CLAIMS

We claim:

1. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads and the second plurality of sequence reads as somatic or germline;

determining, for at least one variant of the plurality of variants classified as somatic, based on a first mutant allele fraction (MAF) and a second MAF, a weighted mean of the first MAFs and a weighted mean of the second MAFs;

determining, for the subject, a ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs;

determining, based on the ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs, a confidence interval; and

outputting, as a molecular response score, the ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs and the confidence interval.

2. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads and the second plurality of sequence reads as somatic or germline;

determining, for at least one variant of the plurality of variants classified as somatic, based on a first mutant allele fraction (MAF) and a second MAF, an MAF ratio;

determining, for the subject, a weighted mean of the MAF ratios;

determining, based on the weighted mean of the MAF ratios, a confidence interval associated with the weighted mean of the MAF ratios; and

outputting, as a molecular response score, the weighted mean of the MAF ratios and the confidence interval.

3. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads as somatic or germline;

classifying the plurality of variants in the second plurality of sequence reads as somatic or germline;

reclassifying at least one variant of the plurality of variants to resolve a classification discrepancy between the first plurality of sequence reads and the second plurality of sequence reads;

determining, for at least one variant of the plurality of variants classified or reclassified as somatic, based on at least a portion of the first plurality of sequence reads, a first mutant allele fraction (MAF);

determining, for the at least one variant of the plurality of variants classified or reclassified as somatic, based on at least a portion of the second plurality of sequence reads, a second MAF; and

determining, based on the first MAF and the second MAF, a molecular response score.

4. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads and the second plurality of sequence reads as somatic or germline;

determining at least one variant of the plurality of variants as a Clonal Hematopoiesis of Indeterminate Potential (CHIP) variant;

removing, from the plurality of variants, the at least one CHIP variant;

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the first plurality of sequence reads, a first mutant allele fraction (MAF);

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the second plurality of sequence reads, a second MAF; and

determining, based on the first MAF and the second MAF, a molecular response score.

5. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads as somatic or germline;

classifying the plurality of variants in the second plurality of sequence reads as somatic or germline;

reclassifying at least one variant of the plurality of variants to resolve a classification discrepancy between the first plurality of sequence reads and the second plurality of sequence reads;

determining at least one variant of the plurality of variants as a Clonal Hematopoiesis of Indeterminate Potential (CHIP) variant;

removing, from the plurality of variants, the at least one CHIP variant;

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the first plurality of sequence reads, a first mutant allele fraction (MAF);

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the second plurality of sequence reads, a second MAF;

determining, for at least one variant of the plurality of variants classified as somatic, based on the first MAF and the second MAFan MAF ratio;

determining, for the subject, a weighted mean of the MAF ratios;

determining, based on the weighted mean of the MAF ratios, a confidence interval associated with the weighted mean of the MAF ratios; and

outputting, as a molecular response score, the weighted mean of the MAF ratios and the confidence interval.

6. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined before a therapy is to be administered and the second plurality of sequence reads are determined after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads as somatic or germline;

classifying the plurality of variants in the second plurality of sequence reads as somatic or germline;

reclassifying at least one variant of the plurality of variants to resolve a classification discrepancy between the first plurality of sequence reads and the second plurality of sequence reads;

determining at least one variant of the plurality of variants as a Clonal Hematopoiesis of Indeterminate Potential (CHIP) variant;

removing, from the plurality of variants, the at least one CHIP variant;

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the first plurality of sequence reads, a first mutant allele fraction (MAF);

determining, for at least one variant of the plurality of variants classified as somatic, based on at least a portion of the second plurality of sequence reads, a second MAF;

determining, for the at least one variant of the plurality of variants classified as somatic, based on the first MAF and the second MAF, a weighted mean of the first MAFs and a weighted mean of the second MAFs;

determining, for the subject, a ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs;

determining, based on the ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs, a confidence interval; and

outputting, as a molecular response score, the ratio of the weighted mean of the first MAFs and the weighted mean of the second MAFs and the confidence interval.

7. A method comprising:

determining a first plurality of sequence reads and a second plurality of sequence reads associated with a subject, wherein the first plurality of sequence reads are determined at a first time point before a therapy is to be administered and the second plurality of sequence reads are determined at a second time point after the therapy has been administered;

classifying a plurality of variants in the first plurality of sequence reads and the second plurality of sequence reads as somatic or germline;

determining, for at least one variant of the plurality of variants classified as somatic, based on a first mutant allele fraction (MAF) at the first time point and a second MAF at the second time point, a first central tendency measure of the first MAFs and a second central tendency measure of the second MAFs;

determining a ratio of the first central tendency measure at the first time point to the second central tendency measure at the second time point; and

outputting, as a molecular response score, the ratio of the first central tendency measure at the first time point to the second central tendency measure at the second time point.

8. The method of claim 7, wherein the central tendency measure is one or more of a: mean, median, or mode.

9. A method of determining a molecular response score for a subject having cancer at least partially using a computer, the method comprising:

(a) determining, by the computer, mutant allele frequencies (MAFs) for a plurality of variants from sequence information generated from targeted nucleic acids associated with

one or more cancer types in samples obtained from the subject at first and second time points to produce sets of first and second MAFs for at least one variant in the plurality of variants;

(b) calculating, by the computer, a ratio of the first and second MAFs for at least one variant in the plurality of variants to produce a set of MAF ratios and a corresponding standard deviation for each MAF ratio in the set of MAF ratios; and,

(c) calculating, by the computer, a weighted mean of the MAF ratios and a confidence interval, thereby determining the molecular response score for the subject having the cancer.

10. A method of identifying clonal hematopoietic variants in a subject having cancer at least partially using a computer, the method comprising:

(a) determining, by the computer, a tumor load change (R) for tumor fraction change P(R) for each of a plurality of variants from sequence information generated from targeted nucleic acids associated with one or more cancer types in samples obtained from the subject at first and second time points to produce a set of tumor load changes; and,

(b) identifying, by the computer, one or more resistance signatures corresponding to one or more clonal hematopoietic variants from the set of tumor load changes, thereby identifying the identifying the clonal hematopoietic variants in the subject having cancer.

11. A method of identifying clonal hematopoietic variants in a subject having cancer at least partially using a computer, the method comprising:

(a) calculating, by the computer, a probability density function for tumor fraction change P(R) for each of a plurality of variants from sequence information generated from targeted nucleic acids associated with one or more cancer types in samples obtained from the subject at first and second time points;

(b) grouping, by the computer, one or more of the variants by P(R) into one or more clones;

(c) generating, by the computer, an updated P(R) for each of the clones; and,

(d) identifying, by the computer, one or more clones having a fractional change between the first and second time points at or above a predetermined threshold value, thereby identifying the identifying the clonal hematopoietic variants in the subject having cancer.

12. The method of claim 11, comprising determining a likelihood that a given pair of variants exhibit an identical fractional change, merging most likely pairs of variants into one clone, and updating the P(R) for the one clone.

13. A method of identifying germline variants in a subject having cancer at least partially using a computer, the method comprising:

(a) determining, by the computer, a mutant allele frequency (MAF) for a given variant from sequence information generated from targeted nucleic acids associated with one or more cancer types in a sample obtained from the subject; and,

(b) identifying, by the computer, that the given variant is a germline variant when the MAF of the given variant increases the max MAF of the sample, which sample comprises a max fraction of diploid genes (max frac_diploid) and/or when the MAF of the given variant is at least about two times greater, three times greater, four times greater, five times greater, six times greater, seven times greater, eight times greater, nine times greater, or more than one or more other MAFs determined from the sample obtained from the subject, thereby identifying the germline variants in the subject having cancer.

14. The method of any one preceding claim, further comprising comparing the molecular response score for the subject having the cancer to a predetermined cutoff point to identify that the subject is a likely responder to one or more therapies for the cancer when the molecular response score is below the predetermined cutoff point or that the subject is a likely non-responder to the one or more therapies for the cancer when the molecular response score is at or above the predetermined cutoff point.

15. The method of any one preceding claim, wherein the one or more therapies comprise one or more immunotherapies.

16. The method of any one preceding claim, wherein one or more therapies for the cancer is to be administered to the subject in view of the molecular response score.

17. The method of any one preceding claim, further comprising discontinuing one or more therapies for the cancer to the subject in view of the molecular response score.

18. The method of any one preceding claim, comprising using the molecular response score as a prognostic biomarker and/or a predictive biomarker for the subject.

19. The method of any one preceding claim, comprising using a molecule count to calculate the standard deviation for each MAF ratio in the set of MAF ratios.

20. The method of any one preceding claim, comprising propagating a variance through each MAF ratio in the set of MAF ratios.

21. The method of any one preceding claim, further comprising excluding one or more germline and/or clonal hematopoietic variants when determining the mutant allele frequencies (MAFs) for the plurality of variants.

22. The method of any one preceding claim, wherein the plurality of variants comprises somatic nucleic acid variants.

23. The method of any one preceding claim, further comprising excluding one or more somatic variants having MAFs that are less than about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, or 0.9% at both the first and second time points.

24. The method of any one preceding claim, wherein the first time point comprises a pre-treatment time point and wherein the second time point comprises an on- or post-treatment time point.

25. The method of any one preceding claim, comprising generating the sequence information from nucleic acid molecules obtained from one or more tissues or cells in the sample.

26. The method of any one preceding claim, comprising generating the sequence information from cell-free nucleic acids (cfNAs) in the samples obtained from the subject.

27. The method of any one preceding claim, wherein the cfNAs comprise circulating tumor DNA (ctDNA).

28. The method of any one preceding claim, wherein the ratio comprises the second MAF to the first MAF for at least one variant in the plurality of variants.

29. The method of any one preceding claim, comprising calculating the weighted mean of the MAF ratios using the formula:

sum[weight * ratio]/sum[weights],

where weight is $1/\text{range}^2$ for a given variant in the plurality of variants, where range is a difference between values of the first and second MAFs for a given variant in the plurality of variants, and ratio is a given MAF ratio in the set of MAF ratios.

30. The method of any one preceding claim, comprising calculating the confidence interval using the formula:

weighted mean of the MAF ratios +/- sqrt[ratio variance],

where ratio variance is 1/sum[weights].

31. The method of any one preceding claim, comprising calculating the ratio of the weighted means of the MAFs comprises using the formula:
(weighted mean of the MAF ratios at time point 2)/(weighted mean of the MAF ratios at time point 1)
where an MAF of a variant that is undetected at a timepoint is set to zero.

32. The method of any one preceding claim, wherein the variants comprise one or more single-nucleotide variants (SNV), insertion/deletion mutations (indels), gene amplifications, and/or gene fusions.

33. The method of any one preceding claim, further comprising using one or more additional genomic data sources to determine the molecular response score for the subject having the cancer.

34. The method of any one preceding claim, wherein the additional genomic data sources comprise one or more of: a coverage, an off-target coverage, an epigenetic signature, and/or a microsatellite instability score.

35. The method of any one preceding claim, wherein the epigenetic signature comprises a cfNA fragment length, position, and/or endpoint density distribution.

36. The method of any one preceding claim, wherein the epigenetic signature comprises an epigenetic state or status exhibited by one or more epigenetic loci in a given targeted genomic region.

37. The method of any one preceding claim, wherein the epigenetic state or status comprises a presence or absence of methylation, hydroxymethylation, acetylation, ubiquitylation, phosphorylation, sumoylation, ribosylation, citrullination, and/or a histone post-translational modification or other histone variation.

38. The method of any one preceding claim, further comprising determining one or more copy number variants before the therapy is to be administered and one or more copy number variants after the therapy has been administered, and removing, from the plurality of variants, the one or more copy number variants based on a number of the one or more copy number variants exceeding a threshold.

39. The method of any one preceding claim, further comprising:

determining a first central tendency measure obtained from MAFs of somatic variants at a first time point and a second central tendency measure obtained from MAFs of the somatic variants at a second time point;

calculating a ratio of the central tendency measure at the first time point to the central tendency measure at the second time point;

calculating a standard deviation of the ratio of the central tendency measure at the first time point to the central tendency measure at the second time point; and

outputting as another molecular response score, the ratio of the central tendency measure at the first time point to the central tendency measure at the second time point.

40. The method of any one preceding claim, further comprising:

obtaining a plurality of sequence reads at each of a plurality of time points; and

determining an MAF ratio between any two time points of the plurality of time points.

41. The method of any one preceding claim, further comprising recommending one or more therapies.

42. An apparatus configured to perform the methods of any preceding claim.

43. A computer readable medium comprising non-transitory computer executable instructions which, when executed by at least one electronic processor, perform the methods of any preceding claim.

44. A system configured to perform the methods of any preceding claim.