

CLAIMS

1. A method of evaluating a subject for the presence of each of a plurality of cancers, comprising:
 - (a) evaluating a biological sample obtained from the subject for the presence of one or more driver gene mutations in each of a plurality of driver genes, wherein each driver gene is associated with the presence of a cancer in the plurality of cancers, and wherein evaluating comprises sequencing a plurality of amplicons comprising the plurality of driver genes such that the sensitivity of detection of each cancer in the plurality of cancers is not increased by increasing the number of amplicons sequenced; and
 - (b) evaluating the level of one or more protein biomarkers in a biological sample,
 - wherein the presence of a cancer of the plurality of cancers is identified when the presence of one or more driver gene mutations and the level of one or more protein biomarkers is detected,
 - thereby evaluating the subject for the presence of each of the plurality of cancers.
2. The method of claim 1, wherein raising the number of amplicons above a threshold level would not detect substantially more cancers but would increase the probability of false positive results.
3. The method of claim 1 or 2, wherein about 60 amplicons (e.g., 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 amplicons) from the driver genes are sequenced.
4. The method of any one of claims 1-3, wherein 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 amplicons from the driver genes are sequenced.
5. The method of any one of claims 1-4, wherein each amplicon comprises about 15 bp to about 1000 bp.

~~6. The method of any one of claims 1-5, wherein each amplicon comprises about 33bp.~~

~~7. The method of any one of claims 1-6, wherein the plurality of cancers comprises 4, 5, 6, 7 or 8 cancers.~~

~~8. The method of any one of claims 1-7, wherein the plurality of cancers comprises a cancer chosen from liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, or breast cancer.~~

~~9. The method of any one of claims 1-8, wherein:~~

- ~~—— (i) the subject has not yet been determined to have a cancer of the plurality of cancers;~~
- ~~—— (ii) the subject has not yet been determined to harbor a cancer cell, or~~
- ~~—— (iii) the subject does not exhibit, or has not exhibited, a symptom associated with a cancer of the plurality of cancers.~~

~~10. The method of any one of claims 1-9, wherein the plurality of driver genes comprises one or more of NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2.~~

~~11. The method of any one of claims 1-10, wherein the plurality of driver genes comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 of NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2.~~

~~12. The method of any one of claims 1-10, wherein the plurality of driver genes comprises all of NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2.~~

~~13. The method of any one of claims 1-12, wherein the one or more protein biomarkers comprise one or more of CA19-9, CEA, HGF, OPN, CA125, prolactin, TIMP-1, or MPO.~~

~~14. The method of any one of claims 1-13, wherein the one or more protein biomarkers comprise 2, 3, 4, 5, 6, 7 or 8 of CA19-9, CEA, HGF, OPN, CA125, prolactin, TIMP-1, or MPO.~~

~~15. The method of any one of claims 1-13, wherein the one or more protein biomarkers comprise all of CA19-9, CEA, HGF, OPN, CA125, prolactin, TIMP-1, or MPO.~~

~~16. The method of any one of claims 1-15, wherein the biological sample comprises one or more of:~~

- ~~—— (i) a tumor sample, a circulating tumor DNA sample, a solid tumor biopsy sample, or a fixed tumor sample;~~
- ~~—— (ii) a blood sample;~~
- ~~—— (iii) an apheresis sample;~~
- ~~—— (iv) a cell-free DNA sample, or~~
- ~~—— (v) a protein sample.~~

~~17. The method of any one of claims 1-16, wherein detecting the presence of one or more driver gene mutations comprises:~~

- ~~a. assigning a unique identifier (UID) to each of a plurality of DNA template molecules present in the sample;~~
- ~~b. amplifying each uniquely tagged DNA template molecule to create UID families of amplification products; and~~
- ~~c. redundantly sequencing the amplification products.~~

~~181. A method of evaluating a subject for the presence of each of a plurality of cancers, comprising:~~

- ~~(a) evaluating a first biological sample obtained from the subject for the presence of one or more driver gene mutations in cell-free tumor DNA derived from a gene selected from the group consisting of in each of a plurality of driver genes, wherein the plurality of driver genes~~

~~comprises one or more of NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2, or combinations thereof,~~

~~(b) detecting in a second biological sample isolated from the subject the absence of one or more gene mutations in DNA derived from a gene selected from the group consisting of: NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2, or combinations thereof, wherein the second biological sample is isolated from white blood cells of the subject; and~~

~~(c) identifying the presence of cancer in the subject when the one or more genetic mutations that are detected in the first sample are not detected in the second sample wherein each driver gene is associated with the presence of a cancer in the plurality of cancers and wherein evaluating comprises sequencing a plurality of amplicons comprising the plurality of driver genes such that the sensitivity of detection of each cancer in the plurality of cancers is not increased by increasing the number of amplicons sequenced; and~~

~~—— (b) evaluating the level of one or more protein biomarkers in a biological sample, wherein the one or more protein biomarkers comprise one or more of CA19-9, CEA, HGF, OPN, CA125, prolactin, TIMP-1, or MPO,~~

~~—— wherein the presence of a cancer of the plurality of cancers is identified when the presence of one or more driver gene mutations and the level of one or more protein biomarkers is detected;~~

~~—— thereby evaluating the subject for the presence of each of the plurality of cancers.~~

~~19. The method of claim 18, wherein raising the number of amplicons above a threshold level would not detect substantially more cancers but would increase the probability of false positive results.~~

~~20. The method of claim 18 or 19, wherein about 60 amplicons (e.g., 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 amplicons) from the driver genes are sequenced.~~

~~21. The method of claim 20, wherein 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 amplicons from the driver genes are sequenced.~~

~~22. The method of any one of claims 18-21, wherein each amplicon comprises about 15 bp to about 1000 bp.~~

~~23. The method of any one of claims 18-22, wherein each amplicon comprises about 33bp.~~

~~24. The method of any one of claims 18-23, wherein the plurality of cancers comprises 4, 5, 6, 7 or 8 cancers.~~

~~25. The method of any one of claims 18-24, wherein the plurality of cancers comprises a cancer chosen from liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, or breast cancer.~~

~~26~~26. The method of ~~any one of claims 18-25~~1, wherein:

- ~~(i) the subject has not yet been determined to have a cancer of the plurality of cancers;~~
- ~~(ii) the subject has not yet been determined to harbor a cancer cell, or~~
- ~~(iii) the subject does not exhibit, or has not exhibited, a symptom associated with a cancer of the plurality of cancers.~~

~~27. The method of any one of claims 1-26, wherein (a) is evaluated in a first biological sample, and/or (b) is evaluated in a second biological sample.~~

~~28~~3. The method of claim ~~27~~1, wherein the first biological sample and the second biological sample are from the same initial sample.

~~29. The method of any one of claims 1-28, wherein the protein biomarker is detected using a method chosen from: an antibody dependent method (e.g., multiplex immunoassay, enzyme-linked immunosorbent assay (ELISA), protein immunoprecipitation, immunoelectrophoresis,~~

western blotting, or protein immunostaining); a spectrometry method (e.g., high performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC/MS)); or an aptamer dependent method.

30. The method of any one of claims 1-29, wherein the subject is administered one or more of the following therapeutic interventions: surgery, adjuvant chemotherapy, neoadjuvant chemotherapy, radiation therapy, immunotherapy, targeted therapy, or an immune checkpoint inhibitor.

31. A kit comprising a population of primer pairs, wherein each primer pair includes a first primer and a second primer, wherein each of the first and second primers comprise a portion complementary to a gene or gene portion chosen from: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 (all) of: NRAS, CTNNB1, PIK3CA, FBXW7, APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1A, or GNAS.

32. The kit of claim 31, for use in evaluating a subject for the presence of a plurality of cancers, optionally wherein the plurality of cancers comprises at least 2, 3, 4, 5, 6, 7, or 8 cancer types.

33. The kit of claim 32, wherein the plurality of cancers comprises a cancer chosen from: liver cancer, ovary cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, or breast cancer.

34. The kit of any one of claims 31-33, which allows for sequencing of a sufficient number of bases to evaluate the presence of a plurality of cancers (e.g., at least 2, 3, 4, 5, 6, 7, or 8 cancers).

35. The kit of any one of claims 31-34, which allows for sequencing of a minimum number of amplicons to detect at least one driver gene in each of 8 cancers.

36. The kit of claim 35, wherein raising the number of amplicons above a threshold level would not detect substantially more cancers but would increase the probability of false positive results.

~~37. The kit of any one of claims 31-36, further comprising means for evaluating the presence of one or more protein biomarkers.~~

~~38. The kit of claim 37, wherein the protein biomarker is chosen from: CA19-9, CEA, HGF, OPN, CA125, prolactin, TIMP-1, or MPO.~~

~~39. The kit of claim 37 or 38, wherein the means of detecting the protein is chosen from: an antibody dependent method (e.g., multiplex immunoassay, enzyme linked immunosorbent assay (ELISA), protein immunoprecipitation, immunoelectrophoresis, western blotting, or protein immunostaining); a spectrometry method (e.g., high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC/MS)); or an aptamer dependent method.~~

~~40. The kit of any one of claims 31-39, wherein the portion of the first and second primer pairs that is complementary to a gene or gene portion comprises 10-100 nucleotides.~~

~~41. The kit of any one of claims 31-40, wherein the first primer pair comprises a second portion comprising a site for hybridization to a third primer.~~

~~42. The kit of any one of claims 31-41, wherein the second primer comprises a second portion comprising a site for hybridization to a fourth primer.~~

~~43. The kit of claim 42, wherein interposed between the first portion and the second portion of the second primer is a third portion consisting of 2 to 4000 nucleotides forming a unique identifier (UID).~~

~~44. A method for identifying the presence of cancer in a subject comprising:
detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: NRAS, CTNNB1, PIK3CA, FBXW7,~~

APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1 A, or GNAS;

detecting a level of one or more of the following protein biomarkers in a second biological sample obtained from the subject: CA19-9, CEA, HGF, OPN, CA125, prolactin, TFMP-1, or MPO;

comparing the detected levels of the one or more protein biomarker to one or more reference levels of the protein biomarkers; and

identifying the presence of cancer in the subject when the presence of one or more genetic biomarkers is detected, the detected levels of the one or more protein biomarkers are higher than the reference levels of the one or more protein biomarkers, or both.

45. A method for identifying the presence of pancreatic cancer in a subject comprising:

detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: KRAS, TP53, CDKN2A, or SMAD4;

detecting a level of one or more of the following protein biomarkers in a second biological sample obtained from the subject: carbohydrate antigen 19-9 (CA19-9),

carcinoembryonic antigen (CEA), hepatocyte growth factor (HGF), or osteopontin (OPN);

comparing the detected levels of the one or more protein biomarker to one or more reference levels of the protein biomarkers; and

identifying the presence of pancreatic cancer in the subject when the presence of one or more genetic biomarkers is detected, the detected levels of the one or more protein biomarkers are higher than the reference levels of the one or more protein biomarkers, or both.

46. A method for identifying the presence of cancer in a subject comprising:

detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: NRAS, CTNNB1, PIK3CA, FBXW7,

APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1 A, or GNAS;

detecting a level of one or more of the following protein biomarkers in a second biological sample obtained from the subject: CA19-9, CEA, HGF, OPN, CA125, AFP, prolactin, TFMP-1, follistatin, G-CSF, or CA15-3;

comparing the detected levels of the one or more protein biomarker to one or more reference levels of the protein biomarkers; and

identifying the presence of cancer in the subject when the presence of one or more genetic biomarkers is detected, the detected levels of the one or more protein biomarkers are higher than the reference levels of the one or more protein biomarkers, or both.

47. A method for identifying the presence of cancer in a subject comprising:

detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: NRAS, CTNNB1, PIK3CA, FBXW7, APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1 A, or GNAS;

detecting a level of one or more of the following protein biomarkers in a second biological sample obtained from the subject: CA19-9, CEA, HGF, OPN, CA125, AFP, prolactin, TFMP-1, or CA15-3;

comparing the detected levels of the one or more protein biomarker to one or more reference levels of the protein biomarkers; and

identifying the presence of cancer in the subject when the presence of one or more genetic biomarkers is detected, the detected levels of the one or more protein biomarkers are higher than the reference levels of the one or more protein biomarkers, or both.

48. A method for identifying the presence of bladder cancer or an upper tract urothelial carcinoma in a subject comprising:

detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: TP53, PIK3CA, FGFR3, KRAS, ERBB2, CDKN2A, MLL, HRAS, MET, or VHL;

~~detecting the presence of at least one mutation in a TERT promoter in a second biological sample obtained from the subject; and~~
~~detecting the presence of aneuploidy in a third biological sample obtained from the subject; and~~
~~identifying the presence of bladder cancer or an upper tract urothelial carcinoma in the subject when the presence of one or more genetic biomarkers is detected, the presence of the at least one mutation in the TERT promoter, the presence of aneuploidy is detected, or combinations thereof.~~

~~49. A method for identifying the presence of ovarian or endometrial cancer in a subject comprising:~~

~~detecting in a first biological sample obtained from the subject the presence of one or more genetic biomarkers in one or more of the following genes: NRAS, PTEN, FGFR2, KRAS, POLE, AKT1, TP53, RNF43, PPP2R1A, MAPK1, CTNNB1, PIK3CA, FBXW7, PIK3R1, APC, EGFR, BRAF, or CDKN2A;~~

~~detecting the presence of aneuploidy in a second biological sample obtained from the subject; and~~

~~identifying the presence of ovarian or endometrial cancer in the subject when the presence of one or more genetic biomarkers is detected, the presence of aneuploidy is detected, or both.~~

4. The method of claim 1, further comprising detecting a level of one or more protein biomarkers in a third biological sample isolated from the subject, wherein the protein biomarker is selected from the group consisting of: carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), hepatocyte growth factor (HGF), osteopontin (OPN), CA125, AFP, prolactin, TIMP-1, follistatin, G-CSF, and CA15-3 and combinations thereof;

_____ comparing the detected levels of the one or more protein biomarkers to one or more reference levels of the protein biomarkers; and

_____ identifying the presence of the cancer in the subject when the presence of one or more genetic mutations in the cell-free tumor DNA is detected in the first sample, the absence of one or more gene mutations is detected in DNA from the second sample, and the detected levels of

the one or more protein biomarkers are higher than the reference levels of the one or more protein biomarkers.

5. The method of claim 4, wherein the one or more protein biomarkers include CA19-9.

6. The method of claim 5, wherein the reference level of the CA19-9 protein biomarker is 92 U/mL.

7. The method of claim 4, wherein the one or more protein biomarkers include CEA.

8. The method of claim 7, wherein the reference level of the CEA protein biomarker is 7.5 ng/mL.

9. The method of claim 4, wherein the one or more protein biomarkers include CA125.

10. The method of claim 9, wherein the reference level of the CA125 protein biomarker is 577 U/mL.

11. The method of claim 4, wherein the one or more protein biomarkers include CA15-3.

12. The method of claim 11, wherein the reference level of the CA15-3 protein biomarker is 98 U/mL.

13. The method of claim 1, wherein the presence of one or more genetic mutations in cell-free tumor DNA in the first biological sample, the absence of one or more genetic mutations in DNA in the second biological sample, or both are detected by amplifying the DNA to form families of amplicons in which each member of a family is derived from a single template molecule in the DNA, wherein each member of a family comprises a common oligonucleotide barcode, and wherein each family comprises a distinct oligonucleotide barcode.

14. The method of claim 13, wherein the oligonucleotide barcode is introduced into the template molecule by a step of amplifying with a population of primers which collectively contain a plurality of oligonucleotide barcodes.

15. The method of claim 13, wherein the oligonucleotide barcode is endogenous to the template molecule, and an adapter comprising a DNA synthesis priming site is ligated to an end of the template molecule adjacent to the oligonucleotide barcode.