

CLAIMS:

1. A method for determining the likelihood that a patient has ~~or will develop~~ pancreatic ductal adenocarcinoma (PDAC) ~~or will in a patient~~, comprising:

(a) ~~providing~~ extracting a cell-free (cf)DNA sample from a blood sample obtained from the patient;

(b) sequencing the patient cfDNA in a manner that identifies 5-hydroxymethylcytosine (5hmC) ~~residues-containing sequences~~ in the cfDNA;

(c) mapping the identified ~~5hmC-residues~~ hydroxymethylated sequences to each of a plurality of reference loci in a reference hydroxymethylation profile, wherein each reference locus:

(i) serves as a hydroxymethylation biomarker that exhibits at least a 1.5-fold differential 5hmC representation between PDAC samples and normal samples and a $\log_2$ [average 5hmC representation] of at least 4, and:

~~comprising a~~ (ii) comprises a sequence contained within a gene feature selected from a 3'UTR, a TTS, an intron, and a promoter, wherein the gene feature is associated with a gene implicated in pancreatic development, with a gene related to cancer development, or with a gene established to exhibit increased or decreased hydroxymethylation density in PDAC;

(d) determining differences in extent ~~and location between~~ of hydroxymethylation between the identified hydroxymethylated sequences in ~~of the patient cfDNA and the reference hydroxymethylation profile at each locus loci;~~ and

(e) using the extent ~~and location of~~ the differences in combination with at least one additional parameter correlated with a risk of developing PDAC, calculating a probability score representing the likelihood that the patient has or is at risk of developing PDAC.

2. ~~The method of claim 1, wherein the probability score is calculated using the extent and location of the differences in combination with at least one additional parameter correlated with a risk of developing PDAC.~~

~~32.~~ The method of ~~claim 2~~ claim 1, wherein the at least one additional parameter is selected from lesion size; lesion location; presence or absence of pancreatic inflammation; jaundice; presence or absence of other symptoms; patient age; weight; gender; ethnicity; family history; genetic mutations; diabetes; physical activity; diet; pro-inflammatory cytokine levels; and smoking status of the patient.

~~4. The method of claims 1, 2, or 3, wherein the reference hydroxymethylation profile is a data set comprising a composite of hydroxymethylation profiles for a population group of individuals who have at least one shared characteristic.~~

~~5. The method of claim 4, wherein the at least one shared characteristic comprises having been diagnosed with PDAC, having been diagnosed with pancreatitis, having been diagnosed with diabetes, a family history of PDAC, cigarette smoking status, and age range.~~

~~6. The method of any one of claims 1 through 5, wherein the cell free DNA sample is extracted from a blood sample.~~

~~7. The method of any one of claims 1 through 5, wherein the cell free DNA sample is extracted from pancreatic cyst fluid.~~

~~83.~~ The method of ~~any one of claims 1 through 7,~~ wherein the hydroxymethylation biomarkers comprise loci that are claim 1 or claim 2, wherein each gene feature is associated with one or more of the following genes:
GATA4, GATA6, PROX1, ONECUT2, YAP1, TEAD1, ONECUT2/ONECUT1-TCGA, IGF1, and IGF2.

~~94.~~ The method of ~~any one of claims 1 through 7,~~ wherein the hydroxymethylation biomarkers comprise loci that are claim 1 or claim 2, wherein each gene feature is associated with a possible mutation in a gene selected from *BRCA2, BRCA1, CDKN2A, ATM, STK11, PRSS1, MLH1, PALB2, KRAS, CDKN2A, TP53, SMAD4,* and combinations thereof.

~~105.~~ The method of claim 1, wherein the patient has had an imaging scan in which a pancreatic lesion was identified and step (c) comprises generating an initial patient hydroxymethylation profile.

~~11. The method of claim 10, wherein the reference hydroxymethylation profile represents a composite of hydroxymethylation profiles for a plurality of individuals who have had a pancreatic lesion identified in an imaging scan.~~

~~126.~~ The method of ~~claim 11~~ claim 5, further including ascertaining a change in the pancreatic lesion by repeating steps (a) through (e) at a later time to generate a later patient hydroxymethylation profile and comparing the later hydroxymethylation profile with the initial patient hydroxymethylation profile to ascertain hydroxymethylation profile changes.

~~137.~~ The method of claim ~~126~~, further including making a determination whether to implement a change in treatment of the patient based on the hydroxymethylation profile changes.

~~148.~~ The method of any one of claims ~~1 through 13~~ 1 to 7, wherein the probability score is calculated using a logistic regression analysis of the differences in hydroxymethylation level at each of the hydroxymethylation biomarkers.

~~159. A method for reducing the risk that a pancreatic lesion surgically removed from a patient is benign, comprising, prior to surgery,~~

- ~~(a) providing a cell-free (cf)DNA sample obtained from the patient;~~
- ~~(b) sequencing the patient cfDNA in a manner that identifies 5-hydroxymethylcytosine (5hmC) residues in the cfDNA;~~
- ~~(c) mapping the identified 5hmC residues to each of a plurality of loci in a reference hydroxymethylation profile, wherein each locus serves as a hydroxymethylation biomarker comprising a gene feature selected from a 3'UTR, a TTS, an intron, and a promoter, the gene feature associated with a gene implicated in pancreatic development, a gene related to cancer development, or a gene established to exhibit hyper-hydroxymethylation in PDAC;~~

~~(d) determining differences in extent and location between hydroxymethylation of the patient cfDNA and the reference hydroxymethylation profile at each locus; and~~

~~(e) using the extent and location of the differences, calculating a probability score representing the likelihood that the pancreatic lesion is benign; and~~

The method of any one of claims 1 to 8, further including:

~~(f) carrying out surgical resection of the pancreatic lesion only if the probability score is greater than a value corresponding to a low risk of cancer, a surgical resection of the pancreatic lesion is to be carried out.~~

~~16. A kit for determining the presence of or likelihood of developing pancreatic ductal adenocarcinoma (PDAC) in a patient, comprising:~~

~~at least one reagent for the determination of hydroxymethylation level at each of a plurality of hydroxymethylation biomarker loci in a cell-free DNA sample;~~

~~a solid support for capturing affinity-tagged 5hmC-containing cell-free DNA in the sample; and~~

~~written instructions for the use of the at least one reagent and the solid support in carrying out the method of claim 1.~~

~~17. The method of claim 1, comprising determining the likelihood that an individual at risk for developing PDAC has PDAC.~~

~~18~~10. The method of ~~claim 17~~ any one of claims 1 to 9, further including determining the likelihood that the individual has at least one additional type of cancer.

~~19~~11. The method of claim ~~18~~10, wherein the at least one additional type of cancer is selected from: bladder cancer; cancers of the blood and bone marrow; brain cancer; breast cancer; cervical cancer; colorectal cancer; esophageal cancer; liver cancer; lung cancer; ovarian cancer; prostate cancer; renal cancer; skin cancer; testicular cancer; thyroid cancer; and uterine cancer.