CLAIMS

What is claimed is:

1. A method comprising:
    a. measuring expression levels of RNA transcripts for at least two of PD-1, TOP2A, PD-L1 and ADAM12 obtained from a tumor specimen from a subject;
    b. measuring tumor mutation burden (TMB) in the biological sample and log2 transforming the TMB measurement to provide a transformed TMB measurement; and
    c. calculating an Immunotherapy Response Score (IRS) from the expression levels of the RNA transcripts of the at least two of PD-1, TOP2A, PD-L1 and ADAM12 obtained in step a), and the transformed TMB measurement, wherein the IRS is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy, and obtaining an IRS having a value indicating a beneficial response to checkpoint inhibitor therapy.

2. The method of claim 1, wherein step a. further comprises ii) measuring expression levels of RNA transcripts for at least one reference gene in the biological sample, and iii) normalizing the measured expression levels of the measured RNA transcripts of the at least two, the at least three, or all of PD-1, TOP2A, PD-L1 and ADAM12 against the level of the RNA transcripts of the at least one reference gene to provide normalized expression levels of the PD-1, TOP2A, PD-L1 and ADAM12 RNA transcripts, and wherein the expression levels of RNA transcripts used to calculate the IRS comprises normalized expression levels of RNA transcripts.

3. The method of claim 2, wherein step a. further comprises iv) median centering the measured expression levels of RNA transcripts of the at least two of PD-1, TOP2A, PD-L1 and ADAM12, prior or after to normalizing the expression levels of the measured RNA transcripts.

4. The method of claim 3, wherein step a. further comprises v) log2 transforming the measured expression levels, the median centered expression levels, the normalized expression levels or the median centered normalized expression levels of RNA transcripts of the at least two, the

at least three, or all of PD-1, TOP2A, PD-L1 and ADAM12, and wherein the expression levels utilized to calculate the IRS in step c are transformed expression levels, transformed median centered expression levels, transformed normalized expression levels or transformed median centered normalized expression levels.

5. The method of claim 2, wherein the one or more reference genes comprise three genes selected from CIAO1, EIF2B1, HMBS, CTCF, GGNBP2, ITGB7, MYC and SLC4A1AP.

6. The method of claim 1, wherein the tumor specimen is a formalin-fixed paraffin-embedded (FFPE) tumor specimen and the tumor specimen contains at least 20% tumor content.

7. The method of claim 1, wherein the tumor specimen is breast cancer, central or peripheral nervous system cancer, cancer of unknown primary, colorectal cancer, endometrial cancer, a gastrointestinal stromal tumor, glioma, hepatobiliary cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer salivary gland cancer, sarcoma, or thyroid cancer.

8. The method of claim 1, wherein the tumor specimen is assessed as having either microsatellite instability-low or microsatellite stability and/or is assessed as having a low tumor mutational burden, wherein low tumor mutational burden is classified as having less than 10 mutations per megabase (muts/Mb).

9. The method of claim 1, wherein the expression levels of RNA transcripts are measured using PCR and next-generation sequencing.

10. The method of claim 1, wherein the checkpoint inhibitor is an anti-PD-1 antibody, an anti-CTLA-4 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody.

11. The method of claim 10, wherein the checkpoint inhibitor is nivolumab, pembrolizumab, atezolizumab, cemiplimab, durvalumab, pidilizumab, atezolimumab, PDR001, BMS- 936559, avelumab, ipilimumab, or SHR-1210.

12. The method of claim 1, wherein step a. comprises measuring expression levels of RNA transcripts for PD-1, TOP2A, PD-L1 and ADAM12, and one or more reference genes in a biological sample obtained from a tumor specimen from the subject, wherein the one or more reference gene comprises three genes selected from CIAO1, EIF2B1, HMBS, CTCF, GGNBP2, ITGB7, MYC and SLC4A1AP, and further log2 transforming, median centering, and normalizing the measured expression levels of the RNA transcripts against the level of the RNA transcripts of the at least one reference gene to provide normalized levels of the RNA transcripts; and wherein step c. comprises calculating an Immunotherapy Response Score (IRS) from the transformed normalized levels of the RNA transcripts of PD-1, TOP2A,

PD-L1 and ADAM12, and the transformed TMB measurement, wherein the IRS is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy.

13. The method of claim 1, wherein the tumor specimen is from a cancer not approved for labeled use of the checkpoint inhibitor therapy.

14. The method of claim 1, wherein the tumor specimen shows a TPS score of 1-49%.

15. A method of identifying a subject that would benefit from checkpoint inhibitor therapy, comprising:
    a. receiving, by a processor, measured expression levels of RNA transcripts for at least two of PD-1, TOP2A, PD-L1 and ADAM12, and at least one reference gene in a biological sample obtained from a tumor specimen from the subject;
    b. log2 transforming, median centering, and normalizing, by a processor, the measured expression levels of the RNA transcripts against the level of the RNA transcripts of at the least one reference gene to provide transformed normalized levels of the RNA transcripts;
    c. receiving, by a processor, measured tumor mutation burden (TMB) in the biological sample;
    d. log2 transforming, by a processor, the TMB measurement to provide a transformed TMB measurement;
    e. calculating, by a processor, an Immunotherapy Response Score (IRS) from the transformed normalized levels of the RNA transcripts of the at least two of PD-1, TOP2A, PD-L1 and ADAM12, and the transformed TMB measurement, that is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy; and
    f. providing a determination if the subject has a checkpoint inhibitor responsive cancer.

16. The method of claim 15, wherein the measured expression levels of RNA transcripts comprise the measured expression level of RNA transcripts of PD-1, TOP2A, PD-L1, ADAM12, and three reference genes selected from CIAO1, EIF2B1, HMBS, CTCF, GGNBP2, ITGB7, MYC and SLC4A1AP.

17. A method comprising:

    a. measuring expression levels of RNA transcripts for PD-1 and PD-L2 obtained from a tumor specimen from a subject;
    b. measuring tumor mutation burden (TMB) in the biological sample and log2 transforming the TMB measurement to provide a transformed TMB measurement; and
    c. calculating an Immunotherapy Response Score (IRS) from the expression levels or normalized levels of the RNA transcripts of PD-1 and PD-L2, and the transformed TMB measurement, wherein the IRS is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy, and obtaining an IRS having a value indicating a beneficial response to checkpoint inhibitor therapy.

18. A method of identifying a subject that would benefit from checkpoint inhibitor therapy, comprising:

    a. measuring expression levels of RNA transcripts for PD-1, PD-L2 and, optionally, expression levels of RNA transcripts for one or more of CD4, ADAM12, PD-L1, and VTCN1, and at least one reference gene in a biological sample obtained from a tumor specimen from the subject;
    b. log2 transforming, median centering, and normalizing the measured expression levels of the RNA transcripts against the level of the RNA transcripts of at the least one reference gene to provide normalized levels of the RNA transcripts;
    c. measuring tumor mutation burden (TMB) in the biological sample and log2 transforming the TMB measurement to provide a transformed TMB measurement;
    d. calculating a Immunotherapy Response Score (IRS) from the normalized levels of the RNA transcripts of PD-1, PD-L2 and, optionally, one or more of CD4, ADAM12, PD-L1, and VTCN1, and the transformed TMB measurement, wherein the IRS is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy, and obtaining an IRS having a value indicating beneficial response to checkpoint inhibitor therapy; and
    e. identifying the subject as benefiting from the checkpoint inhibitor therapy.

19. The method of claim 18, wherein the tumor specimen is from a cancer not approved for labeled use of the checkpoint inhibitor therapy.

20. The method of claims 19, wherein the calculated IRS value indicates that the median time-to-next-treatment (TNTT) is 24 months or greater.

21. A method of identifying a subject that would benefit from checkpoint inhibitor therapy, comprising:
    a. receiving, by a processor, measured expression levels of RNA transcripts for PD-1, PD-L2 and, optionally, expression levels of RNA transcripts for one or more of CD4, ADAM12, PD-L1, and VTCN1, and at least one reference gene in a biological sample obtained from a tumor specimen from the subject;
    b. log2 transforming, median centering, and normalizing, by a processor, the measured expression levels of the RNA transcripts against the level of the RNA transcripts of at the least one reference gene to provide normalized levels of the RNA transcripts;
    c. receiving, by a processor, measured tumor mutation burden (TMB) in the biological sample;
    d. log2 transforming, by a processor, the TMB measurement to provide a transformed TMB measurement;
    e. calculating, by a processor, a Immunotherapy Response Score (IRS) from the normalized levels of the RNA transcripts of PD-1, PD-L2 and, optionally, one or more of CD4, ADAM12, PD-L1, and VTCN1, and the transformed TMB measurement, that is positively correlated with the likelihood that the patient has a beneficial response to checkpoint inhibitor therapy; and
    f. providing a determination if the subject has a checkpoint inhibitor responsive cancer.