

CLAIMS

1. A method of evaluating a subject for the presence 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 NRAS, KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, FGFR2, and combinations thereof,

(b) evaluating in a second biological sample isolated from the subject the absence of one or more driver 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, FGFR2, and combinations thereof, wherein the second biological sample is isolated from white blood cells of the subject;

(c) detecting a level of one or more protein biomarkers in a third biological sample isolated from the subject, wherein the one or more protein biomarkers are 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, CA15-3, and combinations thereof;

(d) comparing the detected levels of the one or more protein biomarkers to one or more reference levels of the protein biomarkers; and

(e) identifying the presence of at least one of the plurality of cancers in the subject when:

(i) at least one driver gene mutation is detected as present in cell-free tumor DNA in the first biological sample and is detected as absent in DNA the second sample; or the detected level of at least one of the one or more protein biomarkers is higher than its reference level, or

(ii) at least one driver gene mutation is detected as present in cell-free tumor DNA in the first biological sample and is detected as absent in DNA in the second sample and the detected level of at least one of the one or more protein biomarkers is higher than its reference level.

2. The method of claim 1, wherein:
 - (i) the subject has not yet been determined to have cancer;
 - (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 cancer.
3. The method of claim 1, wherein the first biological sample and the second biological sample are from the same initial sample, the first biological sample and the third biological sample are from the same initial sample, the second biological sample and the third biological sample are from the same initial sample, or the first biological sample and the second biological sample and the third biological sample are from the same initial sample.
4. The method of claim 1, wherein the one or more protein biomarkers include CA19-9.
5. The method of claim 4, wherein the reference level of the CA19-9 protein biomarker is 92 U/mL.
6. The method of claim 1, wherein the one or more protein biomarkers include CEA.
7. The method of claim 6, wherein the reference level of the CEA protein biomarker is 7.5 ng/mL.
8. The method of claim 1, wherein the one or more protein biomarkers include CA125.
9. The method of claim 8, wherein the reference level of the CA125 protein biomarker is 577 U/mL.
10. The method of claim 1, wherein the one or more protein biomarkers include CA15-3.
11. The method of claim 10, wherein the reference level of the CA15-3 protein biomarker is 98 U/mL.

12. The method of claim 1, wherein the presence of one or more driver gene mutations in cell-free tumor DNA in the first biological sample, the absence of one or more driver gene mutations in DNA in the second biological sample, or both are detected by amplifying 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.

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

14. The method of claim 13, wherein the distinct 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 distinct oligonucleotide barcode.