHE1	KIT	CSF1R	0.896
95	C5 KLK7	CSF1R HAMP	LTF ETHE1	C5a	CTSB	0.895
96	C5-C6 CTSB	KIT THBS4	VEGFA ETHE1	CSF1R	CCL18	0.895
97	C5 THBS4	ALPL KLK7	C5a ETHE1	CCL18	CTSB	0.895
98	CSF1R CTSB	LTF KLK7	C5a ETHE1	CCL23	CCL18	0.895
99	C5 CTSB	KIT THBS4	VEGFA ETHE1	CSF1R	C5a	0.895
100	C5 THBS4	CSF1R CDK5-CDK5R1	C5a ETHE1	CCL18	CTSB	0.895

Table 10: Panels of 9 Biomarkers

	Markers					CV AUC
1	C5 CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	CCL23	0.903
2	C5 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	C5a	0.902
3	C5-C6 CCL18	C5 CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	0.902
4	C5 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	C5a	0.902
5	C5-C6 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	C5a	0.902
6	C5 CCL18	KIT CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.902
7	C5 CCL18	KIT CTSB	CSF1R THBS4	ALPL ETHE1	C5a	0.902
8	C5 CCL18	KIT CTSB	CSF1R THBS4	LTF ETHE1	C5a	0.902
9	C5 CCL18	CSF1R CTSB	ALPL KLK7	KLK8 ETHE1	C5a	0.901
10	PLAT CCL18	C5 CTSB	KIT THBS4	CSF1R ETHE1	C5a	0.901
11	C5 CTSB	CSF1R THBS4	ALPL KLK7	C5a ETHE1	CCL18	0.901
12	C5 CCL18	KIT CTSB	CSF1R IL11RA	LTF ETHE1	C5a	0.901

Table 10 – continued from previous page

Markers						CV AUC
13	C5-C6 CCL18	C5 CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.901
14	C5 CCL18	KIT CTSB	CSF1R THBS4	KLK8 ETHE1	C5a	0.901
15	C5-C6 C5a	C5 CCL18	KIT CTSB	VEGFA ETHE1	CSF1R	0.901
16	C5 CTSB	CSF1R THBS4	LTF KLK7	C5a ETHE1	CCL18	0.901
17	C5 CCL18	KIT CTSB	VEGFA THBS4	CSF1R ETHE1	KLK8	0.901
18	C5 CTSB	VEGFA THBS4	CSF1R KLK7	C5a ETHE1	CCL18	0.901
19	C5-C6 CTSB	VEGFA THBS4	CSF1R KLK7	C5a ETHE1	CCL18	0.901
20	C5 CTSB	CSF1R TFPI	ALPL KLK7	C5a ETHE1	CCL18	0.900
21	C5 CCL18	KIT CTSB	VEGFA THBS4	CSF1R ETHE1	C5a	0.900
22	C5-C6 CCL18	KIT CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	0.900
23	C5-C6 CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	CCL23	0.900
24	PLAT C5a	C5 CCL18	KIT CTSB	CSF1R ETHE1	KLK8	0.900
25	C5 CTSB	CSF1R TFPI	LTF KLK7	C5a ETHE1	CCL18	0.900
26	C5 CCL18	CSF1R CTSB	LTF KLK7	KLK8 ETHE1	C5a	0.900
27	C5 CCL23	KIT CTSB	CSF1R THBS4	LTF ETHE1	C5a	0.900
28	C5-C6 CCL18	C5 CTSB	VEGFA THBS4	CSF1R ETHE1	C5a	0.900
29	C5-C6 CCL18	C5 CTSB	KIT THBS4	CSF1R ETHE1	C5a	0.900
30	C5 CTSB	CSF1R KLK7	LTF IL11RA	C5a ETHE1	CCL18	0.900
31	C5-C6 C5a	C5 CCL18	KIT CTSB	CSF1R ETHE1	LTF	0.900
32	C5 CTSB	CSF1R KLK7	LTF HAMP	C5a ETHE1	CCL18	0.900
33	C5 CCL18	KIT CTSB	CSF1R KLK7	ALPL ETHE1	C5a	0.900
34	C5 CCL18	CSF1R CTSB	LTF KLK7	MMP7 ETHE1	C5a	0.900
35	C5-C6 CCL18	KIT CTSB	VEGFA THBS4	CSF1R ETHE1	C5a	0.900
36	C5 CCL23	KIT CCL18	CSF1R CTSB	LTF ETHE1	C5a	0.900
37	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R ETHE1	KLK8	0.900
38	C5 CTSB	LTF THBS4	C5a KLK7	CCL23 ETHE1	CCL18	0.900

Table 10 – continued from previous page

Markers						CV AUC
39	C5-C6 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	C5a	0.900
40	PLAT CCL18	C5 CTSB	CSF1R KLK7	ALPL ETHE1	C5a	0.900
41	C5-C6 CTSB	CSF1R THBS4	LTF KLK7	C5a ETHE1	CCL18	0.900
42	C5 CTSB	KIT KLK7	LTF HAMP	C5a ETHE1	CCL18	0.899
43	C5 CTSB	KIT THBS4	CSF1R INSR	C5a ETHE1	CCL18	0.899
44	C5 CCL18	KIT CTSB	VEGFA KLK7	CSF1R ETHE1	C5a	0.899
45	C5 CCL18	KIT CTSB	CSF1R IL11RA	ALPL ETHE1	C5a	0.899
46	C5 CCL18	CSF1R CTSB	ALPL KLK7	C5a ETHE1	CCL23	0.899
47	C5 CTSB	CSF1R THBS4	C5a KLK7	CCL23 ETHE1	CCL18	0.899
48	C5 THBS4	CSF1R KLK7	C5a HAMP	CCL18 ETHE1	CTSB	0.899
49	C5 CTSB	CSF1R KLK7	ALPL HAMP	C5a ETHE1	CCL18	0.899
50	C5 CCL18	KIT CTSB	CSF1R THBS4	LTF ETHE1	CCL23	0.899
51	C5 CCL18	KIT CTSB	LTF KLK7	KLK8 ETHE1	C5a	0.899
52	C5-C6 CCL18	C5 CTSB	CSF1R KLK7	ALPL ETHE1	C5a	0.899
53	C5 CTSB	CSF1R KLK7	ALPL IL11RA	C5a ETHE1	CCL18	0.899
54	C5 CCL18	VEGFA CTSB	CSF1R KLK7	C5a ETHE1	CCL23	0.899
55	C5 CCL18	CSF1R CTSB	LTF THBS4	C5a ETHE1	CCL23	0.899
56	C5-C6 CTSB	CSF1R KLK7	MMP7 HAMP	C5a ETHE1	CCL18	0.899
57	C5 CTSB	CSF1R THBS4	MMP7 KLK7	C5a ETHE1	CCL18	0.899
58	C5 CTSB	KIT ACP5	CSF1R THBS4	C5a ETHE1	CCL18	0.899
59	C5 CCL18	KIT CTSB	CSF1R THBS4	MMP7 ETHE1	C5a	0.899
60	C5 CCL18	KIT CTSB	CSF1R TFPI	LTF ETHE1	C5a	0.899
61	C5 CCL18	KIT CTSB	LTF THBS4	C5a ETHE1	CCL23	0.899
62	C5-C6 C5a	PLAT CCL18	C5 CTSB	KIT ETHE1	CSF1R	0.899
63	PLAT CCL18	C5 CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.899
64	C5 CTSB	CSF1R KLK7	MMP7 HAMP	C5a ETHE1	CCL18	0.899

Table 10 – continued from previous page

Markers						CV AUC
65	C5 CCL18	CSF1R CTSB	KLK8 KLK7	C5a ETHE1	CCL23	0.899
66	C9 CCL18	C5 CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.899
67	C5 CTSB	VEGFA KLK7	CSF1R IL11RA	MMP7 ETHE1	C5a	0.899
68	C5 C5a	KIT CCL18	CSF1R CTSB	LTF ETHE1	KLK8	0.899
69	PLAT C5a	C5 CCL23	KIT CTSB	VEGFA ETHE1	CSF1R	0.899
70	C5 CTSB	KIT TFPI	CSF1R THBS4	C5a ETHE1	CCL18	0.899
71	C5-C6 CCL18	KIT CTSB	CSF1R THBS4	C5a ETHE1	CCL23	0.899
72	PLAT C5a	C5 CCL18	KIT CTSB	CSF1R ETHE1	LTF	0.899
73	C5-C6 CCL18	PLAT CTSB	C5 THBS4	CSF1R ETHE1	C5a	0.899
74	C5 CCL18	CSF1R CTSB	MMP7 KLK7	ALPL ETHE1	C5a	0.899
75	C5 CTSB	KIT KLK7	CSF1R HAMP	C5a ETHE1	CCL18	0.899
76	C5 CTSB	KIT TFPI	CSF1R THBS4	LTF ETHE1	CCL18	0.899
77	C5 CCL18	KIT CTSB	VEGFA IL11RA	CSF1R ETHE1	C5a	0.899
78	C5 CCL18	CSF1R CTSB	MMP7 KLK7	KLK8 ETHE1	C5a	0.899
79	PLAT CCL18	C5 CTSB	CSF1R KLK7	C5a ETHE1	CCL23	0.899
80	C5 CTSB	ALPL THBS4	KLK8 KLK7	C5a ETHE1	CCL18	0.899
81	C5-C6 CCL18	KIT CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.899
82	PLAT CCL18	C5 CTSB	CSF1R KLK7	MMP7 ETHE1	C5a	0.899
83	C5 CCL18	IL12A-IL12B CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.899
84	C5 CCL18	KIT CTSB	CSF1R TFPI	ALPL ETHE1	C5a	0.898
85	C5 CTSB	KIT KLK7	VEGFA HAMP	CSF1R ETHE1	C5a	0.898
86	C5 CTSB	KIT TFPI	CSF1R THBS4	ALPL ETHE1	CCL18	0.898
87	PLAT C5a	C5 CCL23	KIT CTSB	CSF1R ETHE1	LTF	0.898
88	C5-C6 CTSB	KIT TFPI	CSF1R THBS4	C5a ETHE1	CCL18	0.898
89	C5 CTSB	VEGFA ACP5	CSF1R KLK7	C5a ETHE1	CCL18	0.898
90	C5 CCL18	VEGFA CTSB	CSF1R KLK7	LTF ETHE1	C5a	0.898

Table 10 – continued from previous page

Markers						CV AUC
91	C5 CTSB	CSF1R TFPI	ALPL THBS4	C5a ETHE1	CCL18	0.898
92	C5 CTSB	CSF1R THBS4	KLK8 KLK7	C5a ETHE1	CCL18	0.898
93	C5 CCL18	KIT CTSB	LTF KLK7	C5a ETHE1	CCL23	0.898
94	PLAT CCL23	C5 CCL18	KIT CTSB	CSF1R ETHE1	C5a	0.898
95	C5 CTSB	VEGFA KLK7	CSF1R ESM1	C5a ETHE1	CCL18	0.898
96	C5 CCL18	KIT CTSB	CSF1R THBS4	C5a ETHE1	CCL23	0.898
97	C5 CTSB	VEGFA KLK7	CSF1R IL11RA	C5a ETHE1	CCL18	0.898
98	C5 CTSB	VEGFA THBS4	CSF1R KLK7	C5a ETHE1	CCL23	0.898
99	C5-C6 CTSB	KIT KLK7	CSF1R HAMP	C5a ETHE1	CCL18	0.898
100	C5-C6 CCL18	CSF1R CTSB	LTF KLK7	MMP7 ETHE1	C5a	0.898

Table 11: Panels of 10 Biomarkers

Markers						CV AUC
1	C5-C6 C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	0.905
2	C5 CCL18	CSF1R CTSB	LTF THBS4	C5a KLK7	CCL23 ETHE1	0.904
3	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	0.904
4	C5-C6 C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	LTF ETHE1	0.904
5	C5 C5a	VEGFA CCL18	CSF1R CTSB	MMP7 KLK7	KLK8 ETHE1	0.904
6	C5-C6 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	0.904
7	C5-C6 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	0.903
8	C5-C6 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 IL11RA	C5a ETHE1	0.903
9	C5 CCL18	VEGFA CTSB	CSF1R THBS4	KLK8 KLK7	C5a ETHE1	0.903
10	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	0.903
11	C5 CCL18	VEGFA CTSB	CSF1R THBS4	MMP7 KLK7	C5a ETHE1	0.903
12	C5 CCL23	KIT CCL18	CSF1R CTSB	LTF THBS4	C5a ETHE1	0.903
13	C5 CCL18	CSF1R CTSB	ALPL THBS4	KLK8 KLK7	C5a ETHE1	0.903

Table 11 – continued from previous page

Markers						CV AUC
14	C5-C6 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	LTF ETHE1	0.903
15	C5-C6 CCL18	VEGFA CTSB	CSF1R THBS4	MMP7 KLK7	C5a ETHE1	0.903
16	C5 CCL23	KIT CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	0.903
17	C5-C6 CCL23	C5 CCL18	VEGFA CTSB	CSF1R KLK7	C5a ETHE1	0.903
18	C5 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 IL11RA	C5a ETHE1	0.903
19	C5-C6 C5a	C5 CCL18	KIT CTSB	VEGFA THBS4	CSF1R ETHE1	0.902
20	C5-C6 CCL18	CSF1R CTSB	LTF THBS4	C5a KLK7	CCL23 ETHE1	0.902
21	C5 C5a	KIT CCL18	CSF1R CTSB	LTF THBS4	KLK8 ETHE1	0.902
22	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R THBS4	KLK8 ETHE1	0.902
23	C5 C5a	KIT CCL18	CSF1R CTSB	ALPL THBS4	KLK8 ETHE1	0.902
24	C5-C6 C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	ALPL ETHE1	0.902
25	C5 C5a	KIT CCL18	CSF1R CTSB	LTF KLK7	KLK8 ETHE1	0.902
26	C5 CCL18	CSF1R CTSB	LTF THBS4	KLK8 KLK7	C5a ETHE1	0.902
27	C5 CCL18	CSF1R CTSB	ALPL THBS4	C5a KLK7	CCL23 ETHE1	0.902
28	C5-C6 C5a	VEGFA CCL18	CSF1R CTSB	MMP7 KLK7	KLK8 ETHE1	0.902
29	C5 CCL23	KIT CCL18	VEGFA CTSB	CSF1R KLK7	C5a ETHE1	0.902
30	C5 CCL18	KIT CTSB	CSF1R KLK7	LTF HAMP	C5a ETHE1	0.902
31	C5 CCL18	KIT CTSB	CSF1R TFPI	LTF THBS4	C5a ETHE1	0.902
32	C5-C6 CCL18	C5 CTSB	VEGFA THBS4	CSF1R KLK7	C5a ETHE1	0.902
33	C5 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ESM1	C5a ETHE1	0.902
34	PLAT C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 ETHE1	0.902
35	C5-C6 CCL18	C5 CTSB	CSF1R THBS4	LTF KLK7	C5a ETHE1	0.902
36	C5 CCL18	VEGFA CTSB	CSF1R THBS4	C5a KLK7	CCL23 ETHE1	0.902
37	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R IL11RA	MMP7 ETHE1	0.902
38	C5 CCL23	CSF1R CCL18	LTF CTSB	KLK8 KLK7	C5a ETHE1	0.902
39	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R IL11RA	KLK8 ETHE1	0.902

Table 11 – continued from previous page

Markers						CV AUC
40	C5-C6 C5a	C5 CCL18	KIT CTSB	CSF1R THBS4	LTF ETHE1	0.902
41	C5 CCL18	CSF1R CTSB	LTF KLK7	MMP7 IL11RA	C5a ETHE1	0.901
42	C5 CCL23	VEGFA CCL18	CSF1R CTSB	KLK8 KLK7	C5a ETHE1	0.901
43	C5 C5a	KIT CCL18	CSF1R CTSB	ALPL KLK7	KLK8 ETHE1	0.901
44	KIT C5a	VEGFA CCL18	CSF1R CTSB	MMP7 KLK7	KLK8 ETHE1	0.901
45	C5 C5a	KIT CCL18	CSF1R CTSB	LTF IL11RA	KLK8 ETHE1	0.901
46	C5-C6 CCL23	C5 CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	0.901
47	C5-C6 CCL23	KIT CCL18	CSF1R CTSB	LTF THBS4	C5a ETHE1	0.901
48	C5-C6 CCL18	VEGFA CTSB	CSF1R THBS4	KLK8 KLK7	C5a ETHE1	0.901
49	PLAT KLK8	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R ETHE1	0.901
50	C5 CCL18	VEGFA CTSB	CSF1R KLK7	LTF IL11RA	C5a ETHE1	0.901
51	C5 CCL18	KIT CTSB	CSF1R TFPI	ALPL THBS4	C5a ETHE1	0.901
52	C5 CCL18	CSF1R CTSB	MMP7 THBS4	KLK8 KLK7	C5a ETHE1	0.901
53	C5 CCL18	CSF1R CTSB	LTF TFPI	KLK8 KLK7	C5a ETHE1	0.901
54	C5 CCL18	CSF1R CTSB	LTF THBS4	MMP7 KLK7	C5a ETHE1	0.901
55	C5 CCL18	CSF1R CTSB	ALPL TFPI	KLK8 KLK7	C5a ETHE1	0.901
56	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R KLK7	LTF ETHE1	0.901
57	C5-C6 CCL18	C5 CTSB	VEGFA ACP5	CSF1R KLK7	C5a ETHE1	0.901
58	PLAT C5a	C5 CCL18	KIT CTSB	CSF1R THBS4	KLK8 ETHE1	0.901
59	C5 CTSB	CSF1R THBS4	MMP7 KLK7	C5a HAMP	CCL18 ETHE1	0.901
60	C5-C6 CCL18	KIT CTSB	CSF1R KLK7	LTF HAMP	C5a ETHE1	0.901
61	PLAT C5a	C5 CCL18	KIT CTSB	VEGFA THBS4	CSF1R ETHE1	0.901
62	C5-C6 CCL18	VEGFA CTSB	CSF1R THBS4	LTF KLK7	C5a ETHE1	0.901
63	C5 CCL23	VEGFA CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	0.901
64	C5 CTSB	CSF1R TFPI	ALPL THBS4	C5a KLK7	CCL18 ETHE1	0.901
65	C5-C6 CCL18	KIT CTSB	VEGFA THBS4	CSF1R KLK7	C5a ETHE1	0.901

Table 11 – continued from previous page

	Markers					CV AUC
66	C5-C6 C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	0.901
67	C5 C5a	KIT CCL18	CSF1R CTSB	LTF KLK7	MMP7 ETHE1	0.901
68	C5-C6 CCL23	C5 CCL18	VEGFA CTSB	CSF1R THBS4	C5a ETHE1	0.901
69	PLAT CSF1R	C9 KLK8	C5 CCL18	KIT CTSB	VEGFA ETHE1	0.901
70	C5-C6 C5a	PLAT CCL18	C5 CTSB	VEGFA KLK7	CSF1R ETHE1	0.901
71	PLAT C5a	C5 CCL18	VEGFA CTSB	CSF1R KLK7	MMP7 ETHE1	0.901
72	C5-C6 C5a	C5 CCL18	CSF1R CTSB	LTF KLK7	KLK8 ETHE1	0.901
73	C5-C6 C5a	C5 CCL18	KIT CTSB	VEGFA KLK7	CSF1R ETHE1	0.901
74	C5-C6 C5a	C5 CCL23	KIT CCL18	VEGFA CTSB	CSF1R ETHE1	0.901
75	C5-C6 C5a	C5 CCL18	VEGFA CTSB	CSF1R THBS4	KLK8 ETHE1	0.901
76	C5-C6 CCL23	KIT CCL18	VEGFA CTSB	CSF1R THBS4	C5a ETHE1	0.901
77	C5 C5a	VEGFA CCL18	CSF1R CTSB	LTF KLK7	KLK8 ETHE1	0.901
78	C5 CTSB	CSF1R TFPI	LTF THBS4	C5a KLK7	CCL18 ETHE1	0.901
79	C5 CCL18	KIT CTSB	CSF1R THBS4	LTF KLK7	C5a ETHE1	0.901
80	C5-C6 KLK8	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R ETHE1	0.901
81	C5 CCL18	KIT CTSB	CSF1R KLK7	MMP7 HAMP	C5a ETHE1	0.901
82	C5 CCL18	CSF1R CTSB	LTF KLK7	MMP7 HAMP	C5a ETHE1	0.901
83	C5 CCL18	KIT CTSB	VEGFA KLK7	CSF1R HAMP	C5a ETHE1	0.901
84	C5 C5a	VEGFA CCL18	CSF1R CTSB	ALPL KLK7	KLK8 ETHE1	0.901
85	C5 CCL23	CSF1R CCL18	LTF CTSB	MMP7 KLK7	C5a ETHE1	0.900
86	C5-C6 CCL18	KIT CTSB	VEGFA KLK7	CSF1R HAMP	C5a ETHE1	0.900
87	C5-C6 CCL23	KIT CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	0.900
88	C5-C6 CSF1R	PLAT C5a	C5 CCL18	KIT CTSB	VEGFA ETHE1	0.900
89	C5 CCL18	VEGFA CTSB	CSF1R KLK7	KLK8 IL11RA	C5a ETHE1	0.900
90	C5 CCL23	CSF1R CCL18	ALPL CTSB	KLK8 KLK7	C5a ETHE1	0.900
91	C5 C5a	KIT CCL18	VEGFA CTSB	CSF1R TFPI	KLK8 ETHE1	0.900

Table 11 – continued from previous page

Markers						CV AUC
92	C5 CCL18	KIT CTSB	VEGFA ACP5	CSF1R KLK7	C5a ETHE1	0.900
93	PLAT CCL18	C5 CTSB	CSF1R THBS4	ALPL KLK7	C5a ETHE1	0.900
94	C5 CCL23	KIT CCL18	VEGFA CTSB	CSF1R THBS4	C5a ETHE1	0.900
95	C5 C5a	VEGFA CCL18	CSF1R CTSB	MMP7 KLK7	ALPL IL11RA	0.900
96	PLAT CCL23	C5 CCL18	CSF1R CTSB	LTF KLK7	C5a ETHE1	0.900
97	C5 C5a	VEGFA CCL18	CSF1R CTSB	LTF KLK7	MMP7 IL11RA	0.900
98	C5 CTSB	CSF1R KLK7	LTF HAMP	C5a IL11RA	CCL18 ETHE1	0.900
99	C5-C6 C5a	KIT CCL18	VEGFA CTSB	CSF1R IL11RA	MMP7 ETHE1	0.900
100	C5-C6 C5a	C5 CCL18	KIT CTSB	CSF1R KLK7	LTF ETHE1	0.900

Table 12: Counts of markers in biomarker panels

	Panel Size							
Biomarker	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ACP5	35	35	40	54	58	60	54	59
ACY1	7	5	2	0	1	0	0	0
AHSG	18	15	3	0	0	0	0	0
ALPL	28	69	109	146	171	179	178	190
APOA1	89	48	22	13	5	3	7	9
APOE	5	1	0	0	0	0	0	0
BMP6	5	1	0	0	0	0	0	0
C2	156	106	100	64	48	41	47	66
C5	256	373	600	731	777	806	819	808
C5a	153	370	552	641	751	862	920	958
C5-C6	45	92	103	114	134	175	217	287
C9	58	119	109	91	73	74	67	71
CCL18	119	157	284	459	605	694	807	893
CCL23	23	35	30	26	26	23	17	17
CCL23	45	57	65	94	115	152	158	182
CDK5-CDK5R1	12	19	19	12	11	14	16	16
CKB-CKM	14	7	1	1	0	0	0	0
CKM	6	0	0	0	0	0	0	0
CRP	65	0	0	0	0	0	0	0
CSF1R	98	74	131	266	442	671	810	913
CTSB	586	963	990	995	999	1000	1000	1000
ENTPD1	4	6	2	0	0	0	0	0
ESM1	14	13	16	17	14	17	27	30
ETHE1	118	237	403	613	778	870	923	955
FCGR3B	34	14	5	2	1	0	0	0
FGFR3	13	10	11	14	10	16	11	8
FSTL3	9	4	1	0	0	0	0	0
GDF11	29	43	31	20	17	20	19	20
GFRA1	10	4	0	0	0	0	0	0
HAMP	93	202	239	218	193	166	154	131
HINT1	14	19	14	14	14	10	8	10
IDUA	4	3	1	0	0	0	0	0
IL11RA	58	78	69	68	73	77	109	150
IL12A-IL12B	18	15	10	13	10	11	13	13
IL18R1	7	1	0	0	0	0	0	0
IL1RL1	17	10	3	0	0	0	0	0
INSR	21	26	24	27	44	42	49	44
KIT	63	142	202	251	306	348	392	445
KLK3-SERPINA3	18	12	2	0	0	0	0	0
KLK7	89	127	231	317	410	511	606	714
KLK8	19	27	40	59	90	137	205	294
KLKB1	12	7	1	0	0	0	0	0
LBP	22	21	5	0	0	0	0	0
LTF	30	66	106	161	202	252	310	347
MCM2	6	4	0	0	0	0	0	0
MDK	14	3	0	0	0	0	0	0
MMP7	56	42	56	73	97	130	194	270
MRC1	19	4	3	1	0	0	1	0
NID1	7	2	0	0	0	0	0	0
NID2	7	0	0	0	0	0	0	0

Table 12 – continued from previous page

Biomarker	3	4	5	6	7	8	9	10
NRP1	44	20	12	5	2	2	2	1
PLAT	48	54	92	123	143	145	165	177
SERPINA5	5	0	0	0	0	0	0	0
SERPINF2	5	3	3	0	0	0	0	0
SGTA	3	2	1	0	0	0	0	0
TFPI	100	60	51	46	57	70	91	111
THBS2	4	0	0	0	0	0	0	0
THBS4	66	110	146	193	243	276	334	354
TIMP1	22	2	0	0	0	0	0	0
TNFRSF18	8	9	3	0	1	3	2	2
TNFRSF1B	20	12	8	6	4	1	0	0
TOP1	6	3	0	0	0	0	0	0
VEGFA	16	33	47	51	75	142	268	455
VEGFC	5	4	2	1	0	0	0	0

Table 13: Analytes in ten marker classifiers

CTSB	C5a
ETHE1	CSF1R
CCL18	C5
KLK7	VEGFA
KIT	THBS4
LTF	

Table 14: Parameters derived from training set for naïve Bayes classifier.

Biomarker	μ_c	μ_d	σ_c	σ_d
CSF1R	10.712	10.995	0.398	0.399
CTSB	8.836	9.398	0.287	0.621
IL1RL1	9.702	10.189	0.533	0.780
GDF11	8.889	8.578	0.291	0.379
ETHE1	7.373	7.443	0.119	0.121
CCL23	8.795	8.975	0.312	0.329
FGFR3	6.992	7.166	0.178	0.225
KIT	9.770	9.623	0.287	0.318
FSTL3	8.787	9.029	0.290	0.374
THBS2	7.481	7.922	0.270	0.633
SERPINF2	9.264	9.175	0.115	0.162
TNFRSF1B	10.748	11.028	0.380	0.452
TNFRSF18	12.308	12.279	0.139	0.168
BMP6	7.958	8.138	0.142	0.239
GFRA1	7.324	7.465	0.182	0.200
CRP	11.965	12.304	0.735	0.233
SERPINA5	10.309	10.101	0.300	0.419
KLKB1	11.802	11.666	0.159	0.211
APOE	8.081	8.314	0.406	0.656
SFRP1	7.096	7.219	0.221	0.309
C2	11.506	11.611	0.100	0.132
CKM	7.313	7.192	0.154	0.116
TFPI	10.179	10.490	0.261	0.352
INSR	8.480	8.633	0.224	0.255
NID2	8.595	8.806	0.213	0.384
HAMP	10.424	11.079	0.788	0.617
MDK	8.034	8.495	0.570	0.578
CDK5-CDK5R1	6.937	6.994	0.108	0.111
NID1	9.771	9.941	0.213	0.357
VEGFC	7.454	7.540	0.118	0.126
C9	11.911	12.076	0.234	0.233
LTF	10.120	9.870	0.442	0.419
IL12A-IL12B	7.311	7.273	0.052	0.057
C5	9.485	9.603	0.119	0.143
IL18R1	7.643	7.845	0.186	0.475
CCL18	11.320	11.616	0.477	0.398
VEGFA	8.532	8.601	0.170	0.134
IDUA	8.428	8.694	0.366	0.558
TOP1	6.892	6.842	0.088	0.091
C5-C6	6.506	6.593	0.133	0.144
TIMP1	9.815	10.148	0.264	0.430
C5a	11.354	11.606	0.254	0.246
THBS4	10.013	9.794	0.359	0.400
ENTPD1	7.225	7.299	0.110	0.103
LBP	9.102	9.489	0.439	0.548
KLK3-SERPINA3	9.034	9.287	0.353	0.422
MCM2	7.794	7.975	0.226	0.359
SGTA	5.920	5.883	0.060	0.079
ESM1	9.715	9.919	0.330	0.476

Table 14 – continued from previous page

Biomarker	μ_c	μ_d	σ_c	σ_d
PLAT	8.517	8.838	0.461	0.502
KLK7	8.322	7.989	0.321	0.391
CCL23	7.909	8.097	0.227	0.267
ACP5	10.198	10.436	0.292	0.343
NRP1	8.832	9.047	0.243	0.256
MMP7	9.084	9.574	0.437	0.706
ACY1	9.898	10.411	0.628	0.919
ALPL	10.577	10.290	0.377	0.417
IL11RA	7.312	7.213	0.110	0.107
APOA1	9.701	9.480	0.171	0.295
CKB-CKM	7.506	7.025	0.653	0.479
KLK8	7.361	7.421	0.100	0.178
AHSG	11.914	11.826	0.133	0.167
HINT1	5.835	5.793	0.086	0.104
MRC1	9.628	9.995	0.370	0.490
FCGR3B	10.920	11.145	0.255	0.269

Table 15: AUC for exemplary combinations of biomarkers

#											AUC
1	CTSB										0.791
2	CTSB	C5a									0.853
3	CTSB	C5a	C5								0.880
4	CTSB	C5a	C5	CCL18							0.890
5	CTSB	C5a	C5	CCL18	CSF1R						0.895
6	CTSB	C5a	C5	CCL18	CSF1R	KLK7					0.895
7	CTSB	C5a	C5	CCL18	CSF1R	KLK7	ETHE1				0.906
8	CTSB	C5a	C5	CCL18	CSF1R	KLK7	ETHE1	C5-C6			0.902
9	CTSB	C5a	C5	CCL18	CSF1R	KLK7	ETHE1	C5-C6	KLK8		0.903
10	CTSB	C5a	C5	CCL18	CSF1R	KLK7	ETHE1	C5-C6	KLK8	VEGFA	0.913

Table 16: Calculations derived from training set for naïve Bayes classifier.

Biomarker	μ_c	μ_d	σ_c	σ_d	$\tilde{x}$	$p(c\|\tilde{x})$	$p(d\|\tilde{x})$	$ln(p(d\|\tilde{x})/p(c\|\tilde{x}))$
CSF1R	10.712	10.995	0.398	0.399	10.751	0.997	0.831	-0.182
CTSB	8.836	9.398	0.287	0.621	9.036	1.091	0.542	-0.700
CCL18	11.320	11.616	0.477	0.398	11.658	0.651	0.996	0.425
KLK7	8.322	7.989	0.321	0.391	8.048	0.862	1.009	0.158
VEGFA	8.532	8.601	0.170	0.134	8.687	1.554	2.425	0.445
ETHE1	7.373	7.443	0.119	0.121	7.313	2.932	1.845	-0.463
C5-C6	6.506	6.593	0.133	0.144	6.349	1.490	0.662	-0.811
C5a	11.354	11.606	0.254	0.246	11.400	1.547	1.139	-0.306
KLK8	7.361	7.421	0.100	0.178	7.420	3.344	2.237	-0.402
C5	9.485	9.603	0.119	0.143	9.306	1.084	0.324	-1.207

Table 17: Clinical characteristics of the training set

Meta Data	Levels	Control	Pancreatic Cancer	p-value
Samples		115	143	
GENDER	F	59	70	
	M	56	73	8.02e-01
AGE	Mean	57.6	68.6	
	SD	13.7	9.7	8.98e-12

Table 18: Ten biomarker classifier proteins

Biomarker	UniProt ID	Direction*	Biological Process (GO)
C5-C6	P01031 P13671	Up	immune system process regulation of immune system process proteolysis response to stress regulation of cell death signaling regulation of signaling pathway
C5	P01031	Up	immune system process regulation of immune system process proteolysis response to stress signaling regulation of signaling pathway
VEGFA	P15692	Down	immune system process regulation of immune system process response to stress regulation of cell death signaling regulation of signaling pathway
CSF1R	P07333	Up	cell proliferation signaling process signaling
KLK8	O60259	Up	proteolysis response to stress cell proliferation
C5a	P01031	Up	immune system process regulation of immune system process proteolysis response to stress signaling regulation of signaling pathway
CCL18	P55774	Up	immune system process response to stress cell communication signaling process signaling
CTSB	P07858	Up	proteolysis response to stress regulation of cell death
KLK7	P49862	Down	proteolysis
ETHE1	O95571	Up	

Table 19: Biomarkers of general cancer

ACY1	APOA1
C5	CCL23
CKB-CKM	CKM
ENTPD1	GDF11
HAMP	HINT1
KIT	KLK3-SERPINA3
LBP	SERPINF2
THBS2	TIMP1
C9	FSTL3
IL12A-IL12B	CDK5-CDK5R1
CCL23	

Table 20: Panels of 1 Biomarker

Markers		Mean CV AUC
1	KIT	0.753
2	CKB-CKM	0.750
3	C9	0.740
4	APOA1	0.740
5	KLK3-SERPINA3	0.732
6	CKM	0.730
7	CCL23	0.713
8	CCL23	0.705
9	TIMP1	0.695
10	LBP	0.691
11	C5	0.690
12	ACY1	0.676
13	HAMP	0.670
14	CDK5-CDK5R1	0.670
15	HINT1	0.669
16	SERPINF2	0.663
17	GDF11	0.656
18	ENTPD1	0.651
19	THBS2	0.650
20	FSTL3	0.643
21	IL12A-IL12B	0.640

Table 21: Panels of 2 Biomarkers

Markers			Mean CV AUC
1	KIT	APOA1	0.808
2	APOA1	CKB-CKM	0.801
3	KIT	CCL23	0.791
4	C9	KIT	0.791
5	KIT	CKB-CKM	0.790
6	C9	CKB-CKM	0.789

Table 21 – continued from previous page

Markers			Mean CV AUC
7	C9	APOA1	0.789
8	KIT	LBP	0.787
9	TIMP1	KIT	0.787
10	C5	KIT	0.787
11	C9	CKM	0.786
12	CKM	APOA1	0.786
13	C5	APOA1	0.785
14	KIT	CCL23	0.784
15	TIMP1	CKB-CKM	0.782
16	CCL23	CKB-CKM	0.781
17	KIT	CKM	0.780
18	KIT	ACY1	0.780
19	KIT	KLK3-SERPINA3	0.780
20	CKM	CCL23	0.778
21	CKB-CKM	HINT1	0.778
22	KIT	CDK5-CDK5R1	0.777
23	SERPINF2	CKB-CKM	0.777
24	APOA1	ACY1	0.777
25	KIT	SERPINF2	0.776
26	LBP	CKB-CKM	0.776
27	CKB-CKM	KLK3-SERPINA3	0.776
28	APOA1	KLK3-SERPINA3	0.776
29	APOA1	CCL23	0.775
30	TIMP1	CKM	0.774
31	C9	ACY1	0.774
32	CDK5-CDK5R1	CKB-CKM	0.773
33	IL12A-IL12B	CKB-CKM	0.773
34	TIMP1	C9	0.773
35	APOA1	HINT1	0.773
36	C5	CCL23	0.772
37	KIT	IINT1	0.772
38	IL12A-IL12B	KIT	0.771
39	CKM	SERPINF2	0.771
40	ACY1	CKB-CKM	0.770
41	APOA1	CCL23	0.770
42	C9	CDK5-CDK5R1	0.769
43	C5	CKB-CKM	0.769
44	C9	HINT1	0.769
45	CKM	CCL23	0.767
46	CCL23	KLK3-SERPINA3	0.767
47	CKM	KLK3-SERPINA3	0.767
48	C9	FSTL3	0.767
49	APOA1	LBP	0.766
50	C9	SERPINF2	0.766
51	C9	CCL23	0.765
52	CKM	LBP	0.765
53	CCL23	CKB-CKM	0.764
54	KIT	ENTPD1	0.764
55	CKM	HINT1	0.764
56	C9	LBP	0.764
57	C9	C5	0.764
58	KIT	HAMP	0.764
59	FSTL3	CKB-CKM	0.763

Table 21 – continued from previous page

Markers			Mean CV AUC
60	KIT	FSTL3	0.763
61	CKM	CKB-CKM	0.762
62	IIAMP	CKB-CKM	0.762
63	CKM	ACY1	0.762
64	TIMP1	APOA1	0.762
65	APOA1	CDK5-CDK5R1	0.761
66	C5	KLK3-SERPINA3	0.761
67	C5	HINT1	0.760
68	C9	GDF11	0.760
69	C9	THBS2	0.760
70	CKM	CDK5-CDK5R1	0.760
71	ENTPD1	CKB-CKM	0.759
72	C5	CCL23	0.759
73	CCL23	ACY1	0.758
74	CCL23	ACY1	0.758
75	C5	CDK5-CDK5R1	0.757
76	C5	CKM	0.757
77	TIMP1	KLK3-SERPINA3	0.757
78	CKM	HAMP	0.757
79	C9	HAMP	0.757
80	C9	CCL23	0.757
81	C9	IL12A-IL12B	0.756
82	LBP	ACY1	0.756
83	C9	ENTPD1	0.754
84	CKM	ENTPD1	0.754
85	APOA1	SERPINF2	0.754
86	LBP	HINT1	0.754
87	CDK5-CDK5R1	KLK3-SERPINA3	0.754
88	APOA1	ENTPD1	0.753
89	TIMP1	CCL23	0.753
90	KIT	GDF11	0.753
91	GDF11	KLK3-SERPINA3	0.753
92	IL12A-IL12B	CKM	0.753
93	C5	SERPINF2	0.752
94	APOA1	GDF11	0.752
95	CCL23	KLK3-SERPINA3	0.751
96	CCL23	CDK5-CDK5R1	0.751
97	ACY1	KLK3-SERPINA3	0.749
98	C9	KLK3-SERPINA3	0.749
99	LBP	CDK5-CDK5R1	0.749
100	APOA1	HAMP	0.748

Table 22: Panels of 3 Biomarkers

Markers				Mean CV AUC
1	C5	KIT	APOA1	0.830
2	KIT	APOA1	CKB-CKM	0.826
3	C9	KIT	APOA1	0.822
4	KIT	APOA1	ACY1	0.820
5	KIT	APOA1	CDK5-CDK5R1	0.819

Table 22 – continued from previous page

Markers				Mean CV AUC
6	APOA1	CCL23	CKB-CKM	0.819
7	APOA1	ACY1	CKB-CKM	0.818
8	C9	KIT	CKB-CKM	0.817
9	C9	KIT	ACY1	0.816
10	KIT	APOA1	LBP	0.816
11	C5	KIT	CCL23	0.816
12	C5	APOA1	CKB-CKM	0.816
13	TIMP1	C9	KIT	0.815
14	C9	APOA1	CKB-CKM	0.815
15	C5	KIT	CDK5-CDK5R1	0.815
16	APOA1	CKB-CKM	HINT1	0.815
17	KIT	CKM	APOA1	0.815
18	KIT	APOA1	CCL23	0.815
19	TIMP1	KIT	APOA1	0.814
20	APOA1	LBP	CKB-CKM	0.813
21	C9	KIT	CKM	0.813
22	APOA1	CDK5-CDK5R1	CKB-CKM	0.812
23	KIT	APOA1	CCL23	0.812
24	C9	CKM	APOA1	0.812
25	TIMP1	KIT	CDK5-CDK5R1	0.812
26	C5	KIT	CKB-CKM	0.812
27	TIMP1	APOA1	CKB-CKM	0.812
28	KIT	APOA1	HINT1	0.812
29	C9	KIT	HINT1	0.811
30	KIT	CDK5-CDK5R1	CKB-CKM	0.811
31	KIT	LBP	CKB-CKM	0.811
32	IL12A-IL12B	KIT	APOA1	0.811
33	C9	C5	KIT	0.811
34	C5	KIT	CCL23	0.811
35	C5	KIT	IIINT1	0.811
36	KIT	CCL23	ACY1	0.809
37	C9	KIT	CCL23	0.809
38	APOA1	CKB-CKM	KLK3-SERPINA3	0.809
39	KIT	CCL23	ACY1	0.809
40	APOA1	SERPINF2	CKB-CKM	0.808
41	C9	ACY1	CKB-CKM	0.808
42	TIMP1	KIT	CKB-CKM	0.808
43	KIT	APOA1	KLK3-SERPINA3	0.808
44	IL12A-IL12B	APOA1	CKB-CKM	0.808
45	KIT	CCL23	CKB-CKM	0.807
46	C5	APOA1	CDK5-CDK5R1	0.807
47	C5	APOA1	CCL23	0.807
48	KIT	ACY1	CKB-CKM	0.807
49	C5	KIT	ACY1	0.807
50	TIMP1	C5	KIT	0.807
51	C9	C5	CKB-CKM	0.806
52	C5	APOA1	HINT1	0.806
53	C9	CDK5-CDK5R1	CKB-CKM	0.806
54	C9	CKB-CKM	HINT1	0.806
55	C5	CCL23	CKB-CKM	0.806
56	C5	KIT	SERPINF2	0.806
57	KIT	CCL23	CKB-CKM	0.806
58	C5	CKM	APOA1	0.806

Table 22 – continued from previous page

Markers				Mean CV AUC
59	CKM	APOA1	CCL23	0.806
60	APOA1	CCL23	CKB-CKM	0.806
61	C5	CKB-CKM	IIINT1	0.806
62	APOA1	IIAMP	CKB-CKM	0.806
63	KIT	LBP	CDK5-CDK5R1	0.805
64	TIMP1	KIT	CCL23	0.805
65	KIT	APOA1	ENTPD1	0.805
66	TIMP1	C9	CKB-CKM	0.805
67	C5	APOA1	ACY1	0.804
68	C9	KIT	CDK5-CDK5R1	0.804
69	TIMP1	KIT	CKM	0.804
70	C9	APOA1	CDK5-CDK5R1	0.804
71	C9	CCL23	CKB-CKM	0.804
72	KIT	CKB-CKM	HINT1	0.804
73	TIMP1	CDK5-CDK5R1	CKB-CKM	0.804
74	KIT	APOA1	SERPINF2	0.804
75	KIT	CKM	LBP	0.803
76	CKM	APOA1	ACY1	0.803
77	C5	CDK5-CDK5R1	CKB-CKM	0.803
78	KIT	APOA1	HAMP	0.803
79	TIMP1	C9	CKM	0.803
80	KIT	LBP	ACY1	0.803
81	C9	CKM	ACY1	0.803
82	C5	IL12A-IL12B	KIT	0.803
83	LBP	CKB-CKM	HINT1	0.803
84	C9	CKM	CDK5-CDK5R1	0.803
85	C9	KIT	FSTL3	0.802
86	LBP	CDK5-CDK5R1	CKB-CKM	0.802
87	C9	KIT	SERPINF2	0.802
88	APOA1	FSTL3	CKB-CKM	0.802
89	C5	KIT	CKM	0.802
90	KIT	CKM	CDK5-CDK5R1	0.802
91	TIMP1	KIT	ACY1	0.802
92	C9	IL12A-IL12B	CKB-CKM	0.801
93	KIT	CCL23	CDK5-CDK5R1	0.801
94	KIT	CCL23	LBP	0.801
95	C9	KIT	LBP	0.801
96	CCL23	CDK5-CDK5R1	CKB-CKM	0.801
97	KIT	SERPINF2	LBP	0.801
98	C5	KIT	ENTPD1	0.801
99	APOA1	ENTPD1	CKB-CKM	0.800
100	KIT	CKM	ACY1	0.800

Table 23: Panels of 4 Biomarkers

Markers					Mean CV AUC
1	C5	KIT	APOA1	CDK5-CDK5R1	0.845
2	C5	KIT	APOA1	CKB-CKM	0.839
3	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.839
4	KIT	APOA1	ACY1	CKB-CKM	0.838

Table 23 – continued from previous page

Markers					Mean CV AUC
5	C5	KIT	APOA1	ACY1	0.837
6	C9	KIT	APOA1	CKB-CKM	0.835
7	C5	KIT	APOA1	IIINT1	0.835
8	C5	APOA1	CKB-CKM	IIINT1	0.835
9	KIT	APOA1	CCL23	CKB-CKM	0.834
10	C5	KIT	APOA1	CCL23	0.834
11	KIT	APOA1	CCL23	CKB-CKM	0.833
12	C9	KIT	APOA1	ACY1	0.833
13	KIT	APOA1	LBP	CKB-CKM	0.833
14	C5	KIT	APOA1	CCL23	0.833
15	C9	KIT	CKM	APOA1	0.833
16	C5	APOA1	CDK5-CDK5R1	CKB-CKM	0.833
17	IL12A-IL12B	KIT	APOA1	CKB-CKM	0.832
18	C5	KIT	CDK5-CDK5R1	CKB-CKM	0.832
19	C5	KIT	CKM	APOA1	0.832
20	TIMP1	KIT	CDK5-CDK5R1	CKB-CKM	0.832
21	C9	C5	KIT	CKB-CKM	0.832
22	TIMP1	KIT	APOA1	CKB-CKM	0.832
23	TIMP1	C5	KIT	CDK5-CDK5R1	0.831
24	C9	KIT	APOA1	CDK5-CDK5R1	0.831
25	C5	KIT	CCL23	ACY1	0.831
26	C5	IL12A-IL12B	KIT	APOA1	0.831
27	C5	APOA1	ACY1	CKB-CKM	0.830
28	C9	C5	KIT	APOA1	0.830
29	KIT	APOA1	CKB-CKM	HINT1	0.830
30	C9	KIT	ACY1	CKB-CKM	0.830
31	C5	KIT	CCL23	CKB-CKM	0.830
32	C5	APOA1	CCL23	CKB-CKM	0.829
33	C9	KIT	CCL23	ACY1	0.829
34	C9	KIT	APOA1	CCL23	0.829
35	C5	KIT	CCL23	CKB-CKM	0.829
36	C5	KIT	APOA1	SERPINF2	0.829
37	APOA1	CCL23	ACY1	CKB-CKM	0.829
38	C5	KIT	APOA1	ENTPD1	0.829
39	KIT	APOA1	LBP	CDK5-CDK5R1	0.829
40	KIT	APOA1	CCL23	ACY1	0.829
41	C9	KIT	APOA1	HINT1	0.829
42	KIT	LBP	CDK5-CDK5R1	CKB-CKM	0.829
43	KIT	APOA1	ACY1	CDK5-CDK5R1	0.828
44	TIMP1	KIT	APOA1	ACY1	0.828
45	C5	KIT	CCL23	CDK5-CDK5R1	0.828
46	KIT	APOA1	CCL23	ACY1	0.828
47	TIMP1	C9	KIT	CDK5-CDK5R1	0.828
48	C5	APOA1	CCL23	CKB-CKM	0.828
49	KIT	CKM	APOA1	ACY1	0.828
50	TIMP1	C5	KIT	APOA1	0.828
51	C5	KIT	CCL23	ACY1	0.828
52	C9	KIT	CCL23	CKB-CKM	0.828
53	APOA1	LBP	ACY1	CKB-CKM	0.827
54	TIMP1	C9	KIT	CKB-CKM	0.827
55	C9	KIT	CDK5-CDK5R1	CKB-CKM	0.827
56	KIT	APOA1	LBP	ACY1	0.827
57	KIT	APOA1	SERPINF2	CKB-CKM	0.827

Table 23 – continued from previous page

Markers					Mean CV AUC
58	APOA1	ACY1	CDK5-CDK5R1	CKB-CKM	0.827
59	C5	KIT	CCL23	CDK5-CDK5R1	0.827
60	C9	KIT	CKM	ACY1	0.827
61	TIMP1	APOA1	ACY1	CKB-CKM	0.827
62	C5	KIT	APOA1	LBP	0.827
63	TIMP1	KIT	APOA1	CDK5-CDK5R1	0.827
64	KIT	CKM	APOA1	CDK5-CDK5R1	0.826
65	KIT	APOA1	HAMP	CKB-CKM	0.826
66	C5	KIT	ACY1	CDK5-CDK5R1	0.826
67	TIMP1	C9	KIT	APOA1	0.826
68	C9	KIT	CKB-CKM	HINT1	0.826
69	APOA1	LBP	CKB-CKM	HINT1	0.826
70	C9	KIT	ACY1	CDK5-CDK5R1	0.826
71	TIMP1	APOA1	CDK5-CDK5R1	CKB-CKM	0.826
72	KIT	APOA1	CCL23	CDK5-CDK5R1	0.826
73	C9	CKM	APOA1	CDK5-CDK5R1	0.826
74	C5	KIT	CKB-CKM	HINT1	0.825
75	C5	KIT	SERPINF2	CDK5-CDK5R1	0.825
76	C9	KIT	CKM	CDK5-CDK5R1	0.825
77	TIMP1	C9	KIT	ACY1	0.825
78	C5	CCL23	CDK5-CDK5R1	CKB-CKM	0.825
79	KIT	APOA1	ENTPD1	CKB-CKM	0.825
80	C9	KIT	APOA1	LBP	0.825
81	C5	KIT	APOA1	KLK3-SERPINA3	0.825
82	C9	KIT	CKM	HINT1	0.825
83	C5	APOA1	LBP	CKB-CKM	0.825
84	KIT	LBP	ACY1	CKB-CKM	0.825
85	APOA1	CCL23	CDK5-CDK5R1	CKB-CKM	0.825
86	C9	CKM	APOA1	ACY1	0.824
87	KIT	CKM	APOA1	LBP	0.824
88	C9	KIT	CKM	CCL23	0.824
89	TIMP1	C5	KIT	ACY1	0.824
90	C9	APOA1	CDK5-CDK5R1	CKB-CKM	0.824
91	KIT	ACY1	CDK5-CDK5R1	CKB-CKM	0.824
92	C5	KIT	ACY1	CKB-CKM	0.824
93	KIT	CCL23	CDK5-CDK5R1	CKB-CKM	0.824
94	APOA1	ACY1	FSTL3	CKB-CKM	0.824
95	C9	C5	KIT	CKM	0.824
96	C5	KIT	CKM	CDK5-CDK5R1	0.824
97	KIT	CCL23	CDK5-CDK5R1	CKB-CKM	0.824
98	C5	APOA1	SERPINF2	CKB-CKM	0.824
99	C5	KIT	APOA1	HAMP	0.824
100	C9	APOA1	CKB-CKM	HINT1	0.824

Table 24: Panels of 5 Biomarkers

Markers						Mean CV AUC
1	C5	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.854
2	C5	KIT	APOA1	ACY1	CKB-CKM	0.851
3	C5	KIT	APOA1	CCL23	CKB-CKM	0.849

Table 24 – continued from previous page

Markers						Mean CV AUC
4	C5	KIT	APOA1	CCL23	CKB-CKM	0.847
5	KIT	APOA1	ACY1	CDK5-CDK5R1	CKB-CKM	0.847
6	KIT	APOA1	LBP	ACY1	CKB-CKM	0.847
7	C5	KIT	APOA1	CKB-CKM	HINT1	0.847
8	TIMP1	KIT	APOA1	ACY1	CKB-CKM	0.847
9	C5	KIT	APOA1	ACY1	CDK5-CDK5R1	0.846
10	C5	KIT	APOA1	CCL23	CDK5-CDK5R1	0.846
11	KIT	APOA1	LBP	CDK5-CDK5R1	CKB-CKM	0.845
12	C5	KIT	CCL23	CDK5-CDK5R1	CKB-CKM	0.845
13	C5	KIT	APOA1	CCL23	CDK5-CDK5R1	0.845
14	TIMP1	C5	KIT	APOA1	CDK5-CDK5R1	0.845
15	TIMP1	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.844
16	KIT	APOA1	CCL23	ACY1	CKB-CKM	0.844
17	KIT	APOA1	CCL23	CDK5-CDK5R1	CKB-CKM	0.844
18	C5	KIT	CCL23	CDK5-CDK5R1	CKB-CKM	0.844
19	C5	KIT	CKM	APOA1	CDK5-CDK5R1	0.844
20	C5	KIT	APOA1	LBP	CDK5-CDK5R1	0.844
21	C5	IL12A-IL12B	KIT	APOA1	CKB-CKM	0.844
22	KIT	APOA1	CCL23	ACY1	CKB-CKM	0.843
23	C5	KIT	APOA1	CCL23	ACY1	0.843
24	KIT	APOA1	CCL23	CDK5-CDK5R1	CKB-CKM	0.843
25	C9	KIT	CKM	APOA1	CDK5-CDK5R1	0.843
26	C5	KIT	APOA1	CCL23	ACY1	0.843
27	C9	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.843
28	C5	KIT	APOA1	LBP	CKB-CKM	0.843
29	IL12A-IL12B	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.843
30	C9	C5	KIT	APOA1	CKB-CKM	0.843
31	C9	KIT	APOA1	ACY1	CKB-CKM	0.843
32	TIMP1	C5	KIT	CDK5-CDK5R1	CKB-CKM	0.843
33	C5	APOA1	CCL23	CDK5-CDK5R1	CKB-CKM	0.843
34	C9	KIT	CKM	APOA1	ACY1	0.843
35	TIMP1	C5	KIT	APOA1	ACY1	0.842
36	C5	KIT	ACY1	CDK5-CDK5R1	CKB-CKM	0.842
37	C5	KIT	APOA1	SERPINF2	CKB-CKM	0.842
38	C5	IL12A-IL12B	KIT	APOA1	CDK5-CDK5R1	0.841
39	KIT	APOA1	CDK5-CDK5R1	HAMP	CKB-CKM	0.841
40	C5	APOA1	CCL23	CKB-CKM	HINT1	0.841
41	C5	APOA1	CDK5-CDK5R1	CKB-CKM	HINT1	0.841
42	C9	KIT	APOA1	CCL23	CKB-CKM	0.841
43	C5	APOA1	ACY1	CDK5-CDK5R1	CKB-CKM	0.841
44	TIMP1	C5	KIT	APOA1	CKB-CKM	0.841
45	C9	KIT	APOA1	CKB-CKM	HINT1	0.841
46	C5	APOA1	ACY1	CKB-CKM	HINT1	0.840
47	TIMP1	KIT	ACY1	CDK5-CDK5R1	CKB-CKM	0.840
48	C9	IL12A-IL12B	KIT	APOA1	CKB-CKM	0.840
49	C5	KIT	APOA1	CDK5-CDK5R1	FSTL3	0.840
50	C9	KIT	CKM	APOA1	HINT1	0.840
51	C9	C5	KIT	ACY1	CKB-CKM	0.840
52	C9	C5	KIT	CKM	APOA1	0.840
53	C5	KIT	CKM	APOA1	ACY1	0.840
54	C5	KIT	CCL23	ACY1	CKB-CKM	0.840
55	IL12A-IL12B	KIT	APOA1	LBP	CKB-CKM	0.840
56	C5	KIT	CCL23	ACY1	CKB-CKM	0.840

Table 24 – continued from previous page

Markers						Mean CV AUC
57	C5	KIT	APOA1	SERPINF2	CDK5-CDK5R1	0.840
58	C9	KIT	CCL23	ACY1	CKB-CKM	0.839
59	KIT	APOA1	LBP	CKB-CKM	IIINT1	0.839
60	IL12A-IL12B	KIT	APOA1	ACY1	CKB-CKM	0.839
61	C5	KIT	APOA1	CDK5-CDK5R1	HAMP	0.839
62	C5	KIT	APOA1	CDK5-CDK5R1	HINT1	0.839
63	C9	KIT	CKM	APOA1	CCL23	0.839
64	C9	C5	KIT	APOA1	CDK5-CDK5R1	0.839
65	C5	KIT	LBP	CDK5-CDK5R1	CKB-CKM	0.839
66	KIT	APOA1	CCL23	LBP	CKB-CKM	0.839
67	KIT	APOA1	CCL23	LBP	CKB-CKM	0.839
68	KIT	APOA1	SERPINF2	ACY1	CKB-CKM	0.838
69	C5	KIT	APOA1	ACY1	FSTL3	0.838
70	C5	IL12A-IL12B	KIT	APOA1	CCL23	0.838
71	KIT	APOA1	ACY1	FSTL3	CKB-CKM	0.838
72	C5	KIT	APOA1	ENTPD1	CKB-CKM	0.838
73	C9	C5	KIT	CCL23	CKB-CKM	0.838
74	C5	APOA1	SERPINF2	CDK5-CDK5R1	CKB-CKM	0.838
75	C9	C5	KIT	CKB-CKM	HINT1	0.838
76	KIT	CKM	APOA1	LBP	CDK5-CDK5R1	0.838
77	C9	KIT	APOA1	CCL23	ACY1	0.838
78	KIT	APOA1	ACY1	CKB-CKM	HINT1	0.838
79	TIMP1	C9	C5	KIT	CKB-CKM	0.838
80	C5	KIT	SERPINF2	CDK5-CDK5R1	CKB-CKM	0.838
81	C5	KIT	CCL23	CKB-CKM	HINT1	0.838
82	C5	KIT	CCL23	ACY1	CDK5-CDK5R1	0.838
83	C5	KIT	CCL23	ACY1	CDK5-CDK5R1	0.838
84	C5	KIT	APOA1	LBP	ACY1	0.838
85	TIMP1	C5	APOA1	CDK5-CDK5R1	CKB-CKM	0.838
86	IL12A-IL12B	KIT	APOA1	CCL23	CKB-CKM	0.838
87	TIMP1	C5	KIT	ACY1	CDK5-CDK5R1	0.838
88	C9	KIT	APOA1	LBP	CKB-CKM	0.838
89	KIT	APOA1	SERPINF2	CDK5-CDK5R1	CKB-CKM	0.838
90	TIMP1	KIT	APOA1	CCL23	CKB-CKM	0.838
91	TIMP1	KIT	LBP	CDK5-CDK5R1	CKB-CKM	0.838
92	C5	KIT	APOA1	CCL23	HINT1	0.838
93	KIT	APOA1	CCL23	CKB-CKM	HINT1	0.838
94	C9	C5	KIT	APOA1	CCL23	0.837
95	C9	C5	KIT	APOA1	ACY1	0.837
96	C5	KIT	LBP	ACY1	CKB-CKM	0.837
97	C5	KIT	CKM	APOA1	CCL23	0.837
98	C5	KIT	CDK5-CDK5R1	CKB-CKM	HINT1	0.837
99	C5	APOA1	CCL23	ACY1	CKB-CKM	0.837
100	C5	KIT	APOA1	FSTL3	CKB-CKM	0.837

Table 25: Panels of 6 Biomarkers

Markers						Mean CV AUC
1	C5 CKB-CKM	KIT	APOA1	ACY1	CDK5-CDK5R1	0.860

Table 25 – continued from previous page

	Markers					Mean CV AUC
2	C5 CKB-CKM	KIT	APOA1	CCL23	CDK5-CDK5R1	0.859
3	C5 CKB-CKM	KIT	APOA1	CCL23	CDK5-CDK5R1	0.858
4	C5 CKB-CKM	KIT	APOA1	CCL23	ACY1	0.857
5	C5 CKB-CKM	KIT	APOA1	CCL23	ACY1	0.856
6	TIMP1 CKB-CKM	C5	KIT	APOA1	CDK5-CDK5R1	0.856
7	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	CDK5-CDK5R1	0.855
8	C5 CKB-CKM	KIT	APOA1	LBP	CDK5-CDK5R1	0.855
9	C5 CKB-CKM	KIT	APOA1	LBP	ACY1	0.855
10	TIMP1 CKB-CKM	KIT	APOA1	ACY1	CDK5-CDK5R1	0.854
11	C5 HINT1	KIT	APOA1	CDK5-CDK5R1	CKB-CKM	0.854
12	C5 HINT1	KIT	APOA1	CCL23	CKB-CKM	0.853
13	C5 CDK5-CDK5R1	KIT	CKM	APOA1	CCL23	0.853
14	C5 CKB-CKM	KIT	APOA1	SERPINF2	CDK5-CDK5R1	0.853
15	C5 HINT1	KIT	APOA1	ACY1	CKB-CKM	0.852
16	KIT CKB-CKM	APOA1	LBP	ACY1	CDK5-CDK5R1	0.852
17	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	ACY1	0.852
18	C9 CDK5-CDK5R1	C5	KIT	CKM	APOA1	0.852
19	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	CCL23	0.852
20	C5 CKB-CKM	KIT	APOA1	SERPINF2	ACY1	0.851
21	C5 CKB-CKM	KIT	APOA1	CDK5-CDK5R1	FSTL3	0.851
22	KIT CKB-CKM	APOA1	CCL23	ACY1	CDK5-CDK5R1	0.851
23	C9 CKB-CKM	KIT	APOA1	CCL23	ACY1	0.851
24	TIMP1 CKB-CKM	C5	KIT	APOA1	ACY1	0.851
25	C5 CDK5-CDK5R1	KIT	APOA1	CCL23	ACY1	0.851
26	KIT CKB-CKM	APOA1	CCL23	ACY1	CDK5-CDK5R1	0.851
27	C5 CKB-CKM	KIT	APOA1	CDK5-CDK5R1	HAMP	0.851

Table 25 – continued from previous page

	Markers					Mean CV AUC
28	C9 CKB-CKM	KIT	APOA1	ACY1	CDK5-CDK5R1	0.850
29	TIMP1 CKB-CKM	C5	KIT	ACY1	CDK5-CDK5R1	0.850
30	C5 CKB-CKM	KIT	APOA1	CCL23	LBP	0.850
31	C9 ACY1	KIT	CKM	APOA1	CCL23	0.850
32	TIMP1 CDK5-CDK5R1	C5	KIT	APOA1	ACY1	0.850
33	C5 CDK5-CDK5R1	KIT	APOA1	CCL23	ACY1	0.850
34	KIT CKB-CKM	APOA1	CCL23	LBP	ACY1	0.850
35	C5 CKB-CKM	APOA1	CCL23	ACY1	CDK5-CDK5R1	0.850
36	C5 CDK5-CDK5R1	KIT	CKM	APOA1	CCL23	0.849
37	C5 CKB-CKM	KIT	LBP	ACY1	CDK5-CDK5R1	0.849
38	IL12A-IL12B CKB-CKM	KIT	APOA1	ACY1	CDK5-CDK5R1	0.849
39	C5 CKB-CKM	KIT	CCL23	ACY1	CDK5-CDK5R1	0.849
40	IL12A-IL12B CKB-CKM	KIT	APOA1	LBP	CDK5-CDK5R1	0.849
41	C5 CKB-CKM	KIT	APOA1	ACY1	FSTL3	0.849
42	C9 CKB-CKM	KIT	APOA1	CCL23	CDK5-CDK5R1	0.849
43	KIT CKB-CKM	APOA1	CCL23	LBP	CDK5-CDK5R1	0.849
44	C9 CKB-CKM	C5	KIT	APOA1	ACY1	0.849
45	C5 CDK5-CDK5R1	KIT	CKM	APOA1	LBP	0.849
46	C5 CKB-CKM	KIT	CCL23	ACY1	CDK5-CDK5R1	0.849
47	C5 CKB-CKM	KIT	APOA1	SERPINF2	CCL23	0.848
48	C9 CKB-CKM	C5	KIT	APOA1	CDK5-CDK5R1	0.848
49	C5 HINT1	KIT	APOA1	LBP	CKB-CKM	0.848
50	C5 CDK5-CDK5R1	KIT	CKM	APOA1	SERPINF2	0.848
51	KIT CKB-CKM	APOA1	ACY1	CDK5-CDK5R1	HAMP	0.848
52	TIMP1 CKB-CKM	C5	KIT	CCL23	CDK5-CDK5R1	0.848
53	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	LBP	0.848

Table 25 – continued from previous page

	Markers					Mean CV AUC
54	C5 CKB-CKM	KIT	APOA1	CCL23	LBP	0.848
55	TIMP1 HINT1	C5	KIT	APOA1	CKB-CKM	0.848
56	C9 CKB-CKM	C5	KIT	APOA1	CCL23	0.848
57	KIT CKB-CKM	APOA1	CCL23	LBP	ACY1	0.848
58	KIT CKB-CKM	APOA1	SERPINF2	ACY1	CDK5-CDK5R1	0.848
59	TIMP1 CKB-CKM	C5	KIT	CCL23	CDK5-CDK5R1	0.848
60	C9 CDK5-CDK5R1	KIT	CKM	APOA1	ACY1	0.848
61	C9 CKB-CKM	C5	KIT	CCL23	ACY1	0.847
62	TIMP1 CKB-CKM	KIT	APOA1	CCL23	CDK5-CDK5R1	0.847
63	KIT CKB-CKM	APOA1	CCL23	LBP	CDK5-CDK5R1	0.847
64	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	CCL23	0.847
65	C5 CKB-CKM	IL12A-IL12B	KIT	CCL23	CDK5-CDK5R1	0.847
66	KIT CKB-CKM	APOA1	ACY1	CDK5-CDK5R1	FSTL3	0.847
67	C5 HINT1	KIT	APOA1	CCL23	CKB-CKM	0.847
68	C5 CDK5-CDK5R1	KIT	CKM	APOA1	ACY1	0.847
69	C5 CDK5-CDK5R1	IL12A-IL12B	KIT	APOA1	CCL23	0.847
70	TIMP1 CKB-CKM	KIT	APOA1	LBP	CDK5-CDK5R1	0.847
71	C5 CDK5-CDK5R1	KIT	APOA1	LBP	ACY1	0.847
72	TIMP1 CKB-CKM	KIT	APOA1	CDK5-CDK5R1	HAMP	0.847
73	TIMP1 CKB-CKM	IL12A-IL12B	KIT	APOA1	CDK5-CDK5R1	0.847
74	C5 CKB-CKM	KIT	SERPINF2	ACY1	CDK5-CDK5R1	0.847
75	TIMP1 CDK5-CDK5R1	C5	KIT	APOA1	CCL23	0.847
76	C5 CDK5-CDK5R1	KIT	APOA1	CCL23	LBP	0.847
77	C9 CDK5-CDK5R1	KIT	CKM	APOA1	CCL23	0.847
78	TIMP1 CKB-CKM	KIT	APOA1	LBP	ACY1	0.847
79	C5 ACY1	KIT	CKM	APOA1	CCL23	0.847

Table 25 – continued from previous page

	Markers					Mean CV AUC
80	TIMP1 CKB-CKM	C5	KIT	APOA1	CCL23	0.847
81	C5 HINT1	APOA1	CCL23	CDK5-CDK5R1	CKB-CKM	0.847
82	C9 HINT1	C5	KIT	CKM	APOA1	0.847
83	TIMP1 CKB-CKM	C9	KIT	APOA1	CDK5-CDK5R1	0.847
84	TIMP1 CDK5-CDK5R1	C5	KIT	APOA1	CCL23	0.846
85	C9 CKB-CKM	KIT	APOA1	CDK5-CDK5R1	HAMP	0.846
86	C5 CKB-CKM	KIT	CCL23	LBP	CDK5-CDK5R1	0.846
87	TIMP1 CKB-CKM	C5	KIT	LBP	CDK5-CDK5R1	0.846
88	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	CDK5-CDK5R1	0.846
89	TIMP1 CDK5-CDK5R1	C5	KIT	CKM	APOA1	0.846
90	C5 CKB-CKM	KIT	APOA1	ACY1	HAMP	0.846
91	TIMP1 CKB-CKM	C5	KIT	APOA1	CCL23	0.846
92	C9 HINT1	C5	KIT	APOA1	CKB-CKM	0.846
93	C5 HINT1	IL12A-IL12B	KIT	APOA1	CKB-CKM	0.846
94	C5 FSTL3	KIT	APOA1	ACY1	CDK5-CDK5R1	0.846
95	C9 CDK5-CDK5R1	C5	KIT	APOA1	CCL23	0.846
96	C5 ACY1	KIT	CKM	APOA1	LBP	0.846
97	C9 CDK5-CDK5R1	KIT	CKM	APOA1	LBP	0.846
98	KIT CKB-CKM	APOA1	LBP	CDK5-CDK5R1	HAMP	0.846
99	C5 CKB-CKM	KIT	APOA1	CCL23	SERPINF2	0.846
100	C9 CKB-CKM	C5	IL12A-IL12B	KIT	APOA1	0.846

Table 26: Panels of 7 Biomarkers

	Markers					Mean CV AUC
1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.864
2	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.863

Table 26 – continued from previous page

Markers						Mean CV AUC
3	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	ACY1	0.863
4	C5 FSTL3	KIT CKB-CKM	APOA1	ACY1	CDK5-CDK5R1	0.861
5	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	ACY1	0.861
6	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	ACY1	0.860
7	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	ACY1	0.860
8	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	0.860
9	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	LBP	0.859
10	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	LBP	0.859
11	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	LBP	0.859
12	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	CDK5-CDK5R1	0.859
13	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	CCL23	0.859
14	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	LBP	0.859
15	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	0.858
16	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	CCL23	0.858
17	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	ACY1	0.858
18	C5 ACY1	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	0.858
19	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	CCL23	0.858
20	C5 HAMP	KIT CKB-CKM	APOA1	ACY1	CDK5-CDK5R1	0.857
21	C9 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.857
22	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.857
23	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	IL12A-IL12B	KIT	APOA1	0.856
24	TIMP1 ACY1	C5 CKB-CKM	KIT	APOA1	CCL23	0.856
25	C5 ACY1	KIT CKB-CKM	APOA1	SERPINF2	CCL23	0.856
26	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	SERPINF2	0.856
27	C9 CCL23	C5 CDK5-CDK5R1	KIT	CKM	APOA1	0.856
28	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	ACY1	0.856

Table 26 – continued from previous page

Markers						Mean CV AUC
29	C5 ACY1	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	0.856
30	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	LBP	0.856
31	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	SERPINF2	0.856
32	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.856
33	C5 CKB-CKM	KIT HINT1	APOA1	ACY1	CDK5-CDK5R1	0.855
34	C9 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	CCL23	0.855
35	TIMP1 CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	ACY1	0.855
36	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	ACY1	0.855
37	C5 ACY1	KIT CKB-CKM	APOA1	SERPINF2	LBP	0.855
38	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	CDK5-CDK5R1	0.855
39	C9 ACY1	C5 CKB-CKM	KIT	APOA1	CCL23	0.855
40	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	CCL23	0.855
41	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	SERPINF2	0.855
42	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	LBP	0.854
43	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	CCL23	0.854
44	C5 CKB-CKM	KIT HINT1	APOA1	LBP	CDK5-CDK5R1	0.854
45	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	LBP	0.854
46	C5 ACY1	IL12A-IL12B CKB-CKM	KIT	APOA1	LBP	0.854
47	C9 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.854
48	C5 SERPINF2	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.854
49	TIMP1 ACY1	C5 CKB-CKM	KIT	APOA1	CCL23	0.854
50	C5 FSTL3	KIT CKB-CKM	APOA1	CCL23	ACY1	0.854
51	C5 HAMP	IL12A-IL12B CKB-CKM	KIT	APOA1	CDK5-CDK5R1	0.854
52	TIMP1 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	ACY1	0.854
53	C5 FSTL3	KIT CKB-CKM	APOA1	CCL23	CDK5-CDK5R1	0.854
54	C5 LBP	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.854

Table 26 – continued from previous page

	Markers					Mean CV AUC
55	C5 HAMP	KIT CKB-CKM	APOA1	CCL23	CDK5-CDK5R1	0.854
56	TIMP1 HAMP	KIT CKB-CKM	APOA1	ACY1	CDK5-CDK5R1	0.854
57	TIMP1 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.854
58	C9 CDK5-CDK5R1	C5 CKB-CKM	KIT	APOA1	ACY1	0.854
59	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	SERPINF2	0.854
60	C5 HAMP	KIT CKB-CKM	APOA1	CCL23	CDK5-CDK5R1	0.854
61	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	ACY1	0.854
62	C9 CDK5-CDK5R1	C5 HINT1	KIT	CKM	APOA1	0.854
63	TIMP1 CKB-CKM	C5 HINT1	KIT	APOA1	CDK5-CDK5R1	0.854
64	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	LBP	0.854
65	TIMP1 CCL23	C5 CDK5-CDK5R1	KIT	CKM	APOA1	0.853
66	C5 FSTL3	KIT CKB-CKM	APOA1	LBP	CDK5-CDK5R1	0.853
67	C9 ACY1	C5 CDK5-CDK5R1	KIT	CKM	APOA1	0.853
68	C5 LBP	IL12A-IL12B CKB-CKM	KIT	APOA1	CCL23	0.853
69	C9 CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	ACY1	0.853
70	C5 CDK5-CDK5R1	KIT CKB-CKM	CCL23	LBP	ACY1	0.853
71	C5 CKB-CKM	KIT HINT1	APOA1	LBP	ACY1	0.853
72	KIT FSTL3	APOA1 CKB-CKM	LBP	ACY1	CDK5-CDK5R1	0.853
73	C5 CDK5-CDK5R1	KIT HINT1	CKM	APOA1	LBP	0.853
74	C9 CKB-CKM	C5 HINT1	KIT	APOA1	CDK5-CDK5R1	0.853
75	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	LBP	0.853
76	C9 CCL23	C5 ACY1	KIT	CKM	APOA1	0.853
77	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.853
78	TIMP1 HAMP	C5 CKB-CKM	KIT	APOA1	CDK5-CDK5R1	0.853
79	C5 FSTL3	KIT CKB-CKM	APOA1	LBP	ACY1	0.853
80	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	SERPINF2	0.853

Table 26 – continued from previous page

	Markers					Mean CV AUC
81	TIMP1 CKB-CKM	C5 HINT1	KIT	APOA1	ACY1	0.853
82	KIT HAMP	APOA1 CKB-CKM	LBP	ACY1	CDK5-CDK5R1	0.852
83	C5 FSTL3	IL12A-IL12B CKB-CKM	KIT	APOA1	CDK5-CDK5R1	0.852
84	C5 CCL23	KIT CDK5-CDK5R1	CKM	APOA1	SERPINF2	0.852
85	C5 CDK5-CDK5R1	KIT CKB-CKM	CCL23	LBP	ACY1	0.852
86	C9 CCL23	C5 CDK5-CDK5R1	KIT	CKM	APOA1	0.852
87	C5 FSTL3	KIT CKB-CKM	APOA1	CCL23	ACY1	0.852
88	C5 FSTL3	KIT CKB-CKM	APOA1	CCL23	CDK5-CDK5R1	0.852
89	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT	LBP	ACY1	0.852
90	C5 LBP	KIT CDK5-CDK5R1	CKM	APOA1	CCL23	0.852
91	C5 CKB-CKM	KIT HINT1	APOA1	SERPINF2	CDK5-CDK5R1	0.852
92	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	LBP	0.852
93	C5 HAMP	KIT CKB-CKM	APOA1	LBP	CDK5-CDK5R1	0.852
94	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.852
95	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	ACY1	0.852
96	C5 CDK5-CDK5R1	KIT HINT1	CKM	APOA1	CCL23	0.852
97	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	LBP	ACY1	0.852
98	TIMP1 ACY1	C5 CKB-CKM	KIT	APOA1	LBP	0.852
99	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT	CKM	APOA1	0.852
100	TIMP1 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	ACY1	0.852

Table 27: Panels of 8 Biomarkers

	Markers					Mean CV AUC
1	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	0.864
2	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.864
3	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	0.864

Table 27 – continued from previous page

	Markers					Mean CV AUC
4	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.863
5	TIMP1 ACY1	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	0.863
6	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.863
7	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	0.862
8	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	0.862
9	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.862
10	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	CCL23	0.862
11	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	SERPINF2	0.861
12	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	0.861
13	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	LBP	ACY1	0.861
14	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.861
15	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	LBP	0.860
16	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	ACY1	0.860
17	C5 CDK5-CDK5R1	IL12A-IL12B FSTL3	KIT CKB-CKM	APOA1	ACY1	0.860
18	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	0.860
19	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	CCL23	0.860
20	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	CCL23	0.859
21	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	ACY1	0.859
22	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	LBP	0.859
23	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	ACY1	0.859
24	TIMP1 CCL23	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	0.859
25	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	ACY1	0.859
26	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.859
27	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	ACY1	0.859
28	TIMP1 CDK5-CDK5R1	C5 HAMP	KIT CKB-CKM	APOA1	ACY1	0.859
29	C5 CCL23	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	0.859

Table 27 – continued from previous page

	Markers					Mean CV AUC
30	C5 ACY1	KIT CKB-CKM	APOA1 HINT1	CCL23	LBP	0.859
31	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.859
32	C9 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	0.859
33	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.859
34	C5 FSTL3	KIT HAMP	APOA1 CKB-CKM	ACY1	CDK5-CDK5R1	0.858
35	TIMP1 LBP	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.858
36	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	LBP	ACY1	0.858
37	C5 CDK5-CDK5R1	IL12A-IL12B HAMP	KIT CKB-CKM	APOA1	ACY1	0.858
38	TIMP1 CCL23	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	0.858
39	TIMP1 CCL23	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	0.858
40	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	SERPINF2	CCL23	0.858
41	TIMP1 LBP	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.858
42	C5 LBP	IL12A-IL12B ACY1	KIT CKB-CKM	APOA1	CCL23	0.858
43	C5 LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	CCL23	0.858
44	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	SERPINF2	0.857
45	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	0.857
46	C5 LBP	KIT ACY1	APOA1 CKB-CKM	CCL23	SERPINF2	0.857
47	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT HINT1	APOA1	ACY1	0.857
48	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT HINT1	APOA1	CCL23	0.857
49	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	ACY1	0.857
50	C5 SERPINF2	KIT ACY1	CKM CDK5-CDK5R1	APOA1	CCL23	0.857
51	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	SERPINF2	ACY1	0.857
52	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11	CCL23	0.857
53	TIMP1 LBP	C5 CDK5-CDK5R1	IL12A-IL12B CKB-CKM	KIT	APOA1	0.857
54	C9 ACY1	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	0.857
55	TIMP1 CDK5-CDK5R1	C5 FSTL3	KIT CKB-CKM	APOA1	ACY1	0.857

Table 27 – continued from previous page

	Markers					Mean CV AUC
56	TIMP1 LBP	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	0.857
57	C9 CCL23	C5 CDK5-CDK5R1	KIT HINT1	CKM	APOA1	0.857
58	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	SERPINF2	ACY1	0.857
59	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	CCL23	0.857
60	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	LBP	ACY1	0.857
61	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	0.857
62	C5 LBP	KIT ACY1	APOA1 CKB-CKM	SERPINF2	CCL23	0.857
63	C9 LBP	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.857
64	TIMP1 ACY1	C9 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.856
65	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.856
66	C9 CCL23	C5 CCL23	KIT CDK5-CDK5R1	CKM	APOA1	0.856
67	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	SERPINF2	0.856
68	C5 CDK5-CDK5R1	IL12A-IL12B HAMP	KIT CKB-CKM	APOA1	LBP	0.856
69	TIMP1 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	0.856
70	C5 CDK5-CDK5R1	IL12A-IL12B HAMP	KIT CKB-CKM	APOA1	CCL23	0.856
71	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	GDF11	0.856
72	C9 CCL23	C5 ACY1	KIT HINT1	CKM	APOA1	0.856
73	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.856
74	TIMP1 ACY1	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	0.856
75	C5 CDK5-CDK5R1	IL12A-IL12B FSTL3	KIT CKB-CKM	APOA1	LBP	0.856
76	TIMP1 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	LBP	0.856
77	TIMP1 APOA1	C9 CCL23	C5 CDK5-CDK5R1	KIT	CKM	0.856
78	C5 LBP	KIT CDK5-CDK5R1	CKM HINT1	APOA1	CCL23	0.856
79	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.856
80	C5 SERPINF2	KIT LBP	CKM CDK5-CDK5R1	APOA1	CCL23	0.856
81	C9 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	CCL23	0.856

Table 27 – continued from previous page

	Markers					Mean CV AUC
82	C5 LBP	IL12A-IL12B ACY1	KIT CKB-CKM	APOA1	CCL23	0.856
83	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	LBP	0.856
84	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	LBP	0.856
85	C5 CCL23	IL12A-IL12B ACY1	KIT CKB-CKM	APOA1	SERPINF2	0.856
86	TIMP1 CDK5-CDK5R1	C5 CKB-CKM	KIT HINT1	APOA1	CCL23	0.856
87	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	LBP	ACY1	0.856
88	TIMP1 CCL23	C9 ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.856
89	C9 ACY1	C5 CDK5-CDK5R1	KIT HINT1	CKM	APOA1	0.856
90	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	CKM	APOA1	0.855
91	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	LBP	0.855
92	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	APOA1	SERPINF2	0.855
93	C9 APOA1	C5 CCL23	IL12A-IL12B CDK5-CDK5R1	KIT	CKM	0.855
94	TIMP1 CDK5-CDK5R1	C5 HAMP	KIT CKB-CKM	APOA1	CCL23	0.855
95	C5 ACY1	KIT CDK5-CDK5R1	CKM FSTL3	APOA1	CCL23	0.855
96	TIMP1 CDK5-CDK5R1	C5 HAMP	IL12A-IL12B CKB-CKM	KIT	APOA1	0.855
97	C9 LBP	C5 ACY1	KIT CKB-CKM	APOA1	CCL23	0.855
98	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	CCL23	0.855
99	C9 CCL23	C5 LBP	KIT CDK5-CDK5R1	CKM	APOA1	0.855
100	C9 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	ACY1	0.855

Table 28: Panels of 9 Biomarkers

	Markers					Mean CV AUC
1	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.864
2	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.864
3	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 CKB-CKM	CCL23	0.863
4	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.863

Table 28 – continued from previous page

	Markers					Mean CV AUC
5	TIMP1 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.863
6	TIMP1 LBP	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.862
7	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	LBP	0.862
8	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	SERPINF2	0.862
9	TIMP1 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.862
10	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.861
11	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.861
12	TIMP1 SERPINF2	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.861
13	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.861
14	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.861
15	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.861
16	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.861
17	C5 CCL23	IL12A-IL12B LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.861
18	TIMP1 ACY1	C5 CDK5-CDK5R1	IL12A-IL12B HAMP	KIT CKB-CKM	APOA1	0.861
19	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	LBP	0.861
20	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.861
21	TIMP1 SERPINF2	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.860
22	C5 SERPINF2	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	CCL23	0.860
23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	LBP	0.860
24	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	LBP	0.860
25	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.860
26	C5 SERPINF2	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.860
27	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	LBP	0.860
28	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.860
29	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	CCL23	0.860
30	TIMP1 CCL23	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.860

Table 28 – continued from previous page

	Markers					Mean CV AUC
31	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM HINT1	APOA1	0.860
32	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	SERPINF2	0.860
33	C9 SERPINF2	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.859
34	TIMP1 CCL23	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.859
35	C5 LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2 HINT1	CCL23	0.859
36	TIMP1 APOA1	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM	0.859
37	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.859
38	C5 CCL23	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	SERPINF2	0.859
39	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.859
40	TIMP1 LBP	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.859
41	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2 HINT1	CCL23	0.859
42	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	CCL23	0.859
43	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	SERPINF2 CKB-CKM	LBP	0.859
44	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	CCL23	0.859
45	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	0.859
46	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	LBP	0.859
47	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.859
48	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.859
49	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.858
50	C9 APOA1	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	CKM	0.858
51	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 HAMP	CCL23 CKB-CKM	ACY1	0.858
52	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.858
53	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	LBP	0.858
54	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	0.858
55	TIMP1 LBP	C9 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.858
56	C9 CCL23	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.858

Table 28 – continued from previous page

	Markers					Mean CV AUC
57	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	LBP	0.858
58	C5 SERPINF2	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	CCL23	0.858
59	TIMP1 SERPINF2	C5 CCL23	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.858
60	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	SERPINF2 CKB-CKM	CCL23	0.858
61	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.858
62	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.858
63	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	SERPINF2 CKB-CKM	CCL23	0.858
64	TIMP1 CCL23	C5 SERPINF2	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.858
65	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 HAMP	LBP CKB-CKM	ACY1	0.858
66	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.858
67	TIMP1 ACY1	C9 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.858
68	TIMP1 CCL23	C5 LBP	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1	0.858
69	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11 HINT1	CCL23	0.858
70	C5 CDK5-CDK5R1	IL12A-IL12B FSTL3	KIT HAMP	APOA1 CKB-CKM	ACY1	0.858
71	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	LBP	0.858
72	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	LBP	0.858
73	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23	0.858
74	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	LBP	0.858
75	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	LBP	0.858
76	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11	0.858
77	C9 LBP	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.858
78	C5 CCL23	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	SERPINF2	0.858
79	C9 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.858
80	TIMP1 CCL23	C5 CDK5-CDK5R1	IL12A-IL12B HAMP	KIT CKB-CKM	APOA1	0.858
81	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	CCL23	0.858
82	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM HAMP	APOA1	0.858

Table 28 – continued from previous page

	Markers					Mean CV AUC
83	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11	0.858
84	C9 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23	0.857
85	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.857
86	C5 LBP	IL12A-IL12B ACY1	KIT CKB-CKM	APOA1 HINT1	CCL23	0.857
87	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	APOA1 CKB-CKM	SERPINF2	0.857
88	C5 CCL23	IL12A-IL12B LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23	0.857
89	C5 CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	CCL23 HINT1	ACY1	0.857
90	TIMP1 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	LBP	0.857
91	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	SERPINF2	0.857
92	TIMP1 SERPINF2	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11	0.857
93	C9 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	CCL23	0.857
94	TIMP1 CCL23	C5 LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2	0.857
95	TIMP1 APOA1	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	CKM	0.857
96	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	CKM HINT1	APOA1	0.857
97	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	SERPINF2	0.857
98	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11	0.857
99	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1	0.857
100	TIMP1 CDK5-CDK5R1	C5 FSTL3	KIT HAMP	APOA1 CKB-CKM	ACY1	0.857

Table 29: Panels of 10 Biomarkers

	Markers					Mean CV AUC
1	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 CKB-CKM	0.863
2	TIMP1 CCL23	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.863
3	TIMP1 CCL23	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.862
4	TIMP1 SERPINF2	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.862
5	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 CKB-CKM	CCL23 HINT1	0.862

Table 29 – continued from previous page

Markers						Mean CV AUC
6	C5 SERPINF2	IL12A-IL12B LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.862
7	TIMP1 SERPINF2	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.862
8	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.862
9	TIMP1 CCL23	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 CKB-CKM	0.861
10	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.861
11	C9 SERPINF2	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	0.861
12	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.861
13	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.861
14	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.861
15	TIMP1 SERPINF2	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.861
16	TIMP1 CCL23	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.860
17	TIMP1 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	0.860
18	C9 SERPINF2	C5 CCL23	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	0.860
19	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2 HINT1	0.860
20	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	CCL23 HINT1	0.860
21	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	0.860
22	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	0.860
23	C9 APOA1	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	CKM CDK5-CDK5R1	0.860
24	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.860
25	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM CKB-CKM	APOA1 HINT1	0.860
26	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.860
27	C9 CCL23	C5 LBP	KIT CDK5-CDK5R1	CKM CKB-CKM	APOA1 HINT1	0.860
28	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 HAMP	CCL23 CKB-CKM	0.860
29	TIMP1 CCL23	C9 ACY1	C5 CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	0.860
30	TIMP1 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	0.860
31	C9 CCL23	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 CKB-CKM	0.860

Table 29 – continued from previous page

Markers						Mean CV AUC
32	TIMP1 SERPINF2	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	0.860
33	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	0.859
34	C5 CCL23	KIT LBP	APOA1 ACY1	CCL23 CDK5-CDK5R1	SERPINF2 CKB-CKM	0.859
35	C9 CCL23	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.859
36	TIMP1 APOA1	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM HINT1	0.859
37	TIMP1 LBP	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	0.859
38	TIMP1 CCL23	C5 SERPINF2	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.859
39	TIMP1 CCL23	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	0.859
40	C9 CCL23	C5 CCL23	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	0.859
41	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.859
42	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	LBP HINT1	0.859
43	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.859
44	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	SERPINF2 FSTL3	CCL23 CKB-CKM	0.859
45	TIMP1 SERPINF2	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.859
46	TIMP1 CKM	C9 APOA1	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	0.859
47	TIMP1 SERPINF2	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.859
48	C5 CCL23	IL12A-IL12B LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2 HINT1	0.859
49	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.859
50	TIMP1 CCL23	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.859
51	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HAMP	0.859
52	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 FSTL3	SERPINF2 CKB-CKM	0.859
53	TIMP1 CCL23	C9 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	0.859
54	TIMP1 CCL23	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.859
55	C9 CCL23	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	0.859
56	TIMP1 GDF11	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.859
57	TIMP1 CCL23	C5 LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	SERPINF2 HINT1	0.858

Table 29 – continued from previous page

Markers						Mean CV AUC
58	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	0.858
59	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	LBP HINT1	0.858
60	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	GDF11 CKB-CKM	0.858
61	TIMP1 APOA1	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	0.858
62	C5 SERPINF2	IL12A-IL12B CCL23	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.858
63	C9 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 CKB-CKM	0.858
64	C9 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 CKB-CKM	0.858
65	TIMP1 ACY1	C5 CDK5-CDK5R1	KIT HAMP	APOA1 CKB-CKM	CCL23 HINT1	0.858
66	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	CCL23 HINT1	0.858
67	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 HAMP	SERPINF2 CKB-CKM	0.858
68	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 HAMP	CCL23 CKB-CKM	0.858
69	TIMP1 LBP	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	SERPINF2 CKB-CKM	0.858
70	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	SERPINF2 HINT1	0.858
71	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	GDF11 CKB-CKM	0.858
72	C5 CCL23	KIT LBP	APOA1 ACY1	GDF11 CDK5-CDK5R1	SERPINF2 CKB-CKM	0.858
73	TIMP1 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	APOA1 HINT1	0.858
74	C9 APOA1	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	CKM HINT1	0.858
75	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	GDF11 HINT1	0.858
76	TIMP1 APOA1	C9 CCL23	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT CKB-CKM	0.858
77	TIMP1 LBP	C9 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	CCL23 CKB-CKM	0.858
78	TIMP1 LBP	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 CKB-CKM	0.858
79	C9 CCL23	C5 LBP	KIT ACY1	APOA1 CDK5-CDK5R1	CCL23 CKB-CKM	0.858
80	TIMP1 CCL23	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.858
81	TIMP1 CCL23	C9 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.858
82	C9 CCL23	C5 SERPINF2	KIT CCL23	CKM ACY1	APOA1 CDK5-CDK5R1	0.858
83	C5 SERPINF2	KIT LBP	APOA1 ACY1	GDF11 CDK5-CDK5R1	CCL23 CKB-CKM	0.858

Table 29 – continued from previous page

	Markers					Mean CV AUC
84	C9 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.858
85	C5 CCL23	IL12A-IL12B LBP	KIT ACY1	APOA1 CDK5-CDK5R1	GDF11 CKB-CKM	0.858
86	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	SERPINF2 CKB-CKM	0.858
87	C5 LBP	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	SERPINF2 CKB-CKM	0.858
88	C5 ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	SERPINF2 CKB-CKM	CCL23 HINT1	0.858
89	C5 CCL23	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	SERPINF2 HINT1	0.858
90	TIMP1 CCL23	C5 ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	CCL23 HINT1	0.858
91	C5 ACY1	KIT CDK5-CDK5R1	APOA1 FSTL3	CCL23 HAMP	LBP CKB-CKM	0.858
92	TIMP1 APOA1	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM CKB-CKM	0.858
93	C9 CCL23	C5 ACY1	KIT CDK5-CDK5R1	CKM HAMP	APOA1 HINT1	0.858
94	TIMP1 GDF11	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.858
95	TIMP1 SERPINF2	C5 CCL23	IL12A-IL12B LBP	KIT CDK5-CDK5R1	APOA1 CKB-CKM	0.858
96	C5 CCL23	IL12A-IL12B ACY1	KIT CDK5-CDK5R1	APOA1 HAMP	SERPINF2 CKB-CKM	0.858
97	C5 ACY1	IL12A-IL12B CDK5-CDK5R1	KIT FSTL3	APOA1 HAMP	GDF11 CKB-CKM	0.858
98	C5 SERPINF2	IL12A-IL12B CCL23	KIT LBP	CKM ACY1	APOA1 CDK5-CDK5R1	0.858
99	TIMP1 APOA1	C9 SERPINF2	C5 CCL23	KIT ACY1	CKM CDK5-CDK5R1	0.858
100	TIMP1 CCL23	C5 LBP	KIT ACY1	CKM CDK5-CDK5R1	APOA1 HINT1	0.858

Table 30: Counts of markers in biomarker panels

	Panel Size							
Biomarker	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ACY1	141	192	308	399	489	590	658	759
APOA1	180	395	598	728	833	919	962	981
C5	163	285	437	559	644	693	773	834
C9	190	314	340	341	359	395	436	511
CCL23	151	168	191	202	238	273	308	363
CCL23	150	160	195	260	332	412	502	587
CDK5-CDK5R1	147	230	359	512	660	785	893	943
CKB-CKM	187	391	473	563	623	654	680	685
CKM	174	227	224	254	298	350	407	476
ENTPD1	107	57	38	31	27	12	8	14
FSTL3	112	89	87	101	107	136	170	190
GDF11	112	62	52	53	73	116	156	228
HAMP	107	67	73	96	134	199	265	322
HINT1	129	156	182	205	240	276	336	421
IL12A-IL12B	116	120	132	169	208	268	320	355
KIT	188	523	728	862	928	977	995	999
KLK3-SERPINA3	166	71	40	28	23	22	21	13
LBP	146	177	208	250	326	383	471	565
SERPINF2	126	134	139	161	206	241	300	351
THBS2	72	7	0	0	0	0	0	0
TIMP1	136	175	196	226	252	299	339	403

Table 31: Parameters derived from cancer datasets set for naïve Bayes classifiers

		Pancreatic Cancer		NSCLC		Mesothelioma	
		Control	Cancer	Control	Cancer	Control	Cancer
ACY1	Mean	9.90	10.41	9.70	9.43	9.29	8.67
	SD	0.63	0.92	0.45	0.46	0.57	0.65
APOA1	Mean	9.70	9.48	8.77	8.65	9.22	8.97
	SD	0.17	0.30	0.21	0.23	0.13	0.24
C5	Mean	9.49	9.60	10.13	10.20	10.05	10.19
	SD	0.12	0.14	0.12	0.14	0.11	0.16
CCL23	Mean	7.91	8.10	7.38	7.45	6.76	6.97
	SD	0.23	0.27	0.15	0.20	0.08	0.23
CDK5-CDK5R1	Mean	6.94	6.99	6.85	6.93	6.72	6.88
	SD	0.11	0.11	0.12	0.15	0.11	0.12
CKB-CKM	Mean	7.51	7.02	7.45	7.06	8.25	7.41
	SD	0.65	0.48	0.49	0.49	0.61	0.49
IL12A-IL12B	Mean	7.31	7.27	8.86	8.80	7.76	7.71
	SD	0.05	0.06	0.11	0.13	0.05	0.07
KIT	Mean	9.77	9.62	8.67	8.46	8.62	8.34
	SD	0.29	0.32	0.22	0.27	0.22	0.17
LBP	Mean	9.10	9.49	8.32	8.47	9.19	9.51
	SD	0.44	0.55	0.32	0.50	0.26	0.68
SERPINF2	Mean	9.26	9.18	8.97	8.85	8.80	8.67
	SD	0.12	0.16	0.21	0.19	0.21	0.26

Table 32: Calculations derived from training set for naïve Bayes classifier.

Biomarker	μ_c	μ_d	σ_c	σ_d	$\tilde{x}$	$p(c\|\tilde{x})$	$p(d\|\tilde{x})$	$ln(p(d\|\tilde{x})/p(c\|\tilde{x}))$
KIT	8.671	8.462	0.222	0.270	8.763	1.652	0.794	-0.732
SERPINF2	8.971	8.852	0.208	0.194	9.085	1.649	0.998	-0.503
CCL23	7.382	7.452	0.146	0.204	7.327	2.539	1.626	-0.445
IL12A-IL12B	8.857	8.798	0.115	0.131	8.863	3.478	2.691	-0.257
CDK5-CDK5R1	6.852	6.931	0.122	0.149	6.688	1.321	0.712	-0.618
ACY1	9.701	9.435	0.449	0.459	9.526	0.823	0.853	0.035
APOA1	8.772	8.648	0.210	0.230	8.805	1.875	1.378	-0.308
CKB-CKM	7.449	7.062	0.495	0.487	7.742	0.676	0.309	-0.782
LBP	8.322	8.472	0.317	0.504	8.215	1.187	0.695	-0.536
C5	10.127	10.201	0.123	0.144	10.086	3.077	2.017	-0.422

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A multiplex aptamer-based method for diagnosing that an individual does or does not have pancreatic cancer, the method comprising:

(a) contacting a biological sample from the individual with at least two aptamers wherein at least one aptamer has specific affinity for CTSB and at least one other aptamer has specific affinity for a protein biomarker selected from HAMP, THBS4, C5, C2, CRP and GDF11; wherein an aptamer protein complex forms when one of the at least two aptamers binds the protein biomarker the aptamer has specific affinity for if the protein biomarker is present in the biological sample,

(b) quantifying the levels of each of the protein biomarkers from step (a); and

(c) classifying said individual as having or not having pancreatic cancer based on said protein biomarker levels.

2. A multiplex aptamer-based method for screening an asymptomatic high risk individual for pancreatic cancer, the method comprising:

(a) contacting a biological sample from the individual with at least two aptamers wherein at least one aptamer has specific affinity for CTSB and at least one other aptamer has specific affinity for a protein biomarker selected from HAMP, THBS4, C5, C2, CRP and GDF11; wherein an aptamer protein complex forms when one of the at least two aptamers binds the protein biomarker the aptamer has specific affinity for if the protein biomarker is present in the biological sample,

(b) quantifying the levels of each of the protein biomarkers from step (a); and

(c) classifying said individual as having or not having pancreatic cancer based on said protein biomarker levels.

3. The method according to claim 1 or 2, wherein the at least two aptamers comprises at least three aptamers and at least one aptamer has specific affinity for a protein biomarker selected from the group consisting of C5a, ETHE1, KIT, C9, KLK7, C5-C6, IL11RA, CCL18, and APOA1.

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4. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for C5, and wherein up-regulation of C5 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

5. The method according to claim 3, wherein the at least one aptamer has specific affinity for C5a, and wherein up-regulation of C5a and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

6. The method according to claim 3, wherein the at least one aptamer has specific affinity for ETHE1, wherein up-regulation of CTSB and up-regulation of ETHE1 are indicative of an increased likelihood of the individual having pancreatic cancer.

7. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for HAMP, and wherein up-regulation of CTSB and up-regulation of HAMP are indicative of an increased likelihood of the individual having pancreatic cancer.

8. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for THBS4, and wherein up-regulation of CTSB and down-regulation of THBS4 are indicative of an increased likelihood of the individual having pancreatic cancer.

9. The method according to claim 3, wherein the at least one aptamer has specific affinity for KIT, and wherein down-regulation of KIT and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

10. The method according to claim 3, wherein the at least one aptamer has specific affinity for C9, and wherein up-regulation of C9 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

11. The method according to claim 3, wherein the at least one aptamer has specific affinity for KLK7 and wherein up-regulation of CTSB and down-regulation of KLK7 are indicative of an increased likelihood of the individual having pancreatic cancer.

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12. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for C2, and wherein up-regulation of CTSB and up-regulation of C2 are indicative of an increased likelihood of the individual having pancreatic cancer.

13. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for CRP, and wherein up-regulation of CTSB and up-regulation of CRP are indicative of an increased likelihood of the individual having pancreatic cancer.

14. The method according to claim 3, wherein the at least one aptamer has specific affinity for C5-C6, and wherein up-regulation of C5-C6 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

15. The method according to claim 3, wherein the at least one aptamer has specific affinity for IL11RA, and wherein up-regulation of CTSB and down-regulation of IL11RA are indicative of an increased likelihood of the individual having pancreatic cancer.

16. The method according to claim 3, wherein the at least one aptamer has specific affinity for CCL18, and wherein up-regulation of CCL18 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

17. The method according to claim 3, wherein the at least one aptamer has specific affinity for APOA1, and wherein down-regulation of APOA1 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

18. The method according to claim 1 or 2, wherein the other aptamer has specific affinity for GDF11, and wherein down-regulation of GDF11 and up-regulation of CTSB are indicative of an increased likelihood of the individual having pancreatic cancer.

19. The method according to any one of claims 1 to 18, wherein the diagnosis comprises the differential diagnosis of pancreatic cancer from benign conditions.

20. The method according to claim 19, wherein the benign condition is pancreatitis or a gastrointestinal disorder.

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21. The method of any one of claims 1 to 20, wherein the individual has an abdominal mass.

22. The method according to any one of claims 1 to 21, wherein the biological sample is selected from the group consisting of whole blood, plasma, serum, pancreatic tissue and pancreatic fluid.

23. The method of claim 22, wherein the biological sample is plasma.

24. The method of claim 22, wherein the biological sample is pancreatic tissue.

25. The method according to any one of claims 1 to 24, wherein the individual is a human.

26. The method of any of claims 1 to 25, wherein the individual is high risk for pancreatic cancer due to smoking, alcohol consumption or family history of pancreatic cancer.

27. The method according to any one of claim 1 to 26, wherein the aptamer protein complex is bound to a solid support.

28. A kit for diagnosing that an individual does or does not have pancreatic cancer using the method of any one of claims 1 to 27, the kit comprising:

at least two aptamers wherein at least one aptamer has specific affinity for CTSB and at least one other aptamer has specific affinity for a protein biomarker selected from HAMP, THBS4, C5, C2, CRP and GDF11; and instructions for use.

29. The kit of claim 28, wherein the kit further comprises reagents for an aptamer-based assay.

30. The kit of claim 28 or 29, wherein the at least two aptamers comprises at least three aptamers and at least one other aptamer has specific affinity for a protein biomarker selected from the group consisting of C5, C5a, ETHE1, KIT, C9, KLK7, C5-C6, IL11RA, CCL18, and APOA1.

Date Reçue/Date Received 2020-04-16

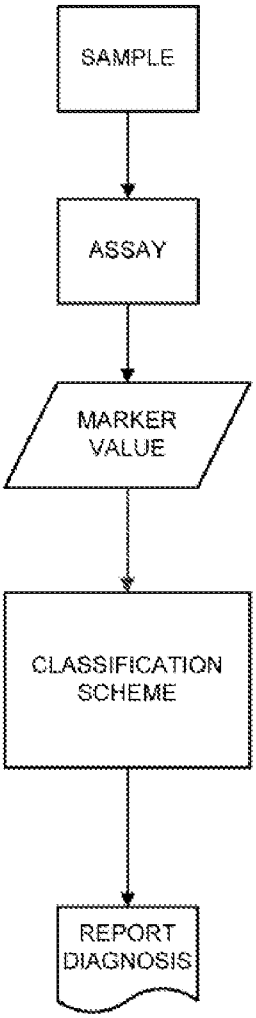

Figure 1A

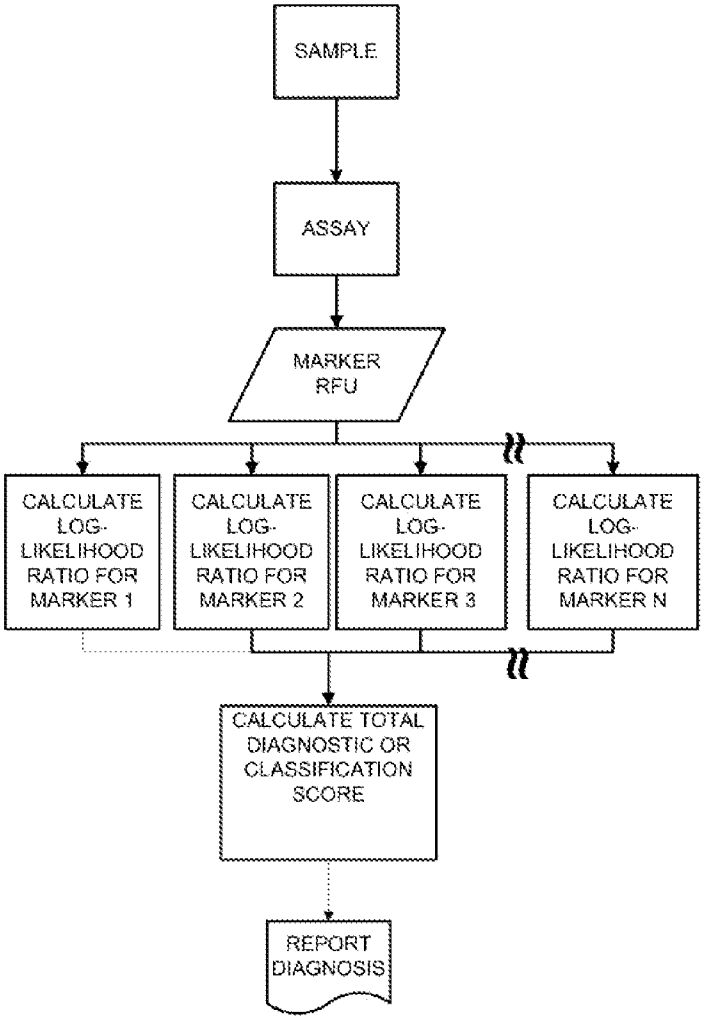

Figure 1B

Figure 1

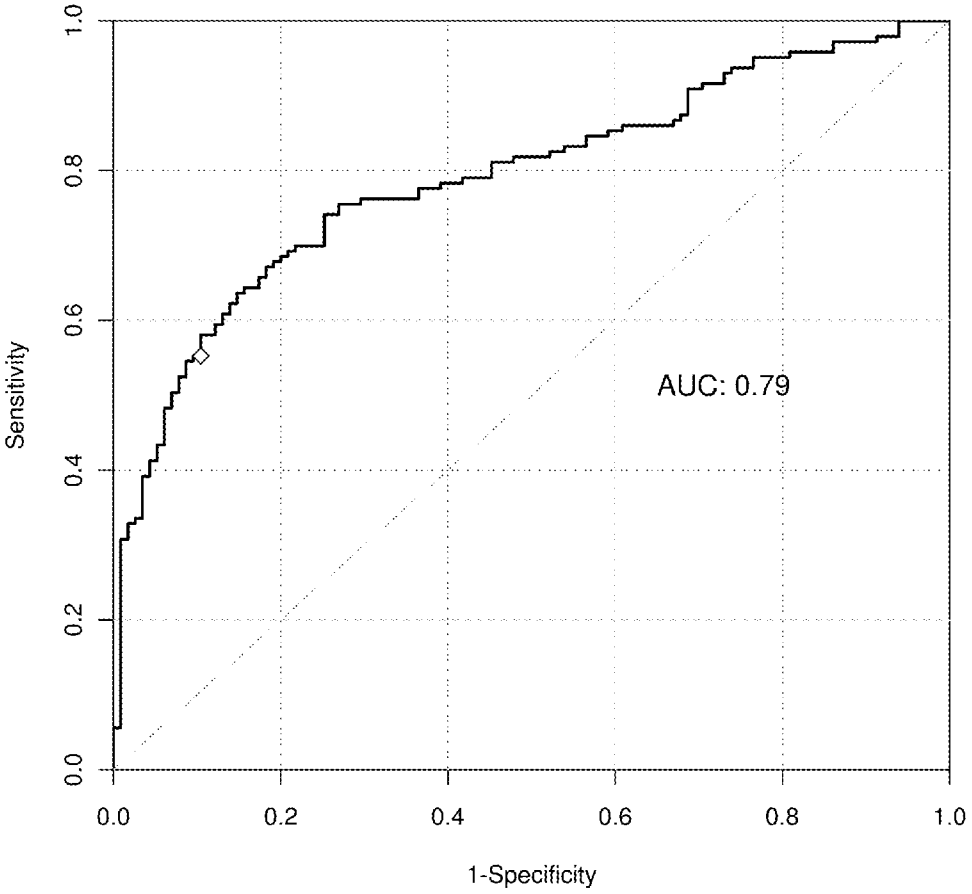

Figure 2

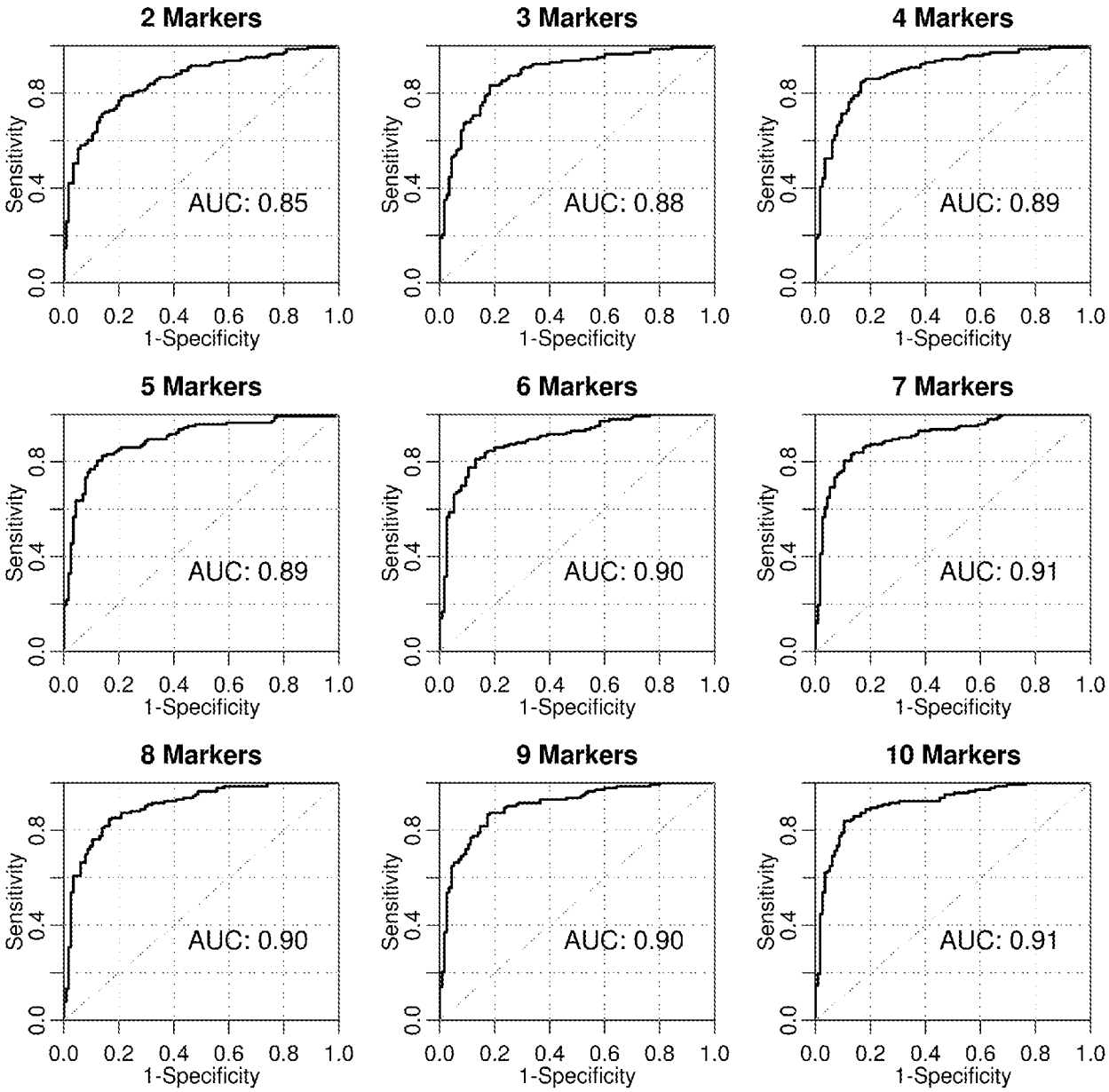
2 Markers
AUC: 0.85
3 Markers
AUC: 0.88
4 Markers
AUC: 0.89
5 Markers
AUC: 0.89
6 Markers
AUC: 0.90
7 Markers
AUC: 0.91
8 Markers
AUC: 0.90
9 Markers
AUC: 0.90
10 Markers
AUC: 0.91
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0

Figure 3

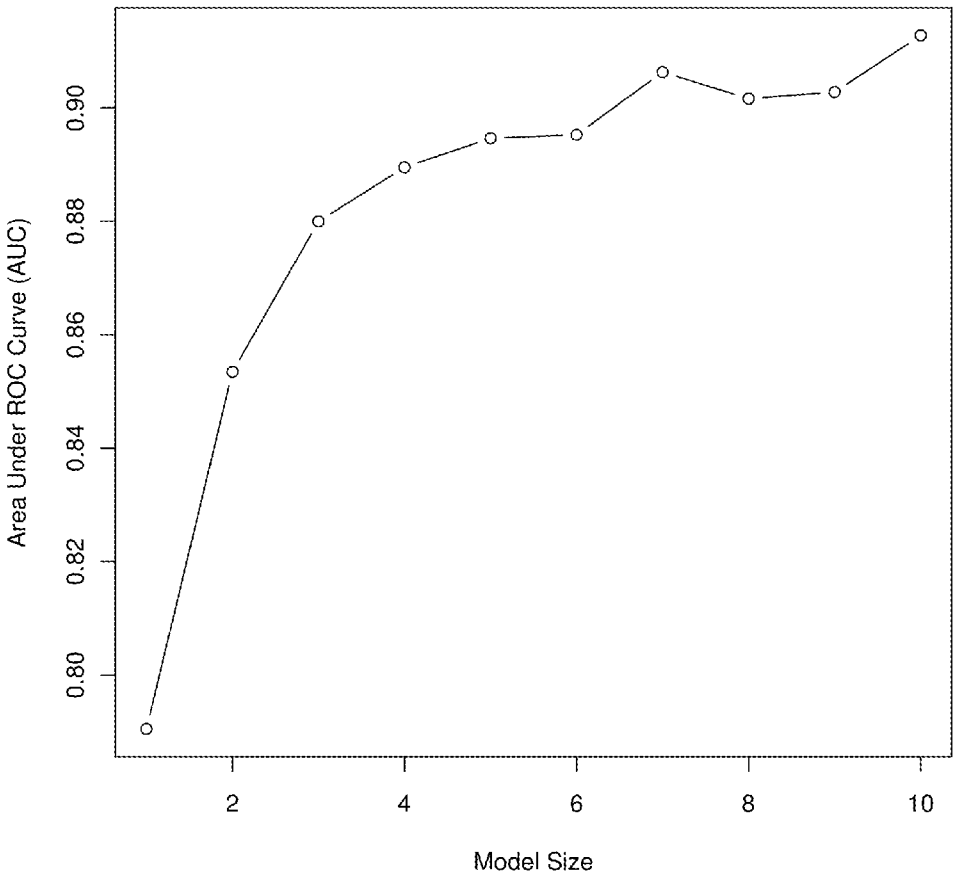
Area Under ROC Curve (AUC)
0.80
0.82
0.84
0.86
0.88
0.90
2
4
6
8
10
Model Size

Figure 4

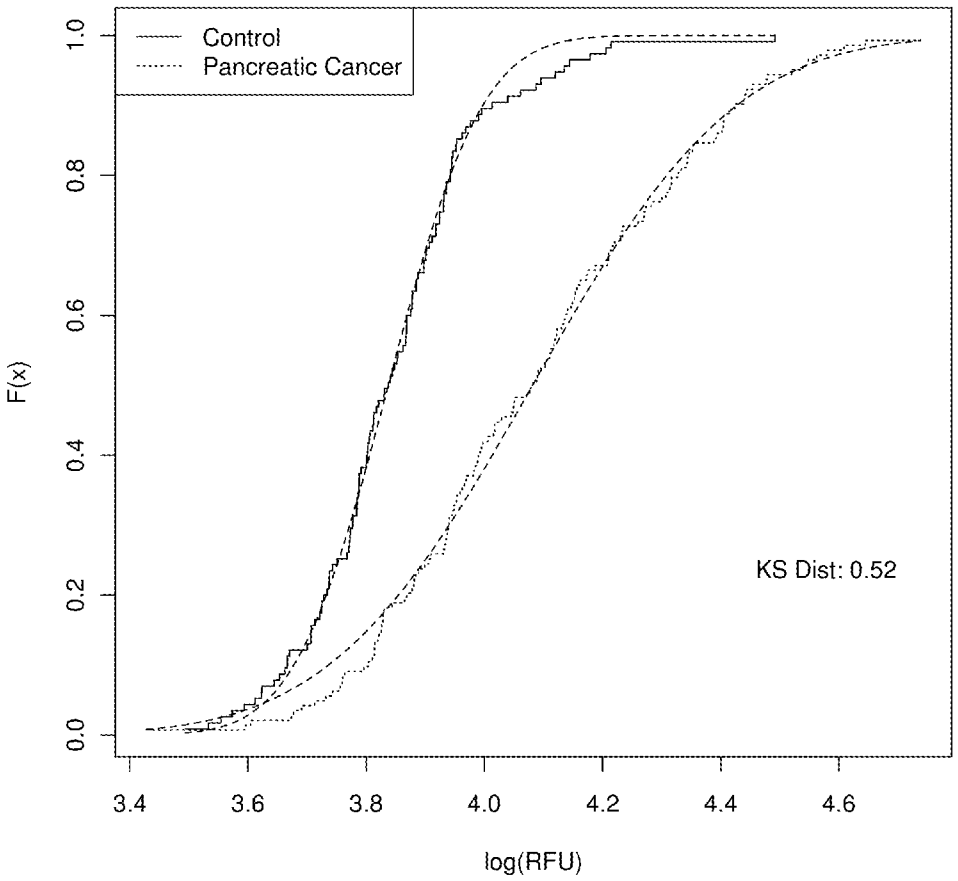
Control
Pancreatic Cancer
KS Dist: 0.52
F(x)
0.0
0.2
0.4
0.6
0.8
1.0
3.4
3.6
3.8
4.0
4.2
4.4
4.6
log(RFU)

Figure 5

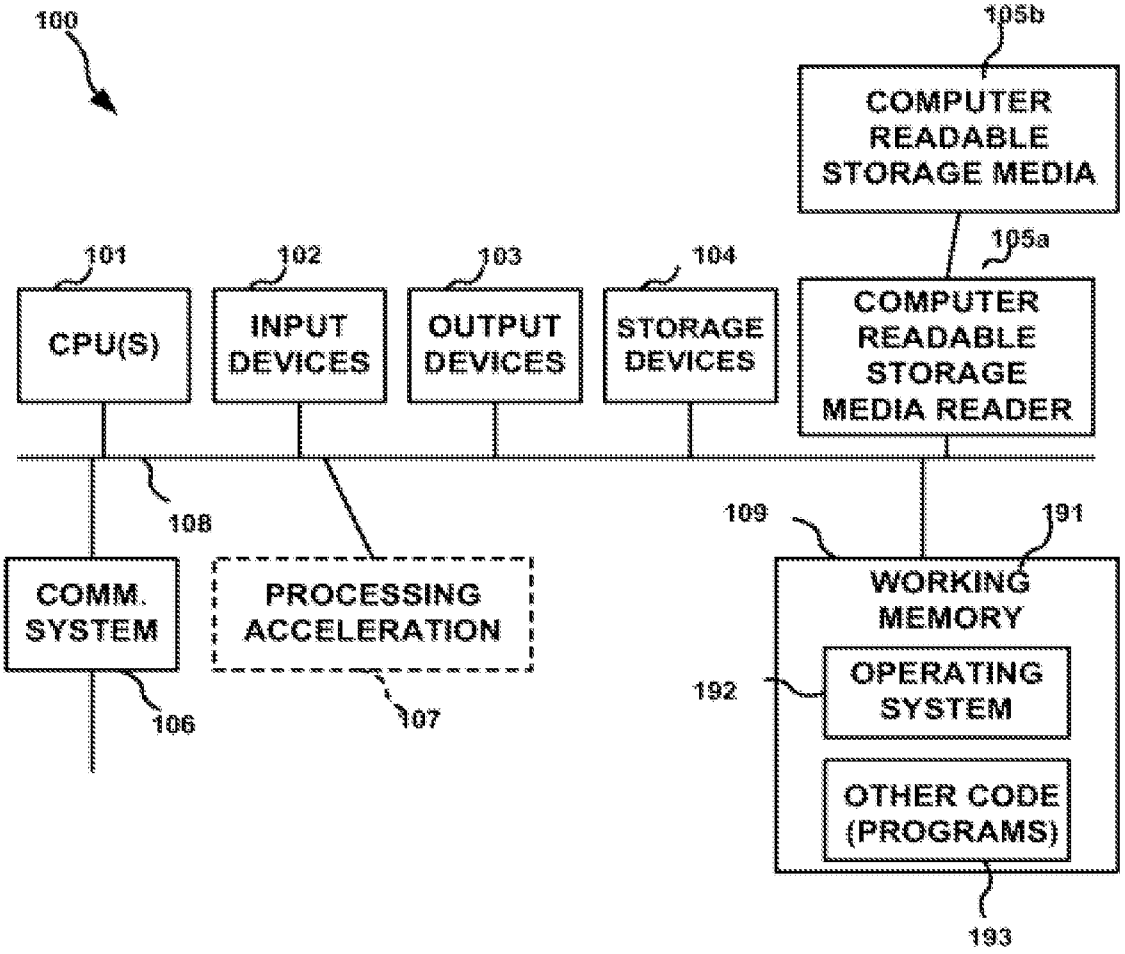
100
105b
COMPUTER READABLE STORAGE MEDIA
105a
COMPUTER READABLE STORAGE MEDIA READER
101
CPU(S)
102
INPUT DEVICES
103
OUTPUT DEVICES
104
STORAGE DEVICES
108
109
191
WORKING MEMORY
192
OPERATING SYSTEM
OTHER CODE (PROGRAMS)
193
COMM. SYSTEM
106
PROCESSING ACCELERATION
107

Figure 6

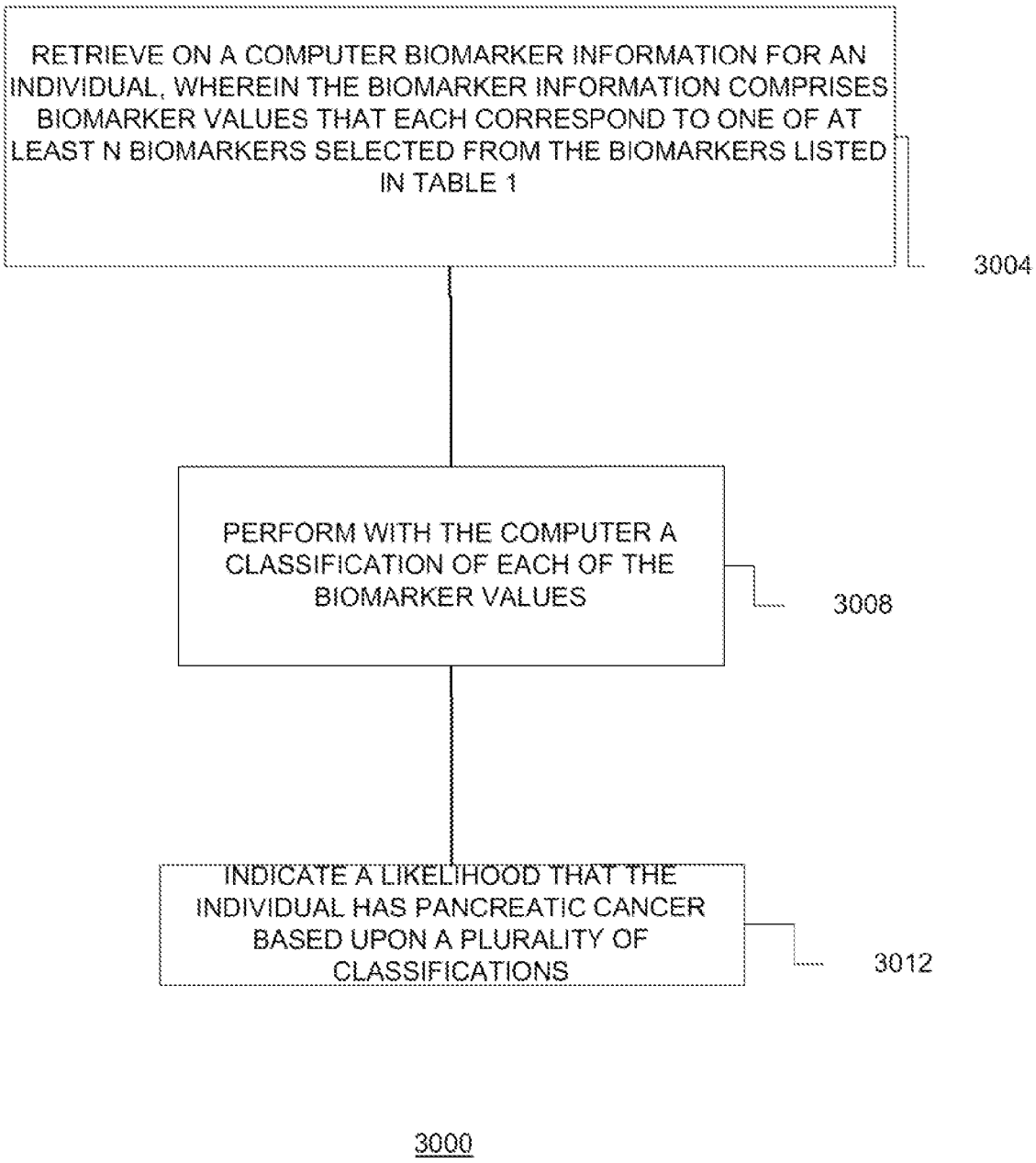
RETRIEVE ON A COMPUTER BIOMARKER INFORMATION FOR AN INDIVIDUAL, WHEREIN THE BIOMARKER INFORMATION COMPRISES BIOMARKER VALUES THAT EACH CORRESPOND TO ONE OF AT LEAST N BIOMARKERS SELECTED FROM THE BIOMARKERS LISTED IN TABLE 1
3004
PERFORM WITH THE COMPUTER A CLASSIFICATION OF EACH OF THE BIOMARKER VALUES
3008
INDICATE A LIKELIHOOD THAT THE INDIVIDUAL HAS PANCREATIC CANCER BASED UPON A PLURALITY OF CLASSIFICATIONS
3012
3000

Figure 7

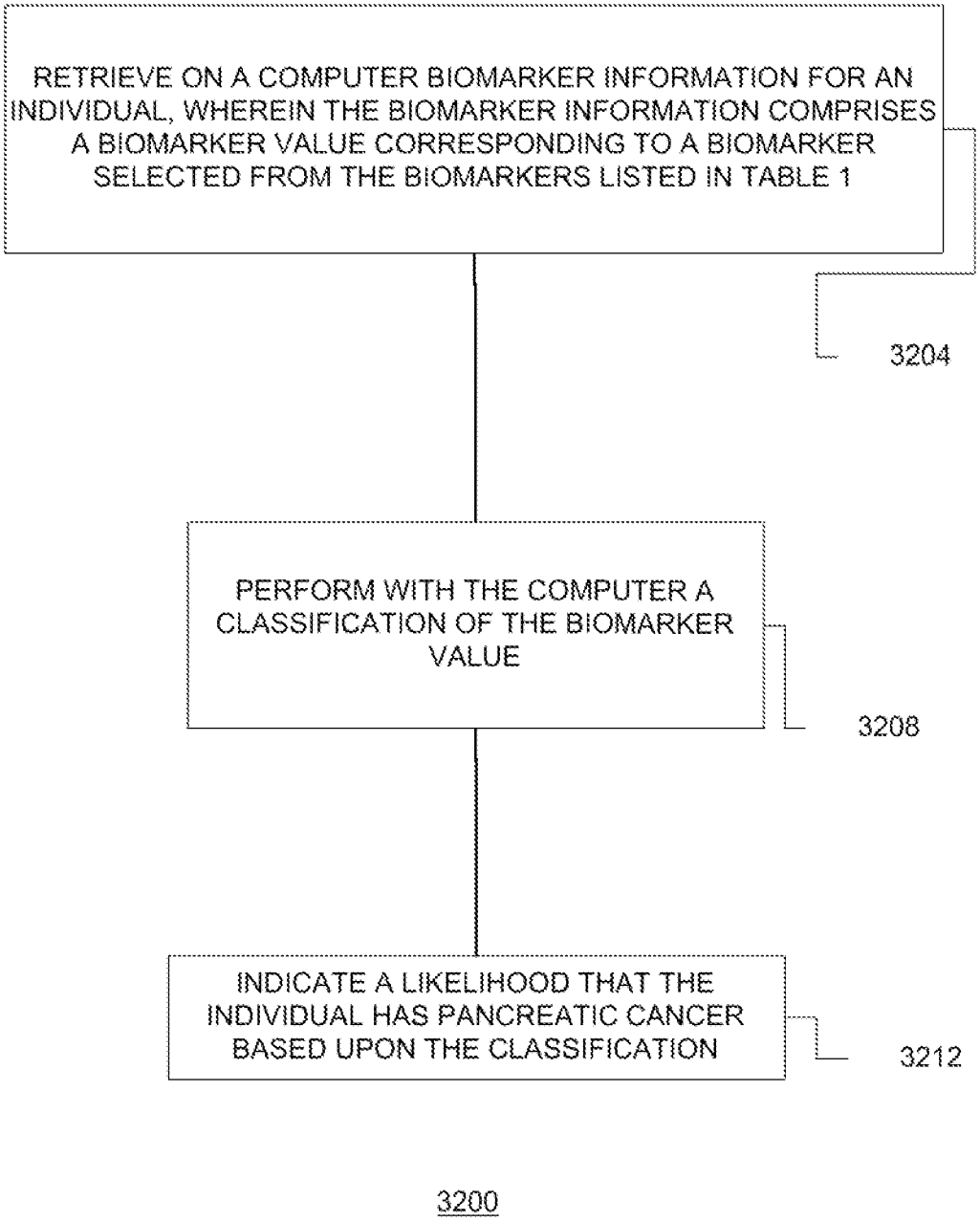
RETRIEVE ON A COMPUTER BIOMARKER INFORMATION FOR AN INDIVIDUAL, WHEREIN THE BIOMARKER INFORMATION COMPRISES A BIOMARKER VALUE CORRESPONDING TO A BIOMARKER SELECTED FROM THE BIOMARKERS LISTED IN TABLE 1
3204
PERFORM WITH THE COMPUTER A CLASSIFICATION OF THE BIOMARKER VALUE
3208
INDICATE A LIKELIHOOD THAT THE INDIVIDUAL HAS PANCREATIC CANCER BASED UPON THE CLASSIFICATION
3212
3200

Figure 8

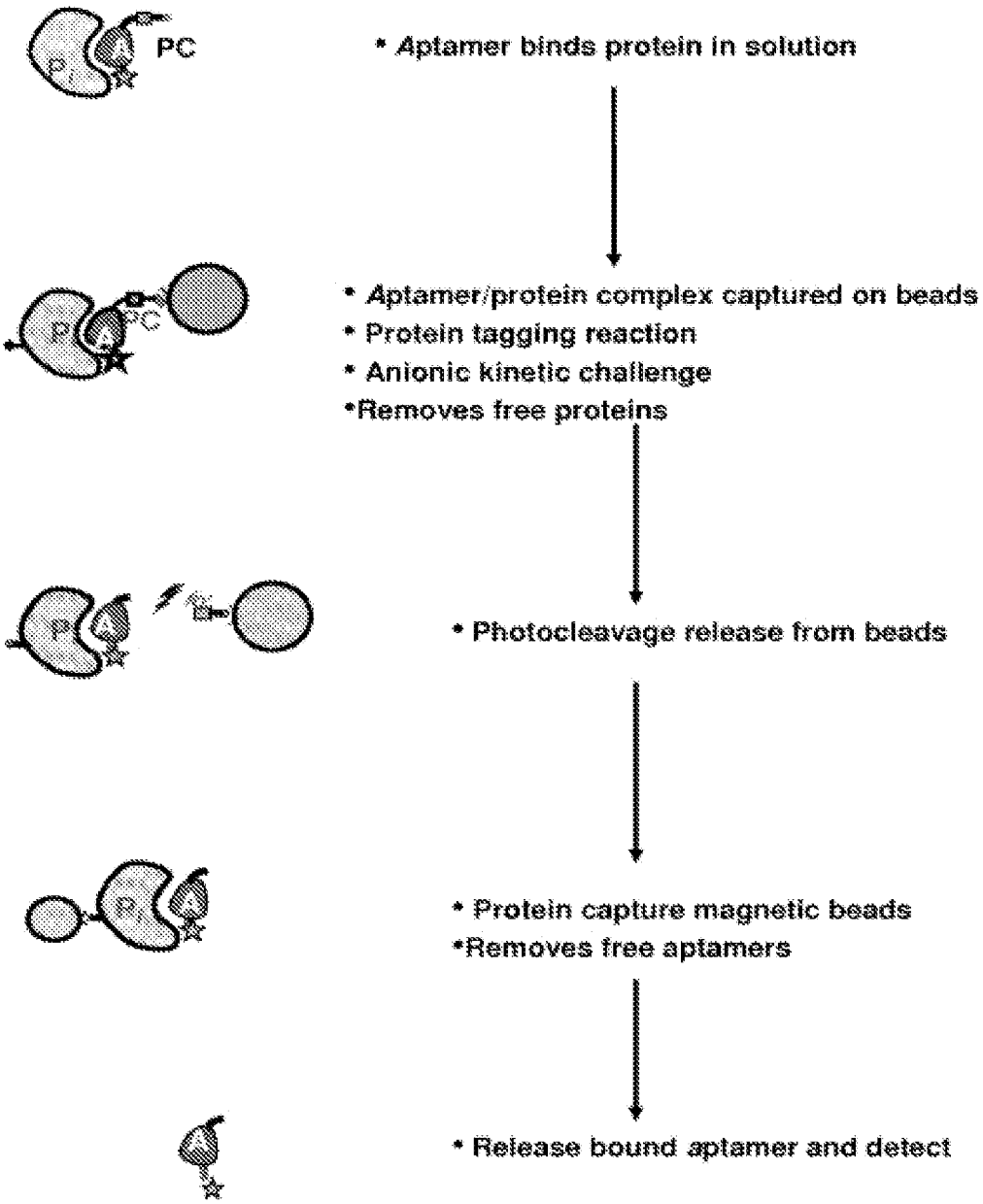
PC
* Aptamer binds protein in solution
* Aptamer/protein complex captured on beads
* Protein tagging reaction
* Anionic kinetic challenge
*Removes free proteins
* Photocleavage release from beads
* Protein capture magnetic beads
*Removes free aptamers
* Release bound aptamer and detect

Figure 9

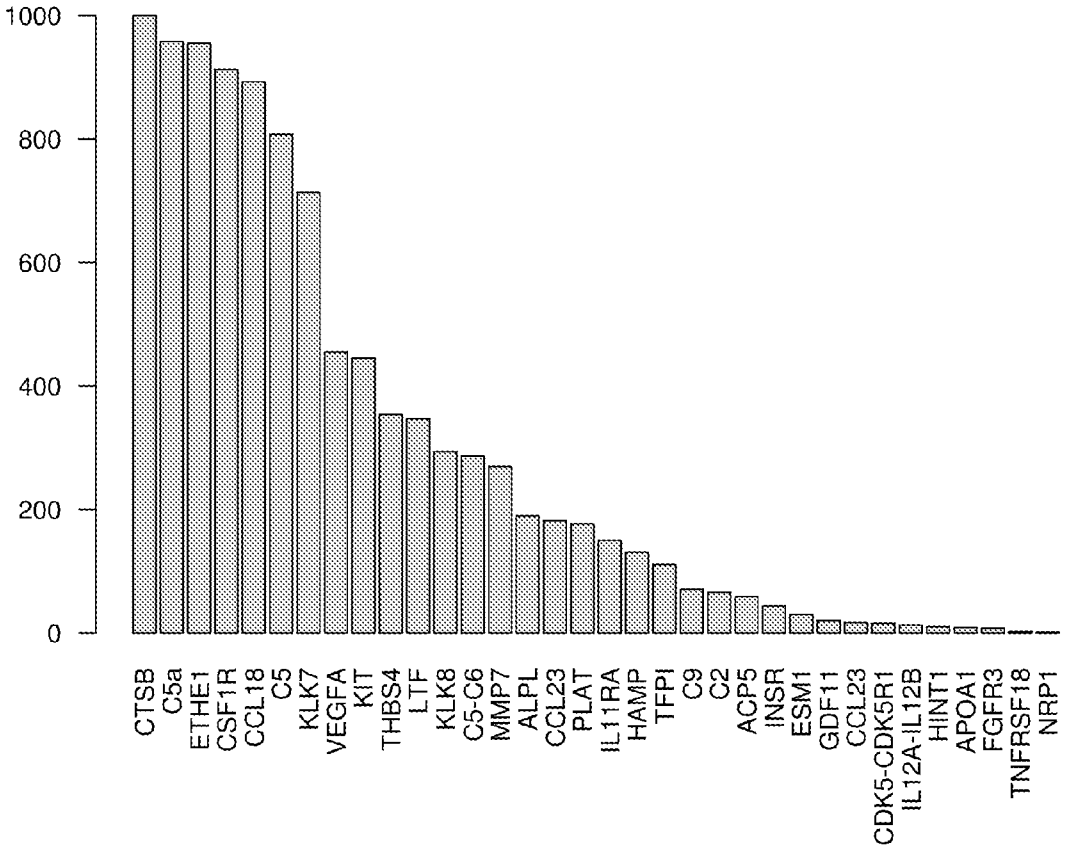
1000
800
600
400
200
0
CTSB
C5a
ETHE1
CSF1R
CCL18
C5
KLK7
VEGFA
KIT
THBS4
LTF
KLK8
C5-C6
MMP7
ALPL
CCL23
PLAT
IL11RA
HAMP
TFPI
C9
C2
ACP5
INSR
ESM1
GDF11
CCL23
CDK5-CDK5R1
IL12A-IL12B
HINT1
APOA1
FGFR3
TNFRSF18
NRP1

Figure 10

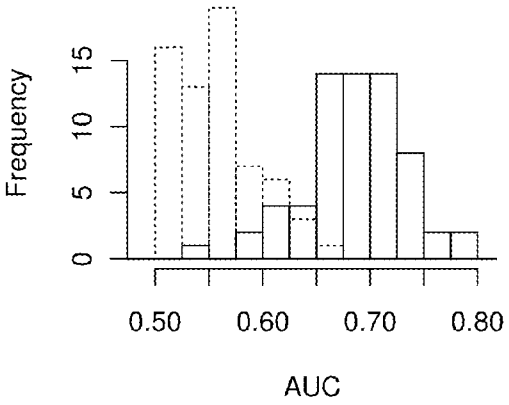

Figure 11A

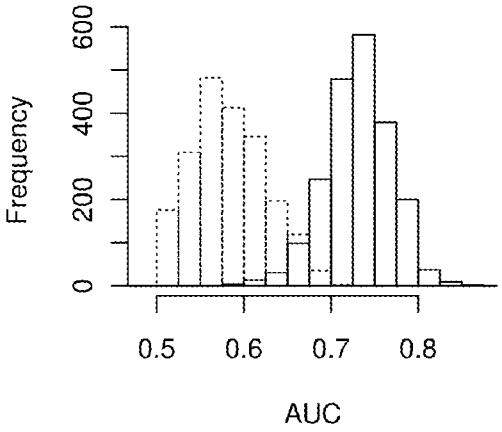

Figure 11B

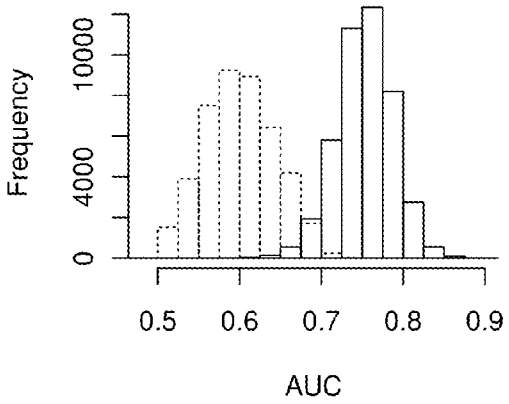

Figure 11C

Figure 11

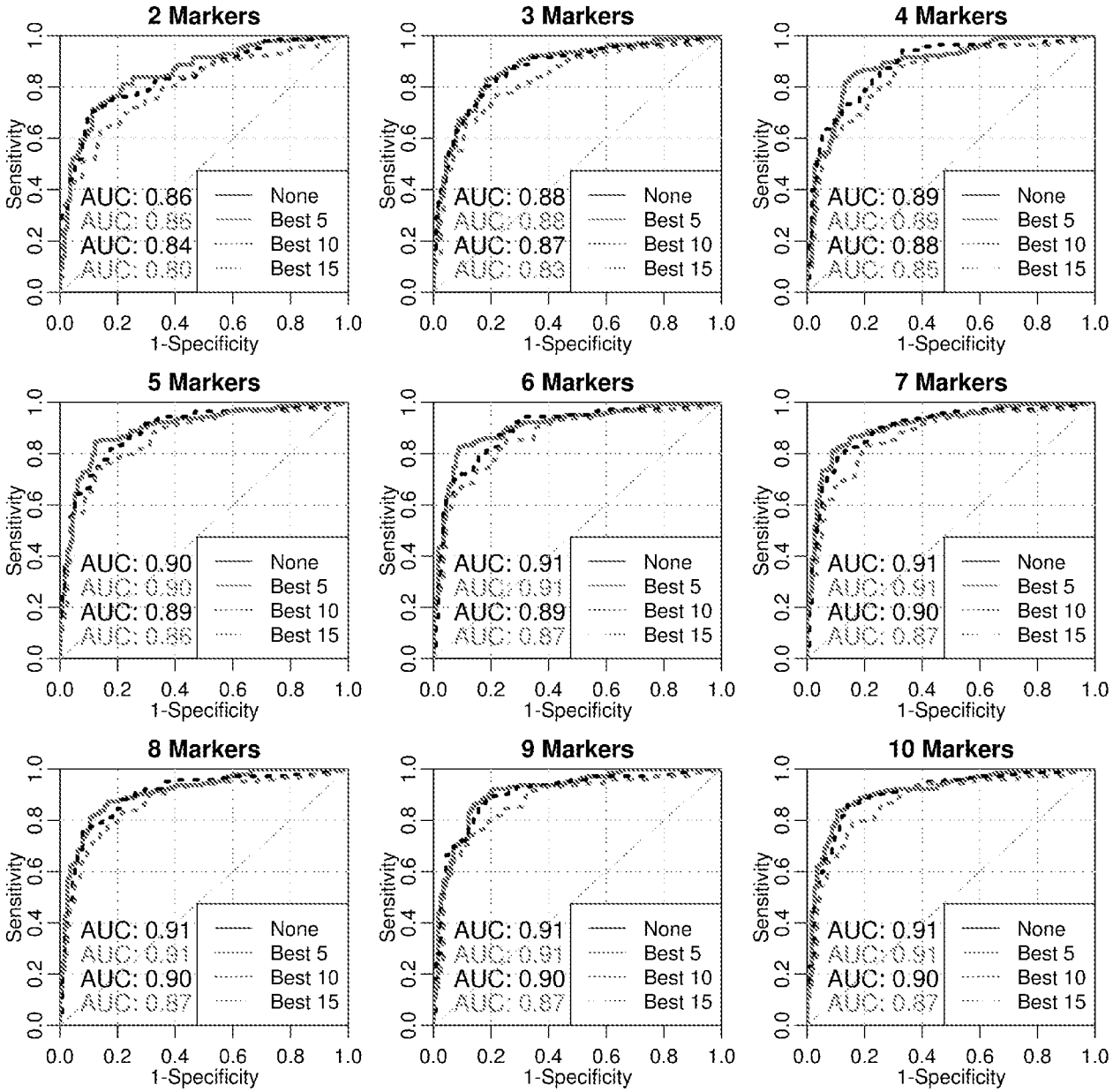
2 Markers
AUC: 0.86
AUC: 0.85
AUC: 0.84
AUC: 0.80
3 Markers
AUC: 0.88
AUC: 0.88
AUC: 0.87
AUC: 0.83
4 Markers
AUC: 0.89
AUC: 0.89
AUC: 0.88
AUC: 0.85
5 Markers
AUC: 0.90
AUC: 0.90
AUC: 0.89
AUC: 0.86
6 Markers
AUC: 0.91
AUC: 0.91
AUC: 0.89
AUC: 0.87
7 Markers
AUC: 0.91
AUC: 0.91
AUC: 0.90
AUC: 0.87
8 Markers
AUC: 0.91
AUC: 0.91
AUC: 0.90
AUC: 0.87
9 Markers
AUC: 0.91
AUC: 0.91
AUC: 0.90
AUC: 0.87
10 Markers
AUC: 0.91
AUC: 0.91
AUC: 0.90
AUC: 0.87
None
Best 5
Best 10
Best 15
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0

Figure 12

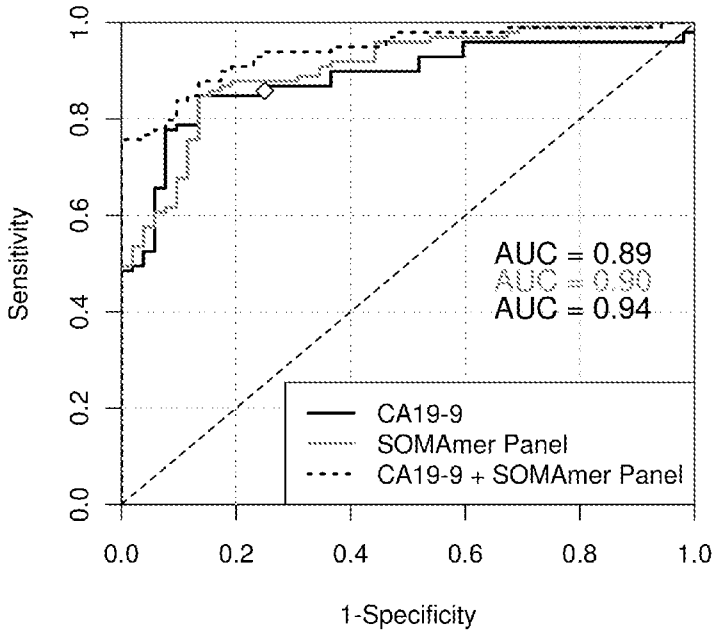

Figure 13

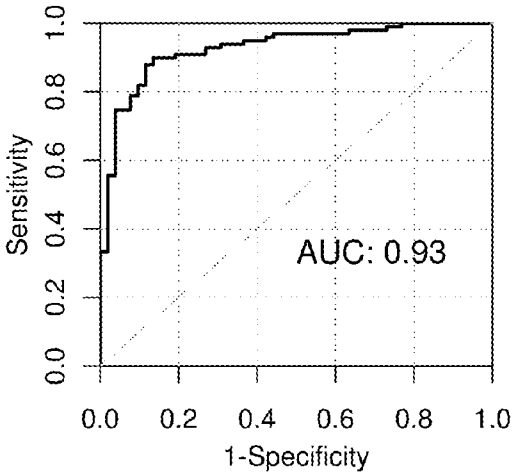

Figure 14A

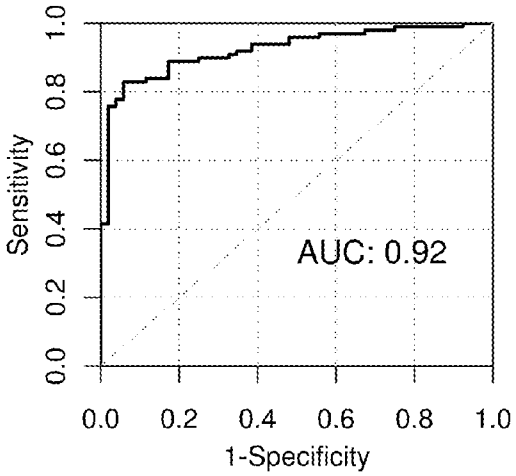

Figure 14B

Figure 14

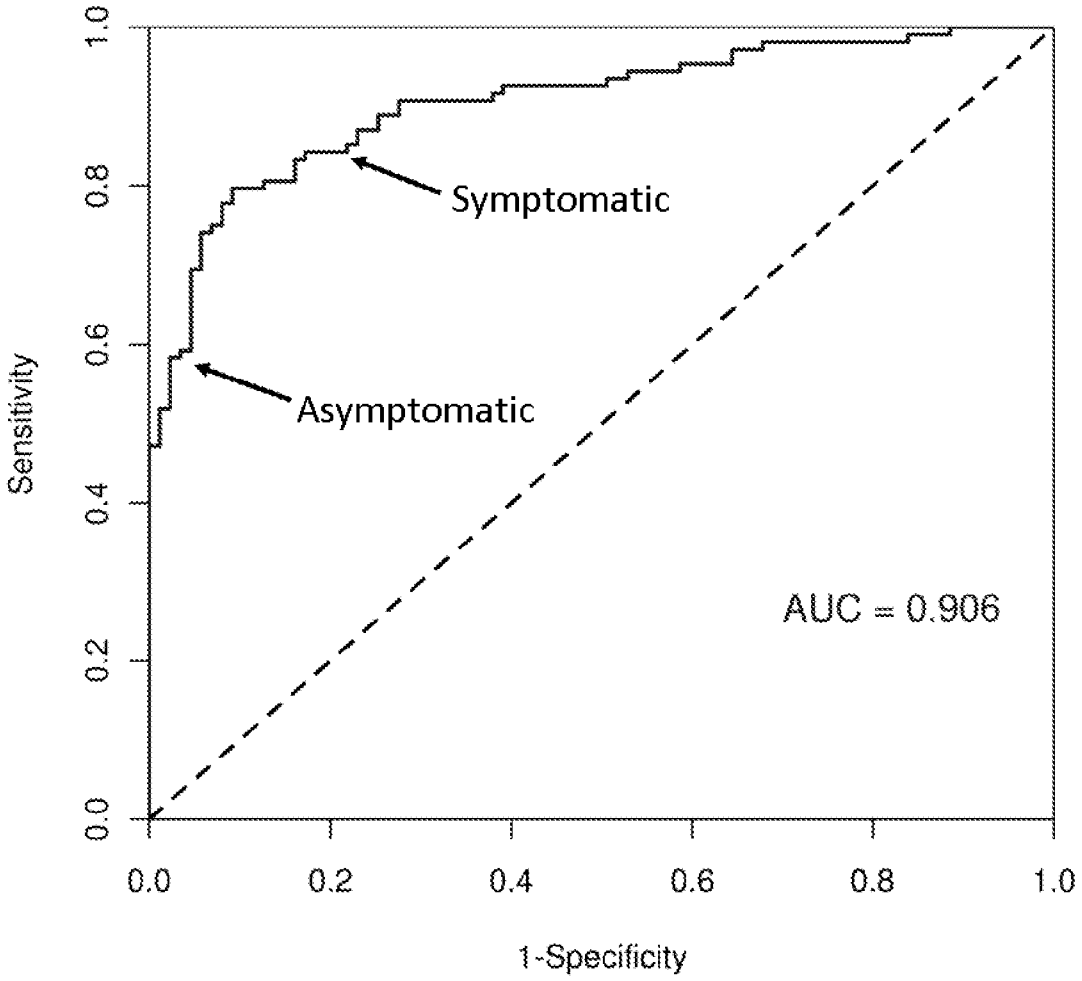
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Symptomatic
Asymptomatic
AUC = 0.906

Figure 15

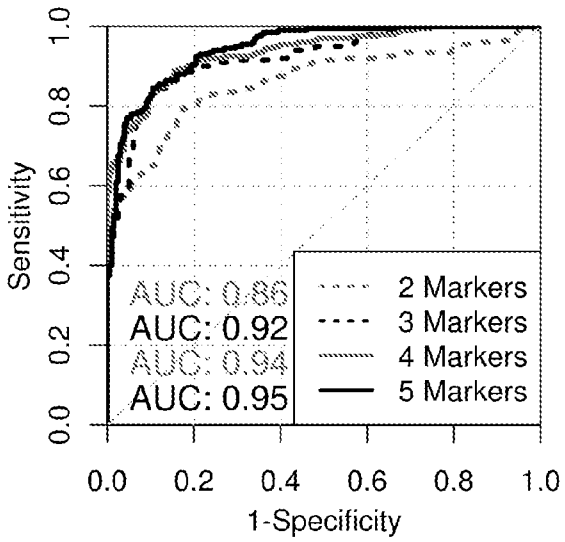

Figure 16A

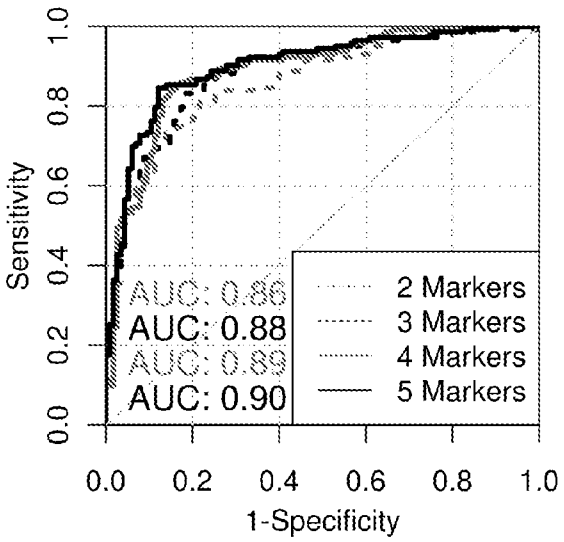

Figure 16B

Figure 16

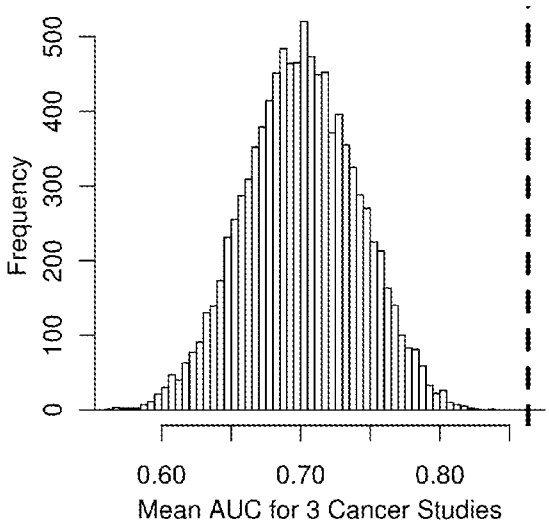

Figure 17A

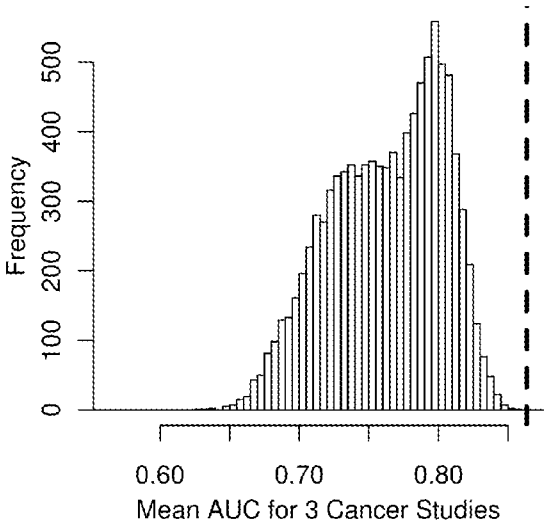

Figure 17B

Figure 17

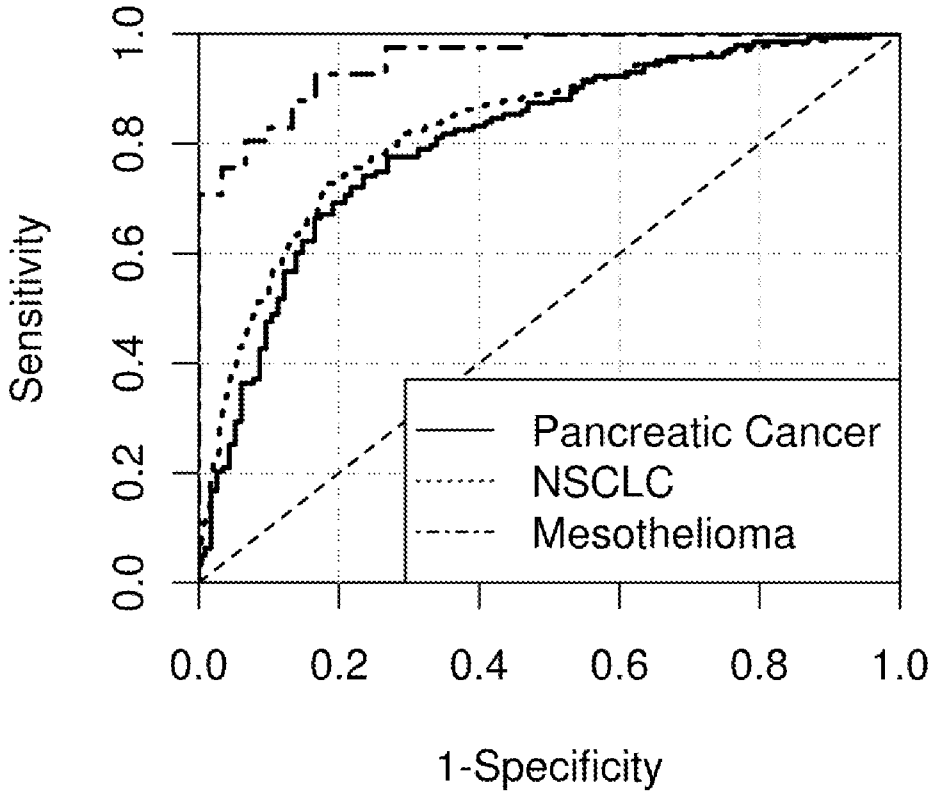
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity
Pancreatic Cancer
NSCLC
Mesothelioma

Figure 18