

CLAIMS

WHAT IS CLAIMED IS:

1. A method for assessing tumor status of a subject with cancer, comprising:
 - obtaining first whole genome sequencing (WGS) data of a first plurality of cell-free DNA (cfDNA) molecules, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes a therapeutic configured to treat the cancer is administered to the subject;
 - determining, based on the first WGS data, (i) a first plurality of copy number aberrations (CNAs) in the first plurality of cfDNA molecules and (ii) a first plurality of fragment lengths of the first plurality of cfDNA molecules;
 - obtaining second whole genome sequencing (WGS) data of a second plurality of cell-free DNA (cfDNA) molecules, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to administration of the therapeutic to the subject;
 - determining, based on the second WGS data, (iii) a second plurality of copy number aberrations (CNAs) in the second plurality of cfDNA molecules and (iv) a second plurality of fragment lengths of the second plurality of cfDNA molecules;
 - comparing the first plurality of CNAs with the second plurality of CNAs to determine a CNA profile change;
 - determining a fragment length profile change based on the first plurality of fragment lengths and the second plurality of fragment lengths;
 - determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the CNA profile change and the fragment length profile change; and
 - detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.
2. The method of claim 1, wherein the first or second bodily fluid sample is selected from the group consisting of: blood, serum, plasma, vitreous, sputum, urine, tears, perspiration, saliva, semen, mucosal excretions, mucus, spinal fluid, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, amniotic fluid, and lymph fluid.

3. The method of claim 1, wherein obtaining the first WGS data comprises sequencing the first plurality of cfDNA molecules to generate a first plurality of sequencing reads, or wherein obtaining the second WGS data comprises sequencing the second plurality of cfDNA molecules to generate a second plurality of sequencing reads.
4. The method of claim 3, wherein the sequencing is performed at a depth of no more than about 25X.
5. The method of claim 3, wherein the sequencing is performed at a depth of no more than about 10X.
6. The method of claim 3, wherein the sequencing is performed at a depth of no more than about 8X.
7. The method of claim 3, wherein the sequencing is performed at a depth of no more than about 6X.
8. The method of claim 3, further comprising aligning the first or second plurality of sequencing reads to a reference genome, thereby producing a plurality of aligned sequencing reads.
9. The method of claim 1, further comprising enriching the first or second plurality of cfDNA molecules for a plurality of genomic regions.
10. The method of claim 9, wherein the enrichment comprises amplifying the first or second plurality of cfDNA molecules.
11. The method of claim 10, wherein the amplification comprises selective amplification.
12. The method of claim 10, wherein the amplification comprises universal amplification.
13. The method of claim 9, wherein the enrichment comprises selectively isolating at least a portion of the first or second plurality of cfDNA molecules.
14. The method of claim 13, wherein selectively isolating the at least the portion of the first or second plurality of cfDNA molecules comprises using a plurality of probes, each of the plurality of probes having sequence complementarity with at least a portion of a genomic region of the plurality of genomic regions.
15. The method of claim 13, wherein the at least the portion comprises a tumor marker locus.
16. The method of claim 15, wherein the at least the portion comprises a plurality of tumor marker loci.

17. The method of claim 16, wherein the plurality of tumor marker loci comprises one or more loci selected from The Cancer Genome Atlas (TCGA) or Catalogue of Somatic Mutations in cancer (COSMIC).
18. The method of claim 3, wherein determining the first plurality of CNAs comprises determining quantitative measures of CNAs at each of at each of a plurality of genomic regions of the first plurality of sequencing reads, and wherein determining the second plurality of CNAs comprises determining quantitative measures of CNAs at each of at each of the plurality of genomic regions of the second plurality of sequencing reads.
19. The method of claim 18, further comprising correcting the first plurality of CNAs or the second plurality of CNAs for GC content and/or mappability bias.
20. The method of claim 19, wherein the correcting comprises using a statistical modeling analysis.
21. The method of claim 20, wherein the statistical modeling analysis comprises LOESS regression or a Bayesian model.
22. The method of claim 18, wherein the plurality of genomic regions comprises non-overlapping genomic regions of a reference genome having a pre-determined size.
23. The method of claim 22, wherein the pre-determined size is about 50 kilobases (kb), about 100 kb, about 200 kb, about 500 kb, about 1 megabases (Mb), about 2 Mb, about 5 Mb, or about 10 Mb.
24. The method of claim 18, wherein the plurality of genomic regions comprises at least about 1,000 distinct genomic regions.
25. The method of claim 24, wherein the plurality of genomic regions comprises at least about 2,000 distinct genomic regions.
26. The method of claim 1, wherein determining the CNA profile change comprises comparing the first plurality of CNAs and the second plurality of CNAs with a plurality of reference CNA values, wherein the plurality of reference CNA values is obtained from additional cfDNA molecules obtained or derived from additional bodily fluid samples of additional subjects.
27. The method of claim 26, wherein the additional subjects comprise one or more subjects without cancer.

28. The method of claim 26, wherein the additional subjects comprise one or more subjects not having tumor progression.
29. The method of claim 26, wherein the plurality of reference CNA values is obtained using additional bodily fluid samples of the subject obtained at one or more subsequent time points after the first timepoint.
30. The method of claim 1, further comprising filtering out a subset of the first plurality of CNAs and the second plurality of CNAs that meet a pre-determined criterion.
31. The method of claim 30, further comprising filtering out a given CNA value of the first plurality of CNAs or the second plurality of CNAs values when the difference between the given CNA value and the corresponding reference CNA value comprises a difference of no more than about 1 standard deviation.
32. The method of claim 31, further comprising filtering out a given CNA value of the first plurality of CNAs or the second plurality of CNAs values when the difference between the given CNA value and the corresponding reference CNA value comprises a difference of no more than about 2 standard deviations.
33. The method of claim 31, further comprising filtering out a given CNA value of the first plurality of CNAs or the second plurality of CNAs values when the difference between the given CNA value and the corresponding reference CNA value comprises a difference of no more than about 3 standard deviations.
34. The method of claim 30, further comprising filtering out a given CNA value of the first plurality of CNAs or the second plurality of CNAs values based on a Spearman's rank correlation between the given CNA value and a corresponding local mean fragment length.
35. The method of claim 34, further comprising filtering out a given CNA value of the first plurality of CNAs or the second plurality of CNAs values when the Spearman's rank correlation coefficient (Spearman's rho) is less than -0.1.
36. The method of claim 1, further comprising normalizing the first plurality of fragment lengths or the second plurality of fragment lengths based on a library or a genomic location.
37. The method of claim 1, further comprising detecting that the tumor status comprises tumor progression of the subject when the first tumor fraction or the second tumor fraction is greater than 1, greater than 1.1, greater than 1.2, greater than 1.3, greater than 1.4, greater than 1.5, greater than

1.6, greater than 1.7, greater than 1.8, greater than 1.9, greater than 2, greater than 3, greater than 4, or greater than 5.

38. The method of claim 1, further comprising detecting a major molecular response (MMR) of the subject when the first tumor fraction or the second tumor fraction is less than 0.01, less than 0.05, less than 0.1, less than 0.2, less than 0.3, less than 0.4, or less than 0.5.

39. The method of any one of claims 1-38, further comprising detecting the tumor status of the subject with a sensitivity of at least about 50%.

40. The method of claim 39, further comprising detecting the tumor status of the subject with a sensitivity of at least about 70%.

41. The method of claim 40, further comprising detecting the tumor status of the subject with a sensitivity of at least about 90%.

42. The method of any one of claims 1-41, further comprising detecting the tumor status of the subject with a specificity of at least about 50%.

43. The method of claim 42, further comprising detecting the tumor status of the subject with a specificity of at least about 70%.

44. The method of claim 43, further comprising detecting the tumor status of the subject with a specificity of at least about 90%.

45. The method of claim 44, further comprising detecting the tumor status of the subject with a specificity of at least about 98%.

46. The method of any one of claims 1-45, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 50%.

47. The method of claim 46, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 70%.

48. The method of claim 47, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 90%.

49. The method of any one of claims 1-48, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 50%.

50. The method of claim 49, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 70%.

51. The method of claim 50, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 90%.
52. The method of any one of claims 1-51, further comprising detecting the tumor status of the subject with an area under the curve (AUC) of at least about 0.60.
53. The method of claim 52, further comprising detecting the tumor status of the subject with an area under the curve (AUC) of at least about 0.75.
54. The method of claim 53, further comprising detecting the tumor status of the subject with an area under the curve (AUC) of at least about 0.90.
55. The method of any one of claims 1-54, further comprising determining a tumor non-progression of the subject when tumor progression is not detected.
56. The method of any one of claims 1-55, further comprising, based on the determined tumor status of the subject, administering a therapeutically effective dose of a treatment to treat the cancer of the subject.
57. The method of claim 56, wherein the treatment comprises surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, cell therapy, an anti-hormonal agent, an antimetabolite chemotherapeutic agent, a kinase inhibitor, a methyltransferase inhibitor, a peptide, a gene therapy, a vaccine, a platinum-based chemotherapeutic agent, an antibody, or a checkpoint inhibitor.
58. The method of any one of claims 1-57, wherein the detected tumor status is indicative of tumor progression, non-progression, regression, or recurrence.
59. The method of any one of claims 1-58, wherein the first and second WGS data are obtained by pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, Nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, massively parallel sequencing, chain termination sequencing, single molecule real-time sequencing, Polony sequencing, combinatorial probe anchor synthesis, or hybrid capture-based sequencing.
60. The method of any one of claims 1-59, wherein the first and second WGS data are obtained by a sequencing device or computer processor.
61. A computer system for assessing tumor status of a subject with cancer, comprising:
a database that is configured to store (i) first whole genome sequencing (WGS) data of a first plurality of cell-free DNA (cfDNA) molecules, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the

first timepoint precedes a therapeutic configured to treat the cancer is administered to the subject, and (ii) second whole genome sequencing (WGS) data of a second plurality of cell-free DNA (cfDNA) molecules, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to administration of the therapeutic to the subject; and

one or more computer processors operatively coupled to the database, wherein the one or more computer processors are individually or collectively programmed to:

determine, based on the first WGS data, (i) a first plurality of copy number aberrations (CNAs) in the first plurality of cfDNA molecules and (ii) a first plurality of fragment lengths of the first plurality of cfDNA molecules;

determine, based on the second WGS data, (iii) a second plurality of copy number aberrations (CNAs) in the second plurality of cfDNA molecules and (iv) a second plurality of fragment lengths of the second plurality of cfDNA molecules;

compare the first plurality of CNAs with the second plurality of CNAs to determine a CNA profile change;

determine a fragment length profile change based on the first plurality of fragment lengths and the second plurality of fragment lengths;

determine a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the CNA profile change and the fragment length profile change; and

detect a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

62. A non-transitory computer-readable medium comprising machine-executable instructions which, upon execution by one or more computer processors, perform a method for assessing tumor status of a subject with cancer, the method comprising:

obtaining first whole genome sequencing (WGS) data of a first plurality of cell-free DNA (cfDNA) molecules, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes a therapeutic configured to treat the cancer is administered to the subject;

determining, based on the first WGS data, (i) a first plurality of copy number aberrations (CNAs) in the first plurality of cfDNA molecules and (ii) a first plurality of fragment lengths of the first plurality of cfDNA molecules;

obtaining second whole genome sequencing (WGS) data of a second plurality of cell-free DNA (cfDNA) molecules, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to administration of the therapeutic to the subject;

determining, based on the second WGS data, (iii) a second plurality of copy number aberrations (CNAs) in the second plurality of cfDNA molecules and (iv) a second plurality of fragment lengths of the second plurality of cfDNA molecules;

comparing the first plurality of CNAs with the second plurality of CNAs to determine a CNA profile change;

determining a fragment length profile change based on the first plurality of fragment lengths and the second plurality of fragment lengths;

determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the CNA profile change and the fragment length profile change; and

detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

63. A method for assessing tumor status of a subject with cancer, comprising:

obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject;

determining, based on the first MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a first average methylation fraction profile;

obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived

from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject;

determining, based on the second MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a second average methylation fraction profile;

comparing the first average methylation fraction profile across the one or more CpG islands and the second average methylation fraction profile across the one or more CpG islands to determine a methylation fraction profile;

determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation fraction profiles; and

detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

64. The method of claim 63, wherein the first or second bodily fluid sample is selected from the group consisting of: blood, serum, plasma, vitreous, sputum, urine, tears, perspiration, saliva, semen, mucosal excretions, mucus, spinal fluid, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, amniotic fluid, and lymph fluid.

65. The method of claim 63, wherein obtaining the first MS data comprises performing methylation sequencing of the first plurality of cfDNA molecules to generate a first plurality of sequencing reads, or wherein obtaining the second WGS data comprises performing methylation sequencing of the second plurality of cfDNA molecules to generate a second plurality of sequencing reads.

66. The method of claim 65, wherein the methylation sequencing comprises whole genome bisulfite sequencing.

67. The method of claim 65, wherein the methylation sequencing comprises whole genome enzymatic methyl-seq.

68. The method of claim 65, wherein the methylation sequencing comprises oxidative bisulfite sequencing, TET-assisted pyridine borane sequencing (TAPS), TET-assisted bisulfite sequencing (TABS), oxidative bisulfite sequencing (oxBS-Seq), APOBEC-coupled epigenetic sequencing (ACE-seq), methylated DNA immunoprecipitation (MeDIP) sequencing, hydroxymethylated DNA

immunoprecipitation (hMeDIP) sequencing, methylation array analysis, reduced representation bisulfite sequencing (RRBS-Seq), or cytosine 5-hydroxymethylation sequencing.

69. The method of claim 65, wherein the methylation sequencing is performed at a depth of no more than about 25X.

70. The method of claim 65, wherein the methylation sequencing is performed at a depth of no more than about 10X.

71. The method of claim 65, wherein the methylation sequencing is performed at a depth of no more than about 8X.

72. The method of claim 65, wherein the methylation sequencing is performed at a depth of no more than about 6X.

73. The method of claim 65, further comprising aligning the first or second plurality of sequencing reads to a reference genome, thereby producing a plurality of aligned sequencing reads.

74. The method of claim 65, further comprising enriching the first or second plurality of cfDNA molecules for the region of the genome.

75. The method of claim 74, wherein the enrichment comprises amplifying the first or second plurality of cfDNA molecules.

76. The method of claim 75, wherein the amplification comprises selective amplification.

77. The method of claim 75, wherein the amplification comprises universal amplification.

78. The method of claim 74, wherein the enrichment comprises selectively isolating at least a portion of the first or second plurality of cfDNA molecules.

79. The method of claim 78, wherein selectively isolating the at least the portion of the first or second plurality of cfDNA molecules comprises using a plurality of probes, each of the plurality of probes having sequence complementarity with at least a portion of the region of the genome.

80. The method of claim 78, wherein the at least the portion comprises a tumor marker locus.

81. The method of claim 80, wherein the at least the portion comprises a plurality of tumor marker loci.

82. The method of claim 81, wherein the plurality of tumor marker loci comprises one or more loci selected from The Cancer Genome Atlas (TCGA) or Catalogue of Somatic Mutations in cancer (COSMIC).

83. The method of claim 63, wherein the region of the genome comprises one or more of: CpG islands, CpG shores, patient-specific partially methylated domains, common partially methylated domains, promoters, gene bodies, evenly spaced genomewide bins, and transposable elements.
84. The method of claim 63, wherein the region of the genome comprises a plurality of non-overlapping regions of the genome.
85. The method of claim 84, wherein the plurality of non-overlapping regions of the genome have a pre-determined size.
86. The method of claim 85, wherein the pre-determined size is about 50 kilobases (kb), about 100 kb, about 200 kb, about 500 kb, about 1 megabases (Mb), about 2 Mb, about 5 Mb, or about 10 Mb.
87. The method of claim 84, wherein the plurality of non-overlapping regions of the genome comprises at least about 1,000 distinct regions.
88. The method of claim 87, wherein the plurality of non-overlapping regions of the genome comprises at least about 2,000 distinct regions.
89. The method of claim 63, wherein determining the first or second tumor fraction comprises comparing the methylation fraction profile with one or more reference methylation fraction profiles, wherein the one or more reference methylation fraction profiles are obtained from additional cfDNA molecules obtained or derived from additional bodily fluid samples of additional subjects.
90. The method of claim 89, wherein the additional subjects comprise one or more subjects with cancer.
91. The method of claim 89, wherein the additional subjects comprise one or more subjects without cancer.
92. The method of claim 89, wherein the additional subjects comprise one or more subjects having tumor progression.
93. The method of claim 89, wherein the additional subjects comprise one or more subjects not having tumor progression.
94. The method of claim 89, wherein the one or more reference methylation fraction profiles are obtained using additional bodily fluid samples of the subject obtained at one or more subsequent time points after the first timepoint.

95. The method of claim 63, further comprising detecting that the tumor status comprises tumor progression of the subject when the first tumor fraction or the second tumor fraction is greater than 1, greater than 1.1, greater than 1.2, greater than 1.3, greater than 1.4, greater than 1.5, greater than 1.6, greater than 1.7, greater than 1.8, greater than 1.9, greater than 2, greater than 3, greater than 4, or greater than 5.
96. The method of claim 63, further comprising detecting a major molecular response (MMR) of the subject when the first tumor fraction or the second tumor fraction is less than 0.01, less than 0.05, less than 0.1, less than 0.2, less than 0.3, less than 0.4, or less than 0.5.
97. The method of any one of claims 63-96, further comprising detecting the tumor status of the subject with a sensitivity of at least about 50%.
98. The method of claim 97, further comprising detecting the tumor status of the subject with a sensitivity of at least about 70%.
99. The method of claim 98, further comprising detecting the tumor status of the subject with a sensitivity of at least about 90%.
100. The method of any one of claims 63-99, further comprising detecting the tumor status of the subject with a specificity of at least about 50%.
101. The method of claim 100, further comprising detecting the tumor status of the subject with a specificity of at least about 70%.
102. The method of claim 101, further comprising detecting the tumor status of the subject with a specificity of at least about 90%.
103. The method of claim 102, further comprising detecting the tumor status of the subject with a specificity of at least about 98%.
104. The method of any one of claims 63-103, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 50%.
105. The method of claim 104, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 70%.
106. The method of claim 105, further comprising detecting the tumor status of the subject with a positive predictive value (PPV) of at least about 90%.
107. The method of any one of claims 63-106, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 50%.

108. The method of claim 107, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 70%.
109. The method of claim 108, further comprising detecting the tumor status of the subject with a negative predictive value (NPV) of at least about 90%.
110. The method of any one of claims 63-109, further comprising detecting the status progression of the subject with an area under the curve (AUC) of at least about 0.60.
111. The method of claim 110, further comprising detecting the tumor status of the subject with an area under the curve (AUC) of at least about 0.75.
112. The method of claim 111, further comprising detecting the tumor status of the subject with an area under the curve (AUC) of at least about 0.90.
113. The method of any one of claims 63-112, further comprising determining a tumor non-progression of the subject when tumor progression is not detected.
114. The method of any one of claims 63-113, further comprising, based on the determined tumor status of the subject, administering a therapeutically effective dose of a second therapeutic to treat the cancer of the subject.
115. The method of claim 114, wherein the second therapeutic comprises surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, cell therapy, an anti-hormonal agent, an antimetabolite chemotherapeutic agent, a kinase inhibitor, a methyltransferase inhibitor, a peptide, a gene therapy, a vaccine, a platinum-based chemotherapeutic agent, an antibody, or a checkpoint inhibitor.
116. The method of any one of claims 63-115, wherein the first and the second pluralities of cfDNA molecules are from immune cells of the subject.
117. The method of any one of claims 63-116, wherein the detected tumor status is indicative of tumor progression, non-progression, regression, or recurrence.
118. The method of any one of claims 63-117, wherein the first and second MS data are obtained by a sequencing device or computer processor.
119. The method of any one of claims 1-60 and 63-118, wherein the subject has brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, kidney cancer, hepatobiliary tract cancer, leukemia, liver cancer, lung cancer,

lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, thyroid cancer, or urinary tract cancer.

120. A computer system for assessing tumor status of a subject with cancer, comprising:

a database that is configured to store (i) first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject, and (ii) second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject; and

one or more computer processors operatively coupled to the database, wherein the one or more computer processors are individually or collectively programmed to:

determine, based on the first MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a first average methylation fraction profile;

determine, based on the second MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a second average methylation fraction profile;

compare the first average methylation fraction profile across the one or more CpG islands and the second average methylation fraction profile across the one or more CpG islands to determine a methylation fraction profile;

determine a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation fraction profiles; and

detect a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

121. A non-transitory computer-readable medium comprising machine-executable instructions which, upon execution by one or more computer processors, perform a method for assessing tumor status of a subject with cancer, the method comprising:

- obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject;

- determining, based on the first MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a first average methylation fraction profile;

- obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject;

- determining, based on the second MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a second average methylation fraction profile;

- comparing the first average methylation fraction profile across the one or more CpG islands and the second average methylation fraction profile across the one or more CpG islands to determine a methylation fraction profile;

- determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation fraction profiles; and

- detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

122. The computer system of claim 120 or the non-transitory computer-readable medium of claim 121, wherein the detected tumor progression is based at least in part on one or more statistical modeling analyses of the respective methylation fraction profiles.

123. The system or medium of claim 122, wherein the one or more statistical modeling analyses comprise linear regression, simple regression, binary regression, Bayesian linear regression, Bayesian modeling, polynomial regression, Gaussian process regression, Gaussian modeling, binary regression, logistic regression, or nonlinear regression.

124. The system or medium of claim 122 or claim 123, wherein the one or more statistical modeling analyses compare the detected tumor progression with MS data derived from a sample having a known tumor fraction, MS data derived from a pure tumor sample, or MS data derived from a healthy sample.

125. A method for assessing tumor status of a subject with cancer, comprising:

- obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject;

- determining, based on the first MS data, a methylation profile for each of one or more loci of the genome, thereby obtaining a first methylation profile;

- obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject;

- determining, based on the second MS data, a methylation profile for each of one or more loci of the genome, thereby obtaining a second methylation profile;

- comparing the first methylation profile across the one or more loci and the second methylation profile across the one or more loci;

- determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation profiles; and

- detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

126. The method of claim 125, wherein the first and the second methylation profiles comprise 5-hydroxymethylcytosine status, 5-methylcytosine status, enrichment-based methylation assessment, median methylation level, mode methylation level, maximum methylation level, or minimum methylation level.

127. The method of claim 125 or claim 126, wherein the first or second bodily fluid sample is selected from the group consisting of: blood, serum, plasma, vitreous, sputum, urine, tears, perspiration, saliva, semen, mucosal excretions, mucus, spinal fluid, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, amniotic fluid, and lymph fluid.

128. The method of claim 125, wherein obtaining the first MS data comprises performing methylation sequencing of the first plurality of cfDNA molecules to generate a first plurality of sequencing reads, or wherein obtaining the second WGS data comprises performing methylation sequencing of the second plurality of cfDNA molecules to generate a second plurality of sequencing reads.

129. The method of claim 128, wherein the methylation sequencing comprises whole genome bisulfite sequencing.

130. The method of claim 128, wherein the methylation sequencing comprises whole genome enzymatic methyl-seq.

131. The method of claim 128, wherein the methylation sequencing comprises oxidative bisulfite sequencing, TET-assisted pyridine borane sequencing (TAPS), TET-assisted bisulfite sequencing (TABS), oxidative bisulfite sequencing (oxBS-Seq), APOBEC-coupled epigenetic sequencing (ACE-seq), methylated DNA immunoprecipitation (MeDIP) sequencing, hydroxymethylated DNA immunoprecipitation (hMeDIP) sequencing, methylation array analysis, reduced representation bisulfite sequencing (RRBS-Seq), or cytosine 5-hydroxymethylation sequencing.

132. The method of claim 128, further comprising aligning the first or second plurality of sequencing reads to a reference genome, thereby producing a plurality of aligned sequencing reads.

133. The method of claim 128, further comprising enriching the first or second plurality of cfDNA molecules for the region of the genome.

134. The method of claim 128, wherein the region of the genome comprises one or more of: CpG islands, CpG shores, patient-specific partially methylated domains, common partially methylated domains, promoters, gene bodies, evenly spaced genomewide bins, and transposable elements.

135. The method of claim 128, wherein the region of the genome comprises a plurality of non-overlapping regions of the genome.
136. The method of claim 128, wherein determining the first or second tumor fraction comprises comparing the methylation fraction profile with one or more reference methylation fraction profiles, wherein the one or more reference methylation fraction profiles are obtained from additional cfDNA molecules obtained or derived from additional bodily fluid samples of additional subjects.
137. The method of claim 128, further comprising detecting that the tumor status comprises tumor progression of the subject when the first tumor fraction or the second tumor fraction is greater than 1, greater than 1.1, greater than 1.2, greater than 1.3, greater than 1.4, greater than 1.5, greater than 1.6, greater than 1.7, greater than 1.8, greater than 1.9, greater than 2, greater than 3, greater than 4, or greater than 5.
138. The method of claim 128, further comprising detecting a major molecular response (MMR) of the subject when the first tumor fraction or the second tumor fraction is less than 0.01, less than 0.05, less than 0.1, less than 0.2, less than 0.3, less than 0.4, or less than 0.5.
139. The method of any one of claims 128-138, further comprising determining a tumor non-progression of the subject when tumor progression is not detected.
140. The method of any one of claims 128-139, further comprising, based on the determined tumor status of the subject, administering a therapeutically effective dose of a second therapeutic to treat the cancer of the subject.
141. The method of any one of claims 128-140, wherein the first and the second pluralities of cfDNA molecules are from immune cells of the subject.
142. The method of any one of claims 128-141, wherein the detected tumor status is indicative of tumor progression, non-progression, regression, or recurrence.
143. The method of any one of claims 128-142, wherein the first and second MS data are obtained by a sequencing device or computer processor.
144. The method of any one of claims 128-143, wherein the subject has brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, kidney cancer, hepatobiliary tract cancer, leukemia, liver cancer, lung cancer, lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, thyroid cancer, or urinary tract cancer.

145. A computer system for assessing tumor status of a subject with cancer, comprising:
a database that is configured to store (i) first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject, and (ii) second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject; and

one or more computer processors operatively coupled to the database, wherein the one or more computer processors are individually or collectively programmed to:

determine, based on the first MS data, a methylation profile for each of one or more CpG islands in the region of the genome, thereby obtaining a first methylation profile;

determine, based on the second MS data, a methylation profile for each of one or more CpG islands in the region of the genome, thereby obtaining a second methylation profile;

compare the first methylation profile across the one or more CpG islands and the second methylation profile across the one or more CpG islands;

determine a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation profiles; and

detect a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

146. A non-transitory computer-readable medium comprising machine-executable instructions which, upon execution by one or more computer processors, perform a method for assessing tumor status of a subject with cancer, the method comprising:

obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the

first timepoint precedes an administration of a therapeutic configured to treat the cancer to the subject;

determining, based on the first MS data, a methylation profile for each of one or more CpG islands in the region of the genome, thereby obtaining a first methylation profile;

obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to the administration of the therapeutic to the subject;

determining, based on the second MS data, a methylation profile for each of one or more CpG islands in the region of the genome, thereby obtaining a second methylation profile;

comparing the first average methylation fraction profile across the one or more CpG islands and the second average methylation fraction profile across the one or more CpG islands;

determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the respective methylation profiles; and

detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

147. A method for assessing tumor status of a subject with cancer, comprising:

obtaining first whole genome sequencing (WGS) data of a first plurality of cell-free DNA (cfDNA) molecules, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes a therapeutic configured to treat the cancer is administered to the subject;

determining, based on the first WGS data, (i) a first plurality of copy number aberrations (CNAs) in the first plurality of cfDNA molecules and (ii) a first plurality of fragment lengths of the first plurality of cfDNA molecules;

obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a bodily fluid sample of the subject at the first timepoint;

determining, based on the first MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a first average methylation fraction profile;

obtaining second whole genome sequencing (WGS) data of a second plurality of cell-free DNA (cfDNA) molecules, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to administration of the therapeutic to the subject;

determining, based on the second WGS data, (iii) a second plurality of copy number aberrations (CNAs) in the second plurality of cfDNA molecules and (iv) a second plurality of fragment lengths of the second plurality of cfDNA molecules;

obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a bodily fluid sample of the subject at the second timepoint;

determining, based on the second MS data, an average methylation fraction for each of one or more CpG islands in the region of the genome, thereby obtaining a second average methylation fraction profile;

comparing the first plurality of CNAs with the second plurality of CNAs to determine a CNA profile change;

determining a fragment length profile change based on the first plurality of fragment lengths and the second plurality of fragment lengths;

comparing the first average methylation fraction profile across the one or more CpG islands and the second average methylation fraction profile across the one or more CpG islands to determine a methylation fraction profile;

determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the CNA profile change, the fragment length profile change, and the respective methylation fraction profiles; and

detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

148. A method for assessing tumor status of a subject with cancer, comprising:

obtaining first whole genome sequencing (WGS) data of a first plurality of cell-free DNA (cfDNA) molecules, wherein the first plurality of cfDNA molecules is obtained or derived from a first bodily fluid sample of the subject at a first timepoint, wherein the first timepoint precedes a therapeutic configured to treat the cancer is administered to the subject;

determining, based on the first WGS data, (i) a first plurality of copy number aberrations (CNAs) in the first plurality of cfDNA molecules and (ii) a first plurality of fragment lengths of the first plurality of cfDNA molecules;

obtaining first methylation sequencing (MS) data of a first plurality of cell-free DNA (cfDNA) molecules across a region of a genome, wherein the first plurality of cfDNA molecules is obtained or derived from a bodily fluid sample of the subject at the first timepoint;

determining, based on the first MS data, a methylation profile for each of one or more loci of the genome, thereby obtaining a first methylation profile;

obtaining second whole genome sequencing (WGS) data of a second plurality of cell-free DNA (cfDNA) molecules, wherein the second plurality of cfDNA molecules is obtained or derived from a second bodily fluid sample of the subject at a second timepoint, wherein the second timepoint is subsequent to administration of the therapeutic to the subject;

determining, based on the second WGS data, (iii) a second plurality of copy number aberrations (CNAs) in the second plurality of cfDNA molecules and (iv) a second plurality of fragment lengths of the second plurality of cfDNA molecules;

obtaining second MS data of a second plurality of cell-free DNA (cfDNA) molecules across the region of the genome, wherein the second plurality of cfDNA molecules is obtained or derived from a bodily fluid sample of the subject at the second timepoint;

determining, based on the second MS data, a methylation profile for each of one or more loci of the genome, thereby obtaining a second methylation profile;

comparing the first plurality of CNAs with the second plurality of CNAs to determine a CNA profile change;

determining a fragment length profile change based on the first plurality of fragment lengths and the second plurality of fragment lengths;

comparing the first methylation profile across the one or more loci and the second methylation profile across the one or more loci;

determining a first tumor fraction of the subject at the first timepoint or a second tumor fraction of the subject at the second timepoint, based at least in part on the CNA profile change, the fragment length profile change, and the respective methylation fraction profiles; and

detecting a tumor status of the subject based at least in part on the first tumor fraction or the second tumor fraction.

149. The method of claim 148, wherein the first and the second methylation profiles comprise 5-hydroxymethylcytosine status, 5-methylcytosine status, enrichment-based methylation assessment, median methylation level, mode methylation level, maximum methylation level, or minimum methylation level.

150. The method of any one of claims 147-149, wherein the first WGS data and the first MS data are obtained from the same sample.

151. The method of any one of claims 147-149, wherein the first WGS data and the first MS data are obtained from different samples.

152. The method of any one of claims 147-151, wherein the second WGS data and the second MS data are obtained from the same sample.

153. The method of any one of claims 147-151, wherein the second WGS data and the second MS data are obtained from different samples.

154. The method of any one of claims 147-153, wherein the first or second bodily fluid sample is selected from the group consisting of: blood, serum, plasma, vitreous, sputum, urine, tears, perspiration, saliva, semen, mucosal excretions, mucus, spinal fluid, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, amniotic fluid, and lymph fluid.

155. The method of any one of claims 147-154, wherein obtaining the first WGS data comprises sequencing the first plurality of cfDNA molecules to generate a first plurality of sequencing reads, or wherein obtaining the second WGS data comprises sequencing the second plurality of cfDNA molecules to generate a second plurality of sequencing reads.

156. The method of any one of claims 147-155, further comprising enriching the first or second plurality of cfDNA molecules for a plurality of genomic regions.

157. The method of claim 155 or claim 156, wherein determining the first plurality of CNAs comprises determining quantitative measures of CNAs at each of a plurality of genomic regions of the first plurality of sequencing reads, and wherein determining the second plurality of

CNAs comprises determining quantitative measures of CNAs at each of at each of the plurality of genomic regions of the second plurality of sequencing reads.

158. The method of any one of claims 147-157, wherein determining the CNA profile change comprises comparing the first plurality of CNAs and the second plurality of CNAs with a plurality of reference CNA values, wherein the plurality of reference CNA values is obtained from additional cfDNA molecules obtained or derived from additional bodily fluid samples of additional subjects.

159. The method of any one of claims 147-158, wherein the first and second WGS data are obtained by pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, Nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, massively parallel sequencing, chain termination sequencing, single molecule real-time sequencing, Polony sequencing, combinatorial probe anchor synthesis, or hybrid capture-based sequencing.

160. The method of any one of claims 147-159, wherein obtaining the first MS data comprises performing methylation sequencing of the first plurality of cfDNA molecules to generate a first plurality of sequencing reads, or wherein obtaining the second WGS data comprises performing methylation sequencing of the second plurality of cfDNA molecules to generate a second plurality of sequencing reads.

161. The method of any one of claims 147-160, further comprising enriching the first or second plurality of cfDNA molecules for the region of the genome.

162. The method of any one of claims 147-161, wherein the region of the genome comprises one or more of: CpG islands, CpG shores, patient-specific partially methylated domains, common partially methylated domains, promoters, gene bodies, evenly spaced genomewide bins, and transposable elements.

163. The method of any one of claims 147-162, wherein determining the first or second tumor fraction comprises comparing the methylation fraction profile with one or more reference methylation fraction profiles, wherein the one or more reference methylation fraction profiles are obtained from additional cfDNA molecules obtained or derived from additional bodily fluid samples of additional subjects.

164. The method of any one of claims 147-163, further comprising, based on the determined tumor status of the subject, administering a therapeutically effective dose of a treatment to treat the cancer of the subject.

165. The method of claim 164, wherein the treatment comprises surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, cell therapy, an anti-hormonal agent, an antimetabolite chemotherapeutic agent, a kinase inhibitor, a methyltransferase inhibitor, a peptide, a gene therapy, a vaccine, a platinum-based chemotherapeutic agent, an antibody, or a checkpoint inhibitor.

166. The method of any one of claims 147-165, wherein the first and the second pluralities of cfDNA molecules are from immune cells of the subject.

167. The method of any one of claims 147-166, wherein the detected tumor status is indicative of tumor progression, non-progression, regression, or recurrence.

168. The method of any one of claims 147-167, wherein the first and second MS data are obtained by a sequencing device or computer processor.

169. The method of any one of claims 147-168, wherein the subject has brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, kidney cancer, hepatobiliary tract cancer, leukemia, liver cancer, lung cancer, lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, thyroid cancer, or urinary tract cancer.