

CLAIMS

What is claimed is:

1. A method of predicting treatment response of an individual with cancer to treatment with a checkpoint inhibitor, comprising:
 - isolating cfRNA from a blood sample obtained from the individual, wherein the cfRNA encodes a checkpoint inhibition gene;
 - quantifying the cfRNA using quantitative PCR method; and
 - predicting a positive treatment response when the quantity of the cfRNA is above a threshold level.
2. The method of claim 1 wherein the step of isolating the cfRNA uses RNA stabilization and cell preservation.
3. The method of claim 1 further comprising a step of quantifying at least a second cfRNA using the quantitative PCR method.
4. The method of claim 3 wherein the at least a second cfRNA encodes TIM3 or LAG3.
5. The method of claim 1 further comprising a step of quantifying at least a second cfRNA, wherein the at least second cfRNA encodes a gene having a tumor and patient specific mutation, a tumor associated gene, or a cancer specific gene.
6. A method of monitoring treatment of an individual with cancer, comprising:
 - isolating cfRNA from a blood sample obtained from the individual, wherein the cfRNA encodes a checkpoint inhibition gene, or wherein the cfRNA encodes a tumor associated or cancer specific gene, or wherein the cfRNA encodes a gene having a tumor and patient specific mutation;
 - quantifying the cfRNA using quantitative PCR method; and
 - updating a patient record using the quantity of the cfRNA.
7. The method of claim 6 wherein the step of isolating the cfRNA uses RNA stabilization and cell preservation.
8. The method of claim 1 or claim 6 wherein the quantitative PCR method includes real time PCR.

9. The method of claim 1 or claim 6 wherein the step of quantifying uses β -actin as an internal standard.
10. The method of claim 6 wherein the patient record is updated when the quantity of the cfRNA is $\Delta\Delta CT > 5$ for HER2 relative to β -actin or $\Delta\Delta CT > 10$ for PCA3 relative to β -actin.
11. A method of detecting prostate cancer, comprising:
 - isolating cfRNA from a blood sample obtained from an individual, wherein the cfRNA encodes PCA3 or a splice variant 7 of an androgen receptor;
 - quantifying the cfRNA using quantitative PCR method; and
 - detecting the cancer when the cfRNA quantity is above a threshold level.
12. The method of claim 11 wherein the cancer is detected when the quantity of PCA3 cfRNA is $\Delta\Delta CT > 10$ relative to β -actin.
13. The method of claim 11 further comprising a step of quantifying at least a second cfRNA, wherein the at least second cfRNA encodes a gene having a tumor and patient specific mutation, a tumor associated gene, a cancer specific gene, or a checkpoint inhibition gene.
14. The method of claim 13 wherein the second cfRNA encodes PD-L1, LAG3, TIM3, AR-V7, PSA, and PSMA.
15. Use of afinitor in the preparation of a medicament in the treatment of cancer, wherein the medicament is to be administered to an individual diagnosed with a PD-L1 negative cancer;
 - treatment of the individual is to be monitored by isolating cfRNA from the blood,
 - wherein the cfRNA encodes PD-L1;
 - the cfRNA is to be quantified using quantitative PCR method; and
 - a checkpoint inhibitor is to be included in the treatment upon detection of the cfRNA.
16. The use of claim 15 wherein the PD-L1 negative cancer is a solid cancer.
17. The use of claim 16 wherein the solid cancer is breast cancer.
18. The use of claim 15 wherein the cfRNA is to be quantified using real-time PCR.

19. The use of claim 15 wherein the checkpoint inhibitor is included when the cfRNA is detected and increases over time.
20. The use of claim 15 wherein the checkpoint inhibitor is included when the cfRNA is detected and the cfRNA level is $\Delta\Delta CT > 10$ relative to β -actin.
21. A method of determining an immune signature in a patient, comprising:
 - determining quantities of distinct cfRNA molecules in a blood sample obtained from an individual, wherein the cfRNA molecules encode distinct checkpoint inhibition genes;
 - wherein the step of determining is performed prior to or during treatment with at least one of a checkpoint inhibitor, a chemotherapeutic drug, an immune therapeutic drug, and radiation treatment.
22. The method of claim 21 wherein at least one of the distinct cfRNA molecules encodes PD-L1, LAG3, or TIM3.
23. The method of claim 21 wherein the step of determining quantities comprises real time PCR.
24. Use of cfRNA to predict a treatment response of an individual with cancer to treatment with a checkpoint inhibitor, wherein the cfRNA encodes a checkpoint inhibition gene, and wherein the cfRNA is above a threshold level.
25. The method of claim 1 or the use of claim 24 wherein the checkpoint inhibitor is an antibody against PD1 or PD-L1 and wherein the cfRNA is PD-L1 cfRNA.
26. The method of claim 1 or the use of claim 24 wherein the threshold level is $\Delta\Delta CT > 10$ for PD-L1 relative to β -actin.
27. Use of cfRNA to monitor treatment of an individual with cancer, wherein the cfRNA encodes a checkpoint inhibition gene, or wherein the cfRNA encodes a tumor associated or cancer specific gene, or wherein the cfRNA encodes a gene having a tumor and patient specific mutation.
28. The method of claim 6 or the use of claim 27 wherein the checkpoint inhibition gene is PD-L1, TIM3, or LAG3.

29. The method of claim 6 or the use of claim 27 wherein the tumor associated or cancer specific gene is CEA, MUC1, brachyury, HER2, PCA3, or AR-V7.
30. The method of claim 6 or the use of claim 27 wherein the gene having a tumor and patient specific mutation encodes a neoepitope.
31. Use of a cfRNA encoding PCA3 or a splice variant 7 of an androgen receptor in the detection of prostate cancer in an individual.
32. The use of claim 31 wherein the individual is diagnosed as having prostate cancer when the cfRNA quantity is above a threshold level.
33. The use of claim 32 wherein the threshold level for PCA3 cfRNA is $\Delta\Delta CT > 10$ relative to β -actin.