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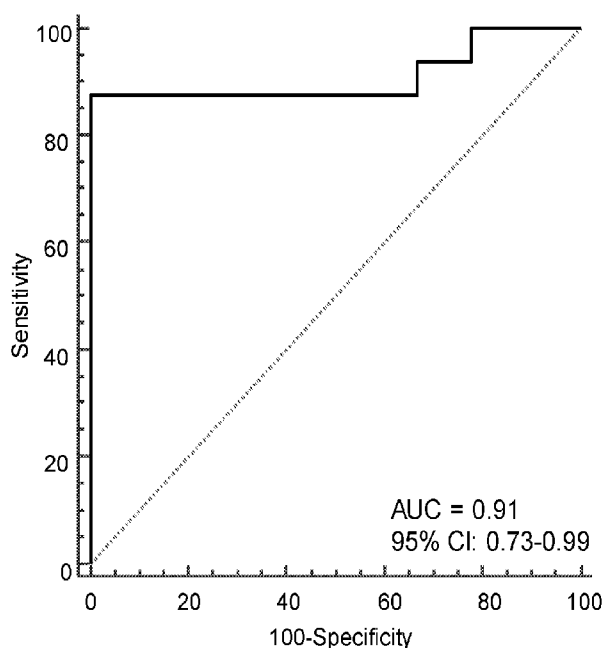


FIG. 1C

(57) Abstract: Provided herein are methods and compositions for detecting and treating a micrometastatic disease in a subject who has or is suspected of having a cancer.



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METHODS AND COMPOSITIONS FOR DETERMINING CANCER RISK

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/191,251, filed May 20, 2021, the content of which is incorporated herein by reference in its entirety and for all purposes.

REFERENCE TO A “SEQUENCE LISTING,” A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII FILE

[0002] The Sequence Listing written in file 048440-807001WO_SL_ST25.TXT, created May 16, 2022, 1,505 bytes in size, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference.

BACKGROUND

[0003] Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers, and is projected to become the second most common cause of cancer-related deaths in the United States by 2030¹⁻³. Despite an improved understanding of the molecular and genetic basis of PDAC and recent advancements in treatments, including newly introduced chemotherapy and radiotherapy regimens, the 5-year survival rates have remained low, well under 10%^{3, 4}. Surgical resection (i.e. pancreatectomy) for localized tumors is an invasive procedure with high complication and mortality rates; nonetheless, it is still considered the only treatment option for a potential cure or long-term survival in patients with PDAC. However, even for the most favorable cohort of patients with resectable PDAC, up to 80% of patients experience a recurrence following surgery, with a short recurrence-free interval^{1, 5, 6}. These high recurrence rates are primarily attributed to the presence of occult micrometastatic disease at the time of resection^{7, 8}. In hindsight, these patients should perhaps have been spared an ineffective invasive surgery. Unfortunately, this problem is exacerbated by a lack of clinically useful biomarkers that can predict the risk of post-surgical recurrence prior to PDAC patients receive such treatments.

[0004] Given that overall, surgery alone offers only minimal survival benefits, multidisciplinary treatment strategies, including neoadjuvant therapy (NAT), are being aggressively investigated and becoming increasingly more common, especially in western countries⁹⁻¹⁵. However, because there are no established criteria to implement specific NAT regimens, and lack of clinical tools for risk assessment following NAT, physicians and

patients often have to make difficult decisions on whether to continue chemotherapy or to proceed with surgery. Currently, as per the National Comprehensive Cancer Network (NCCN) guidelines, such pre-treatment risk predictions are often estimated based on clinicopathological factors, including carbohydrate antigen 19-9 (CA19-9) levels, tumor size, co-morbidities, nutritional status, and others features^{1, 5, 14, 16-22}. Unfortunately, however, all of these prognostic tools remain clinically challenging and inadequate for predicting recurrence in PDAC patients following surgery, due to their poor sensitivity and specificity; highlighting the need to develop novel, preferably noninvasive molecular biomarkers, which can accurately predict cancer recurrence prior to any treatment. Translational research efforts to develop such risk-stratification biomarkers have been limited. Although a handful of studies have reported that preoperative levels of serum duke pancreatic monoclonal antigen type 2, gamma-glutamyltransferase-to-albumin ratio, or a kirsten rat sarcoma (KRAS)-mutated circulating tumor deoxyribonucleic acid (DNA) might help predict recurrence in PDAC, the accuracy of these biomarkers remains insufficient for an effective clinical decision-making^{16, 23, 24}.

[0005] Recent technological advances have enabled innovative genomic and epigenomic profiling in various malignancies and have facilitated identification of previously unrecognized molecular biomarkers²⁵⁻³³. MicroRNAs (miRNAs) are small noncoding ribonucleic acids (RNAs) that play a pivotal role in gene regulation^{34, 35} and are frequently deregulated in human cancers. The expression patterns of miRNAs have been shown to have diagnostic, prognostic, and therapeutic potential, in various cancers, including PDAC^{26, 31, 32, 34, 36}. Although miRNAs offer high detection sensitivity, the heterogeneity associated with sources of circulating, cell-free miRNAs (cf-miRNAs) appear to limit their overall detection accuracy. It is well-recognized that cf-miRNAs may be produced from multiple sources, including apoptotic bodies, and immune cells. One type of small (40–200 nm) membranous microvesicles are exosomes, which inherit molecular cargo from their cell-of-origin³⁷⁻⁴². Accordingly, exosomes secreted by cancer cells appear to possess cancer-specific exosomal miRNA (exo-miRNA) signatures⁴³⁻⁴⁵ that may result in greater representation of cancer-derived miRNAs compared to cf-miRNAs, hence providing more specific biomarkers in systemic circulation.

[0006] Hence, there is an unmet need of developing some individualized treatment strategies and genome-guided prospective for the management of patients with the PDAC.

BRIEF SUMMARY

[0007] Provided herein, inter alia, are methods of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer. The disclosed methods comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and administering to the subject neoadjuvant therapy.

[0008] In embodiments, provided herein are methods of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer. The disclosed methods comprise administering to the subject neoadjuvant therapy, if a biological sample obtained from the subject comprises an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

[0009] Additionally provided herein are methods of identifying an increased risk of developing an occult micrometastatic disease or detecting an occult micrometastatic disease in a subject with cancer. The disclosed methods comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates an increased risk of developing an occult micrometastatic disease or the presence of an occult micrometastatic disease in the subject.

[0010] Also provided herein are methods of diagnosing a subject with cancer as having an increased risk of developing an occult micrometastatic disease. The disclosed methods comprise detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and diagnosing the subject as having an increased risk of developing an occult micrometastatic disease if an elevated microRNA expression level, relative to a control, is detected in the biological sample. In embodiments, the disclosed methods further comprise administering to the subject neoadjuvant therapy. In embodiments, the neoadjuvant therapy comprises an

anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof. In embodiments, the disclosed methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0011] Additionally provided herein are methods of monitoring a subject having cancer for an increased risk of developing an occult micrometastatic disease. The disclosed methods comprise (i) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; and (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a second time point subsequent to the first time point; wherein detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease.

[0012] In embodiments, monitoring further comprises the use of ultrasound, computerized tomography (CT) scans, magnetic resonance imaging (MRI), positron emission tomography (PET) scans, and any combinations thereof. In embodiments, the disclosed methods further comprise proposing neoadjuvant therapy to a subject with an increased risk of developing an occult micrometastatic disease. In embodiments, the disclosed methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0013] In embodiments, the microRNA is exosomal microRNA.

[0014] In embodiments, the cancer is pancreatic cancer. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). In embodiments, the occult micrometastatic disease is pancreatic metastases.

[0015] In embodiments, provided herein is a method of treating occult micrometastatic disease in a subject who has or is suspected of having a pancreatic cancer. The disclosed method comprises administering to the subject neoadjuvant therapy, if a biological sample

obtained from the subject has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof. In embodiments, the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0016] In embodiments, a subject with no indication of metastatic disease is monitored every 3 to 6 months for two years after testing, and thereafter every six months for three additional years.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIGS. 1A-1F illustrate small ribonucleic acid (RNA) sequencing identifies a blood-based exo-microRNA panel (EMP) for predicting cancer recurrence after surgery in patients with pancreatic ductal adenocarcinoma (PDAC). FIG. 1A, Volcano plot showing significant and differentially upregulated exosomal microRNAs (exo-miRNAs) selected from sequence data. miRNAs selected for further study are depicted in gray shaded area (upper right quadrant). FIG. 1B, Heatmap representing the 12 significantly differentiated exo-miRNAs in pre-treatment plasma specimens from a discovery cohort of patients who had recurrence (+) after surgery (n = 16) compared to non-recurrent patients (-) (n = 9). FIG. 1C, Receiver operating characteristic (ROC) curve of a 12-exo-miRNA EMP for predicting recurrence in the discovery cohort (area under the Curve (AUC) = 0.91). FIG. 1D, Risk score distribution plot in the discovery cohort. Modified risk score was obtained by subtracting individual risk score from Youden's index value of risk model. FIG. 1E, Comparison of relapse-free survival (RFS) between high- and low-risk groups estimated by 12-exo-miRNA EMP. FIG. 1F, Distribution of risk scores according to recurrence status ($P < 0.01$, Mann Whitney test).

[0018] FIGS. 2A-2E illustrate clinical training which establishes an EMP for predicting recurrence and cancer prognosis in patients with PDAC. FIG. 2A, ROC curve of the pre-treatment 6-exo-miRNA EMP recurrence prediction model constructed using cox regression with backward elimination for feature selection using the training cohort (AUC = 0.81). FIG. 2B, Distribution of risk scores according to recurrence status ($P < 0.01$, Mann Whitney test). FIG. 2C, Kaplan-Meier plot showing RFS between high- and low-risk groups estimated by the 6-exo-miRNA EMP. FIG. 2D, Forest plots showing hazard ratios of clinicopathological variables and panel risk score status in uni- and multivariate cox proportional analyses of

RFS in the training cohort. FIG.2E, the new combination model, EMP and CA19-9, outperformed the prediction accuracy of other variables in the training cohort (AUC = 0.84).

[0019] FIGS. 3A-3E illustrate validation of the EMP in an independent clinical cohort demonstrates its translational potential. FIG.3A, ROC curve of the 6-exo-miRNA EMP derived from the training cohort for recurrence prediction in an independent validation cohort (AUC = 0.78). FIG.3B, Distribution of risk scores according to recurrence status ($P < 0.01$, Mann Whitney test). FIG.3C, Kaplan-Meier plot showing RFS between high- and low-risk groups estimated by the EMP. FIG.3D, Forest plots showing hazard ratios of clinicopathological variables and panel risk score status in uni- and multivariate cox proportional analyses of RFS in the validation cohort. FIG.3E, ROC curves showing the recurrence predictive performance of the combination model, EMP and CA19-9, compared to conventional clinical factors in the validation cohort (AUC = 0.82).

[0020] FIGS.4A-4E illustrate additional clinical validation of the EMP predicts PDAC recurrence in patients after neoadjuvant therapy (NAT). FIG.4A, ROC curve of the 6-exo-miRNA EMP derived from the training cohort for recurrence prediction in an additional validation cohort, comprised of post-NAT blood samples from patients with PDAC who underwent NAT followed by curative surgery (AUC = 0.72). FIG.4B, Distribution of risk scores according to recurrence status ($P < 0.01$, Mann Whitney test). FIG.4C, Kaplan-Meier plot showing RFS between high- and low-risk groups estimated by the EMP. FIG.4D, Forest plots showing hazard ratios of clinicopathological variables and panel risk score status in uni- and multivariate cox proportional analyses of RFS in the post-NAT validation cohort. FIG.4E, ROC curves showing the recurrence predictive performance of the combination model, EMP and CA19-9, compared to conventional clinical factors in the post-NAT validation cohort (AUC = 0.79).

[0021] FIG.5 illustrates miRNA–mRNA (messenger RNA) regulatory network analysis and pathway enrichment analysis of candidate miRNAs reveals biologically meaningful pathways. A miRNA–mRNA regulatory network analysis to determine the downstream gene targets of the discovered miRNAs. Subsequently, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to perform pathway analysis of the identified downstream 96 gene targets from network analysis.

[0022] FIG. 6 illustrates a flowchart of determining exosomal microRNAs for the EMP and training and validation of the EMP.

DETAILED DESCRIPTION

DEFINITIONS

[0023] Before the present invention is further described, it is to be understood that this invention is not strictly limited to particular embodiments described, as such may of course vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the claims.

[0024] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. It should further be understood that as used herein, the term “a” entity or “an” entity refers to one or more of that entity. For example, a nucleic acid molecule refers to one or more nucleic acid molecules. As such, the terms “a,” “an,” “one or more” and “at least one” can be used interchangeably. Similarly, the terms “comprising,” “including” and “having” can be used interchangeably.

[0025] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, e.g., Singleton et al., Dictionary of Microbiology and Molecular Biology, 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this disclosure. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0026] As used herein, the term “about” means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, about means within a standard deviation using measurements generally acceptable in the art. In embodiments, about means a range extending to +/- 10% of the specified value. In embodiments, about means the specified value.

[0027] A “cell” refers to a cell carrying out metabolic or other function sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaryotic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian (e.g. human) cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0028] As used herein, the term “tumor-derived exosome” or “exosome” refers to a small (between 20-300 nm in diameter) vesicle comprising a lipid bilayer membrane that encloses an internal space, and which is generated from a cancer cell by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane. The components of tumor-derived exosomes include proteins, DNA, mRNA, microRNA, long noncoding RNA, circular RNA, and the like, which play a role in regulating tumor growth, metastasis, and angiogenesis in the process of cancer development.

[0029] “Exosomal RNA” refers to RNA within a tumor-derived exosome or RNA obtained from within a tumor-derived exosome. In embodiments, “exosomal RNA” is exosomal miRNA. In embodiments, “exosomal RNA” is exosomal mRNA. Exosomal RNA can be detected and measured by methods known in the art, such as those described in Example 2 herein.

[0030] “Cell-free RNA” or “cf-RNA” refers to RNA that is not within a tumor-derived exosome or RNA that has not been obtained from within a tumor-derived exosome.

[0031] “Nucleic acid” refers to nucleotides (e.g., deoxyribonucleotides or ribonucleotides) and polymers thereof in either single-, double- or multiple-stranded form, or complements thereof; or nucleosides (e.g., deoxyribonucleosides or ribonucleosides). In embodiments, “nucleic acid” does not include nucleosides. The terms “polynucleotide,” “oligonucleotide,” “oligo” or the like refer, in the usual and customary sense, to a linear sequence of nucleotides. The term “nucleoside” refers, in the usual and customary sense, to a glycosylamine including a nucleobase and a five-carbon sugar (ribose or deoxyribose). Non limiting examples, of nucleosides include, cytidine, uridine, adenosine, guanosine, thymidine and inosine. The term

“nucleotide” refers, in the usual and customary sense, to a single unit of a polynucleotide, i.e., a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof. Examples of polynucleotides contemplated herein include single and double stranded DNA, single and double stranded RNA, and hybrid molecules having mixtures of single and double stranded DNA and RNA. Examples of nucleic acid, e.g. polynucleotides, contemplated herein include any types of RNA, e.g. mRNA, siRNA, miRNA, and guide RNA and any types of DNA, genomic DNA, plasmid DNA, and minicircle DNA, and any fragments thereof. The term “duplex” in the context of polynucleotides refers, in the usual and customary sense, to double strandedness. Nucleic acids can be linear or branched. For example, nucleic acids can be a linear chain of nucleotides or the nucleic acids can be branched, e.g., such that the nucleic acids comprise one or more arms or branches of nucleotides. Optionally, the branched nucleic acids are repetitively branched to form higher ordered structures such as dendrimers and the like.

[0032] A polynucleotide is typically composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); and thymine (T) (uracil (U) for thymine (T) when the polynucleotide is RNA). Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule; alternatively, the term may be applied to the polynucleotide molecule itself. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. Polynucleotides may optionally include one or more non-standard nucleotide(s), nucleotide analog(s) and/or modified nucleotides.

[0033] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, “conservatively modified variants” refers to those nucleic acids that encode identical or essentially identical amino acid sequences. Because of the degeneracy of the genetic code, a number of nucleic acid sequences will encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid.

One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0034] An “antisense nucleic acid” as referred to herein is a nucleic acid (e.g., DNA or RNA molecule) that is complementary to at least a portion of a specific target nucleic acid and is capable of reducing transcription of the target nucleic acid (e.g. mRNA from DNA), reducing the translation of the target nucleic acid (e.g. mRNA), altering transcript splicing (e.g. single stranded morpholino oligo), or interfering with the endogenous activity of the target nucleic acid. See, e.g., Weintraub, *Scientific American*, 262:40 (1990). Typically, synthetic antisense nucleic acids (e.g. oligonucleotides) are generally between 15 and 25 bases in length. Thus, antisense nucleic acids are capable of hybridizing to (e.g. selectively hybridizing to) a target nucleic acid. In aspects, the antisense nucleic acid hybridizes to the target nucleic acid *in vitro*. In aspects, the antisense nucleic acid hybridizes to the target nucleic acid in a cell. In embodiments, the antisense nucleic acid hybridizes to the target nucleic acid in an organism. In aspects, the antisense nucleic acid hybridizes to the target nucleic acid under physiological conditions. Antisense nucleic acids may comprise naturally occurring nucleotides or modified nucleotides such as, e.g., phosphorothioate, methylphosphonate, and anomeric sugar-phosphate, backbone-modified nucleotides.

[0035] In the cell, the antisense nucleic acids hybridize to the corresponding RNA forming a double-stranded molecule. The antisense nucleic acids interfere with the endogenous behavior of the RNA and inhibit its function relative to the absence of the antisense nucleic acid. Furthermore, the double-stranded molecule may be degraded via the RNAi pathway. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, (1988)). Further, antisense molecules which bind directly to the DNA may be used. Antisense nucleic acids may be single or double stranded nucleic acids. Non-limiting examples of antisense nucleic acids include siRNAs (including their derivatives or pre-cursors, such as nucleotide analogs), short hairpin RNAs (shRNA), micro RNAs (miRNA), saRNAs (small activating RNAs) and small nucleolar RNAs (snoRNA) or certain of their derivatives or pre-cursors.

[0036] A “microRNA,” “microRNA nucleic acid sequence,” “miR,” “miRNA” as used herein, refers to a nucleic acid that functions in RNA silencing and post-transcriptional regulation of gene expression. The term includes all forms of a miRNA, such as the pri-, pre-, and mature forms of the miRNA. In embodiments, microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs. miRNAs are transcribed by RNA polymerase II as part of capped and polyadenylated primary transcripts (pri-miRNAs) that can be either protein-coding or non-coding. The primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA*) products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA. As a result, these mRNA molecules are silenced, by one or more of the following processes: (1) Cleavage of the mRNA strand into two pieces, (2) Destabilization of the mRNA through shortening of its poly(A) tail, and (3) Less efficient translation of the mRNA into proteins by ribosomes. miRNAs resemble the small interfering RNAs (siRNAs) of the RNA interference (RNAi) pathway, except miRNAs derive from regions of RNA transcripts that fold back on themselves to form short hairpins, whereas siRNAs derive from longer regions of double-stranded RNA. miRNAs are abundant in many mammalian cell types and as extracellular circulating miRNAs. Circulating miRNAs are released into body fluids including blood and cerebrospinal fluid and have the potential to be available as biomarkers in a number of diseases. MiRNAs appear to target about 60% of the genes of humans and other mammals.

[0037] The terms “messenger RNA” or “mRNA” refer a single-stranded molecule of RNA that corresponds to the genetic sequence of a gene, and is read by a ribosome in the process of synthesizing a protein.

[0038] Nucleic acids can include nonspecific sequences. As used herein, the term “nonspecific sequence” refers to a nucleic acid sequence that contains a series of residues that are not designed to be complementary to or are only partially complementary to any other nucleic acid sequence. By way of example, a nonspecific nucleic acid sequence is a sequence

of nucleic acid residues that does not function as an inhibitory nucleic acid when contacted with a cell or organism.

[0039] The term “complement,” as used herein, refers to a nucleotide (e.g., RNA or DNA) or a sequence of nucleotides capable of base pairing with a complementary nucleotide or sequence of nucleotides. As described herein and commonly known in the art the complementary (matching) nucleotide of adenosine is thymidine and the complementary (matching) nucleotide of guanosine is cytosine. Thus, a complement may include a sequence of nucleotides that base pair with corresponding complementary nucleotides of a second nucleic acid sequence. The nucleotides of a complement may partially or completely match the nucleotides of the second nucleic acid sequence. Where the nucleotides of the complement completely match each nucleotide of the second nucleic acid sequence, the complement forms base pairs with each nucleotide of the second nucleic acid sequence. Where the nucleotides of the complement partially match the nucleotides of the second nucleic acid sequence only some of the nucleotides of the complement form base pairs with nucleotides of the second nucleic acid sequence. Examples of complementary sequences include coding and a non-coding sequences, wherein the non-coding sequence contains complementary nucleotides to the coding sequence and thus forms the complement of the coding sequence. A further example of complementary sequences are sense and antisense sequences, wherein the sense sequence contains complementary nucleotides to the antisense sequence and thus forms the complement of the antisense sequence.

[0040] The term “gene” means the segment of DNA involved in producing a protein; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer as well as the introns include regulatory elements that are necessary during the transcription and the translation of a gene. Further, a “protein gene product” is a protein expressed from a particular gene.

[0041] The word “expression” or “expressed” as used herein in reference to a gene means the transcriptional and/or translational product of that gene. The level of expression of a DNA molecule in a cell may be determined on the basis of either the amount of corresponding RNA that is present within the cell or the amount of protein encoded by that DNA produced by the cell. The level of expression of non-coding nucleic acid molecules (e.g., miRNA,

mRNA) may be detected by standard PCR or Northern blot methods well known in the art. See, Sambrook et al., 1989 Molecular Cloning: A Laboratory Manual, 18.1-18.88.

[0042] The terms an “elevated expression level” or “elevated level” of gene expression is an expression level of the gene that is higher than the expression level of the gene in a control. The control may be any suitable control, as described herein.

[0043] The terms “does not have an elevated expression level” or an expression level that is “not elevated” is an expression level of the gene that is about the same as (or lower than) the expression level of the gene in a control. The control may be any suitable control, as described herein.

[0044] “Control” is used in accordance with its plain ordinary meaning and refers to an assay, comparison, or experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In embodiments, the control is used as a standard of comparison in evaluating experimental effects. In embodiments, a control is the measurement of the activity or level of RNA. In embodiments, a control is a level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a healthy patient or a healthy population of patients. In embodiments, a control is a level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a patient having a cancer who does not have an occult micrometastatic disease or a population of patients having a cancer who do not have an occult micrometastatic disease. For example, a test sample can be taken from a patient suspected of having occult pancreatic metastases and compared to samples from a patient having pancreatic cancer and no occult micrometastatic disease, or a known normal (non-disease) individual. A control can also represent an average level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, gathered from a population of similar individuals, e.g., cancer patients or healthy individuals with a similar medical background, age, weight, etc. A control can also be obtained from the same individual, e.g., from an earlier-obtained sample, prior to disease, or prior to treatment. One of skill will recognize that controls can be designed for assessment of any number of parameters. In embodiments, a control is a negative control. In embodiments, such as some embodiments

relating to detecting the level of expression of a gene/protein or a subset of genes/proteins, a control comprises the average amount of expression (e.g., protein or mRNA) in a population of subjects (e.g., with cancer) or in a healthy or general population. In embodiments, the control comprises an average amount (e.g. amount of expression) in a population in which the number of subjects (n) is 5 or more, 20 or more, 50 or more, 100 or more, 1,000 or more, and the like. In embodiments, the control is a standard control. In embodiments, the control is a level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a population of pancreatic cancer subjects that do not have occult micrometastatic disease. One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0045] The term “recombinant” when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all. Transgenic cells and plants are those that express a heterologous gene or coding sequence, typically as a result of recombinant methods.

[0046] The term “heterologous” when used with reference to portions of a nucleic acid indicates that the nucleic acid including two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein including two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0047] The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous

population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only a subset of antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, *Using Antibodies, A Laboratory Manual* (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[0048] The terms “isolate” or “isolated”, when applied to a nucleic acid, virus, or protein, denotes that the nucleic acid, virus, or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. An RNA that is the predominant species present in a preparation is substantially purified.

[0049] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. The

terms “non-naturally occurring amino acid” and “unnatural amino acid” refer to amino acid analogs, synthetic amino acids, and amino acid mimetics which are not found in nature.

[0050] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0051] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0052] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the disclosure.

[0053] The following eight groups each contain amino acids that are conservative substitutions for one another: (1) Alanine (A), Glycine (G); (2) Aspartic acid (D), Glutamic acid (E); (3) Asparagine (N), Glutamine (Q); (4) Arginine (R), Lysine (K); (5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); (6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); (7) Serine (S), Threonine (T); and (8) Cysteine (C), Methionine (M) (*see, e.g.,* Creighton, *Proteins* (1984)).

[0054] “Percentage of sequence identity” is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.,* gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the

number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0055] [0001] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (e.g., www.ncbi.nlm.nih.gov/BLAST/ or the like). Such sequences are then considered to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0056] An amino acid or nucleotide base “position” is denoted by a number that sequentially identifies each amino acid (or nucleotide base) in the reference sequence based on its position relative to the N-terminus (or 5'-end). Due to deletions, insertions, truncations, fusions, and the like that must be taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence determined by simply counting from the N-terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where a variant has a deletion relative to an aligned reference sequence, there will be no amino acid in the variant that corresponds to a position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference sequence, that insertion will not correspond to a numbered amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

[0057] The terms “numbered with reference to” or “corresponding to,” when used in the context of the numbering of a given amino acid or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence.

[0058] As used herein, the term “about” means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, “about” means within a standard deviation using measurements generally acceptable in the art. In embodiments, “about” means a range extending to +/- 10% of the specified value. In embodiments, “about” includes the specified value.

[0059] A “detectable agent” or “detectable moiety” is a compound or composition detectable by appropriate means such as spectroscopic, photochemical, biochemical, immunochemical, chemical, magnetic resonance imaging, or other physical means. The RNA described herein and the expression level of the RNA described herein may be accomplished through the use of a detectable moiety in an assay or kit. A detectable moiety is a monovalent detectable agent or a detectable agent bound (e.g. covalently and directly or via a linking group) with another compound, e.g., a nucleic acid. Exemplary detectable agents/moieties for use in the present disclosure include an antibody ligand, a peptide, a nucleic acid, radioisotopes, paramagnetic metal ions, fluorophore (e.g. fluorescent dyes), electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, a biotin-avidin complex, a biotin-streptavidin complex, digoxigenin, magnetic beads (e.g., DYNABEADS® by ThermoFisher, encompassing functionalized magnetic beads such as DYNABEADS® M-270 amine by ThermoFisher), paramagnetic molecules, paramagnetic nanoparticles, ultrasmall superparamagnetic iron oxide nanoparticles, ultrasmall superparamagnetic iron oxide nanoparticle aggregates, superparamagnetic iron oxide nanoparticles, superparamagnetic iron oxide nanoparticle aggregates, monocrySTALLINE iron oxide nanoparticles, monocrySTALLINE iron oxide, nanoparticle contrast agents, liposomes or other delivery vehicles containing Gadolinium chelate molecules, gadolinium, radionuclides (e.g. carbon-11, nitrogen-13, oxygen-15, fluorine-18, rubidium-82), fluorodeoxyglucose (e.g. fluorine-18 labeled), any gamma ray emitting radionuclides, positron-emitting radionuclide, radiolabeled glucose, radiolabeled water, radiolabeled ammonia, biocolloids, microbubbles (e.g. including microbubble shells including albumin, galactose, lipid, and/or polymers; microbubble gas

core including air, heavy gas(es), perfluorocarbon, nitrogen, octafluoropropane, perflerane lipid microsphere, perflutren, etc.), iodinated contrast agents (e.g. iohexol, iodixanol, ioversol, iopamidol, ioxilan, iopromide, diatrizoate, metrizoate, ioxaglate), barium sulfate, thorium dioxide, gold, gold nanoparticles, gold nanoparticle aggregates, fluorophores, two-photon fluorophores, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide.

[0060] “Biological sample” or “sample” refer to materials obtained from or derived from a subject or patient. A biological sample includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histological purposes. Such samples include bodily fluids such as blood and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like), sputum, tissue, cultured cells (e.g., primary cultures, explants, and transformed cells) stool, urine, synovial fluid, joint tissue, synovial tissue, synoviocytes, fibroblast-like synoviocytes, macrophage-like synoviocytes, immune cells, hematopoietic cells, fibroblasts, macrophages, T cells, etc. In embodiments, a biological sample is blood. In embodiments, a biological sample is a serum sample (e.g., the fluid and solute component of blood without the clotting factors). In embodiments, a biological sample is a plasma sample (e.g., the liquid portion of blood). In embodiments, a biological sample is cell-free RNA obtained from blood. In embodiments, a biological sample is an exosome obtained from a blood sample, wherein the exosome comprises RNA. In embodiments, a biological sample is an exosome obtained from a serum sample, wherein the exosome comprises RNA. In embodiments, a biological sample is an exosome obtained from a plasma sample, wherein the exosome comprises RNA.

[0061] “Liquid biological sample” refers to liquid materials obtained or derived from a subject or patient. Liquid biological samples include bodily fluids such as blood and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like), sputum, urine, synovial fluid, and the like. In embodiments, a liquid biological sample is a blood sample.

[0062] The term “diagnosis” is used in accordance with its plain and ordinary meaning and refers to an identification or likelihood of the presence of a disease (e.g., colorectal cancer with or without lymph node metastases) or outcome in a subject.

[0063] The terms “treating” or “treatment” are used in accordance with their plain and ordinary meaning and broadly includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (i.e., not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, “treatment” as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease’s spread; relieve the disease’s symptoms, fully or partially remove the disease’s underlying cause, shorten a disease’s duration, or do a combination of these things.

[0064] The terms “treating” and “treatment” may include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the risk or condition, the age of the patient, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In embodiments, chronic administration is required. For example, the therapeutic agents are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0065] The term “prevent” is used in accordance with its plain and ordinary meaning and refers to a decrease in the occurrence of disease symptoms in a patient. The prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment.

[0066] An “effective amount” is an amount sufficient to accomplish a stated purpose (e.g. achieve the effect for which it is administered, treat a disease). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a “therapeutically

effective amount.” A “reduction” of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A “prophylactically effective amount” of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of a disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of a disease, pathology, or condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques.

[0067] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0068] As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0069] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present disclosure, should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound

effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0070] As used herein, the term “administering” means oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc. In embodiments, the administering does not include administration of any active agent other than the recited active agent.

[0071] The terms “patient” or “subject” are used in accordance with its plain and ordinary meaning and refer to a living organism suffering from or prone to a disease that can be treated by administration of a pharmaceutical composition, such as anti-cancer agents and chemotherapeutic agents. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, cats, monkeys, and other non-mammalian animals. In embodiments, a patient is human. In embodiments, the patient is a human with colorectal cancer. In embodiments, the subject is a human with invasive submucosal colorectal cancer.

[0072] The terms “marker,” “RNA marker,” and “biomarker” are used interchangeably throughout the disclosure, and are used in accordance with their plain and ordinary meaning. A marker refers generally to RNA (e.g., miRNA or mRNA), the level or concentration of which is associated with a particular biological state, particularly a state associated with occult micrometastatic disease. Panels, assays, kits and methods described herein may comprise antibodies, binding fragments thereof or other types of target-binding agents, which are specific for the RNA markers described herein (e.g., miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f).

[0073] The terms “metastasis,” “metastatic,” and “metastatic cancer” can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating

site, e.g., pancreas, which site is referred to as a primary tumor, e.g., primary pancreatic cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if pancreatic cancer metastasizes to the lymph nodes, the secondary tumor at the site of the lymph nodes consist of pancreatic cells and not abnormal lymph node cells. The secondary tumor in the lymph nodes is referred to as lymph node metastasis. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors.

[0074] The term “antibody” is used in the broadest sense and includes fully assembled antibodies, tetrameric antibodies, monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), antibody fragments that can bind an antigen (e.g., Fab’, F’(ab)₂, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the forgoing as long as they exhibit the desired biological activity. An “immunoglobulin” or “tetrameric antibody” is a tetrameric glycoprotein that consists of two heavy chains and two light chains, each comprising a variable region and a constant region. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antibody fragments or antigen-binding portions include, inter alia, Fab, Fab’, F(ab’)₂, Fv, domain antibody (dAb), complementarity determining region (CDR) fragments, CDR-grafted antibodies, single-chain antibodies (scFv), single chain antibody fragments, chimeric antibodies, diabodies, triabodies, tetrabodies, minibody, linear antibody; chelating recombinant antibody, a tribody or bibody, an intrabody, a nanobody, a small modular immunopharmaceutical (SMIP), an antigen-binding-domain immunoglobulin fusion protein, a camelized antibody, a VHH containing antibody, or a variant or a derivative thereof, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide, such as one, two, three, four, five or six CDR sequences, as long as the antibody retains the desired biological activity.

[0075] Monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

[0076] “Antibody variant” as used herein refers to an antibody polypeptide sequence that contains at least one amino acid substitution, deletion, or insertion in the variable region of the reference antibody variable region domains. Variants may be substantially homologous or substantially identical to the unmodified antibody.

[0077] The terms “biological fluids”, “body fluids”, “bodily fluids” or “biofluids” refer to liquids within the human body. Such liquids can be blood, serum, plasma, saliva, ascites fluid, peritoneal fluid, and urine. In embodiments, the fluid is blood. In embodiment, the fluid is serum. In embodiments, the fluid is plasma. In embodiments, the fluid is saliva. In embodiments, the fluid is ascites fluid. In embodiments, the fluid is peritoneal fluid. In embodiments, the fluid is urine.

[0078] The term “confirmatory diagnostic procedure” as used herein refers to medical tests or procedures used to confirm a medical diagnosis. A confirmatory diagnostic procedure can be, *e.g.* an angiography, an alfa-fetoprotein (AFP) protein blood test, a tumor marker test, a microsatellite instability (MSI) test, a colonoscopy, an esophagus-gastric-duodenoscopy (EGD), an abdominal ultrasound, an endoscopic ultrasound, a bronchoscopy, a tissue biopsy, a fine needle aspiration, an esophagogastroduodenoscopy (EGD), a tissue biopsy, a CA19-9 antigen test, a fine needle aspiration, an endoscopy, biopsy collection, a blood test, a fecal test, a fecal occult blood test, a magnetic resonance imaging scan (MRI scan) (*e.g.* a cholangiopancreatography), a computed tomography scan (CT scan), a positron emission tomography scan (PET scan), or a carcinoembryonic antigen (CEA) test.

[0079] The terms “computed tomography scan” or “CT scan” refer to a medical imaging technique that uses computer-processed combinations of multiple X-ray measurements taken from different angles to produce tomographic (cross-sectional) images (virtual “slices”) of a body, allowing the user to see inside the body without cutting. CT imaging is an extremely valuable tool for the evaluation of pancreatic lesions. The CT can provide the precise location of the lesion, size, and, most importantly, any involvement of any surrounding structures. CT imaging can really help determine the resectability of the tumor. A pancreatic CT protocol

involves triphasic cross-sectional imaging with arterial, late arterial, and venous phases. This type of imaging allows identification of the majority of pancreatic cancers in the delayed arterial phase/venous phase, as many are hypovascular in nature. The arterial phase can identify many other pancreatic lesions, as well. CT imaging has a reported sensitivity ranging from 76-92% for diagnosing pancreatic cancer and surgical resectability up to 90%.

[0080] The term “X-ray” or “X-radiation” refers to a penetrating form of high-energy electromagnetic radiation. Most X-rays have a wavelength ranging from 10 picometers to 10 nanometers, corresponding to frequencies in the range 30 petahertz to 30 exahertz (30×10^{15} Hz to 30×10^{18} Hz) and energies in the range 124 eV to 124 keV. X-ray wavelengths are shorter than those of UV rays and typically longer than those of gamma rays.

[0081] The terms “PET”, “PET scan”, “positron emission tomography”, or “positron emission tomography scan” refer to a functional imaging technique that uses radioactive substances known as radiotracers to visualize and measure changes in metabolic processes, and in other physiological activities including blood flow, regional chemical composition, and absorption. Different tracers are used for various imaging purposes, depending on the target process within the body. PET scan is a common imaging technique, a medical scintillography technique used in nuclear medicine. A radiopharmaceutical – a radioisotope attached to a drug is injected into the body as a tracer. Gamma rays are emitted and detected by gamma cameras to form a three-dimensional image, in a similar way that an X-ray image is captured. PET scans have conflicting evidence for the regular use in diagnosing and staging of pancreatic disease. The sensitivity depends on the lesion’s size, so it is susceptible to miss small areas of disease. Elevated serum glucose levels can cause false negatives and cause missed metastatic disease. When CT and PET scans are combined, this can increase the overall accuracy while increasing the sensitivity and specificity, especially for lesions that were missed on CT imaging. Overall, combined CT and PET scans are more useful in finding and confirming occult disease post-resection with CA 19-9 increases.

[0082] The term “MRI” or “magnetic resonance imaging” refer to a medical imaging technique used in radiology to form pictures of the anatomy and the physiological processes of the body. MRI scanners use strong magnetic fields, magnetic field gradients, and radio waves to generate images of the organs in the body. MRI does not involve X-rays or the use of ionizing radiation, which distinguishes it from CT and PET scans. MRI is a medical application of nuclear magnetic resonance (NMR) which can also be used for imaging in

other NMR applications, such as NMR spectroscopy. MRI also has a role in diagnosing pancreatic lesions. MRI is an excellent modality for determining pancreaticobiliary anatomy and pathology. MRCP can identify pancreatic duct abnormalities, such as strictures. It is better overall at identifying the biliary and pancreatic ductal systems. MRI is also used for diagnosing and following lesions post-operatively as well.

[0083] The term “endoscopic ultrasound” refers to a minimally invasive procedure that uses a camera device (endoscope) together with high frequency sound waves (ultrasound) to examine the GI tract and beyond. A regular endoscope is a thin, lighted tube that can be inserted through the mouth or anus to view the inside of the esophagus, stomach or intestines. Endoscopic ultrasound can be used in the preoperative and operative settings. The ultrasound can be used to look for vascular invasion and resectability of the mass. Endoscopic is best used to obtain a tissue biopsy. Endoscopic ultrasound is very useful in small pancreatic lesions such as cysts. Another useful advantage for ultrasound is the use of helping diagnose nodal involvement and metastatic diseases, such as with large lymph nodes in the celiac or mediastinal positions.

[0084] The term “disease” or “condition” refers to a state of being or health status of a patient or subject that is being treated with the compounds or methods provided herein. The disease may be a cancer. The disease may be an autoimmune disease. The disease may be an inflammatory disease. The disease may be an infectious disease. In some further instances, “cancer” refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin’s lymphomas (e.g., Burkitt’s, Small Cell, and Large Cell lymphomas), Hodgkin’s lymphoma, leukemia (including AML, ALL, and CML), or multiple myeloma.

[0085] As used herein, the term “cancer” is used in accordance with its plain ordinary meaning and refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemias, lymphomas, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, Medulloblastoma, melanoma, cervical cancer, gastric cancer, ovarian cancer, lung cancer,

cancer of the head, Hodgkin's Disease, and Non-Hodgkin's Lymphomas. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, ovary, pancreas, rectum, stomach, and uterus. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, non-small cell lung carcinoma, mesothelioma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0086] The term “carcinoma” refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound or method provided herein include, for example, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriiform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epierrmoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniformi carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ,

intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberos carcinoma, verrucous carcinoma, or carcinoma villosum.

[0087] The term “pancreatic ductal adenocarcinoma” or “PDAC” refers to a malignant epithelial neoplasm with pancreatic ductal differentiation and mucin production. PDAC is the most common pancreatic malignancy, accounting for more than 85% of pancreatic tumors. It is typically a disease of elderly patients, with a mean age at presentation of 68 years and a male-to-female ratio of 1.6:1. Abdominal pain is the most frequently reported clinical symptom, even when the tumor is small (<2 cm). Other symptoms include weight loss, pruritus, and jaundice, which are typically present when the tumor invades adjacent tissue or seeds distant organ metastases. A hypercoagulable state frequently accompanies PDAC, leading to a high incidence of both venous and arterial thromboembolism. Approximately 60%–70% of PDACs are located in the head of the pancreas, with the remaining in the body and tail. PDAC is generally a solitary lesion. At gross pathologic examination, PDACs are firm multinodular and sclerotic tumors with indistinct margins and a whitish cut surface. The pathogenesis of PDAC follows a series of stepwise mutations from normal pancreatic tissue that first forms a precursor lesion and eventually mutates to an invasive malignancy. The most common neoplastic precursor lesions of PDAC are pancreatic intraepithelial neoplasms, which are microscopic tumors (<5 mm) that are not directly visible at pancreatic imaging. Less frequently, PDAC can evolve from intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms. At histopathologic evaluation, most tumors are well differentiated to moderately differentiated with infiltrating glandular and ductlike structures.

Typically, a desmoplastic stromal reaction is associated with these tumors and corresponds to the firm consistency, haphazard growth pattern, and obstructive features of the tumor.

[0088] The term “metastasis” or the plural form “metastases” refers to the development of secondary malignant growths at a distance from a primary site of cancer. The condition refers to when cancer cells break away from the main tumor and enter the bloodstream or lymphatic system. The terms “metastasis,” “metastatic,” and “metastatic cancer” can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating site, e.g., colon, which site is referred to as a primary tumor, e.g., primary colon cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if colorectal cancer metastasizes to the lymph nodes, the secondary tumor at the site of the lymph nodes consist of colorectal cells and not abnormal lymph node cells. The secondary tumor in the lymph nodes is referred to as lymph node metastasis. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors.

[0089] The term “diagnosis” refers to the identification of the nature and cause of a certain phenomenon. Diagnosis is used in many different disciplines, with variations in the use of logic, analytics, and experience, to determine “cause and effect”. In medicine, a “diagnosis”, a “diagnostic procedure” or “medical diagnosis” is the process of determining which disease or condition explains a person’s symptoms and signs. It is most often referred to as diagnosis with the medical context being implicit. The information required for diagnosis is typically collected from a history and physical examination of the person seeking medical care. Often, one or more diagnostic procedures, such as medical tests, are also done during the process. Sometimes posthumous diagnosis is considered a kind of medical diagnosis. The term “to diagnose” refers to the act of realizing a diagnosis. The terms “confirmatory diagnostic

procedure” or “confirmatory diagnosis procedure” refer to a process of confirming a diagnosis.

[0090] The terms “fine-needle aspiration” refer to diagnostic procedure used to investigate lumps or masses. In this procedure a thin, hollow needle and a syringe are used to extract cells, fluid or tissue from a suspicious lump or other abnormal area of the body. The material is then examined under a microscope or tested in the laboratory to determine the cause of the abnormality. The sampling and biopsy considered together are called fine-needle aspiration biopsy or fine-needle aspiration cytology (the latter to emphasize that any aspiration biopsy involves cytopathology, not histopathology).

[0091] The term “biopsy” refers to a medical test which involves extraction of sample cells or tissues for examination to determine the presence or extent of a disease in a subject. The extracted tissue is generally examined under a microscope by a pathologist, and it may also be analyzed chemically. When an entire lump or suspicious area is removed, the procedure is called an excisional biopsy. An incisional biopsy or core biopsy samples a portion of the abnormal tissue without attempting to remove the entire lesion or tumor. When a sample of tissue or fluid is removed with a needle in such a way that cells are removed without preserving the histological architecture of the tissue cells, the procedure is called a needle aspiration biopsy. The terms “biopsy material” refer to the sample extracted from the subject. The terms “tissue biopsy” refer to the extraction of tissue from a subject.

[0092] The terms “DNA test” or “genetic test” refer to test of DNA material obtained from a subject or sample, which is used to identify changes in DNA sequence or chromosome structure. Genetic testing can also include measuring the results of genetic changes, such as RNA analysis as an output of gene expression, or through biochemical analysis to measure specific protein output. In a medical setting, genetic testing can be used to diagnose or rule out suspected genetic disorders, predict risks for specific conditions, or gain information that can be used to customize medical treatments based on an individual’s genetic makeup. Genetic testing can also be used to determine biological relatives, such as a child’s parentage (genetic mother and father) through DNA paternity testing, or be used to broadly predict an individual’s ancestry.

[0093] The terms “blood test” refer to a laboratory analysis performed on a blood sample. Blood tests are often used in health care to determine physiological and biochemical states,

such as disease, mineral content, pharmaceutical drug effectiveness, and organ function. Blood tests can involve different tests on the blood sample, such as biochemical analyses, molecular profiling, and cellular evaluation.

[0094] The term “distant metastasis” or “distant metastasis tumor” refers to a cancer that has spread from the original (primary) tumor to distant organs or distant lymph nodes.

[0095] As used herein, the term “neoadjuvant therapy” is used in accordance with its plain ordinary meaning and refers to a treatment given as a first step to shrink a tumor before the main treatment, which is usually surgery, is given. In embodiments, the main treatment is surgery to remove the tumor. In embodiments, the main treatment is surgery to remove the primary tumor in a patient that has occult micrometastatic disease. Neoadjuvant therapy aims to increase the likelihood of success of the main treatment and reduce the consequences of more extensive treatments, which would be required if the tumor were not reduced in size or extent. Examples of the first step of a neoadjuvant therapy include chemotherapy, radiation therapy, hormone therapy, and targeted therapy. Neoadjuvant therapy may also be used to reduce the number or volumes of micrometastatic tumors. The downstaging is then a surrogate marker of efficacy on undetected dissemination, resulting in improved longtime survival compared to the surgery-alone strategy. Neoadjuvant therapy is commonly used in advanced cancers, such as pancreatic cancer. The use of such therapy can effectively reduce the difficulty and morbidity of more extensive procedures. In embodiments, neoadjuvant therapy is administered to a subject with a cancer or who is suspected of having cancer before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, neoadjuvant therapy is administered to a subject with a cancer who has or is suspected of having an occult micrometastatic disease before the subject undergoes surgery to remove all or portions of the cancer and micrometastatic tumors. In embodiments, a subject with a cancer or who is suspected of having cancer completes one or more cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes one cycle of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes two cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes three cycles of neoadjuvant therapy before the subject undergoes

surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes four cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes five cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes six cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes seven cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with a cancer or who is suspected of having cancer completes eight or more cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the cancer. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes one or more cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease a cancer or who is suspected of having an occult metastatic disease completes one cycle of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes two cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes three cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes four cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes five cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes six cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes seven cycles of neoadjuvant therapy before the subject undergoes surgery

to remove all or a portion of the micrometastatic tumors. In embodiments, a subject with an occult metastatic disease or who is suspected of having an occult metastatic disease completes eight or more cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or a portion of the micrometastatic tumors. In embodiments, when a biological sample obtained from a subject who is suspected to have a cancer and/or a micrometastatic disease has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f, and a combination of two or more thereof, the subject completes one or more cycles of neoadjuvant therapy before the subject undergoes surgery to remove all or portions of the cancer or micrometastatic tumors. In embodiments, the subject never undergoes surgery to remove all or portions of the cancer or micrometastatic tumors before completing one or more rounds of neoadjuvant therapy, if a biological sample obtained from the subject who is suspected to have a cancer and/or a micrometastatic disease has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f, and a combination of two or more thereof.

[0096] The term “radiation therapy” or “radiotherapy” refers to a therapy that uses ionizing radiation, and is generally provided as part of cancer treatment to control or kill malignant cells. Radiation therapy is normally delivered by a linear accelerator. Radiation therapy may be curative in a number of types of cancer if they are localized to one area of the body. It may also be used as part of adjuvant therapy, to prevent tumor recurrence after surgery that removes a primary malignant tumor. Radiation therapy is synergistic with chemotherapy, and has been used before, during, and after chemotherapy in susceptible cancers.

[0097] The term “chemotherapy” refers to a type of cancer treatment that uses one or more anti-cancer drugs (chemotherapeutic agents) as part of a standardized chemotherapy regimen. Chemotherapy may be given with a curative intent (which almost always involves combinations of drugs), or it may aim to prolong life or to reduce symptoms (palliative chemotherapy). Chemotherapy drugs include, but are not limited to, alkylating agents, nitrosoureas, antimetabolites, alkaloids, antitumor antibiotics, hormonal agents and biological response modifiers. Examples of chemotherapy drugs include, but are not limited to, cyclophosphamide, melphalan, temozolomide, carboplatin, cisplatin, oxaliplatin, 5-fluorouracil, 6-mercaptopurine, cytarabine, gemcitabine, methotrexate, actimycin-D,

blemycin, daunorubicin, doxorubicin, docetaxel, estramustine, paclitaxel, vinblastine, etoposide, irinotecan, teniposide, topotecan, prednisone, methylprednisolone and dexamethasone. Traditional chemotherapeutic agents are cytotoxic by means of interfering with cell division (mitosis) but cancer cells vary widely in their susceptibility to these agents. Many of the side effects of chemotherapy can be traced to damage to normal cells that divide rapidly and are thus sensitive to anti-mitotic drugs such as, but not limited to, cells in the bone marrow, digestive tract and hair follicles. In embodiments, the chemotherapy includes administration of an effective amount of an anticancer agent as set forth herein.

[0098] The term “targeted therapy” refers to a method for treating cancer that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with all rapidly dividing cells. Exemplary forms of targeted therapy include, but are not limited to, antibody-drug conjugates, nano-engineered enzymes that bind to a tumor cell, and chemical entities that target or preferentially target a protein or enzyme that carries a mutation or other genetic alteration that is specific to cancer cells and is not found in normal host tissue. Exemplary drugs that are used in targeted therapy include, but are not limited to, trastuzumab and bevacizumab. In embodiments, the targeted therapy includes administration of an effective amount of an anticancer agent as set forth herein.

[0099] The term “immunotherapy” refers to methods of treating cancer that are based on the stimulation of the patient’s immune system. Cancer immunotherapy exploits the fact that cancer cells often have tumor antigens that can be detected and bound by the antibodies of the immune system. Clinical success of cancer immunotherapy is highly variable between different forms of cancer. Examples of immunotherapy include, but are not limited to, therapeutic cancer vaccines, CAR-T cell, and targeted antibody therapies. Examples of drugs used in immunotherapy include, but are not limited to, ipilimumab, pembrolizumab, nivolumab and atezolizumab. In embodiments, the immunotherapy includes administration of an effective amount of an anticancer agent as set forth herein.

[0100] The term “hormonal therapy” refers to a type of a cancer treatment that slows or stops the growth of cancer that uses hormones to grow. Hormonal therapy may be used alone as the main treatment or with other treatments. It may be used before surgery or radiation therapy to shrink the tumor. Hormonal therapy may be given in addition to main treatments such as surgery, radiation therapy or chemotherapy to lower the risk of cancer recurrence.

Hormonal therapy includes, but is not limited to, removing the gland or organ that makes the hormone, irradiating the gland or organ to destroy hormone-producing cells, and administration of drugs that suppress hormonal production. In embodiments, the hormonal therapy includes administration of an effective amount of an anticancer agent as set forth herein.

[0101] As used herein, the term “synthetic lethality” or “synthetic lethality therapy” is used in accordance with its plain ordinary meaning and refers to a combination of deficiencies in the expression of two or more genes that lead to cell death, whereas a deficiency in only one of these genes does not. The deficiencies can arise through mutations, epigenetic alterations or inhibitors of one of the genes. “Synthetic lethality therapy” has utility for purposes of molecular targeted cancer therapy, with the first example of a molecular targeted therapeutic exploiting a synthetic lethal exposed by an inactivated tumor suppressor gene (BRCA1 and 2) receiving FDA approval in 2016 (PARP inhibitor).[1] A sub-case of synthetic lethality, where vulnerabilities are exposed by the deletion of passenger genes rather than tumor suppressor is the so-called collateral lethality.

[0102] The term “angiogenesis inhibitor administration therapy” refers to methods of treating cancer that block the growth of blood vessels that support tumor growth rather than blocking the growth of tumor cells themselves. Exemplary angiogenesis inhibitors include, but are not limited to, monoclonal antibodies that specifically recognize and bind to vascular endothelial growth factor (VEGF) and thus block activation of the VEGF receptor, and immunomodulatory drugs that stimulate or suppress the immune system. For some cancers, angiogenesis inhibitors are most effective when combined with additional therapies. The term “synthetic lethality therapy” refers to a type of cancer treatment in which the simultaneous mutation of two genes leads to cell death, whereas mutation of only one of the genes is not lethal. For most cancer mutations caused by a loss-of-function, there are no targeted therapies available, and synthetic lethal therapy provides additional opportunities. It requires identification of inactive genes in a cancer and the targeting of their synthetic lethal partner genes. Examples of targeted therapies exploiting the synthetic lethality (SL) principle include, but are not limited to, the use of poly-ADP ribose polymerase (PARP) inhibitors in breast and ovarian cancer that harbor mutations in the breast cancer gene (BRCA). Treatment of BRCA-deficient tumors with PARP inhibitors generally selectively kills the cancer cells in

breast and ovarian cancer. In embodiments, the angiogenesis inhibitor administration therapy includes administration of an effective amount of an anticancer agent as set forth herein.

[0103] “Anti-cancer agent” and “anticancer agent” are used in accordance with their plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechlorethamine, cyclophosphamide, chlorambucil, melphalan), ethylenimine and methylmelamines (e.g., hexamethylmelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomustine, semustine, streptozocin), triazines (decarbazine)), anti-metabolites (e.g., 5- azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), etc.), plant alkaloids (e.g., vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, etc.), topoisomerase inhibitors (e.g., irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, etc.), antitumor antibiotics (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, etc.), platinum-based compounds (e.g. cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossypol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin,

tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatan; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrididemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid;

idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod;
 immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon
 agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact;
 irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F;
 lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin;
 letrozole; leukemia inhibiting factor; leukocyte alpha interferon;
 leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine
 analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7;
 lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine;
 lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A;
 marimastat; masoprocil; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors;
 menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor;
 mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone;
 mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin;
 mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic
 gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple
 drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer
 agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-
 substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin;
 nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide;
 nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine;
 octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral
 cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine;
 palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine;
 pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron;
 perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors;
 picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B;
 plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine
 complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2;
 proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor;
 protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine
 nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin
 polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein
 transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium

Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; tricinibine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; broprimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitucin; enloplatin; enpromate; epiropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate;

fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin II (including recombinant interleukin II, or rIL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedapa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; tretolone acetate; tricyriline phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinyglicinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e.

Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, Inanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tularik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyeleutherobin, Isoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (e.g., dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethylstilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (e.g., Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-

interferon, etc.), monoclonal antibodies (e.g., anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (e.g., anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, etc.), immunotherapy (e.g., cellular immunotherapy, antibody therapy, cytokine therapy, combination immunotherapy, etc.), radioimmunotherapy (e.g., anti-CD20 monoclonal antibody conjugated to ^{111}In , ^{90}Y , or ^{131}I , etc.), immune checkpoint inhibitors (e.g., CTLA4 blockade, PD-1 inhibitors, PD-L1 inhibitors, etc.), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (IressaTM), erlotinib (TarcevaTM), cetuximab (ErbixTM), lapatinib (TykerbTM), panitumumab (VectibixTM), vandetanib (CaprelsaTM), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0104] “Remission” means that the clinical signs and symptoms of cancer have been significantly diminished or have disappeared entirely based on clinical diagnostics, although cancerous cells can still exist in the body. Thus, it is contemplated that remission encompasses partial and complete remission. Remission can occur for any period of time, such as from one month to several years or more.

[0105] “Relapse” or “Recurrence” refers to the clinical diagnosis of a return of cancer after a period of remission.

[0106] “Relapse-free survival” or “Recurrence-free survival” or “RFS” refers to the length of time from the date of primary treatment for a cancer ends that the patient survives without any signs or symptoms of that cancer until the date of relapse. In clinical trials, measuring the relapse-free survival is a way to determine the efficacy of a new treatment.

[0107] “Good prognosis” refers to a normal risk of relapse, a reduced risk of relapse, an increased chance for remission, an increased relapse-free survival time, or a high survival rate. In embodiments, a “good prognosis” refers to a reduced risk of relapse, an increased

chance for remission, an increased relapse-free survival time, or a high survival rate. In embodiments, a “good prognosis” refers to a reduced risk of relapse. In embodiments, a “good prognosis” refers to an increased chance for remission. In embodiments, a “good prognosis” refers to an increased relapse-free survival time. In embodiments, a “good prognosis” refers to a high survival rate. In embodiments, a high survival rate refers to a 5-year survival rate greater than 50%. In embodiments, a high survival rate refers to a 5-year survival rate greater than 60%, greater than 70%, greater than 80%, or greater than 90%. In embodiments, “good prognosis” is an increased likelihood of a good prognosis.

[0108] The terms an “elevated level” or an “increased level” or a “high level” of gene expression is an expression level of the gene or protein that is higher than the expression level of the gene or protein in a standard control or in a control with no or very low risk of recurrence (e.g. a control biological sample derived from a subject or subjects with no or low risk of recurrence). The standard control may be any suitable control, examples of which are described herein. The control with no risk of recurrence may be a patient or subject who has undergone pancreatectomy for treatment of pancreatic cancer and is at no risk or very low risk of developing cancer recurrence within the first 5 years after surgery, examples of which are described herein.

[0109] The terms a “reduced level” or a “decreased expression level” or a “low level” of gene expression is an expression level of the gene or protein that is lower than the expression level of the gene or protein in a standard control or in a control with no risk of recurrence. The standard control may be any suitable control, examples of which are described herein. The control with no risk of recurrence is a patient or subject who has undergone pancreatectomy for treatment of pancreatic cancer and is at no risk or very low risk of developing cancer recurrence within the first 5 years after surgery, examples of which are described herein.

[0110] “Pathway” refers to a set of system components involved in two or more sequential molecular interactions that result in the production of a product or activity. A pathway can produce a variety of products or activities that can include, for example, intermolecular interactions, changes in expression of a nucleic acid or polypeptide, the formation or dissociation of a complex between two or more molecules, accumulation or destruction of a metabolic product, activation or deactivation of an enzyme or binding activity. Thus, the term “pathway” includes a variety of pathway types, such as, for example, a biochemical pathway,

a gene expression pathway, and a regulatory pathway. Similarly, a pathway can include a combination of these exemplary pathway types.

[0111] As used herein, the term “occult micrometastatic disease” refers to a small collection of cancer cells (e.g. a small tumor or tumors) derived from a primary tumor that are initially not detected by an imaging test (e.g. cross-sectional imaging) that have spread to a different part of the body than the primary tumor. In embodiments, the identity of the primary tumor is not known. In embodiments, the occult micrometastatic disease is an occult lymph node micrometastatic disease which is an occult micrometastatic disease in which the small collection of cancer cells (e.g. small tumor) is found in the lymph nodes. In embodiments, the small tumor formed from the collection of cancer cells has a longest dimension (e.g. diameter) of is less than 2 mm. In embodiments, the small tumor formed from the collection of cancer cells has a longest dimension (e.g. diameter) of is less than 1 mm. In embodiments, the small tumor formed from the collection of cancer cells has a longest dimension (e.g. diameter) of is less than 0.5 mm. In embodiments, the small tumor formed from the collection of cancer cells has a longest dimension (e.g. diameter) of is less than 0.1 mm.

[0112] As used herein, the term miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f is used in accordance with its plain ordinary meaning and refers to list of mRNA targets.

[0113] As used herein, the term “miR-130b-5p” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 1. Diseases associated with the microRNA 130b gene include, but are not limited to, T-cell lymphoblastic leukemia/lymphoma and pancreatic ductal adenocarcinoma. MiR-130b, which is situated in the 22q11 locus, has dual characteristics in the progression of tumors. For example, miR-130b is an oncogene in gastric cancer, liver cancer, and endometrial cancer, but it has a protective effect against ovarian and thyroid papillary carcinoma.

[0114] As used herein, the term “miR-133a-3p” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 2. Evidence suggests that miR-133a-3p is a tumor suppressor in human colorectal cancer.

[0115] As used herein, the term “miR-195-5p” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 3. Evidence suggests that miR-195-5p is a tumor suppressor in cervical cancer.

[0116] As used herein, the term “miR-432-5p” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 4. miR-432-5p has been found to induce angiogenesis in osteosarcoma.

[0117] As used herein, the term “miR-1229-3p” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 5. miR-1229 has been indicated as a potential biomarker to predict resistance to 5-fluorouracil-based chemotherapy in gastric cancer patients.

[0118] As used herein, the term “miR-1273f” refers to a microRNA (miRNA) corresponding to SEQ ID NO: 6. miR-1273f is strongly expressed in non-small cell lung cancer.

[0119] The term “pancreas” refers to an oblong glandular abdominal organ that has both endocrine and exocrine functions. The organ lies in the retroperitoneum while crossing the body of L1 and L2 along the posterior aspect of the abdominal wall. The pancreas lies in a transverse plane between the C loop of the duodenum while the tail of the pancreas touches the hilum of the spleen. The pancreas is divided into five regions, which consist of a head, uncinate process, neck, body, and tail. The head of the pancreas is the widest part of the organ, while the uncinate lies underneath the body of the pancreas, coming off the posteromedial area of the pancreatic head and curving just posterior to the superior mesenteric artery and vein. The head of the pancreas lies just to the right of the superior mesenteric vessels, and it is attached to the second and third portions of the duodenum. The uncinate process lies adjacent to the third and fourth portions of the duodenum. The uncinate is absent in some people, can vary in size and thickness, and has also been noted to encircle the superior mesenteric vessels in others. The neck begins at the passage of the superior mesenteric vessels and spleno-portal vein confluence posterior to the gland. The neck is the thinnest portion of the pancreas and can be crushed in blunt trauma by being compressed against the second lumbar vertebrae, which sit just posterior to the neck. The gastroduodenal artery runs in a superior to inferior fashion just to the right of the neck of the pancreas. The body of the pancreas is covered anteriorly by the omental bursa, which separates the stomach from the pancreas. The body lies just to the left of the aorta. The tail of the pancreas is the

most mobile aspect of the gland. The tail usually rests in the hilum of the spleen or just below in the majority of people. The endocrine portion is responsible for glucose homeostasis. The exocrine function is involved with the secretion of enzymes involved in digestion. The exocrine pancreas has a functional unit called an acinus. The acinus ultimately has cells that drain zymogen granules that subsequently drain into the main pancreatic duct. The acinar cells secrete either amylases, lipases, or proteases. Stimulation for the secretion of these enzymes is due to the parasympathetic system, secretin, and CCK. Usually, around 50% of the pancreatic acinar cells have to be damaged before there is an effect on their digestion function. The acinar cells can secrete each of these enzymes, which ultimately appears as an alkaline fluid that is colorless and has no odor. The pancreas drains between 1 to 2 liters of exocrine fluid per day, which has an alkalotic pH due to the high bicarbonate concentration in the fluid. As pancreatic stimulation increases, so do the rate of bicarbonate, but chloride concentration decreases. The islets of Langerhans perform the pancreatic endocrine function. There are nearly one million islets in the normal functioning adult pancreas. These islets consist of 5 cell types- alpha cells that secrete glucagon, beta cells that secrete insulin, delta cells that secrete somatostatin, epsilon cells that secrete ghrelin, and F or PP cells that secrete pancreatic polypeptide. The hormones are released in a balanced mixture into the portal vein in reaction to changes in the plasma levels. The majority of the endocrine cells are located in the islet cell periphery, while the beta cells are located in the center and comprise 70% of the islet mass. Alpha cells are predominantly seen in the islet cells in the superior aspect of the pancreatic head, body, and tail; it accounts for 10% of the islet mass. Delta cells are like beta cells, which are present in all islets throughout the pancreas. Delta cells make up only 5% of the islet cell mass. PP cells are mostly seen in the islets in the pancreas' head and account for 15% of the islet mass. Endocrine and exocrine functions are extremely complex, so surgical planning for the patient must take into account the physiological function of the pancreas that will remain. Some patients may require exogenous administration of pancreatic digestive enzymes after surgery or insulin administration with frequent glucose monitoring. Roughly 20 % of the pancreas volume is needed in the remnant to avoid endocrine or exocrine insufficiency. This may not be the case for a more diseased or older fat replaced pancreas.

[0120] As used herein, the term “pancreatectomy” refers to the surgical removal of all or part of the pancreas. Pancreaticoduodenectomy (Whipple procedure) is the most common surgical procedure involving the removal of a portion of the pancreas. It consists of the removal of cancerous parts of the pancreas, duodenum, common bile duct, and if required,

portions of the stomach. Other types of pancreatectomy include, distal pancreatectomy, segmental pancreatectomy, and total pancreatectomy. In recent years, the TP-IAT (Total Pancreatectomy with Islet Autotransplantation) has also gained respectable traction within the medical community. These procedures are used in the management of several conditions involving the pancreas, such as benign pancreatic tumors, pancreatic cancer, and pancreatitis.

[0121] As used herein, the term “metastases” or “pancreatic metastases” is used in accordance with its plain ordinary meaning and refers to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. “Metastatic cancer” is also called “Stage IV cancer.” Cancer occurs at an originating site, e.g., breast, which site is referred to as a primary tumor, e.g., primary breast cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if lung cancer metastasizes to the breast, the secondary tumor at the site of the breast consists of abnormal lung cells and not abnormal breast cells. The secondary tumor in the breast is referred to a metastatic lung cancer. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors. For example, “pancreatic metastases” refers to a disease in a subject with or with a history of a primary pancreatic tumor and spreads to other organs, most commonly within the abdomen and to the liver, lungs, bones and brain.

[0122] As used herein, the term “tissue sample” is used in accordance with its plain ordinary meaning and refers to a piece of tissue removed from an organism for examination, analysis, or propagation.

[0123] As used herein, the term “formalin fixed paraffin -embedded (FFPE)” is used in accordance with its plain ordinary meaning and refers to a long-term tissue preservation approach and preparation for biopsy specimens that aids in examination, experimental research, and diagnostic/drug development and is widely used in pathology. In FFPE a tissue

specimen is preserved by formalin fixing. This step helps to preserve the vital structures and protein within the tissue. It is then embedded into a paraffin wax block and sliced into the required slices.

[0124] As used herein, the term “cancer recurrence” is used in accordance with its plain ordinary meaning and refers to cancer that has recurred (come back), usually after a period of time during which the cancer could not be detected. The cancer may come back to the same place as the original (primary) tumor or to another place in the body.

[0125] As used herein, the term “level” is used in accordance with its plain ordinary meaning and refers to a position on a scale of amount, quantity, extent, or quality. Here, level is in the context of expression level of a gene which can be calculated by measuring the transcribed mRNA (northern blot), the expressed protein (Western Blot), or by directly staining the protein or mRNA when it is still in the cell.

[0126] The term “subject” refer to any living or non-living organism, including but not limited to a human, non-human animal, plant, bacterium, fungus, virus or protist. A subject may be any age (*e.g.*, an embryo, a fetus, infant, child, adult). A subject can be of any sex (*e.g.*, male, female, or combination thereof). A subject may be pregnant. In some embodiments, a subject is a mammal. In some embodiments, a subject is a human subject. A subject can be a patient (*e.g.*, a human patient). In some embodiments a subject is at risk of developing a cancer.

[0127] “Patient” or “subject in need thereof” refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0128] As used herein, the term “periodically monitored” is used in accordance with its plain ordinary meaning and refers to direct observation and assessment of the circumstances at least once a day or the time period assigned.

[0129] As used herein, the term “surgery” is used in accordance with its plain ordinary meaning and refers to medical intervention using operative manual and instrumental

techniques on a person to investigate or treat a pathological condition such as a disease or injury, to help improve bodily function, appearance, or to repair unwanted ruptured areas.

[0130] As used herein, the term “sensitivity” is used in accordance with its plain ordinary meaning and refers to measures the proportion of positives that are correctly identified (i.e. the proportion of those who have some condition (affected) who are correctly identified as having the condition).

[0131] As used herein, the term “specificity” is used in accordance with its plain ordinary meaning and refers to measures the proportion of negatives that are correctly identified (i.e. the proportion of those who do not have the condition (unaffected) who are correctly identified as not having the condition).

[0132] As used herein, the term “primer” is used in accordance with its plain ordinary meaning and refers to a short nucleic acid sequence that provides a starting point for DNA synthesis.

[0133] As used herein, the term “probe” is used in accordance with its plain ordinary meaning and refers to a single-stranded sequence of DNA or RNA used to search for its complementary sequence in a sample genome. The probe is placed into contact with the sample under conditions that allow the probe sequence to hybridize with its complementary sequence. A probe can be labeled with a radioactive or chemical tag that allows its binding to be detected.

Methods of Treatment

[0134] Provided herein are methods of treating an occult micrometastatic disease in a subject with a cancer or suspected of having a cancer. The disclosed methods comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and administering to the subject neoadjuvant therapy. In embodiments, the subject has cancer. In embodiments, the subject is suspected of having a cancer. In embodiments, the methods of treating an occult micrometastatic disease in a subject in need thereof comprises administering to the subject neoadjuvant therapy, if a biological sample obtained from the subject comprises an elevated expression level, relative to a control, of a

microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

[0135] In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy (i.e. as a first step to shrink a tumor before the main treatment, which is usually surgery, is given). In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Flurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0136] In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). In embodiments, the occult micrometastatic disease is pancreatic metastases. In embodiments, an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f indicates that the subject is at high risk of cancer recurrence. In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the microRNA is exosomal microRNA. In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and

exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments of the methods provided herein, a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f is/are analyzed and not other microRNA molecules are analyzed.

[0137] Provided herein are methods of treating an occult micrometastatic disease in a subject who has or is suspected of having a pancreatic cancer, the method comprising administering to the subject neoadjuvant therapy, wherein a biological sample obtained from the subject has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof. In embodiments, the subject has cancer. In embodiments, the subject is suspected of having a cancer. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma.

[0138] In embodiments, the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof. In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering

to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0139] In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). In embodiments, the occult micrometastatic disease is pancreatic metastases. In embodiments, an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f indicates that the subject is at high risk of cancer recurrence. In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In

embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the microRNA is exosomal microRNA.

[0140] In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f.

Methods of Detecting an Occult Micrometastatic Disease or Identifying an Increased Risk of Occult Micrometastatic Disease.

[0141] Provided herein are methods of identifying an increased risk of developing an occult micrometastatic disease or detecting an occult micrometastatic disease in a subject with cancer or suspected of having a cancer. In embodiments the subject has a cancer. In embodiments the subject is suspected of having a cancer. The disclosed methods comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates an increased risk of developing an occult micrometastatic disease or the presence of an occult micrometastatic disease in the subject. In embodiments, the disclosure provides methods of identifying an increased risk of developing an occult micrometastatic disease, which comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates an increased risk of developing an occult micrometastatic disease. In embodiments, the disclosure provides methods of detecting an occult micrometastatic disease in a subject with cancer, which comprise detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates the presence of an occult micrometastatic disease in the subject. In embodiments, the occult micrometastatic disease is pancreatic metastases.

[0142] In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the microRNA is exosomal microRNA. In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-

3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the methods provided herein further comprise administering adjuvant therapy to a subject if a biological sample obtained from the subject has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof. In embodiments, the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof. In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen. In embodiments, the methods further comprise surgically

removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy.

Methods of Diagnosing an Increased Risk for Occult Micrometastatic Disease

[0143] Provided herein are methods of diagnosing a subject with a cancer or suspected of having a cancer as having an increased risk of developing an occult micrometastatic disease, wherein the method comprises detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and diagnosing the subject as having an increased risk of developing an occult micrometastatic disease if an elevated microRNA expression level, relative to a control, is detected in the biological sample. In embodiments, the subject has cancer. In embodiments, the subject is suspected of having a cancer. In embodiments, the cancer is pancreatic cancer. In embodiments, the occult micrometastatic disease is pancreatic metastases. In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f.

[0144] In embodiments, the microRNA is exosomal microRNA. In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f.

[0145] In embodiments, the methods provided herein further comprise administering adjuvant therapy to a subject if a biological sample obtained from the subject has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof. In embodiments, the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof. In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise

administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Flurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen. In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy.

[0146] In embodiments, the subject has a low risk of developing an occult micrometastatic disease if the microRNA expression level in the biological sample is not elevated relative to a control. In embodiments, the disclosed methods further comprise monitoring the subject for an increased risk of developing an occult micrometastatic disease every 3 to 6 months for two years therefrom, and thereafter every six months for three additional years.

Methods of Monitoring for Occult Micrometastatic Disease

[0147] Provided herein are methods of monitoring a subject having cancer or suspected of having a cancer for an increased risk of developing an occult micrometastatic disease, wherein the methods comprise detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; and (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p,

miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a second time point subsequent to the first time point. In embodiments, the subject has a cancer. In embodiments, the subject is suspected of having a cancer. In embodiments, detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease. In embodiments, the disclosed methods further comprise the use of one or more of ultrasound, computerized tomography (CT) scans, magnetic resonance imaging (MRI), positron emission tomography (PET) scans, or any combinations thereof, to monitor the subject. In embodiments, the cancer is pancreatic cancer. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). In embodiments, the occult micrometastatic disease is pancreatic metastases. In embodiments, an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f indicates that the subject is at high risk of cancer recurrence. In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f.

[0148] In embodiments, the microRNA is exosomal microRNA. In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f.

[0149] In embodiments, the disclosed methods of monitoring a subject comprise administering to the subject neoadjuvant therapy, if a biological sample obtained from the subject comprises an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof. In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to

the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen. In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy.

[0150] In embodiments, the disclosed methods further comprise monitoring a subject with no elevated microRNA expression level at the second time point for an increased risk of developing a micrometastatic disease every 3 to 6 months for two years therefrom, and thereafter every six months for three additional years.

Methods of Monitoring for Occult Micrometastatic Disease and Treating Occult Micrometastatic Disease.

[0151] Provided herein are methods of monitoring a subject having cancer or suspected of having a cancer for an increased risk of developing an occult micrometastatic disease, and treating an occult micrometastatic disease in the subject. The disclosed methods comprise (i) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the

subject, at a second time point subsequent to the first time point; and (iii) treating the subject with neoadjuvant therapy if an elevated microRNA expression level in the biological sample is detected at the second time point compared to the microRNA expression level at the first time.

[0152] In embodiments, the subject has a cancer. In embodiments, the subject is suspected of having a cancer. In embodiments, detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease. In embodiments, the disclosed methods further comprise the use of one or more of ultrasound, computerized tomography (CT) scans, magnetic resonance imaging (MRI), positron emission tomography (PET) scans, or any combinations thereof, to monitor the subject. In embodiments, the cancer is pancreatic cancer. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). In embodiments, the occult micrometastatic disease is pancreatic metastases. In embodiments, an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f indicates that the subject is at high risk of cancer recurrence. In embodiments, the biological sample is a liquid biological sample. In embodiments, the liquid biological sample is blood, plasma, serum, urine or saliva. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five microRNAs selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six microRNAs

selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f.

[0153] In embodiments, the microRNA is exosomal microRNA. In embodiments, the microRNA is cell-free microRNA. In embodiments, the microRNA is exosomal microRNA and cell-free microRNA. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of one exosomal microRNA selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of two exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of three exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of four exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of five exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f. In embodiments, the biological sample comprises an elevated expression level, relative to a control, of six exosomal microRNAs selected from the group consisting of exosomal miR-130b-5p, exosomal miR-133a-3p, exosomal miR-195-5p, exosomal miR-432-5p, exosomal miR-1229-3p, and exosomal miR-1273f.

[0154] In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of

immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Flurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen. In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy.

[0155] In embodiments, the disclosed methods further comprise monitoring a subject with no elevated microRNA expression level at the second time point for an increased risk of developing a micrometastatic disease every 3 to 6 months for two years therefrom, and thereafter every six months for three additional years.

[0156] In embodiments, the methods provided herein can be used to determine whether a subject is at high risk for cancer recurrence. In embodiments, the subject is at high risk of cancer recurrence if an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, is detected in a biological sample obtained from the subject. In embodiments, a panel risk score for cancer recurrence is determined by the formula: $\text{logit}(-0.3305 \cdot \text{miR-130b-5p}) + (-0.2089 \cdot \text{miR-133a-3p}) + (1.1546 \cdot \text{miR-195-5p}) + (0.2297 \cdot \text{miR-432-5p}) + (0.4848 \cdot \text{miR-1229-3p}) + (-0.3591 \cdot \text{miR-1273f})$. In embodiments, a panel risk score above a threshold is indicative of a high risk of cancer recurrence. In embodiments, the threshold is greater than -6. In embodiments, the threshold is greater than -5.5. In embodiments, the threshold is greater than -5.38. In embodiments, the threshold is greater than -5. In embodiments, the threshold is greater than -4.5. In embodiments, the threshold is greater than -4. The disclosed methods

comprise monitoring the subject for increases in risk of cancer recurrence by detecting the expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject. In embodiments, the method of detection is repeated at least daily. In embodiments, the method of detection is repeated at least weekly. In embodiments, the method of detection is repeated at least monthly. In embodiments, the method of detection is repeated at least every six months. In embodiments, the method of detection is repeated at least yearly. In embodiments, the monitoring period is at least a week. In embodiments, the monitoring period is at least a month. In embodiments, the monitoring period is at least 3 months. In embodiments, the monitoring period is at least 6 months. In embodiments, the monitoring period is at least 9 months. In embodiments, the monitoring period is at least 1 year. In embodiments, the monitoring period is at least 2 years. In embodiments, the monitoring period is at least 3 years. In embodiments, the monitoring period is at least 4 years. In embodiments, the monitoring period is at least 5 years.

[0157] The methods provided herein further comprise administering adjuvant therapy to a subject if the subject has an increased risk of cancer recurrence, as determined by an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject. In embodiments, the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof. In embodiments, the methods comprise administering to the subject an effective amount of an anticancer agent. In embodiments, the methods comprise administering to the subject an effective amount of radiation therapy. In embodiments, the methods comprise administering to the subject an effective amount of chemotherapy. In embodiments, the methods comprise administering to the subject an effective amount of targeted therapy. In embodiments, the methods comprise administering to the subject an effective amount of immunotherapy. In embodiments, the methods comprise administering to the subject an effective amount of hormonal therapy. In embodiments, the methods comprise administering to the subject an effective amount of angiogenesis inhibitor. In embodiments, the methods comprise administering to the subject an effective amount of synthetic lethality therapy. In

embodiments, the methods comprise administering to the subject an effective amount of any combination of an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, and synthetic lethality therapy. In embodiments, the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5f-Flurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV). In embodiments, the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen. In embodiments, the methods further comprise surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of one or more rounds of neoadjuvant therapy.

Kits

[0158] Provided herein are kits comprising reagents and reaction mixtures for the detection, analysis and measurement of the expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from a subject. As part of the kit, materials and instruction are provided, e.g., for storage and use of kit components.

[0159] “Assaying” or “detecting” means using an analytical procedure to qualitatively assess or quantitatively measure the level of methylation of the genes as described herein such as, for example, detecting a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, using an analytic procedure (such as an *in vitro* procedure) to qualitatively assess or quantitatively measure the level of the selected microRNAs. In embodiments, the detecting includes or is assaying, which includes wet lab analysis, physical steps and/or physical manipulation of the sample, for example in a laboratory setting involving physical assaying techniques.

[0160] In embodiments, the kit comprises one or more of a probe that can hybridize to a biomarker, pairs of primers for PCR amplification, instructions on how to use the kit, and a label or insert indicating regulatory approval for diagnostic use.

[0161] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

Embodiments 1-66

[0162] Embodiment 1. A method of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer, the method comprising detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, miR-1273f, and a combination of two or more thereof, in a biological sample obtained from the subject, and administering to the subject neoadjuvant therapy.

[0163] Embodiment 2. A method of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer, the method comprising administering to the subject neoadjuvant therapy, wherein a biological sample obtained from the subject comprises an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

[0164] Embodiment 3. The method of Embodiment 1 or Embodiment 2, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

[0165] Embodiment 4. The method of Embodiment 3, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5fFlurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

[0166] Embodiment 5. The method of Embodiment claim 3, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0167] Embodiment 6. The method of anyone of Embodiments 1-5, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of the treatment.

[0168] Embodiment 7. A method of identifying an increased risk of developing an occult micrometastatic disease or detecting an occult micrometastatic disease in a subject with cancer, the method comprising detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates an increased risk of developing an occult micrometastatic disease or the presence of an occult micrometastatic disease in the subject.

[0169] Embodiment 8. The method of Embodiment 7, wherein the method comprises identifying an increased risk of developing an occult micrometastatic disease in a subject with cancer.

[0170] Embodiment 9. The method of Embodiment 7, wherein the method comprises detecting an occult micrometastatic disease in a subject with cancer.

[0171] Embodiment 10. A method of diagnosing a subject with cancer as having an increased risk of developing an occult micrometastatic disease, wherein the method comprises detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and diagnosing the subject as having an increased risk of developing an occult micrometastatic disease if an elevated microRNA expression level, relative to a control, is detected in the biological sample.

[0172] Embodiment 11. The method of anyone of Embodiments 7-10, wherein the method further comprises administering to the subject neoadjuvant therapy.

[0173] Embodiment 12. The method of Embodiment 11, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy,

immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

[0174] Embodiment 13. The method of Embodiment 12, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

[0175] Embodiment 14. The method of Embodiment 12, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0176] Embodiment 15. The method of any one of Embodiments 7-14, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0177] Embodiment 16. A method of monitoring a subject having cancer for an increased risk of developing an occult micrometastatic disease, wherein the method comprises detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; and (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a second time point subsequent to the first time point; wherein detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease.

[0178] Embodiment 17. The method of Embodiment claim 16, wherein the monitoring further comprises the use of ultrasound, computerized tomography (CT) scans, magnetic resonance imaging (MRI), positron emission tomography (PET) scans, and any combinations thereof.

[0179] Embodiment 18. The method of Embodiment 16 or Embodiment claim 17, wherein a subject with no elevated microRNA expression level at the second time point is monitored

every 3 to 6 months for two years therefrom, and thereafter every six months for three additional years.

[0180] Embodiment 19. The method of Embodiment 16 or Embodiment 17, wherein the method further comprises proposing neoadjuvant therapy to a subject with an increased risk of developing an occult micrometastatic disease.

[0181] Embodiment 20. The method of Embodiment 19, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

[0182] Embodiment 21. The method of Embodiment 20, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

[0183] Embodiment 22. The method of Embodiment 20, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0184] Embodiment 23. The method of any one of Embodiments 19-22, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0185] Embodiment 24. The method of any one of Embodiments 1-23, wherein the microRNA is exosomal microRNA.

[0186] Embodiment 25. The method of any one of Embodiments 1-24, wherein the cancer is pancreatic cancer.

[0187] Embodiment 26. The method of Embodiment 25, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).

[0188] Embodiment 27. The method of Embodiment 26, wherein the occult micrometastatic disease is pancreatic metastases.

[0189] Embodiment 28. The method of anyone of Embodiments 1-27, wherein an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f or a combination of two or more thereof indicates that the subject is at high risk of cancer recurrence.

[0190] Embodiment 29. The method of anyone of Embodiments 1-28, wherein the biological sample is a liquid biological sample.

[0191] Embodiment 30. The method of Embodiment 29, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

[0192] Embodiment 31. A method of treating an occult micrometastatic disease in a subject who has or is suspected of having a pancreatic cancer, the method comprising administering to the subject neoadjuvant therapy, wherein a biological sample obtained from the subject has an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

[0193] Embodiment 32. The method of Embodiment 31, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

[0194] Embodiment 33. The method of Embodiment 31 or Embodiment claim 32, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

[0195] Embodiment 34. The method of Embodiment 33, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5fFlurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

[0196] Embodiment 35. The method of Embodiment 34, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0197] Embodiment 36. The method of any one of Embodiments 31-35, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0198] Embodiment 37. The method of any one of Embodiments 31-36, wherein the biological sample is a liquid biological sample.

[0199] Embodiment 38. The method of Embodiment 37, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

[0200] Embodiment 39. A kit comprising reagents capable of detecting an expression level of microRNA in a biological sample, wherein the microRNA is selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or any combination of two or more thereof.

[0201] Embodiment 40. A method of detecting microRNA expression in a subject that has or is suspected of having cancer, wherein the method comprises measuring the expression of one or more microRNAs in a biological sample obtained from the subject in comparison to control, wherein the subject has, or is suspected of having occult micrometastatic disease, and wherein the one or more microRNAs are miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

[0202] Embodiment 41. The method of Embodiment 40, wherein the one or more microRNAs are exosomal microRNAs.

[0203] Embodiment 42. The method of Embodiment 40 or Embodiment 41, wherein the control is a healthy subject.

[0204] Embodiment 43. The method of any of Embodiments 40 to 42, wherein the subject had previously undergone a pancreatectomy.

[0205] Embodiment 44. The method of Embodiment 43, wherein metastases of the occult micrometastatic disease are pancreatic metastases.

[0206] Embodiment 45. The method of Embodiment 40, wherein the biological sample is a liquid biological sample.

[0207] Embodiment 46. The method of Embodiment 45, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

[0208] Embodiment 47. The method of any of Embodiments 40 to 46, wherein the subject is at high risk of cancer recurrence if the expression level of the one or more microRNAs are above that of said control.

[0209] Embodiment 48. The method of Embodiment 47, wherein the subject at high risk of cancer recurrence is proposed or provided further treatment or treatments.

[0210] Embodiment 49. The method of Embodiment 48, wherein the treatment or treatments are radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or a combination of two or more thereof.

[0211] Embodiment 50. The method of Embodiment 49, wherein the therapy is a neoadjuvant therapy.

[0212] Embodiment 51. The method of Embodiment 40, wherein the subject is a low risk of cancer recurrence if the expression level of the one or more microRNAs are equal or below that of a control.

[0213] Embodiment 52. The method of Embodiment 51, wherein the subject at low risk of cancer recurrence is periodically monitored for the development of cancer.

[0214] Embodiment 53. The method of Embodiment 51, further comprising treating the subject by surgery.

[0215] Embodiment 54. A method of monitoring a subject having cancer or suspected of having a cancer for an increased risk of developing an occult micrometastatic disease and treating occult micrometastatic disease in the subject, wherein the method comprises (i) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the

subject, at a second time point subsequent to the first time point; and (iii) treating the subject with neoadjuvant therapy if an elevated microRNA expression level in the biological sample is detected at the second time point compared to the microRNA expression level at the first time,.

[0216] Embodiment 55. The method of Embodiment 54, wherein detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease.

[0217] Embodiment 56. The method of Embodiment 54 or Embodiment 55, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

[0218] Embodiment 57. The method of Embodiment 56, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

[0219] Embodiment 58. The method of Embodiment 56, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

[0220] Embodiment 59. The method of any one of Embodiments 54-58, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

[0221] Embodiment 60. The method of any one of Embodiment 54-59, wherein the microRNA is exosomal microRNA.

[0222] Embodiment 61. The method of any one of Embodiment 54-60, wherein the cancer is pancreatic cancer.

[0223] Embodiment 62. The method of Embodiment 61, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).

[0224] Embodiment 63. The method of Embodiment 62, wherein the occult micrometastatic disease is pancreatic metastases.

[0225] Embodiment 64. The method of any one of Embodiments 54-63, wherein an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates that the subject is at high risk of cancer recurrence.

[0226] Embodiment 65. The method of any one of Embodiments 54-65, wherein the biological sample is a liquid biological sample.

[0227] Embodiment 66. The method of Embodiment 65, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

EXAMPLES

EXAMPLE 1: RESULTS AND DISCUSSION

[0228] Herein, for the first time, we performed comprehensive biomarker discovery to identify a pre-operative, blood-based, exo-miRNA panel (EMP) for risk prediction of recurrence following surgery in patients with PDAC. This panel was initially developed using genome-wide sequencing, followed by rigorous validation and performance evaluation in a total of three large, independent clinical cohorts including a post-NAT cohort, highlighting its potential clinical significance for the management of patients with PDAC.

[0229] Genome-wide miRNA expression profiling identifies a novel exosomal miRNA panel for predicting recurrence following surgery in patients with PDAC.

[0230] To identify exosomal microRNAs (exo-miRNAs) specifically dysregulated in patients with pancreatic ductal adenocarcinoma (PDAC) recurrence, miRNA expression profiling was performed in exosomes harvested from plasma before any treatment in a discovery cohort of 25 patients. This cohort of 25 patients included 16 patients with early recurrence within 6 months after surgery and 9 PDAC patients without recurrence for more than 3 years following curative surgery (Table 1). Among 2265 expressed miRNAs, candidate exo-miRNAs were selected if they were significantly and differentially expressed between early recurrence and non-recurrent patients (upregulated miRNAs in recurrent patients, absolute log₂ fold change > 1.0, P < 0.05) and were expressed in at least half of the

samples; immature miRNAs were excluded (FIG. 6). Subsequently, 12 candidate miRNAs were identified: hsa-miR-130b-5p, hsa-miR-133a-3p, hsa-miR-184-3p, hsa-miR-195-5p, hsa-miR-320c, hsa-miR-432-5p, hsa-miR-548q, hsa-miR-766-5p, hsa-miR-1229-3p, hsa-miR-1261, hsa-miR-1273f, and hsa-miR-3177-3p, which were upregulated in the exosomes of patients with early PDAC recurrence (FIGS. 1A-1B).

[0231] Finally, a cox regression model was constructed with these 12 miRNAs in the discovery cohort, which demonstrated excellent performance for recurrence prediction and relapse-free survival (RFS) following surgery (FIGS. 1C-1E). Furthermore, when the distribution of risk scores and recurrence status were assessed, it was observed that patients with recurrence had a significantly higher risk score than non-recurrent patients ($P < 0.01$, FIG. 1F); highlighting the performance of this model for the recurrence prediction following surgery in patients with PDAC.

Table 1: Clinicopathological characteristics of the preoperative clinical cohorts

Characteristics	Discovery cohort (n = 25)	Training cohort (n = 82)	Validation cohort (n = 57)
Age			
< 65 years	15 (60.0)	31 (37.8)	16 (28.1)
≥ 65 years	10 (40.0)	51 (62.2)	41 (71.9)
Gender			
Male	16 (64.0)	48 (58.5)	41 (71.9)
Female	9 (36.0)	34 (41.5)	16 (28.1)
Pre-treatment CA19-9			
≤ 37 U/mL	5 (20.0)	25 (30.5)	24 (42.1)
> 37 U/mL	20 (80.0)	57 (69.5)	33 (57.9)
Tumor location			
Ph	19 (76.0)	48 (58.5)	37 (64.9)
Pbt	6 (24.0)	34 (41.5)	20 (45.1)
Pre-treatment tumor size (CT)			
≤ 4 cm	15 (60.0)	41 (50.0)	52 (91.2)
> 4 cm	10 (40.0)	41 (50.0)	5 (8.8)
Resectability status			
Resectable	NA	47 (57.3)	NA
Borderline-resectable	NA	33 (40.2)	NA
Unresectable	NA	2 (2.4)	NA
Nodal metastasis			
Present	14 (56.0)	59 (72.0)	32 (56.1)
Absent	11 (44.0)	22 (26.8)	25 (43.9)
Not Available	0 (0.0)	1 (1.2)	0 (0.0)
UICC pStage (ver.7)			
IA	0 (0.0)	NA	3 (5.3)
IB	3 (12.0)	NA	0 (0.0)
IIA	8 (32.0)	NA	22 (38.6)
IIB	14 (56.0)	NA	31 (54.4)

Characteristics	Discovery cohort (n = 25)	Training cohort (n = 82)	Validation cohort (n = 57)
III	0 (0.0)	NA	1 (1.8)
IV	0 (0.0)	NA	0 (0.0)
UICC pStage (ver.8)			
IA	NA	4 (4.9)	NA
IB	NA	5 (6.1)	NA
IIA	NA	12 (14.6)	NA
IIB	NA	33 (40.2)	NA
III	NA	26 (31.7)	NA
IV	NA	2 (2.4)	NA
Adjuvant therapy			
Yes	19 (76.0)	57 (66.3)	43 (75.4)
No	5 (20.0)	29 (33.7)	13 (22.8)
Not Available	1 (4.0)	1 (1.2)	1 (1.8)
Recurrence			
Present	16 (64.0)	68 (82.9)	25 (43.9)
Absent	9 (36.0)	14 (17.1)	32 (56.1)

UICC, International Union Against Cancer

Clinical training establishes an EMP for predicting recurrence and cancer prognosis in patients with PDAC.

[0232] To evaluate the predictive robustness of the discovered exosomal miRNAs, training and validation of selected exo-miRNAs was performed using quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays in blood specimens from two large, independent clinical cohorts (Table 1). Eliminating redundancy and minimizing the number of candidate miRNAs might allow an easier translation of the exo-miRNA Panel (EMP) into clinical practice, firstly, optimization of the model using cox regression was performed with backward elimination for feature selection in the training cohort cases (n = 82; 68 recurrence and 14 non-recurrence). As a result, successful establishment of a final pre-treatment recurrence prediction EMP model was observed which comprised of a reduced 6 miRNA panel: miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f. Subsequently, a risk score was developed based on the coefficients derived from individual exo-miRNAs and the constant derived from this analysis as follows: $\text{logit}(-0.3305 \cdot \text{miR-130b-5p}) + (-0.2089 \cdot \text{miR-133a-3p}) + (1.1546 \cdot \text{miR-195-5p}) + (0.2297 \cdot \text{miR-432-5p}) + (0.4848 \cdot \text{miR-1229-3p}) + (-0.3591 \cdot \text{miR-1273f})$. The risk-assessment EMP demonstrated excellent performance for predicting recurrence, with an area under the curve (AUC) value of 0.81 (95% confidence interval [CI] = 0.70–0.89; FIG. 2A), and a corresponding specificity of 0.85 and a sensitivity of 0.72. Based on the distribution of risk scores and recurrence status, patients with recurrence after surgery demonstrated significantly higher risk scores vs. non-recurrent patients ($P < 0.01$; FIG. 2B). Subsequently, patients were

dichotomized into low- and high-risk groups based on EMP risk scores obtained from Youden's index-derived cutoff thresholds^{49, 58}. Importantly, high-risk patients exhibited a significantly worse RFS compared to low-risk patients, with a hazard ratio (HR) of 2.20 (95% CI = 1.36–3.55; FIG. 2C).

[0233] Next, univariate and multivariate cox regression analyses was performed to evaluate the efficacy of various preoperative clinicopathological variables (age, gender, carbohydrate antigen 19-9 (CA19-9), tumor location, tumor size) and the EMP for its ability to predict a patient's cancer prognosis. In multivariate analysis, high-risk patients defined by our EMP emerged as an independent predictor for worse RFS in patients with PDAC (HR = 1.81; 95% CI = 1.07–3.07; P = 0.02; FIG. 2D). These results demonstrate that the EMP was successfully developed as a blood-based, pre-treatment recurrence prediction assay, which has significant recurrence prediction potential for cancer prognosis in patients with PDAC.

[0234] A combined signature including EMP and CA19-9 levels demonstrates significantly superior accuracy for predicting recurrence in PDAC patients.

[0235] The glycoprotein CA19-9 is a widely established and important biomarker in PDAC. In addition, the multivariate cox regression model demonstrated that both EMP and CA19-9 were selected as independent risk factors for predicting RFS in the training cohort. Accordingly, a model combining the EMP with CA19-9 expression levels was examined. The combination signature demonstrated an improved predictive performance for patient prognosis (AUC= 0.84; FIG. 2E). Furthermore, the combination signature also demonstrated significantly superior recurrence predictive accuracy compared to other classic preoperative clinicopathological features, including tumor location and size (FIG. 2E); highlighting potentially utility of this signature for more accurate prediction of recurrence following surgery in PDAC.

[0236] To evaluate the translational potential of the EMP in identifying high-risk patients with PDAC, the performance was evaluated in an independent clinical validation cohort (n = 57; 25 recurrence and 32 non-recurrence; FIG.6). To this end, the EMP was applied using the same statistical model, coefficients, and cutoff values derived from the training cohort to the validation cohort, which once again confirmed the robustness of our risk-assessment model in predicting PDAC recurrence, with an AUC value of 0.78 (95% CI = 0.65–0.88; FIG. 3A). Moreover, the EMP demonstrated consistent results in the same statistical analysis with the

training cohort, including in multivariate analysis for RFS (FIGS. 3B-3E), underscoring the clinical significance of the EMP in predicting recurrence and cancer prognosis in patients with PDAC.

Additional validation of the EMP predicts PDAC recurrence in patients after NAT

[0237] For patients with PDAC, multidisciplinary treatment strategies, including neoadjuvant therapy (NAT), are being actively explored and becoming increasingly common treatment options, especially in western countries. Therefore, if the EMP may also discriminate which patients will have worse prognosis after NAT, it might potentially facilitate a more informed decision-making by physicians and patients, to determine which patients should continue with chemo-/chemoradiotherapy and who should proceed to surgery following NAT. Therefore, to further advance the translation of the noninvasive miRNA marker into clinical practice, post-NAT blood samples were collected from an additional validation cohort (n = 46; 25 recurrence and 21 non-recurrence) of patients with PDAC who underwent NAT followed by curative surgery (Table 2) and evaluated the performance of EMP for predicting recurrence and cancer prognosis after surgery. Recurrence was predicted with an AUC value of 0.72 (95% CI = 0.57–0.85, FIG. 4A). Patients with recurrence had significantly higher risk scores than patients without recurrence ($P < 0.01$; FIG. 4B). Moreover, patients who were classified as high-risk for recurrence by EMP showed significantly worse RFS than the low-risk patients (FIG. 4C). Also, in a multivariate cox regression analysis including EMP and other conventional post-NAT clinicopathological variables, the EMP emerged as an independent feature for RFS (HR = 2.84; 95% CI = 1.30–6.20; $P < 0.01$, FIG. 4D). In addition, a combination signature constructed from the EMP and CA19-9 demonstrated significantly superior performance compared to other clinicopathological factors (FIG. 4E). These results demonstrate that the EMP may be applied to post-NAT patients, which indicates medical practitioners could re-evaluate potential risk for recurrence and poor prognosis in patients with PDAC even after NAT.

Table 2: Clinicopathological characteristics of the post-NAT validation cohort

Characteristics	Additional validation cohort (Post-NAT)
	(n = 46)
Age	
< 65 years	18 (39.1)
≥ 65 years	28 (60.9)

Characteristics	Additional validation cohort (Post-NAT)
	(n = 46)
Gender	
Male	25 (54.3)
Female	21 (45.7)
Post-NAT CA19-9	
≤ 37 U/mL	22 (47.8)
> 37 U/mL	24 (52.2)
Tumor location	
Ph	36 (78.3)
Pbt	10 (21.7)
Post-NAT tumor size (CT)	
≤ 4 cm	42 (91.3)
> 4 cm	4 (8.7)
Nodal metastasis	
Present	14 (30.4)
Absent	32 (69.6)
UICC ypStage (ver.7)	
0	2 (4.3)
IA	3 (6.5)
IB	5 (10.9)
IIA	21 (45.7)
IIB	13 (28.3)
III	2 (4.3)
IV	0 (0.0)
Neoadjuvant therapy	
Neoadjuvant chemotherapy	17 (37.0)
Neoadjuvant chemoradiotherapy	29 (63.0)
Adjuvant therapy	
Yes	33 (71.7)
No	8 (17.4)
Data not available	5 (10.9)
Recurrence	
Present	25 (54.3)
Absent	21 (45.7)

NAT, Neoadjuvant therapy; UICC, International Union Against Cancer

The miRNA–mRNA regulatory network analysis identifies that candidate miRNAs are involved in key cancer-related signaling pathways.

[0238] To determine the downstream gene targets of the discovered miRNAs, a miRNA–mRNA regulatory network analysis was conducted and identified 96 gene targets with predicted mechanistic involvement in gene regulatory pathways (FIG. 5). KEGG analysis was used to perform pathway analysis of the validated downstream gene targets, which

revealed biologically meaningful pathways, including several specific signaling pathways related to PDAC, such as PI3K-Akt (phosphatidylinositol 3-kinase-protein kinase B) signaling, pancreatic cancer, and miRNAs in cancer (FIG. 5). These results further validate the EMP and its biological significance in the pathogenesis of PDAC.

Discussion

[0239] Recent innovations in medical technologies have led cancer treatment into a new era of precision medicine and development of individualized treatment strategies for cancer patients. However, especially in PDAC, which remains one of the most lethal malignancies worldwide, further advances including medical technologies, treatment strategies and basic/translational researches are essential³. In the quest to develop a robust pre-treatment, noninvasive risk-stratification model in PDAC, a systemic and comprehensive biomarker discovery approach was used to successfully develop a blood-based, 6-exo-miRNA panel that performed excellently in predicting tumor recurrence in patients with PDAC, which was subsequently validated in two independent clinical cohorts. Furthermore, the predictive potential of the EMP was confirmed even in post-NAT blood specimens, which was comparable to the performance of these biomarkers in blood from pre-treatment cohorts. Considering the current clinical strategies, in which NAT is becoming a common therapeutic option, this highlights the potential significance of the EMP for clinical translation for improving risk assessment and survival in patients with PDAC. Moreover, when compared with other conventional clinicopathological risk factors, the EMP remained a significant prognostic indicator in all independent clinical cohorts, including post-NAT specimens. Furthermore, a model combining the EMP with CA19-9, the most commonly used tumor marker in PDAC, demonstrated an even further improved predictive accuracy. To further highlight the clinical significance of the findings, although the National Comprehensive Cancer Network (NCCN) guidelines are insufficient to identify high-risk patients with PDAC, the EMP robustly stratified patients from all clinical cohorts into significantly distinct high- and low-risk subgroups.

[0240] From a clinical perspective, the EMP has the potential to enable several significant improvements upon translation into clinical practice: (1) it can facilitate a reduction of ineffective and invasive surgeries currently being performed in patients with PDAC, (2) it might provide an optimal indication for NAT in patients with resectable status, and (3) it could potentially help physicians and patients in making decisions to continue chemotherapy

or proceed to surgery after NAT. Further, the EMP panel can evaluate the risk status at multiple timepoints in a patient's clinical course.

[0241] In conclusion, using genome-wide expression profiling, a novel noninvasive EMP for predicting recurrence in patients with PDAC was identified and validated, which will have clinical significance for the selection of optimized treatment strategies.

EXAMPLE 2: METHODS

Study design and patient cohorts

[0242] The workflow for the discovery of candidate miRNAs and the development of a miRNA signature is shown in FIG.6. For the identification, clinical training, and validation of the EMP, biospecimens were analyzed from four independent cohorts of patients with PDAC. A total of 210 plasma or serum specimens were examined, which included a biomarker discovery cohort that was subjected to small RNA sequencing (n = 25; 16 with recurrence within 6 months after surgery and 9 without recurrence for more than 3 years). The patients within this cohort were enrolled at the Asan Medical Center, Korea between 2012 and 2015. Likewise, the training cohort (n = 82; 68 recurrence and 14 non-recurrence) of patients were enrolled at the Samsung Medical Center, Korea between 2008 and 2017; and a validation cohort (n = 57; 25 recurrence and 32 non-recurrence) of patients enrolled at the Nagoya University, Japan and Medical College of Wisconsin between 2012 and 2017. None of these patients received preoperative cancer treatment, all blood specimens were obtained prior to initiation of any treatment, and all patients were diagnosed as having PDAC by pathological examination following surgery. Post-NAT blood samples were collected for an additional validation cohort (n = 46; 25 recurrence and 21 non-recurrence) of patients who underwent NAT followed by surgery at Nagoya University, Japan and Medical College of Wisconsin between 2012 and 2018. In this additional validation cohort, all blood specimens were obtained during the waiting period for surgery following NAT. Tumors were classified according to the TNM (Tumor, Nodes and Metastases) staging system of the International Union Against Cancer (UICC) version 7 or 8 by pathological examination. Patients who had positive peritoneal washing cytology or para-aortic lymph node metastases-without other distant metastases were included in this study. Exclusion criteria included macroscopically incomplete resection, a tumor histology other than diagnosis of PDAC, or insufficient survival information (follow-up periods $\leq$ 6 months after surgery). The study was conducted in accordance with the Declaration of Helsinki. A written informed consent was obtained

from all patients, and the study was approved by the institutional review boards of all participating institutions.

Exosomal RNA extraction

[0243] For small RNA sequencing, total exosomal RNA was isolated from 400 μ L plasma, using an exoRNeasy Midi Kit (Qiagen, Valencia, California (CA)) according to the manufacturer's instructions. For real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR), total exosomal RNA was extracted from 200 μ L plasma or serum using a Total Exosome Isolation Kit (Cat no: 4484450, Invitrogen) and an miRNeasy serum/plasma Kit (Qiagen, Valencia, California (CA)).

Small RNA sequencing and biomarker discovery

[0244] A small RNA sequencing library was prepared from extracted exosomal RNA, using a NEXTflex™ Small RNA-Seq Kit v3 (PerkinElmer). Following the size selection and quality check, all libraries were pooled together and sequenced on an Illumina NovoSeq using paired-end sequencing. For data analysis, cutadapt (v2.2) was used to move adapters and low-quality bases in reads. Thereafter the reads were aligned to human ribosomal RNA (rRNA) precursor sequences from National Center for Biotechnology Information (NCBI) using Bowtie2 (v2.3.5) and matching reads were discarded from further analysis. A sRNAAnalyzer pipeline was used to align the remaining reads and quantify miRNA expression. The quantified small RNA sequencing data was analyzed to identify differentially expressed miRNAs between patients in the discovery cohort who had early PDAC recurrence (within 6 months after surgery, $n = 16$) and those who had long-term (more than 3 years) non-recurrence ($n = 9$) after curative surgery. Differential expression analysis was conducted using DESeq2 (v1.26.0). A Benjamini-Hochberg false discovery rate (FDR) of 5% was used to account for multiple testing corrections⁴⁸. Finally, differentially expressed miRNAs were used to build classifiers that distinguished early recurrence patients from non-recurrent patients. To evaluate the recurrence predictive potential of the discovered exosomal miRNAs, a multivariate cox regression model was first established using selected biomarkers. Thereafter, the resulting risk scores were used to determine the area under the curve (AUC) values for each of the receiver operator characteristic (ROC) plots^{33, 49-51}.

Real-time quantitative reverse transcription polymerase chain reaction

[0245] Synthesis of complementary DNA from total exosomal RNA was performed using a miRCURY LNA RT Kit (Qiagen, Valencia, California (CA)). RT-qPCR analysis was

performed using a SensiFAST™ sybr Lo-ROX Kit (Bioline, London, United Kingdom (UK)) on the Quantstudio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, California (CA)), and expression levels were evaluated using Applied Biosystems QuantStudio 6 Flex Real-Time PCR System Software. The relative abundance of target transcripts was evaluated and normalized to the expression of miR-16-5p as an internal control using the $2^{-\Delta\Delta C_t}$ method. Normalized values were further log2 transformed^{50, 51}. All of the primers for miRNAs used in this study were purchased from Qiagen (miRCURY LNA miRNA PCR Assays). Catalog numbers of each miRNA are as follows: has-miR-16-5p: YP00205702, hsa-miR-130b-5p: YP00204456, hsa-miR-133a-3p: YP00204788, hsa-miR-184-3p: YP00204601, hsa-miR-195-5p: YP00205869, hsa-miR-320c: YP00205706, hsa-miR-432-5p: YP00204776, hsa-miR-548q: YP02103735, hsa-miR-766-5p: YP02113546, hsa-miR-1229-3p: YP00206036, hsa-miR-1261: YP00205716, hsa-miR-1273f: YP02102122, and hsa-miR-3177-3p: YP02103017.

MicroRNA regulatory network analysis

[0246] The miRNA:mRNA regulatory network was constructed using the validated miRNAs to elucidate perturbed pathways, through data analysis using miRTarBase version 7.0⁵²⁻⁵⁵. miRNA–mRNA pairs with weak evidence in the miRTarBase database were excluded from the analysis. Pathway enrichment analysis for selected target genes was performed using KEGG pathways.

Statistical analysis

[0247] Data pre-processing and handling were performed using R/Bioconductor. Univariate and multivariate cox regression analyses were employed to evaluate various preoperative clinicopathological variables (age, gender, carbohydrate antigen 19-9 [CA19-9], tumor location, tumor size evaluated by computed tomography) and the EMP for predicting a patient's cancer prognosis. Relapse-free survival (RFS) times were calculated from the date of surgery to the date of death from any cause or recurrence, or last follow-up date. RFS was estimated using the Kaplan-Meier method. The primary endpoint was analyzed using a stratified log-rank test^{12, 56}. Median follow-up was calculated using the reverse Kaplan-Meier method^{12, 57}. A multivariate Cox proportional hazard regression model was established and a P value <0.05 was considered statistically significant. Statistical analyses were performed using Medcalc statistical software, GraphPad Prism V8.0 (GraphPad Software, San Diego, California (CA)), and R (3.5.0, R Development Core Team).

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INFORMAL SEQUENCE LISTING

miR-130b-5p: ACUCUUUCCCUGUUGCACUAC (SEQ ID NO: 1)

miR-133a-3p: UUUGGUCCCCUUAACCAGCUG (SEQ ID NO: 2)

miR-195-5p: UAGCAGCACAGAAAUAUUGGC (SEQ ID NO: 3)

miR-432-5p: UCUUGGAGUAGGUCAUUGGGUGG (SEQ ID NO: 4)

miR-1229-3p: CUCUCACCACUGCCCCUCCCACAG (SEQ ID NO: 5)

miR-1273f: GGAGAUGGAGGUUGCAGUG (SEQ ID NO: 6)

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer, the method comprising detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and administering to the subject a neoadjuvant therapy.

2. A method of treating occult micrometastatic disease in a subject with a cancer or suspected of having a cancer, the method comprising administering to the subject neoadjuvant therapy, wherein a biological sample obtained from the subject comprises an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

3. The method of claim 1 or claim 2, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

4. The method of claim 3, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5fFlurouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

5. The method of claim 3, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

6. The method of anyone of claims 1-5, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of the treatment.

7. A method of identifying an increased risk of developing an occult micrometastatic disease or detecting an occult micrometastatic disease in a subject with cancer or suspected of having a cancer, the method comprising detecting an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-

5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, wherein an elevated expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates an increased risk of developing an occult micrometastatic disease or the presence of an occult micrometastatic disease in the subject.

8. The method of claim 7, wherein the method comprises identifying an increased risk of developing an occult micrometastatic disease in a subject with cancer.

9. The method of claim 7, wherein the method comprises detecting an occult micrometastatic disease in a subject with cancer.

10. A method of diagnosing a subject with cancer as having an increased risk of developing an occult micrometastatic disease, wherein the method comprises detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, and diagnosing the subject as having an increased risk of developing an occult micrometastatic disease if an elevated microRNA expression level, relative to a control, is detected in the biological sample.

11. The method of anyone of claims 7-10, wherein the method further comprises administering to the subject neoadjuvant therapy.

12. The method of claim 11, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

13. The method of claim 12, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

14. The method of claim 12, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

15. The method of anyone of claims 7-14, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

16. A method of monitoring a subject having cancer for an increased risk of developing an occult micrometastatic disease, wherein the method comprises

- (i) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point; and
- (ii) (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a second time point subsequent to the first time point.

17. The method of claim 16, wherein the monitoring further comprises the use of ultrasound, computerized tomography (CT) scans, magnetic resonance imaging (MRI), positron emission tomography (PET) scans, and any combinations thereof.

18. The method of claim 16 or claim 17, wherein a subject with no elevated microRNA expression level at the second time point is monitored every 3 to 6 months for two years therefrom, and thereafter every six months for three additional years.

19. The method of claim 16 or claim 17, wherein detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease.

20. The method of claim 19, wherein if an elevated microRNA expression level in the biological sample is detected at the second time point compared to the microRNA expression level at the first time point, the method further comprises proposing neoadjuvant therapy to a subject with an increased risk of developing an occult micrometastatic disease.

21. The method of claim 20, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy,

hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

22. The method of claim 21, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

23. The method of claim 21, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

24. The method of anyone of claims 20-23, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

25. The method of anyone of claims 1-24, wherein the microRNA is exosomal microRNA.

26. The method of anyone of claims 1-25, wherein the cancer is pancreatic cancer.

27. The method of claim 26, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).

28. The method of claim 27, wherein the occult micrometastatic disease is pancreatic metastases.

29. The method of anyone of claims 1-28, wherein an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates that the subject is at high risk of cancer recurrence.

30. The method of anyone of claims 1-29, wherein the biological sample is a liquid biological sample.

31. The method of claim 30, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

32. A method of treating occult micrometastatic disease in a subject who has or is suspected of having a pancreatic cancer, the method comprising administering to the subject neoadjuvant therapy, wherein a biological sample obtained from the subject has an

elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof.

33. The method of claim 31, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

34. The method of claim 32 or claim 33, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

35. The method of claim 34, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

36. The method of claim 34, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

37. The method of anyone of claims 32-36, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

38. The method of anyone of claims 32-37, wherein the biological sample is a liquid biological sample.

39. The method of claim 38, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

40. A method of monitoring a subject having cancer or suspected of having a cancer for an increased risk of developing an occult micrometastatic disease and treating occult micrometastatic disease in the subject, wherein the method comprises

- (i) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a first time point;

- (ii) detecting an expression level of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof, in a biological sample obtained from the subject, at a second time point subsequent to the first time point; and
- (iii) treating the subject with neoadjuvant therapy if the microRNA expression level at the second time point is elevated relative to the microRNA expression level at the first time point.

41. The method of claim 40, wherein detection of an elevated microRNA expression level in the biological sample at the second time point compared to the microRNA expression level at the first time point indicates that the subject has an increased risk of developing an occult micrometastatic disease.

42. The method of claim 40 or claim 41, wherein the neoadjuvant therapy comprises an anticancer agent, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibitor administration therapy, synthetic lethality therapy, or any combination thereof.

43. The method of claim 42, wherein the chemotherapy comprises a regimen comprising gemcitabine plus nab-paclitaxel (GA); a combination of folinic acid, 5-Fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX); or nanoliposomal irinotecan (nal-IRI) in combination with 5-FU and leucovorin (LV).

44. The method of claim 42, wherein the targeted therapy comprises the use of an anti-VEGF antibody or antibodies combined to a modified FOLFIRINOX regimen.

45. The method of anyone of claims 40-44, wherein the method further comprises surgically removing all or portions of the cancer and micrometastatic tumors in the subject after completion of therapy.

46. The method of anyone of claims 40-45, wherein the microRNA is exosomal microRNA.

47. The method of anyone of claims 40-46, wherein the cancer is pancreatic cancer.

48. The method of claim 47, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).

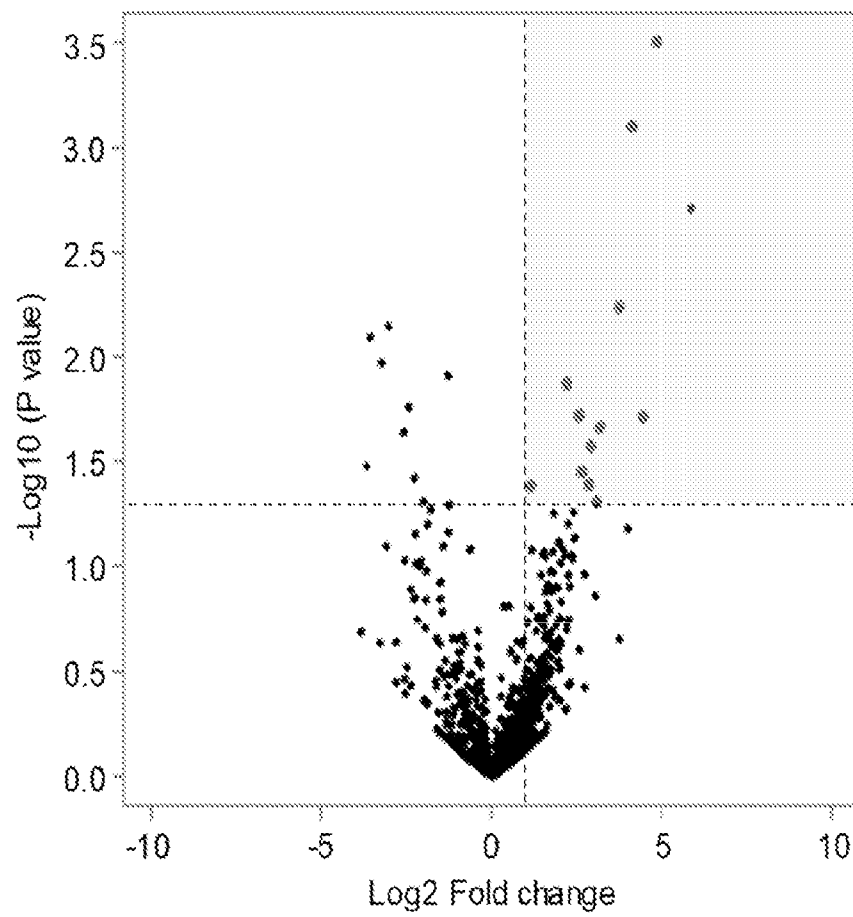
49. The method of claim 48, wherein the occult micrometastatic disease is pancreatic metastases.

50. The method of anyone of claims 40-49, wherein an elevated expression level, relative to a control, of a microRNA selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or a combination of two or more thereof indicates that the subject is at high risk of cancer recurrence.

51. The method of anyone of claims 40-50, wherein the biological sample is a liquid biological sample.

52. The method of claim 51, wherein the liquid biological sample is blood, plasma, serum, urine or saliva.

53. A kit comprising reagents capable of detecting an expression level of microRNA in a biological sample, wherein the microRNA is selected from the group consisting of miR-130b-5p, miR-133a-3p, miR-195-5p, miR-432-5p, miR-1229-3p, and miR-1273f, or any combination of two or more thereof.

**FIG. 1A**

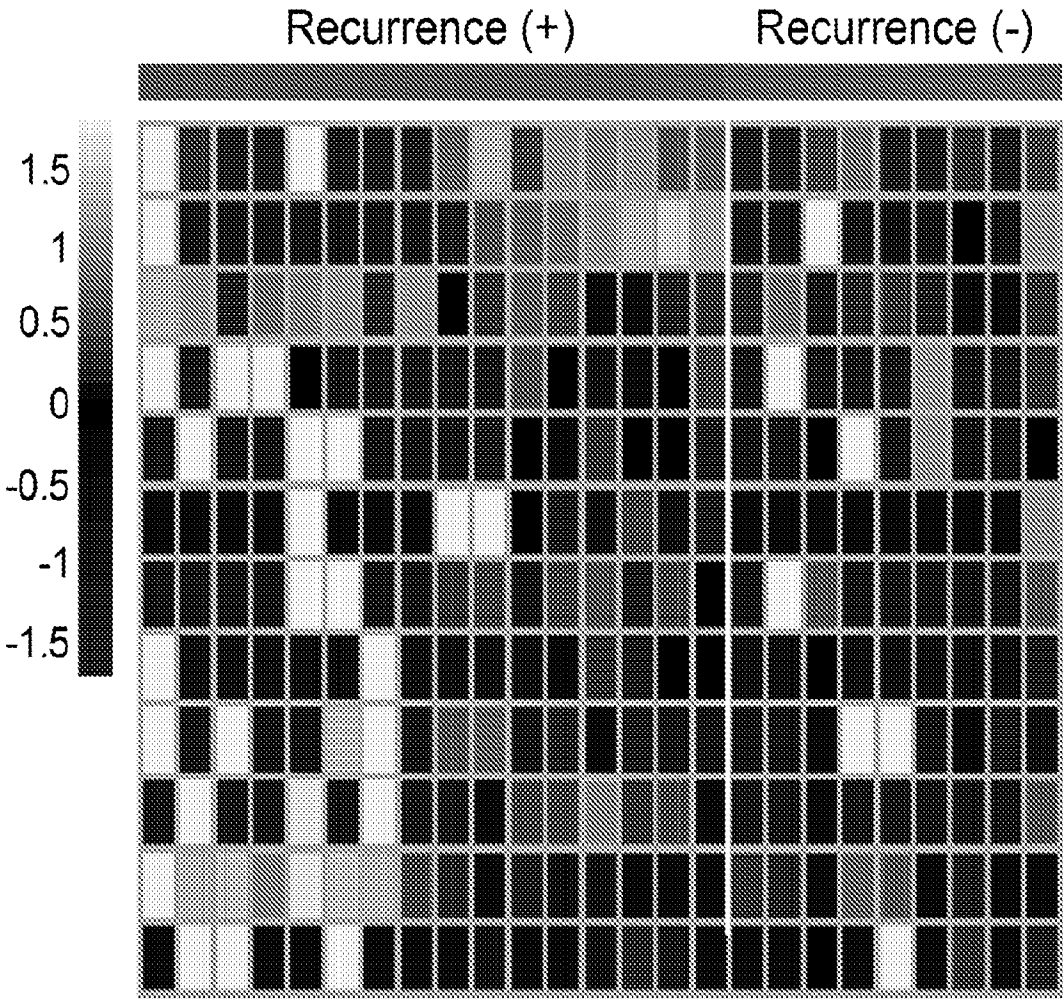
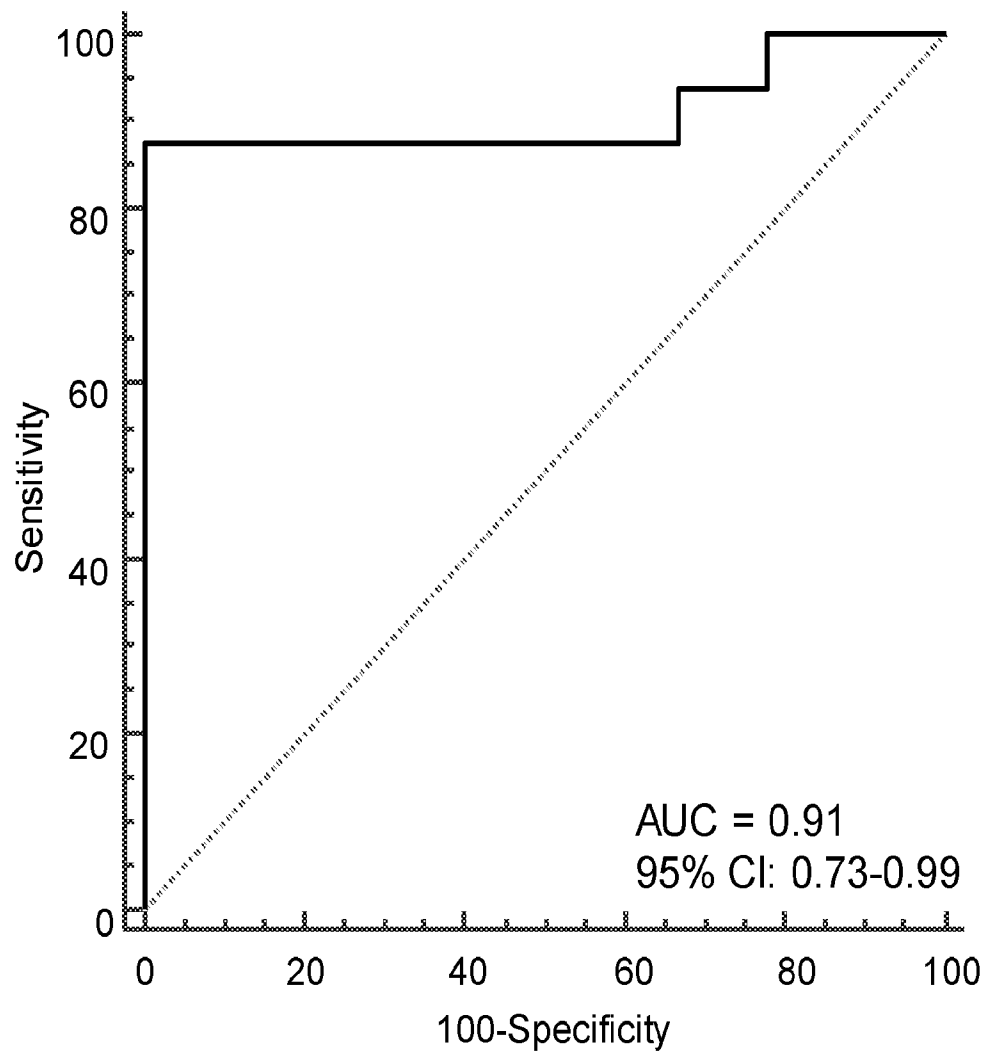


FIG. 1B

**FIG. 1C**

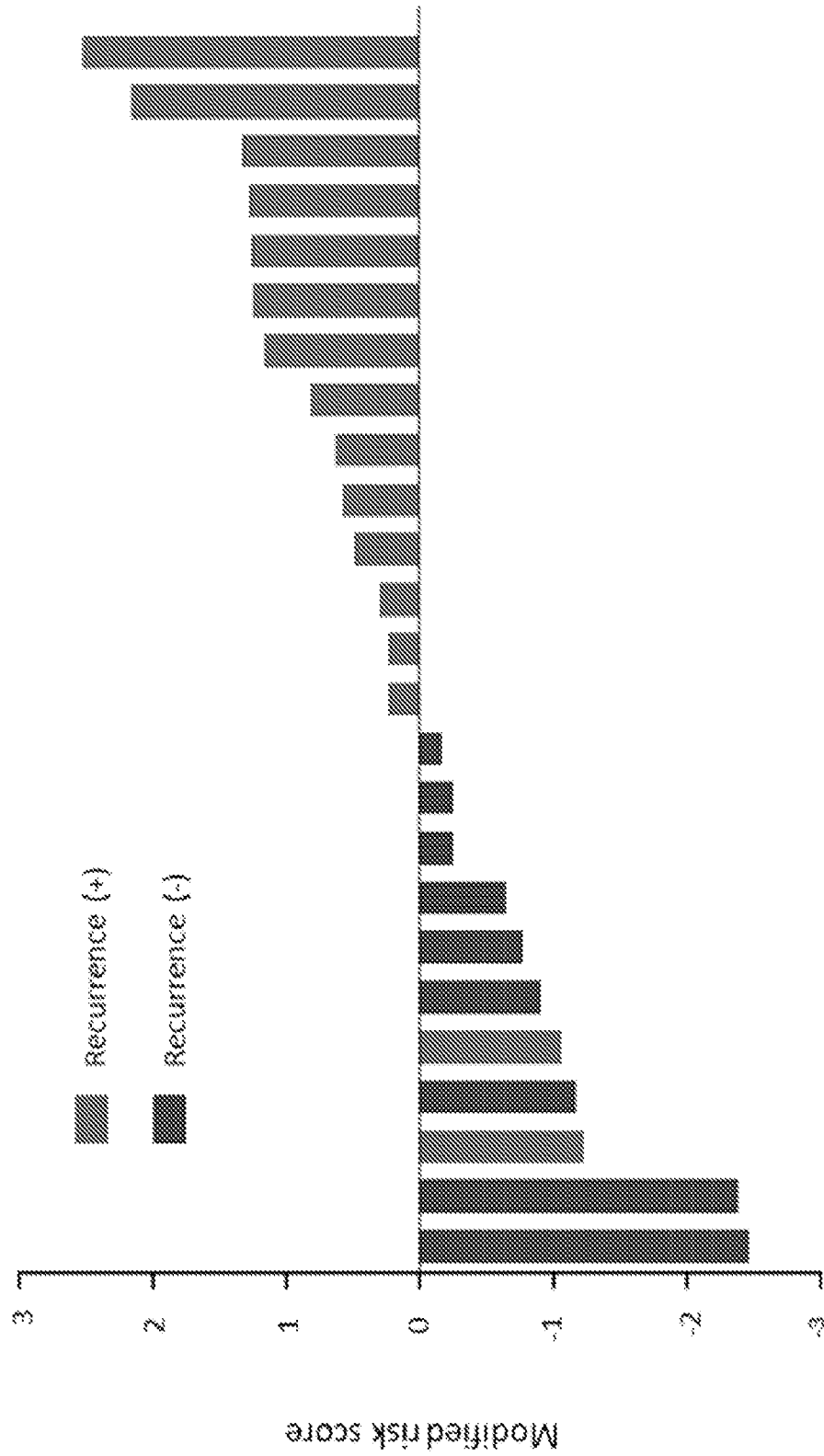


FIG. 1D

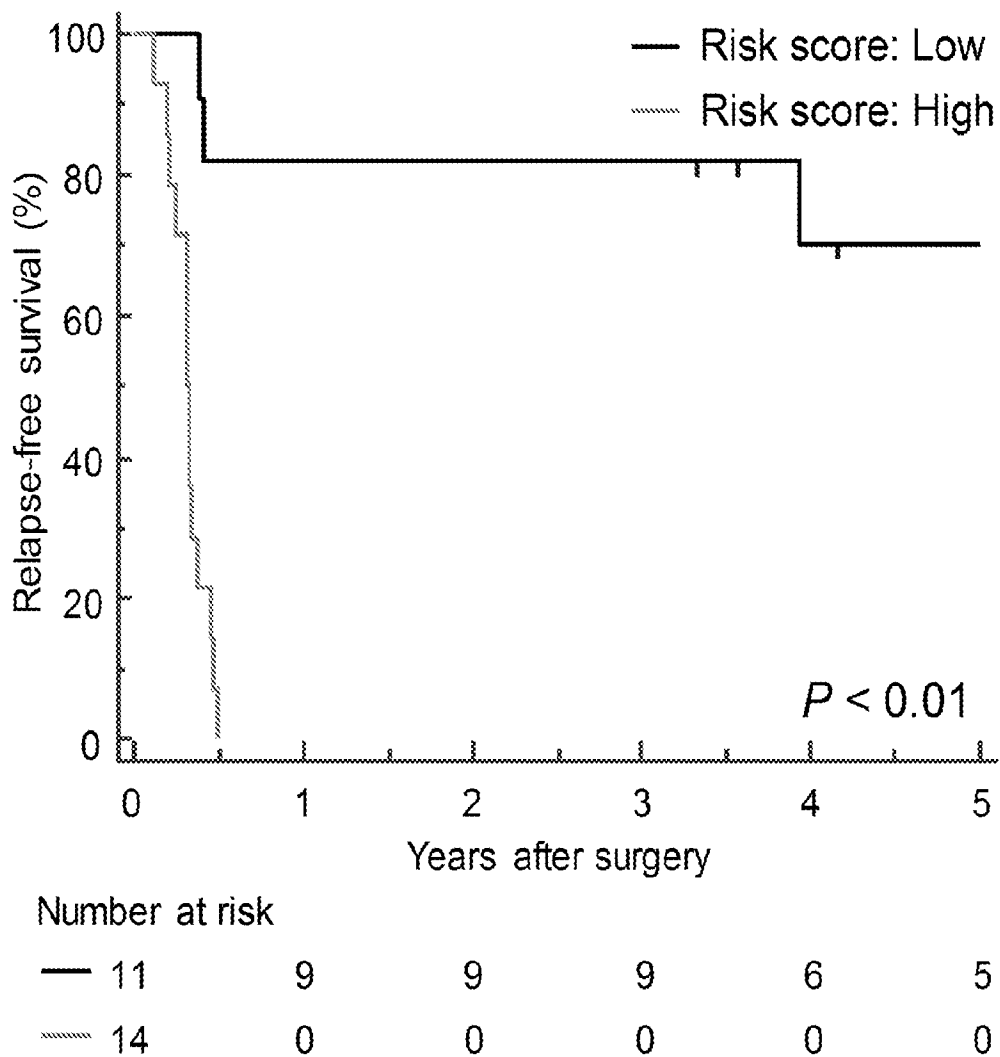
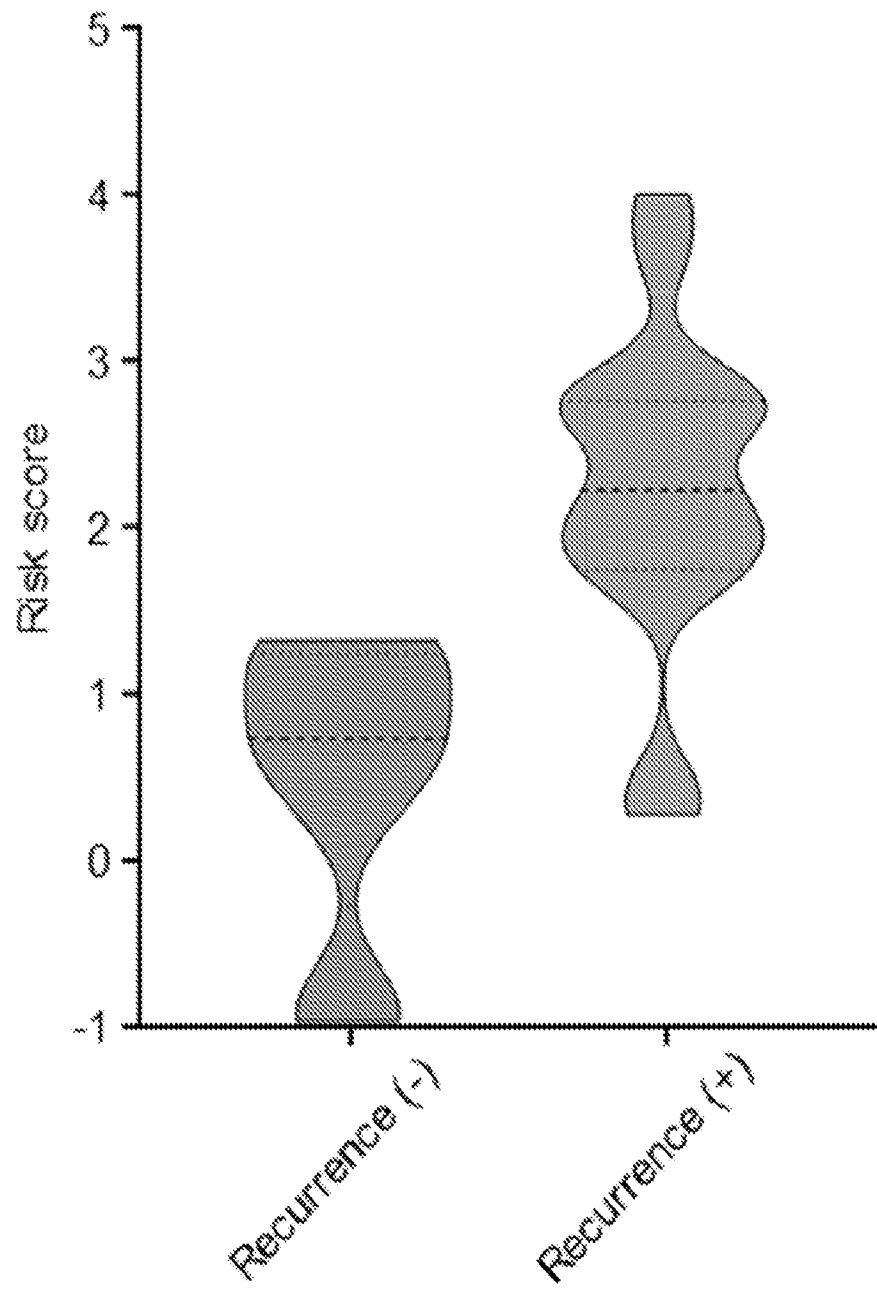
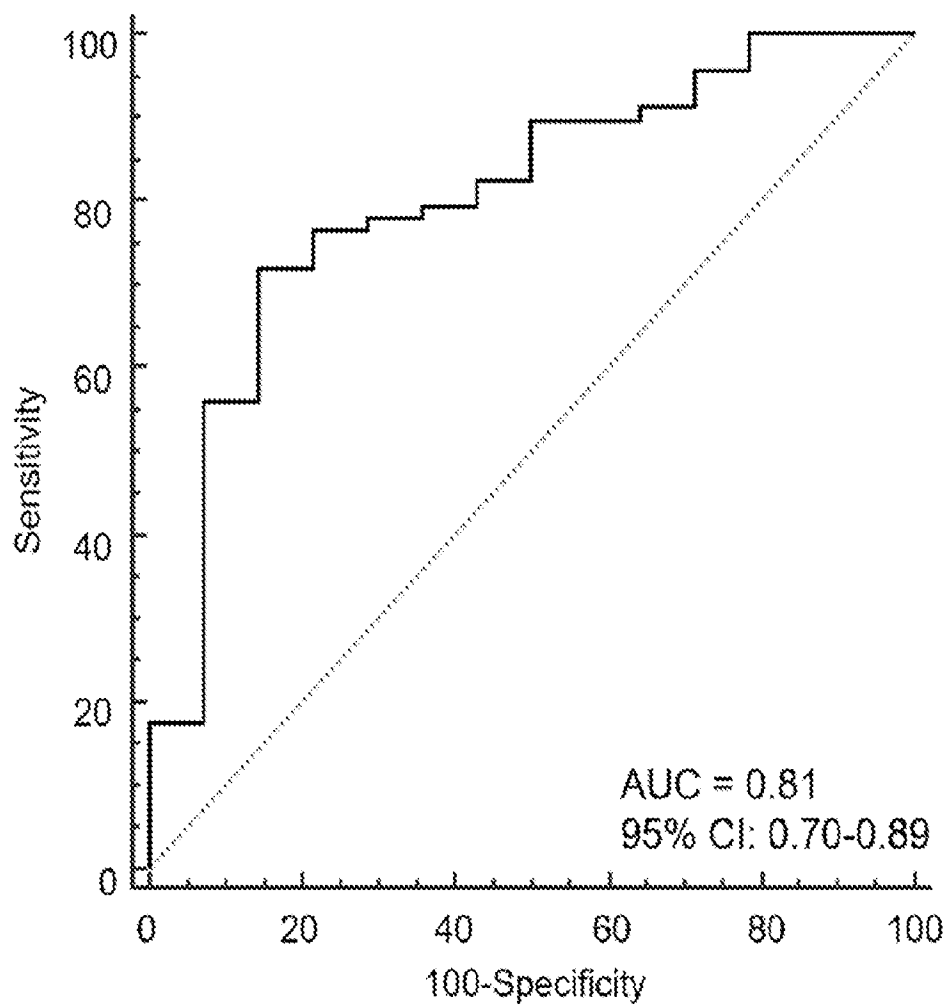
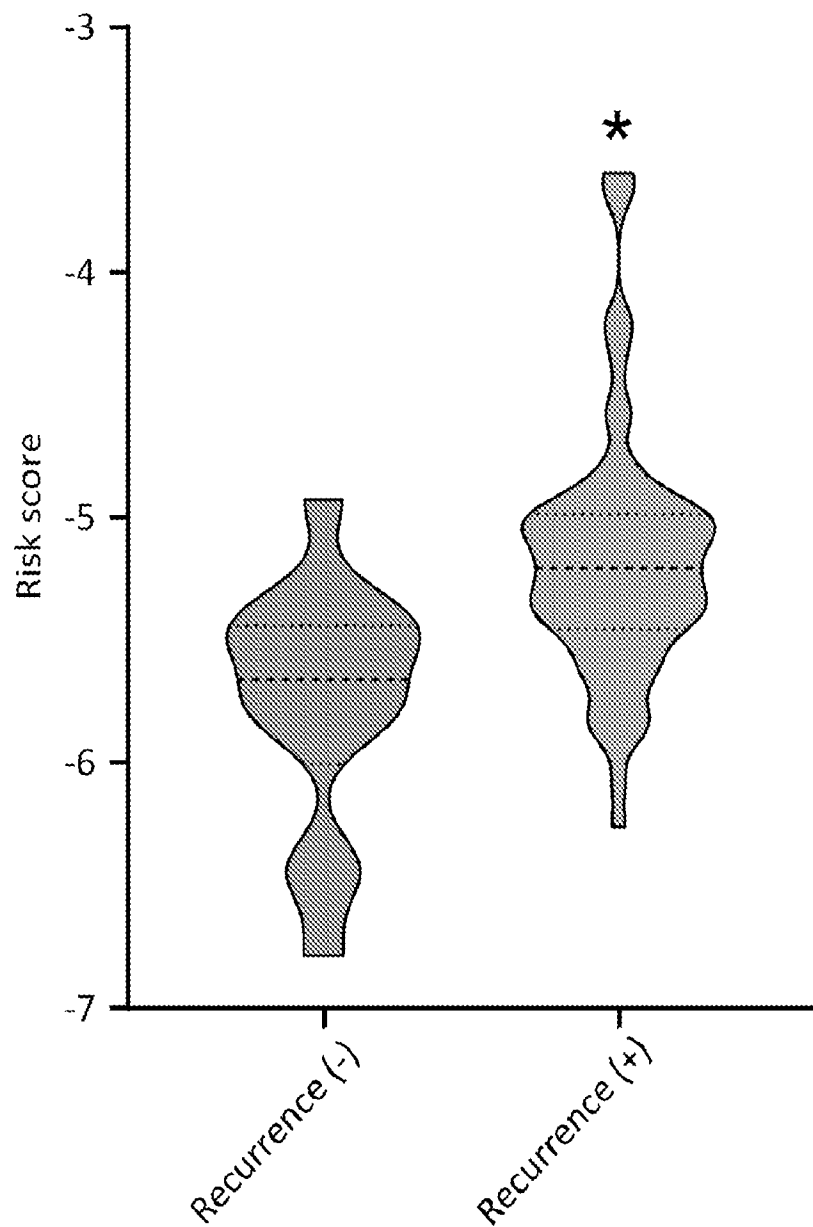


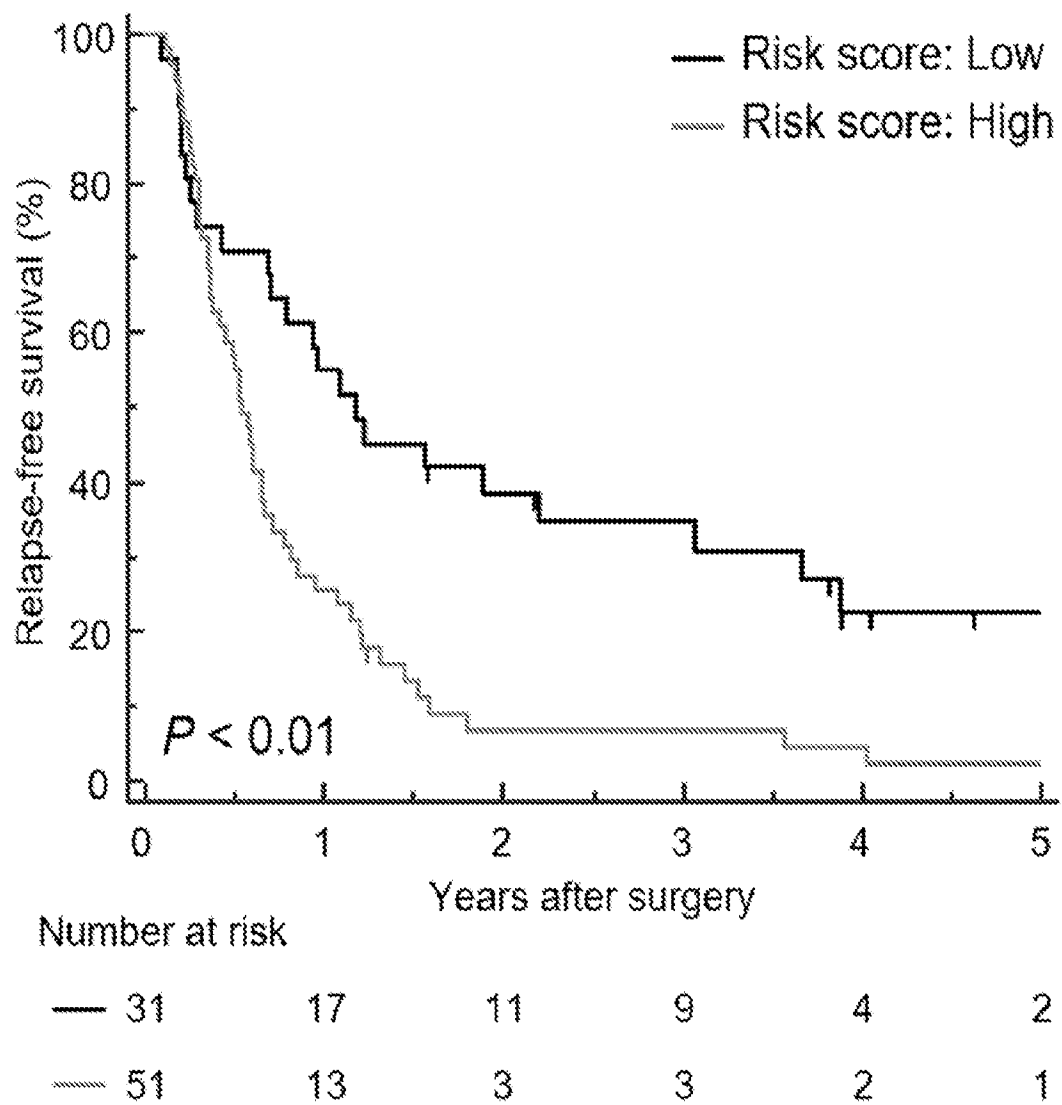
FIG. 1E

**FIG. 1F**

**FIG. 2A**

**FIG. 2B**

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**FIG. 2C**

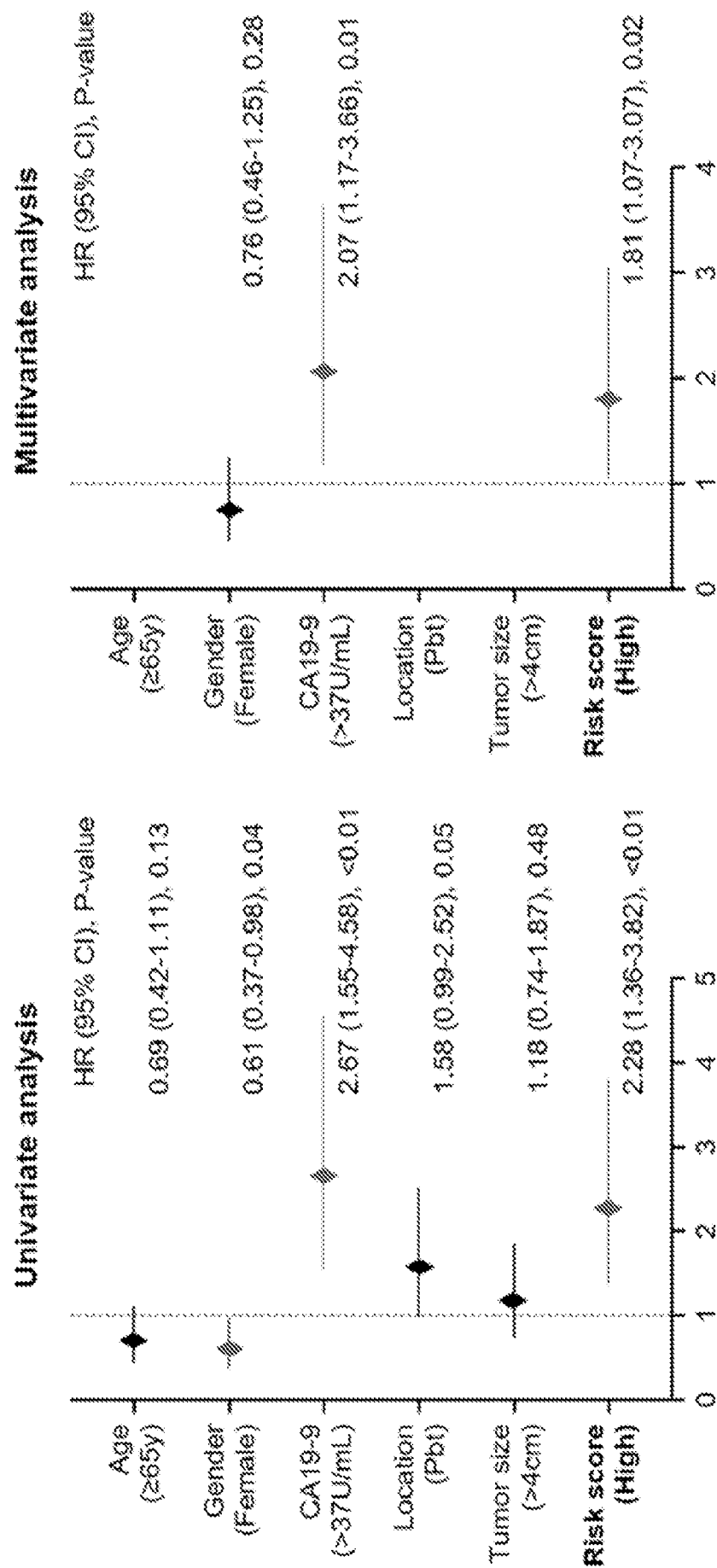
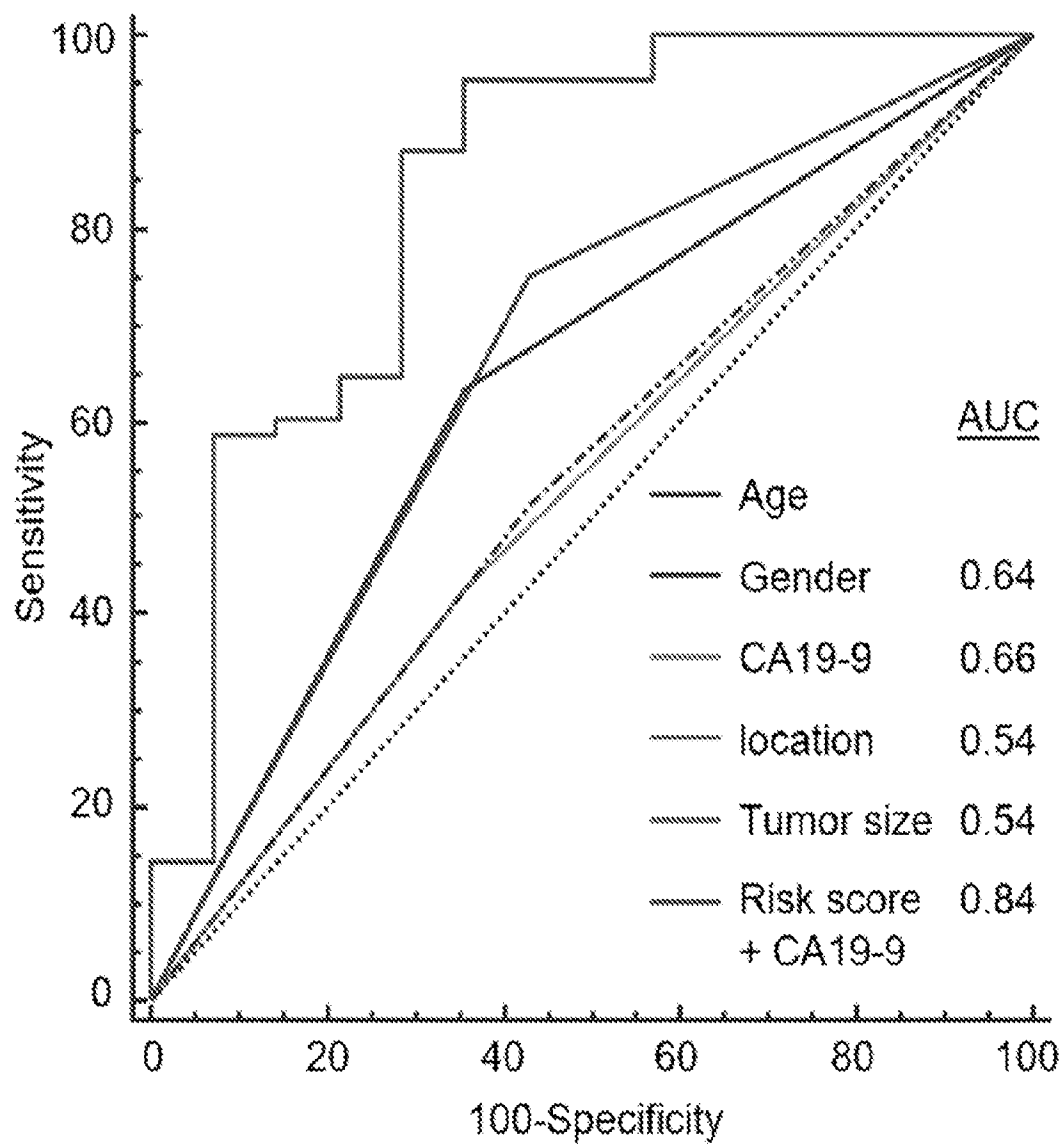
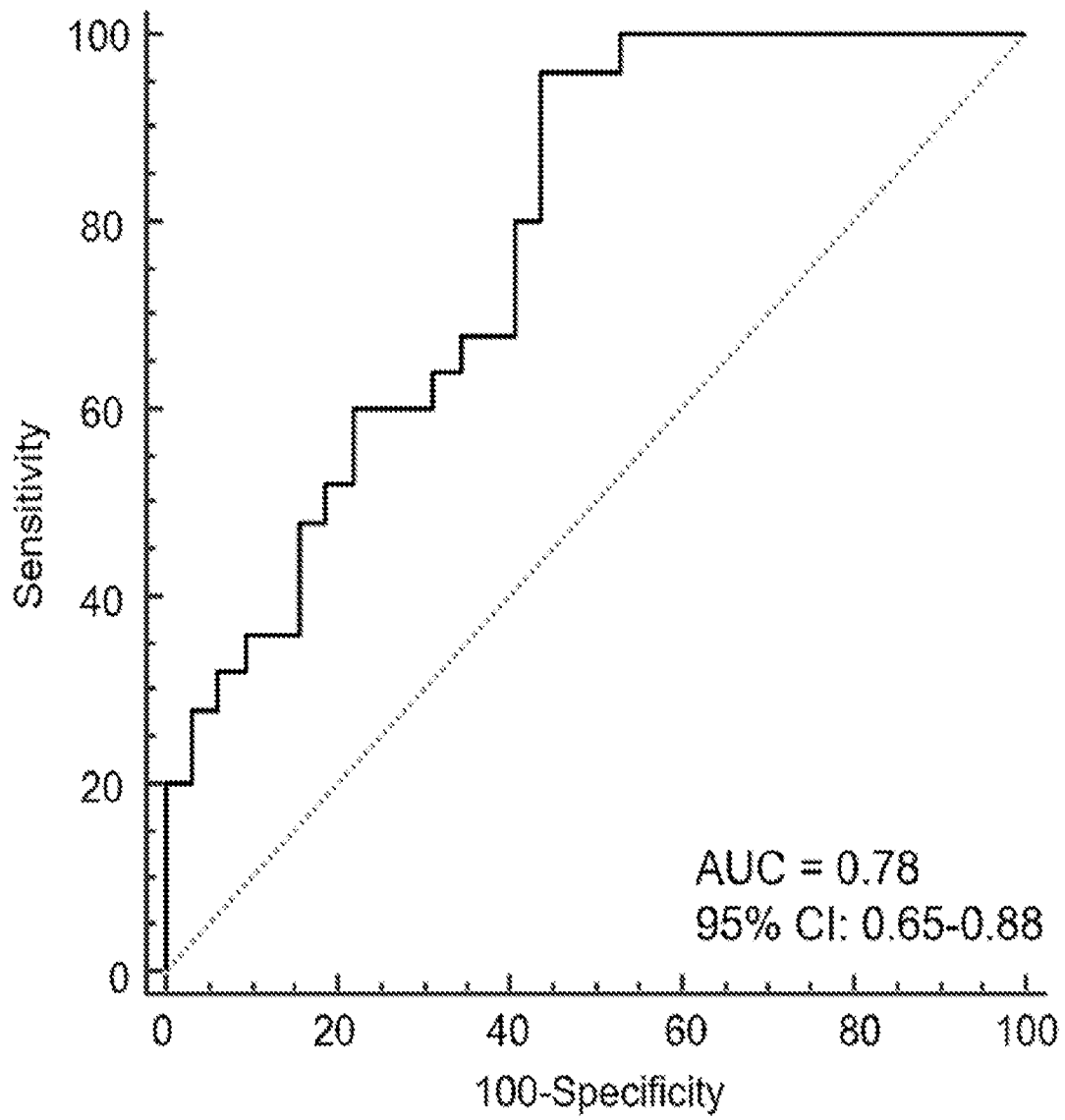
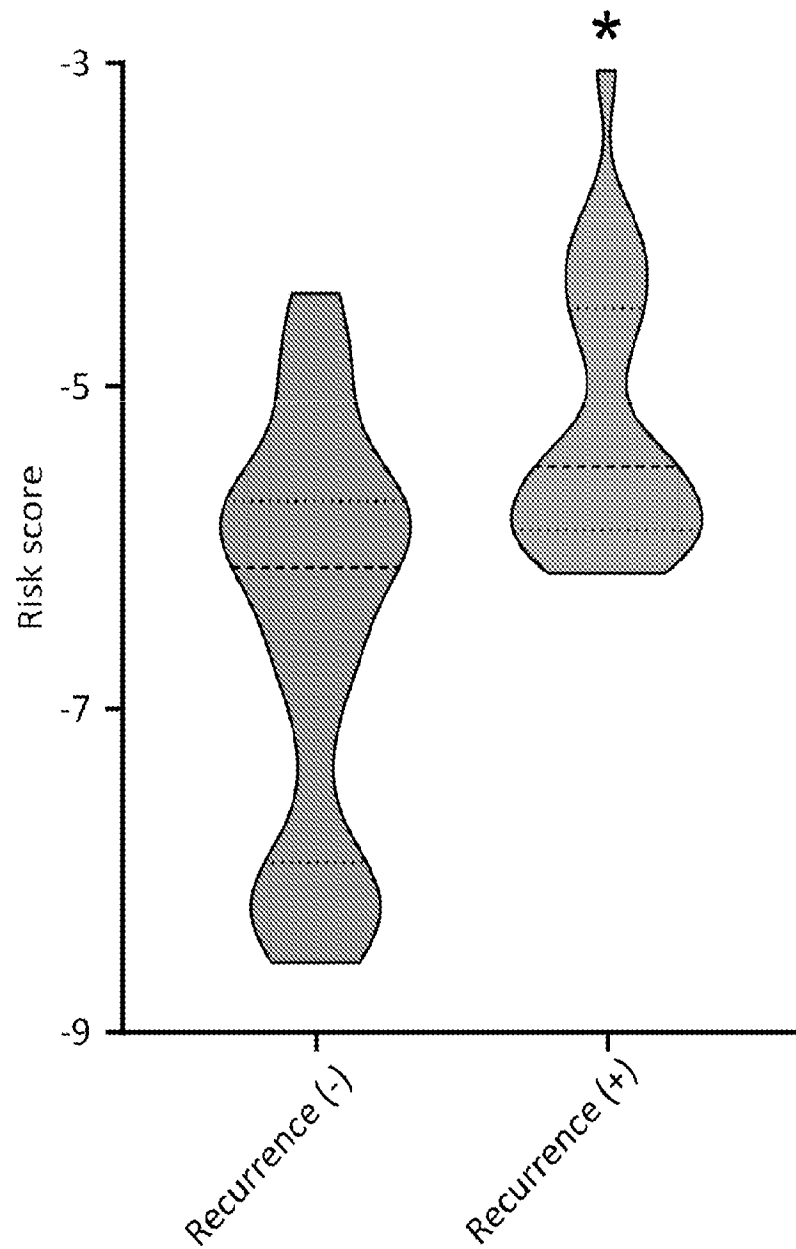


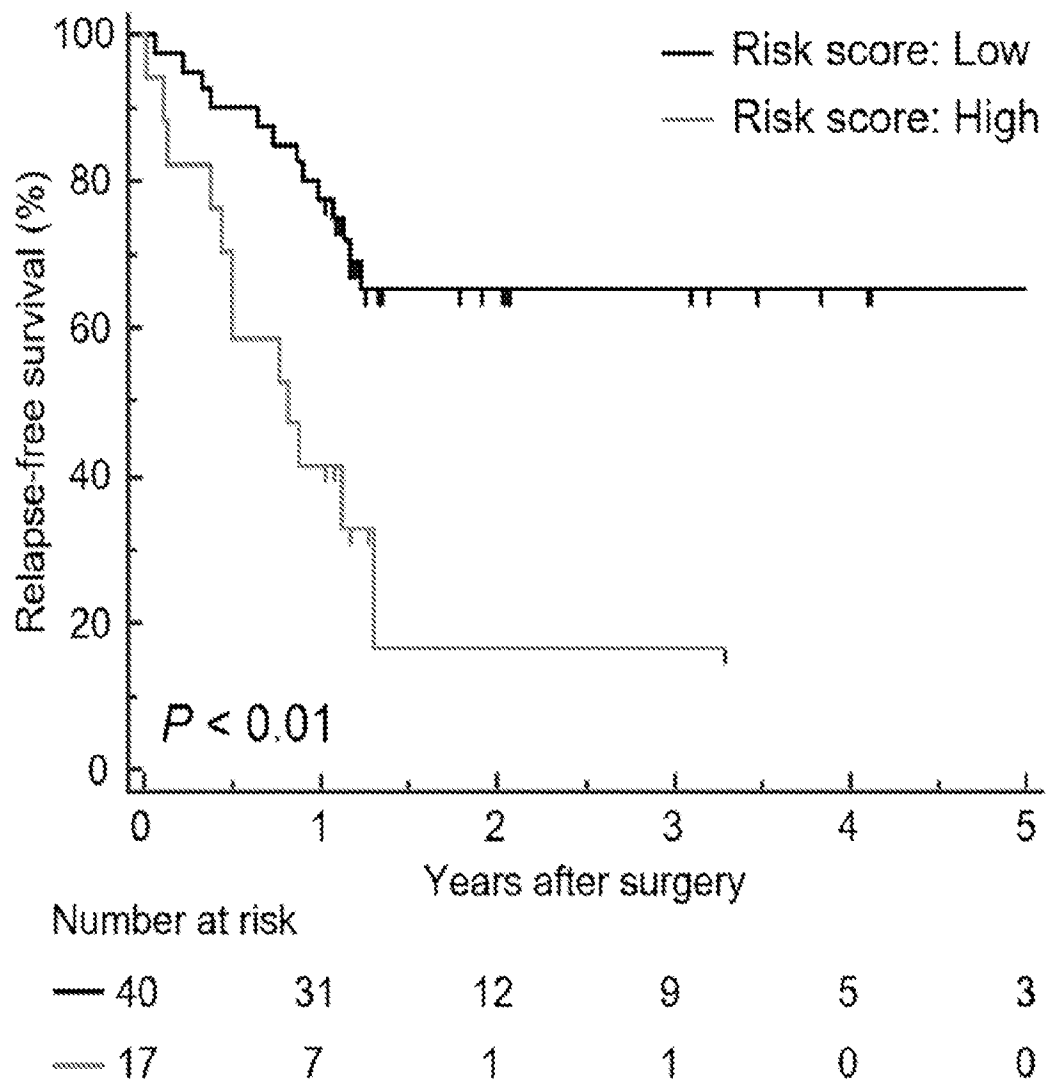
FIG. 2D

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**FIG. 2E**

**FIG. 3A**

**FIG. 3B**

**FIG. 3C**

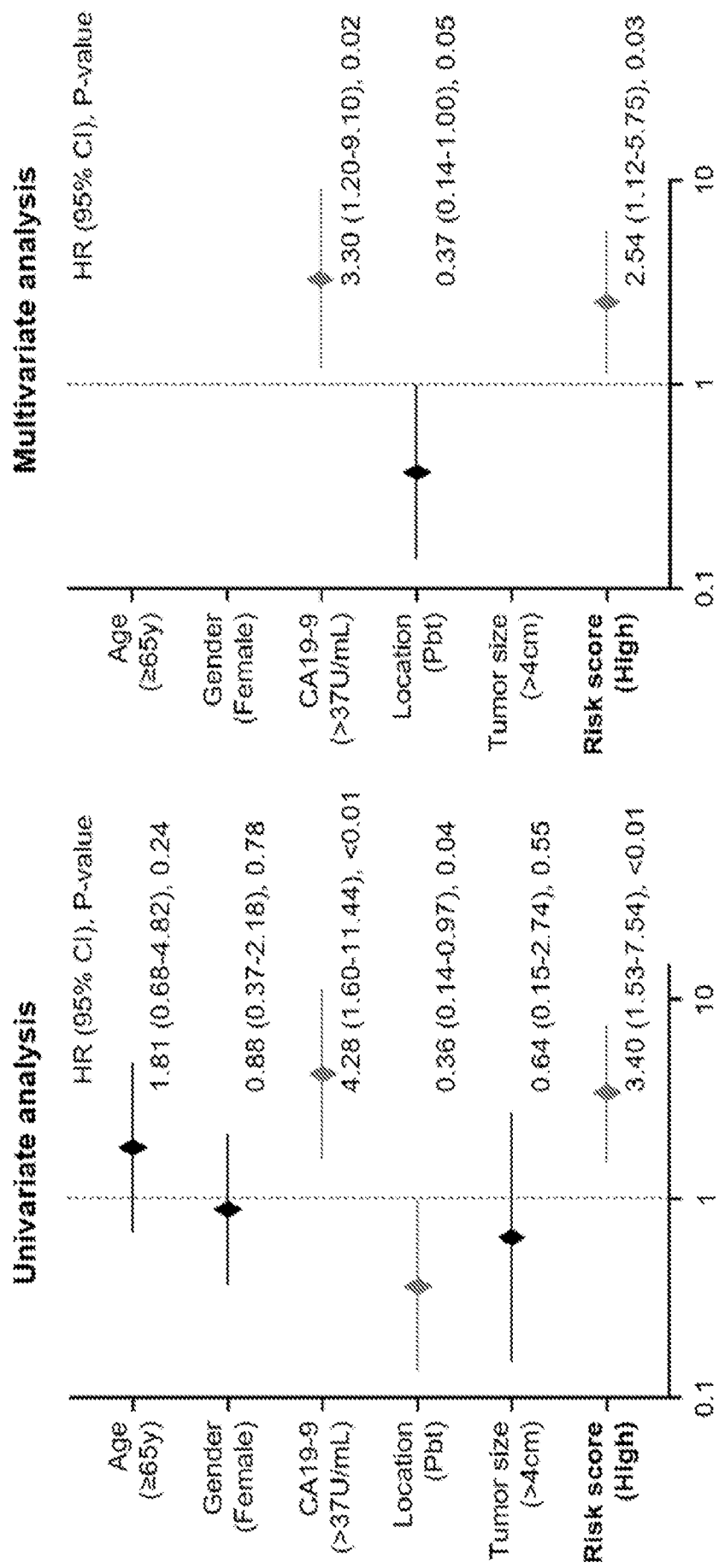


FIG. 3D

FIG. 3E

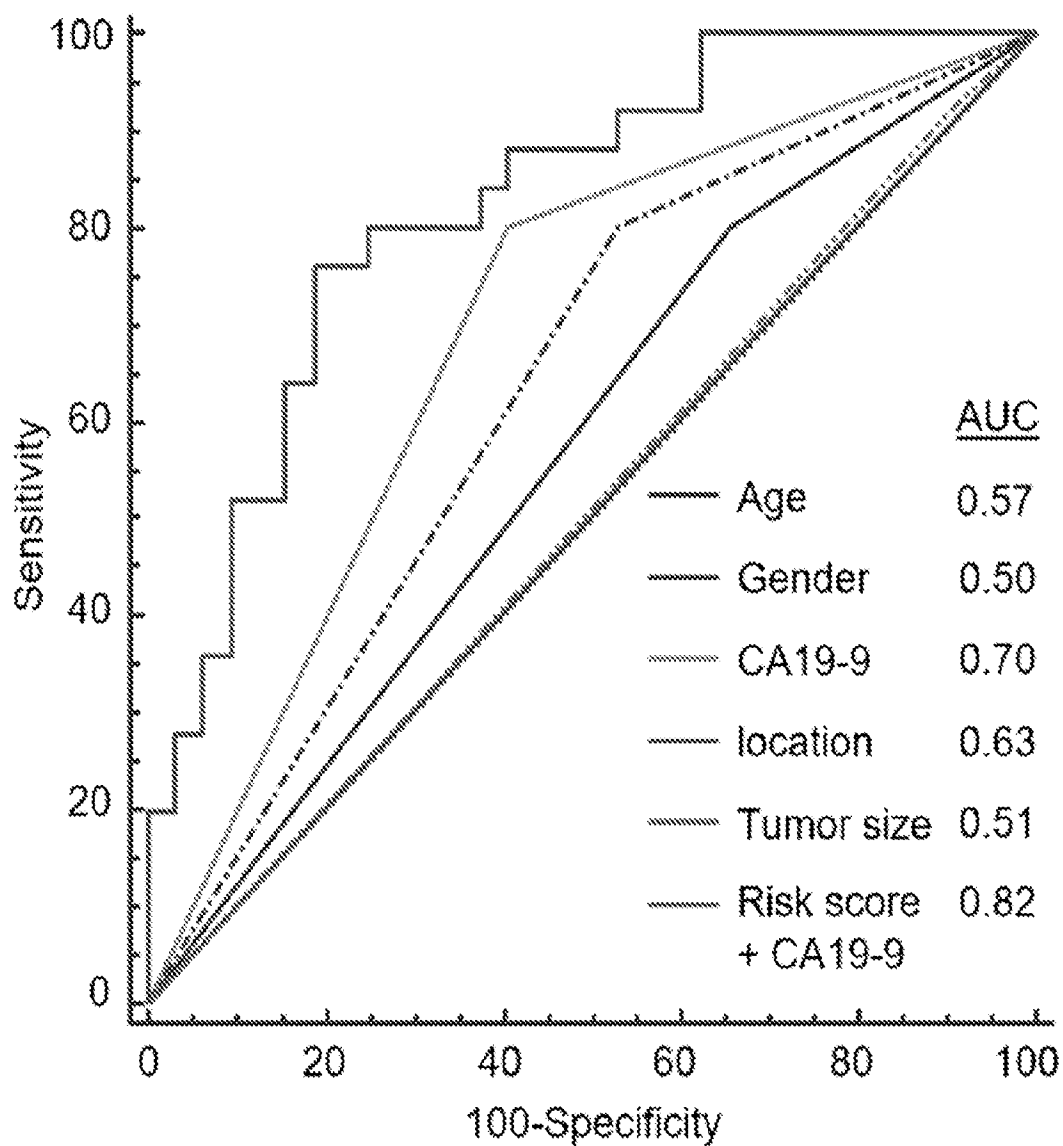


FIG. 3E

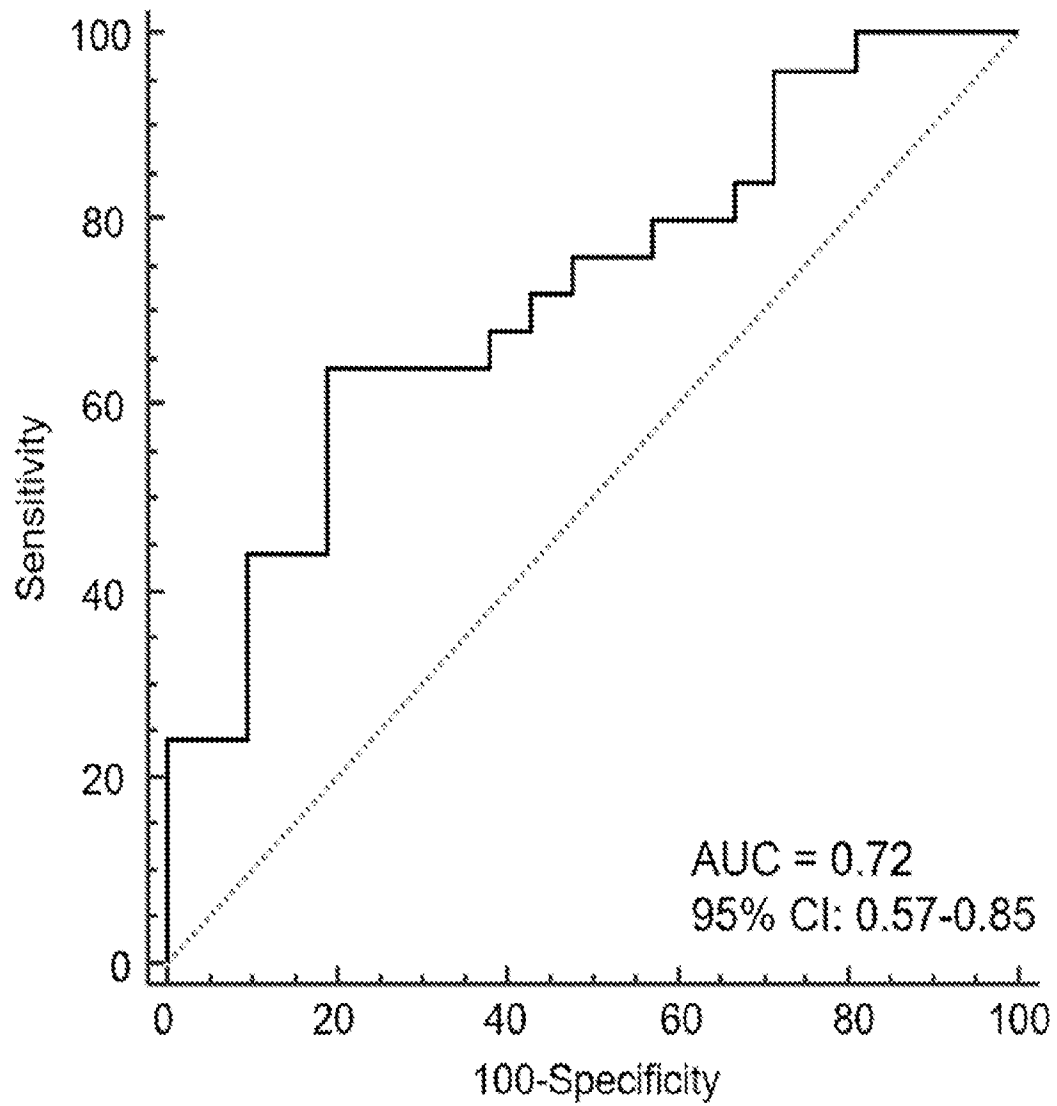
FIG. 4A**FIG. 4A**

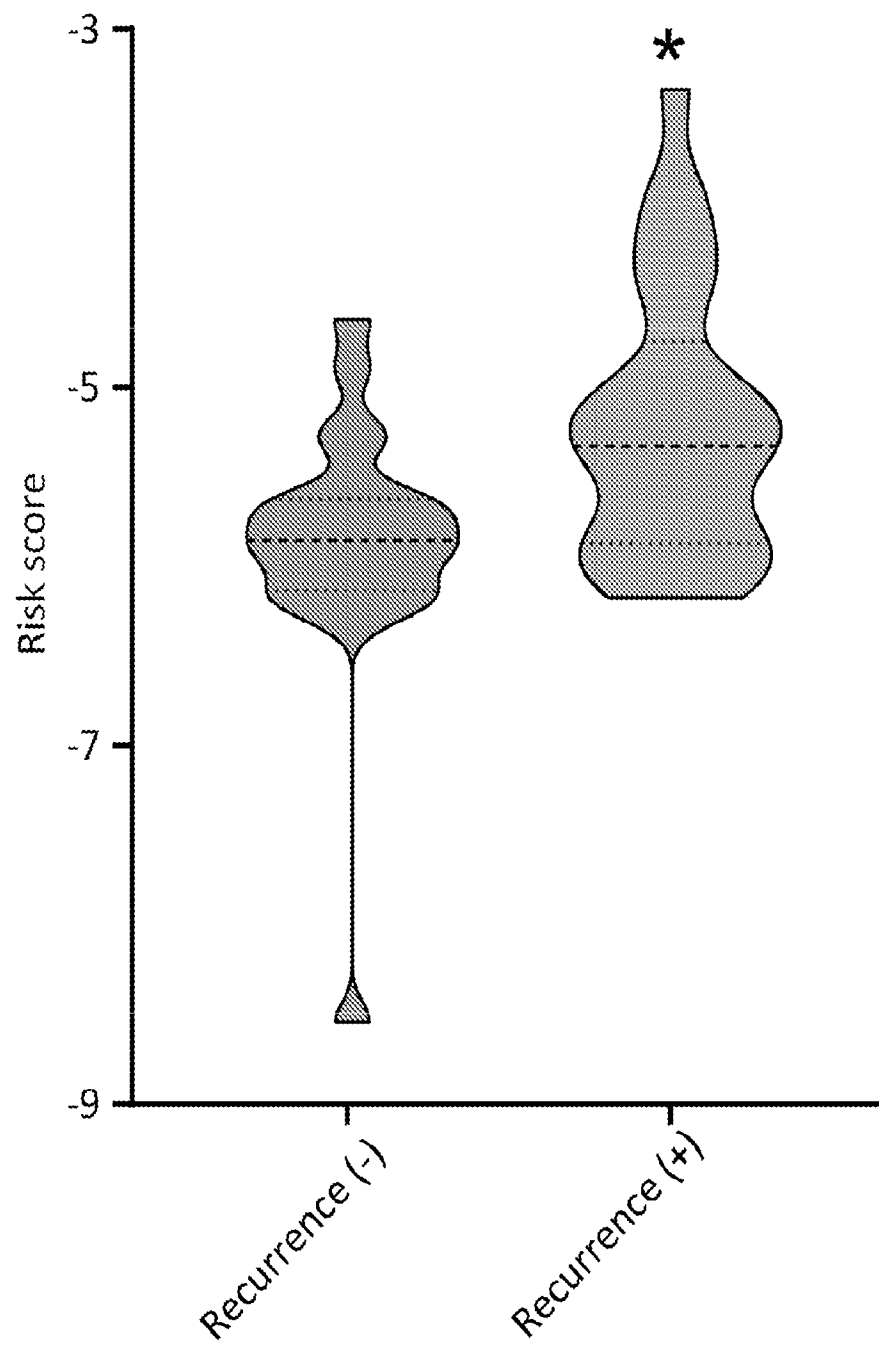
FIG. 4B**FIG. 4B**

FIG. 4C

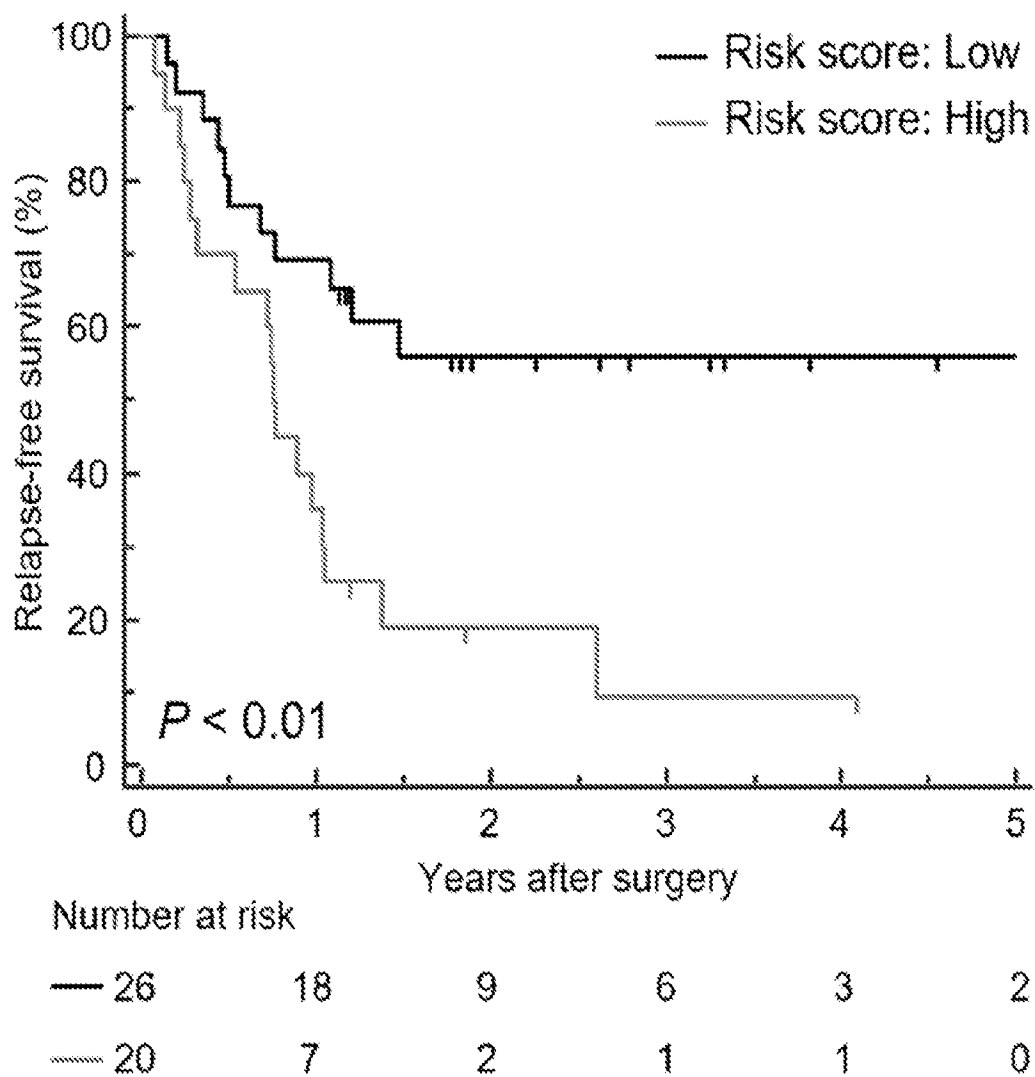


FIG. 4C

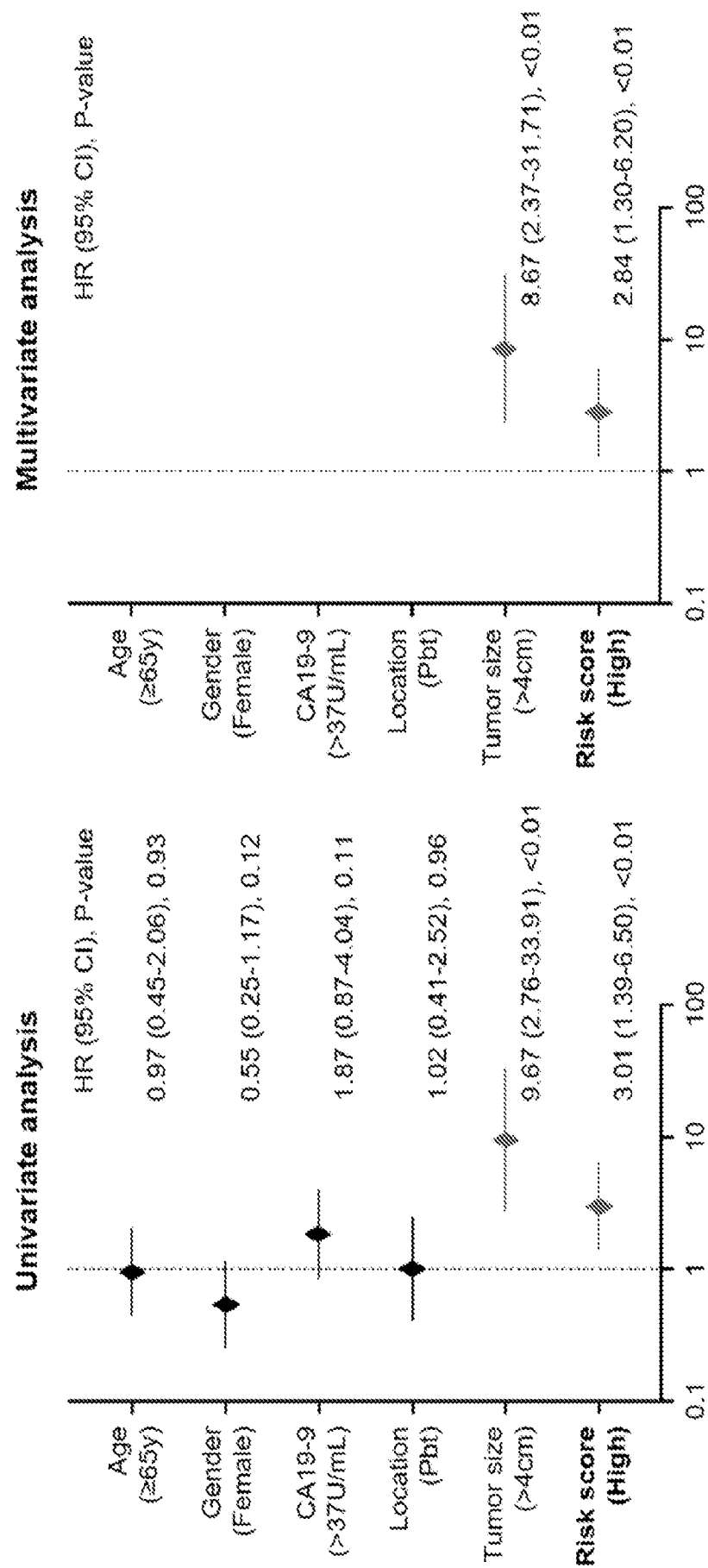


FIG. 4D

FIG. 4E

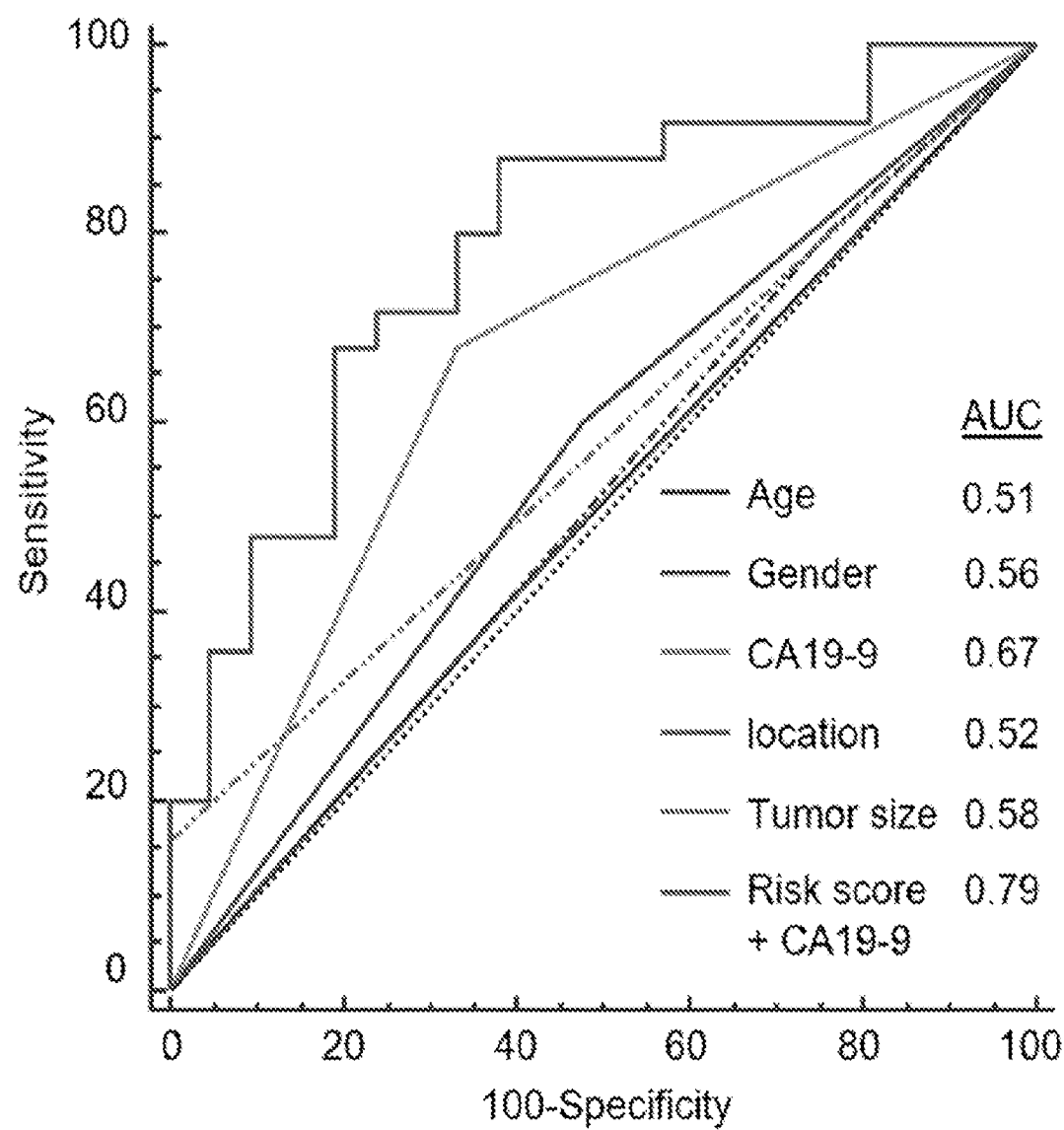


FIG. 4E

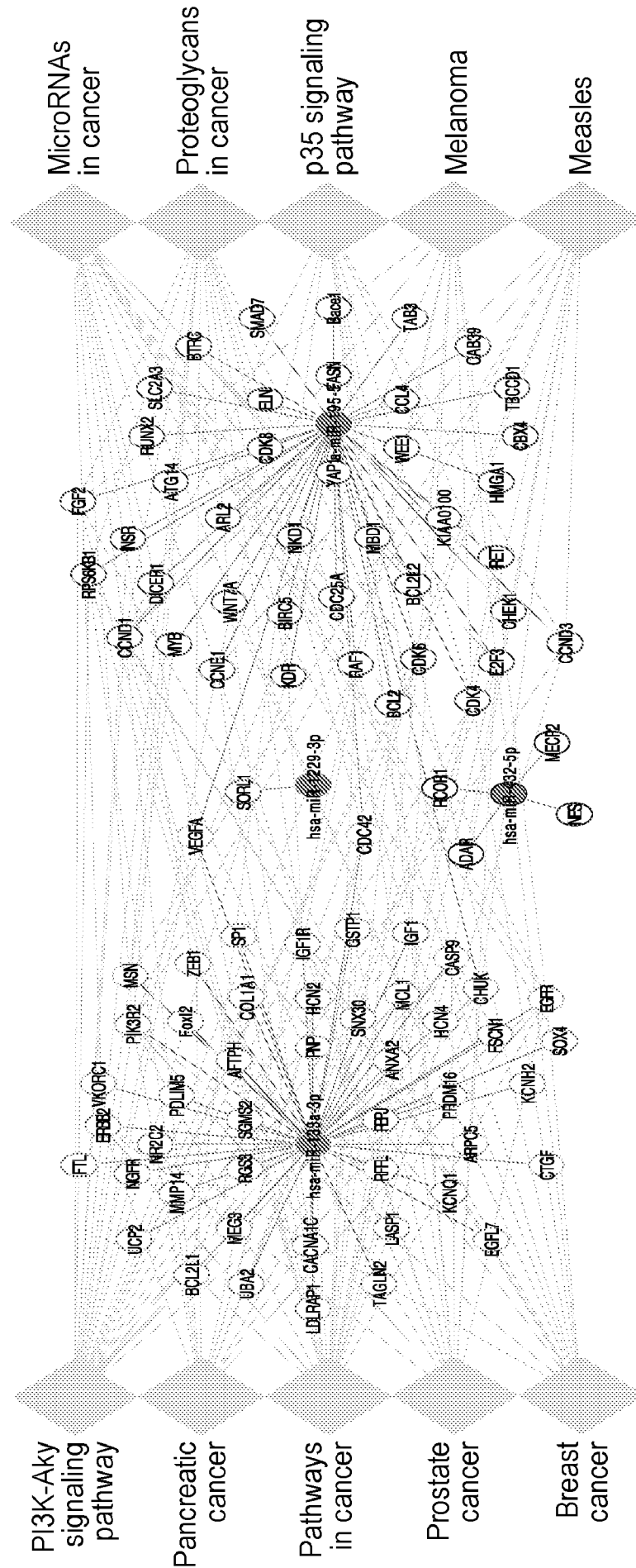
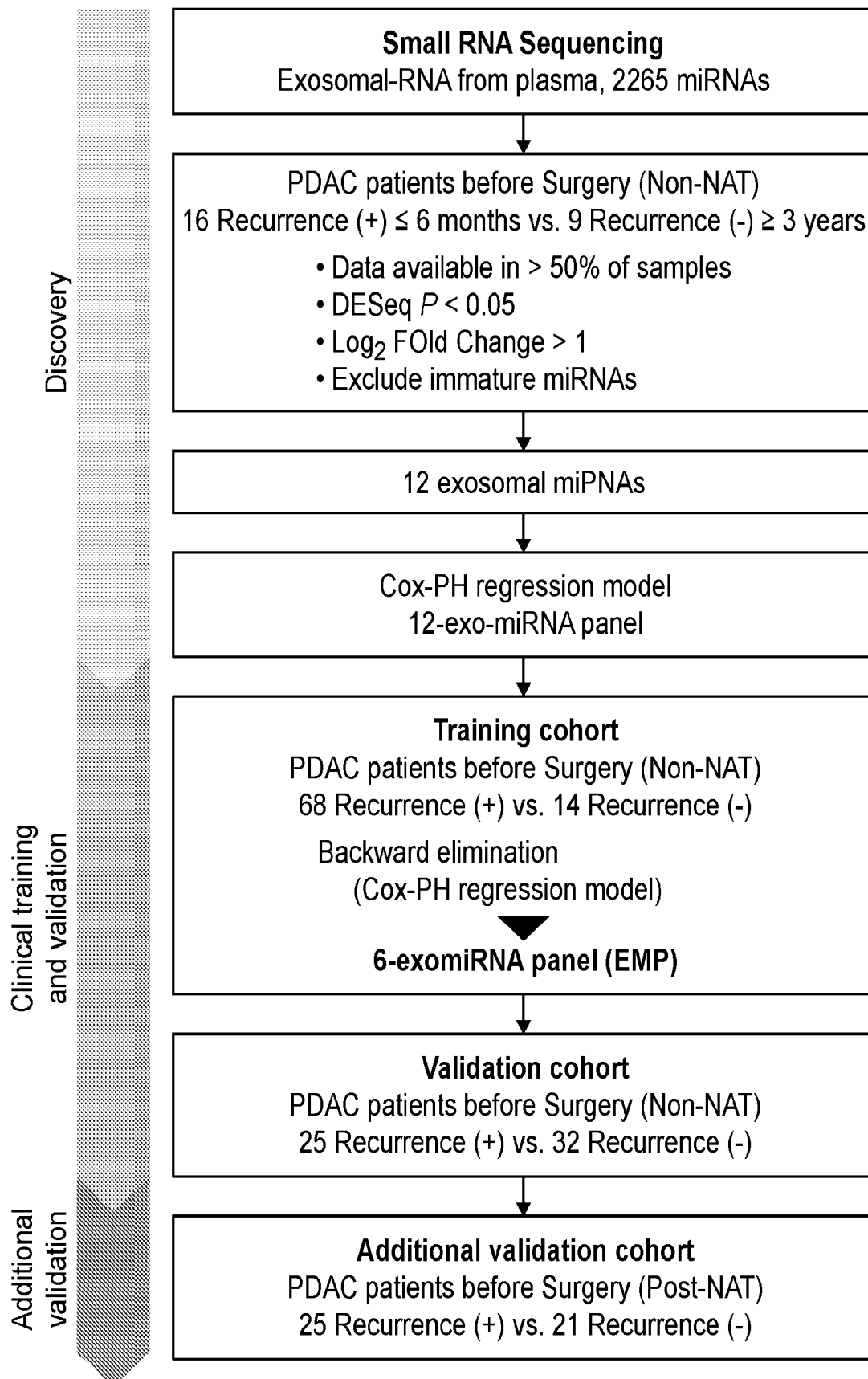


FIG. 5

**FIG. 6**