



US 20250266127A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2025/0266127 A1  
BLOOM et al. (43) Pub. Date: Aug. 21, 2025(54) IMMUNO-ONCOLOGY APPLICATIONS  
USING NEXT GENERATION SEQUENCING*G06F 18/2111* (2023.01)*G06F 18/2134* (2023.01)(71) Applicant: Cofactor Genomics, Inc., St. Louis,  
MO (US)*G16B 5/00* (2019.01)(72) Inventors: Ryan J. BLOOM, San Francisco, CA  
(US); Jon R. ARMSTRONG, San  
Diego, CA (US)*G16B 20/00* (2019.01)*G16B 20/20* (2019.01)

(21) Appl. No.: 19/204,154

*G16B 25/00* (2019.01)

(22) Filed: May 9, 2025

*G16B 25/10* (2019.01)*G16B 40/00* (2019.01)

**Related U.S. Application Data**

(63) Continuation of application No. 16/823,195, filed on Mar. 18, 2020, now abandoned, which is a continuation of application No. 16/056,406, filed on Aug. 6, 2018, now Pat. No. 10,636,512, which is a continuation of application No. PCT/US2018/042176, filed on Jul. 13, 2018.

(52) U.S. Cl.  
CPC ..... *G16B 30/00* (2019.02); *G06F 18/2111* (2023.01); *G06F 18/21343* (2023.01); *G16B 20/00* (2019.02); *G16B 20/20* (2019.02);  
*G16B 25/00* (2019.02); *G16B 40/00* (2019.02); *G01N 33/574* (2013.01); *G01N 2800/52* (2013.01);  
*G16B 5/00* (2019.02)

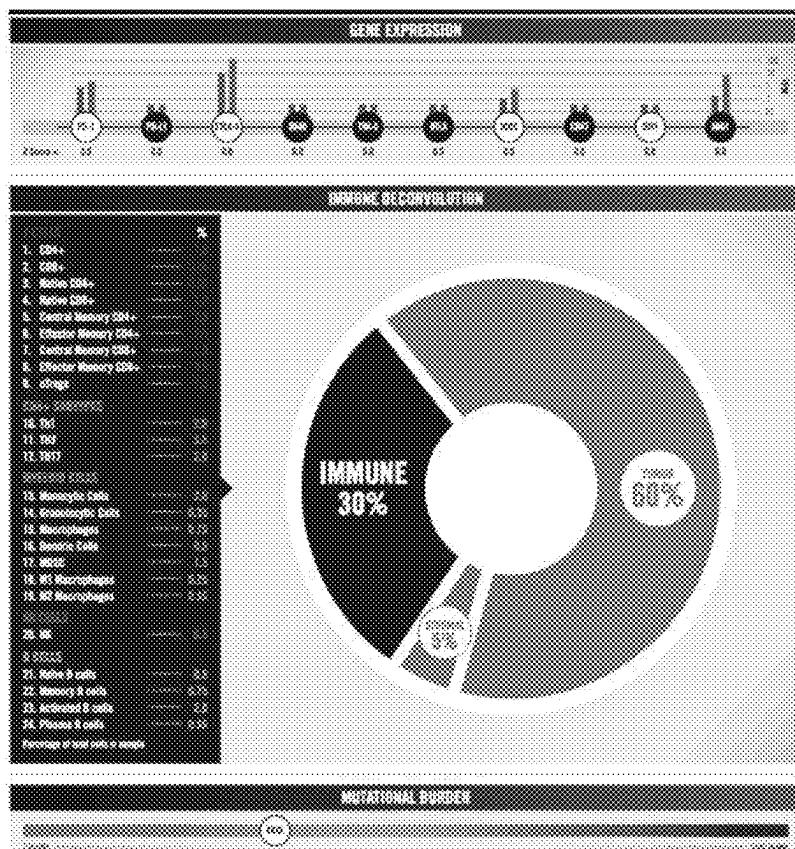
(60) Provisional application No. 62/658,418, filed on Apr. 16, 2018, provisional application No. 62/532,921, filed on Jul. 14, 2017.

#### Publication Classification

(51) Int. Cl.  
*G16B 30/00* (2019.01)  
*G01N 33/574* (2006.01)

#### (57) ABSTRACT

Provided herein are systems and methods for generating an immune-oncology profile from a biological sample. The immune-oncology profile can include the proportion or percentage of immune cells, expression of immune escape genes, and/or mutational burden. The immune-oncology profile may allow the generation of classifiers for making prognostic or diagnostic predictions.



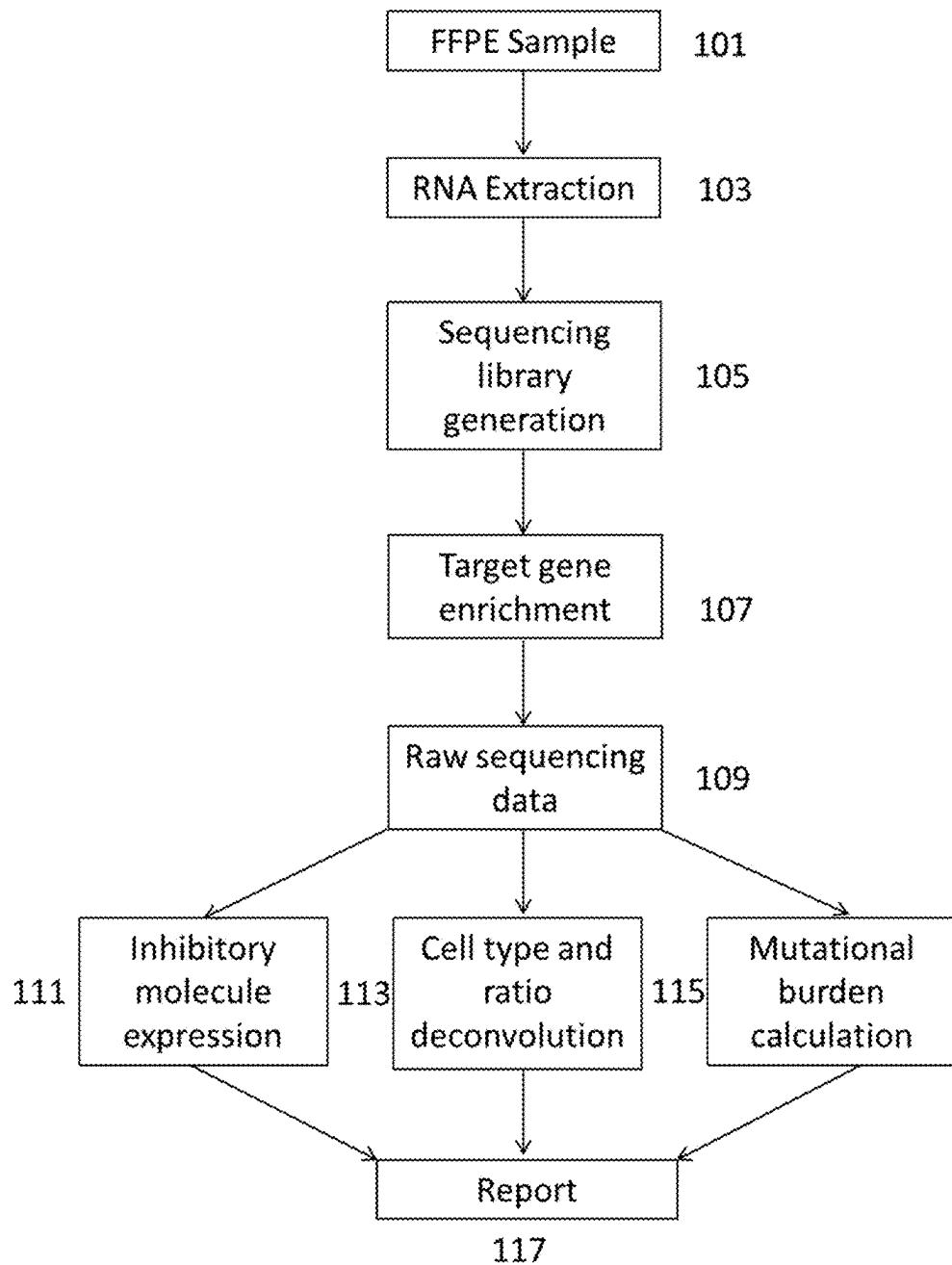


FIG. 1

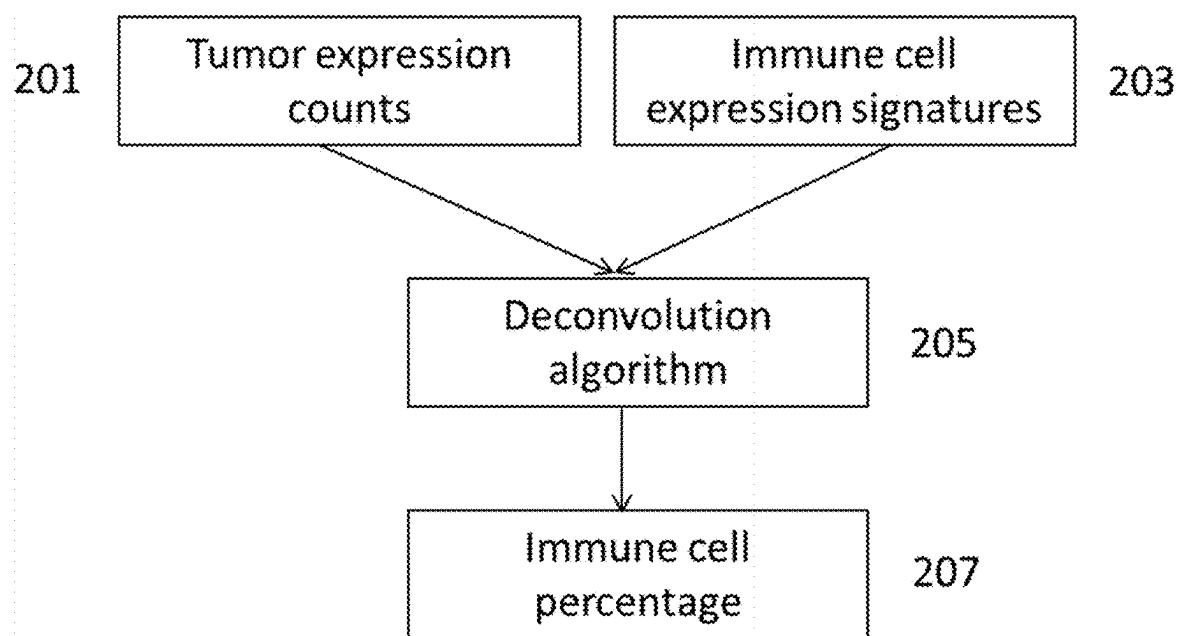


FIG. 2

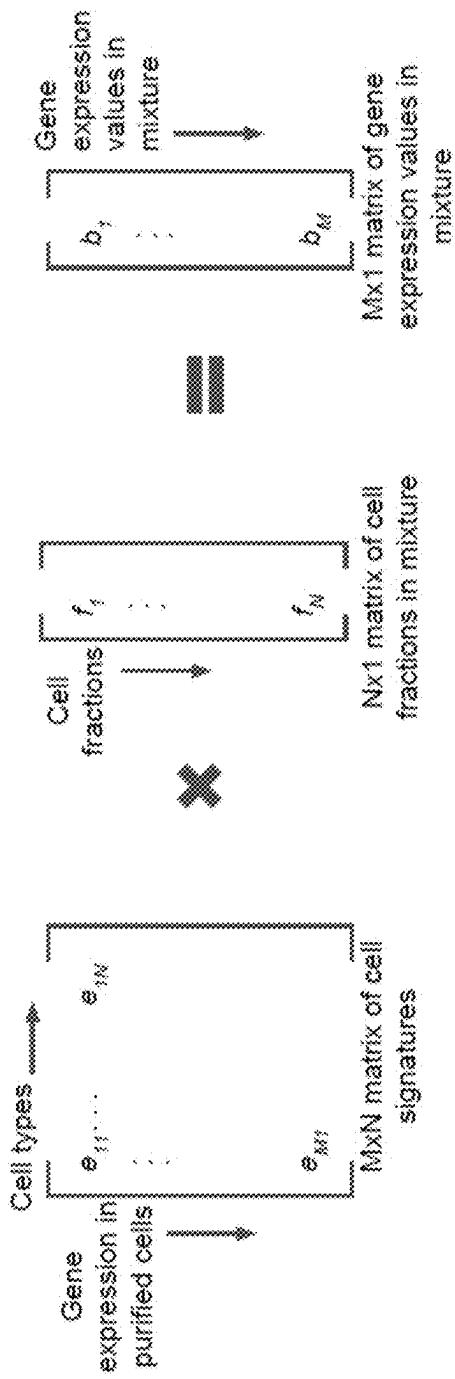


FIG. 3

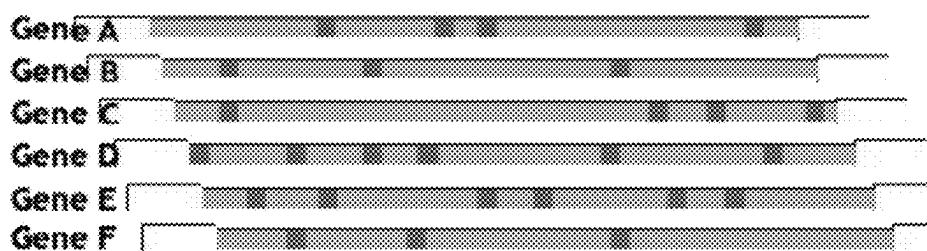


FIG. 4A

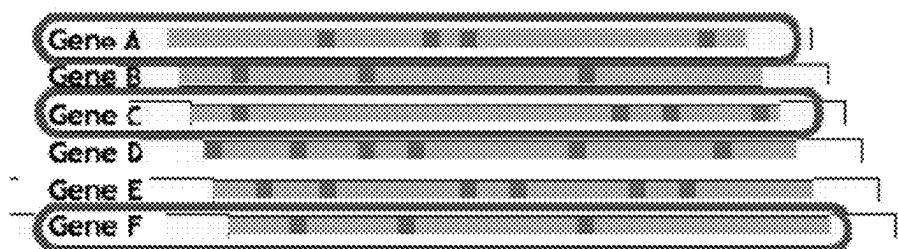


FIG. 4B

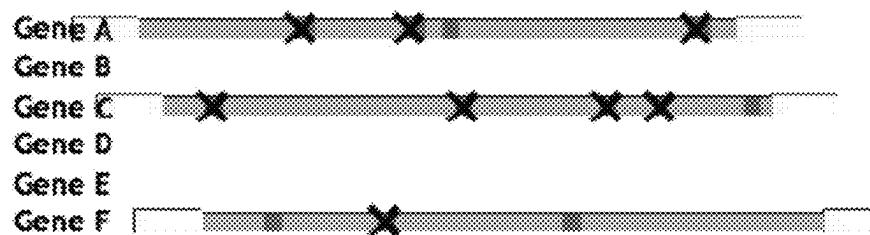


FIG. 4C

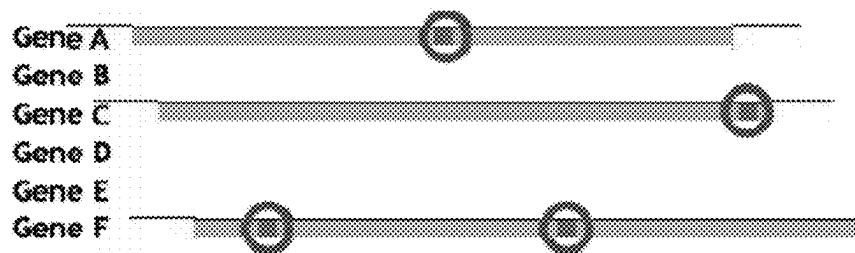


FIG. 4D

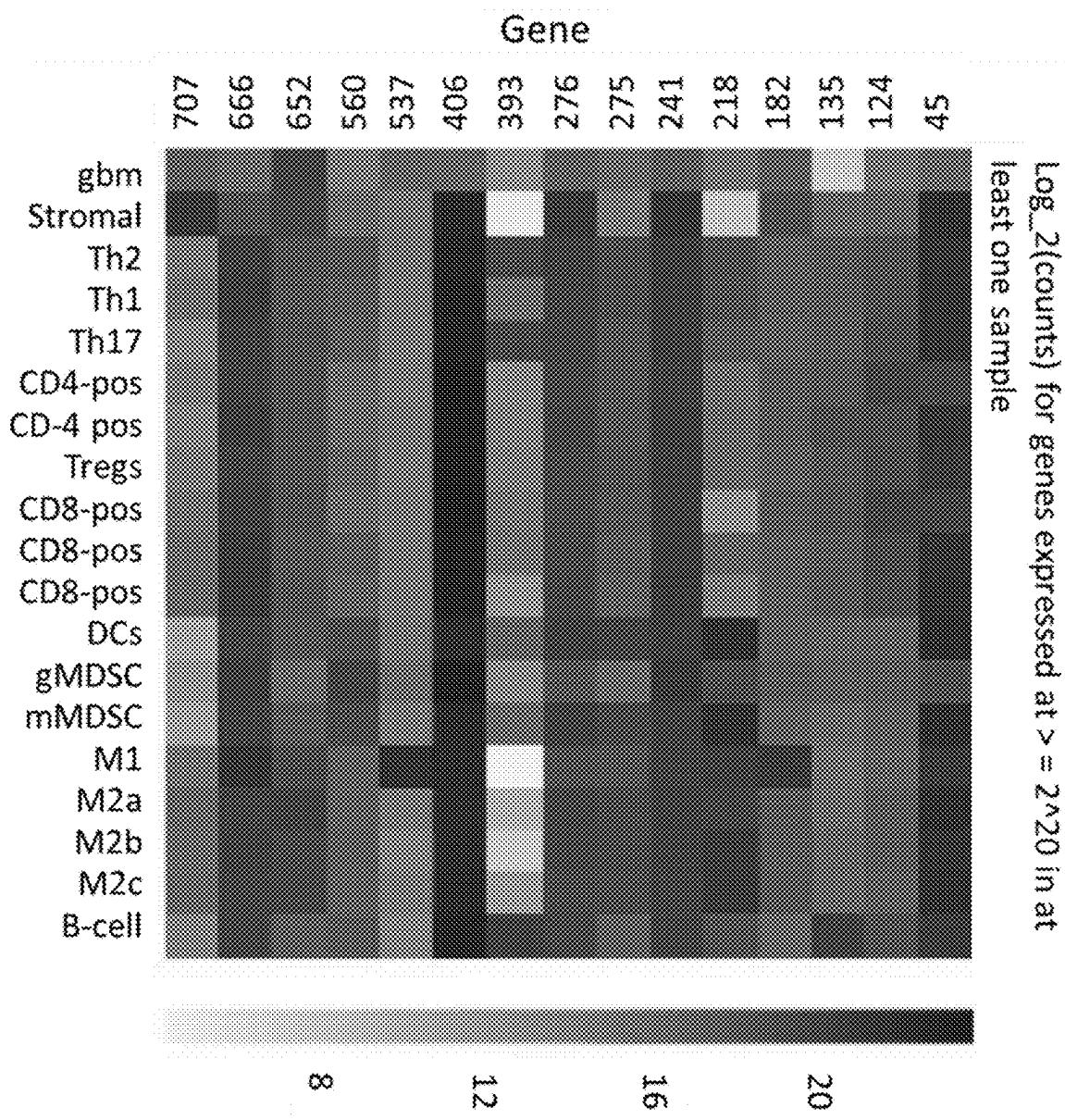


FIG. 5A

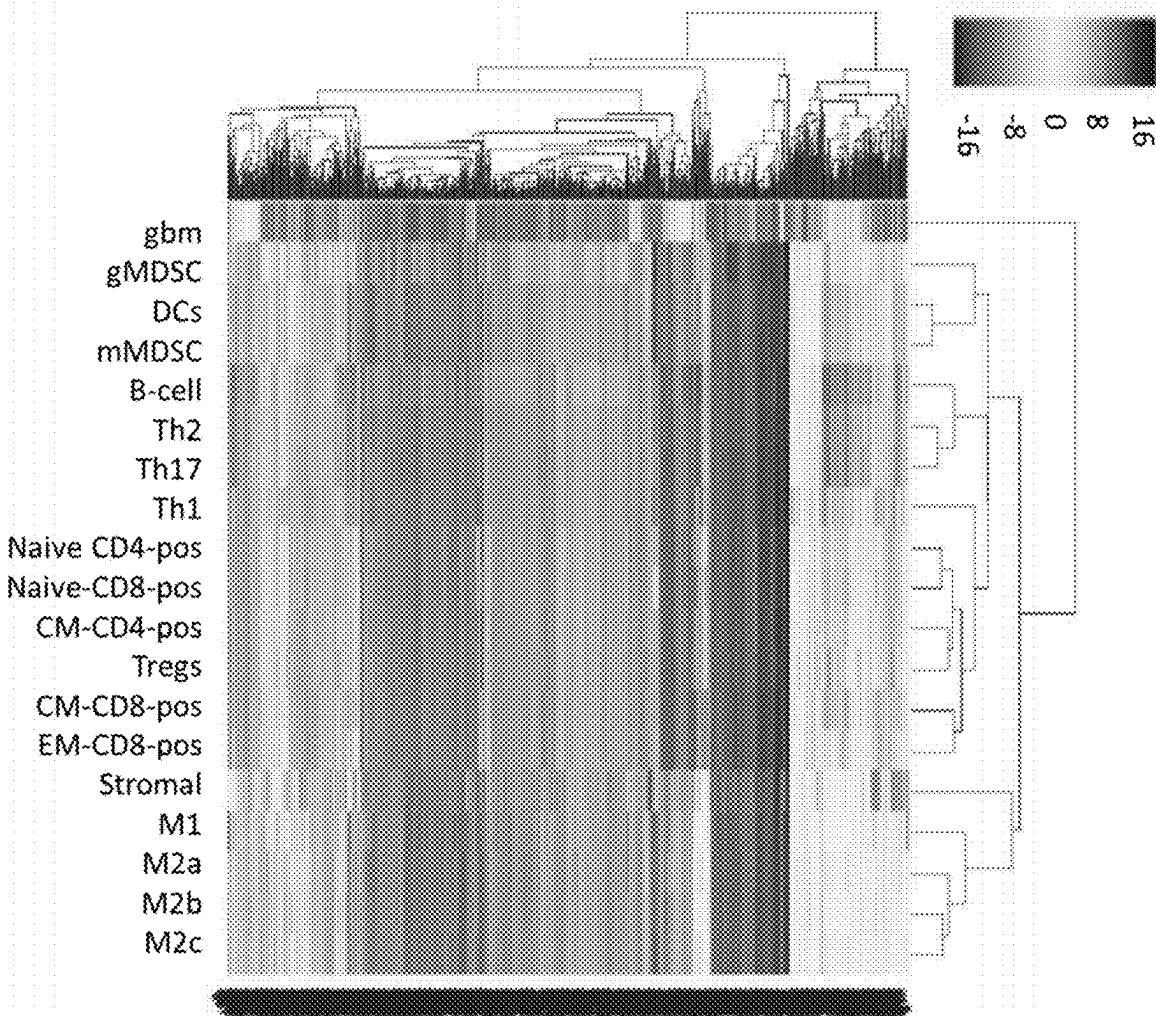


FIG. 5B

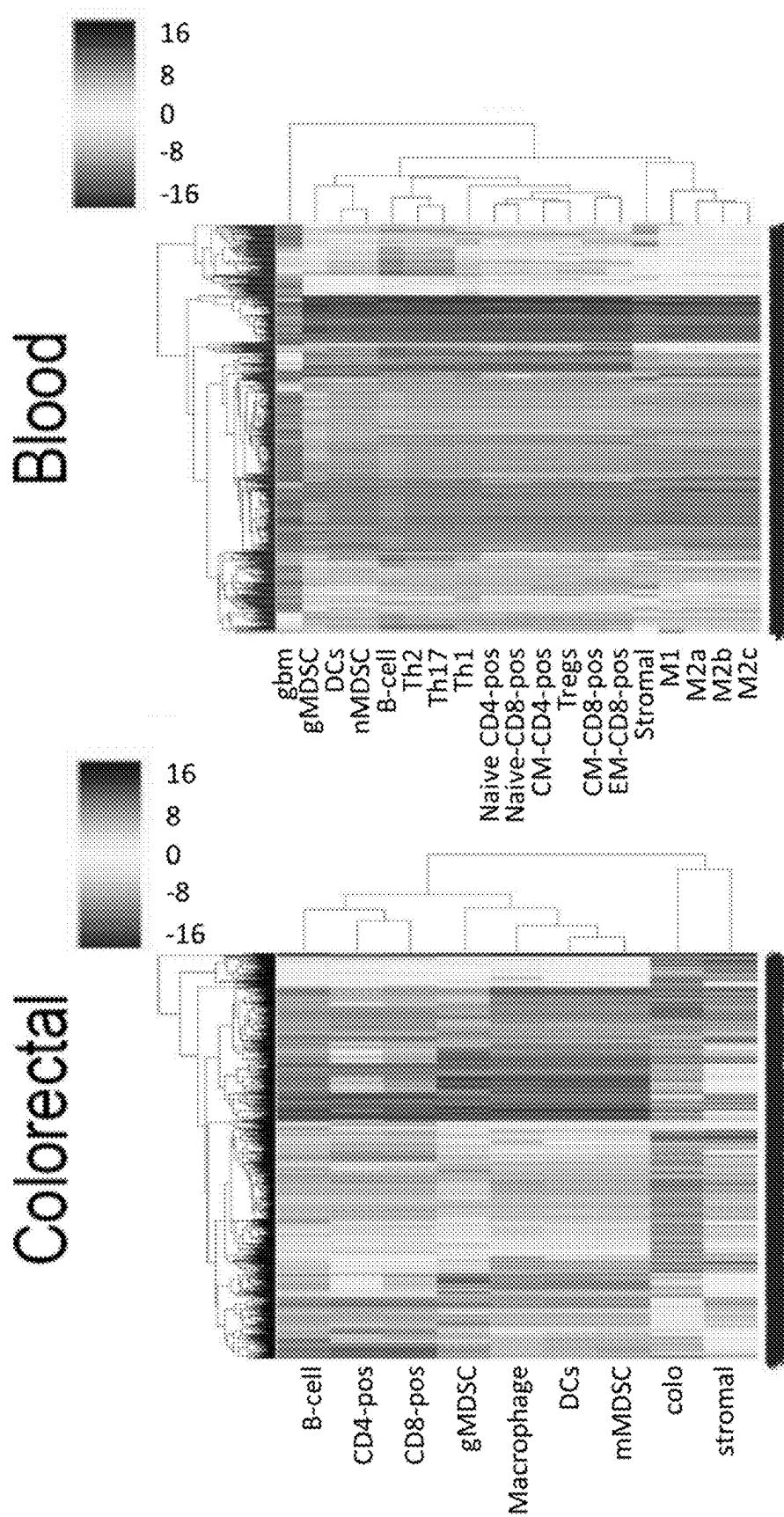


FIG. 6

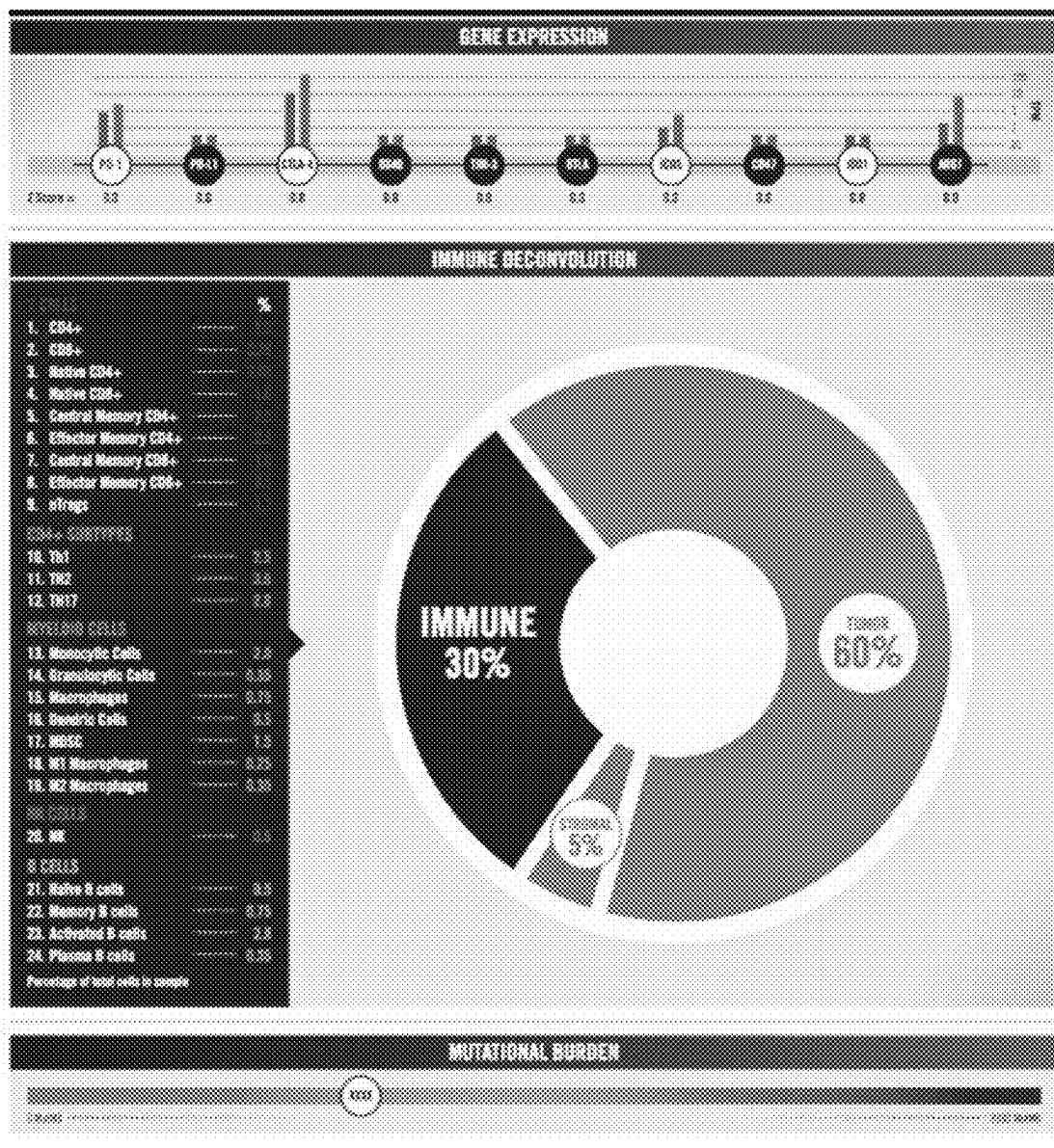


FIG. 7

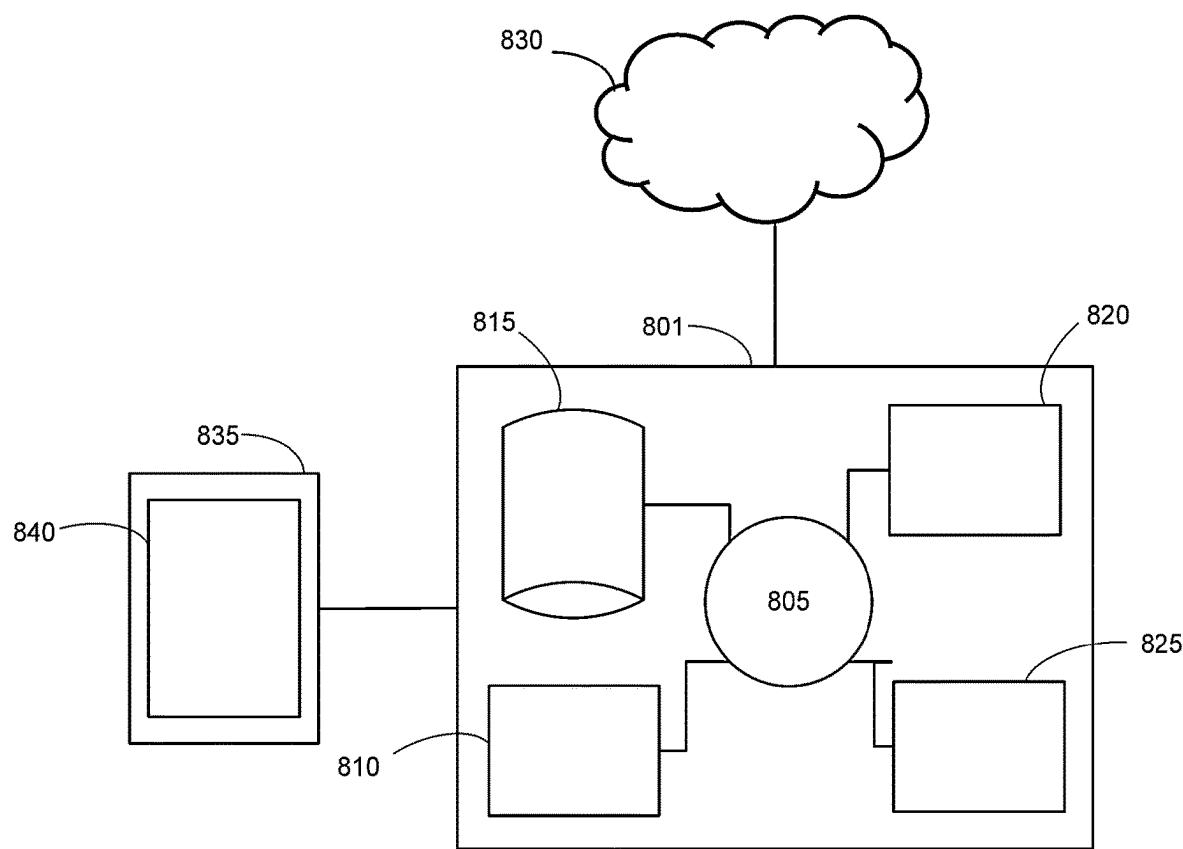


FIG. 8

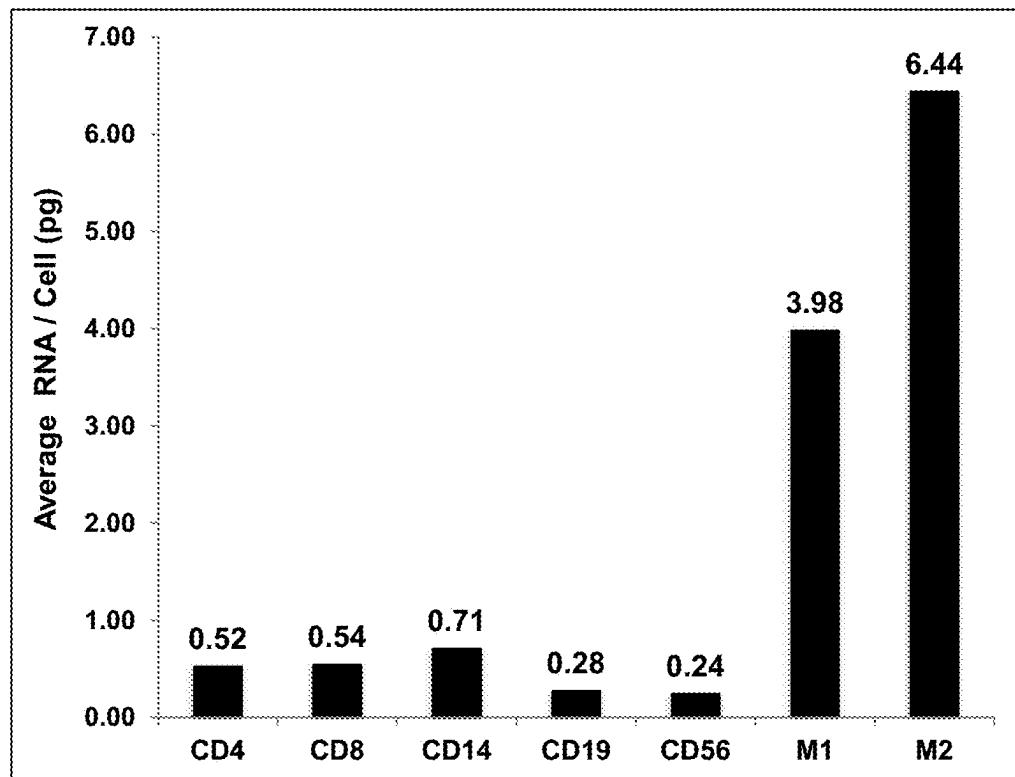


FIG. 9

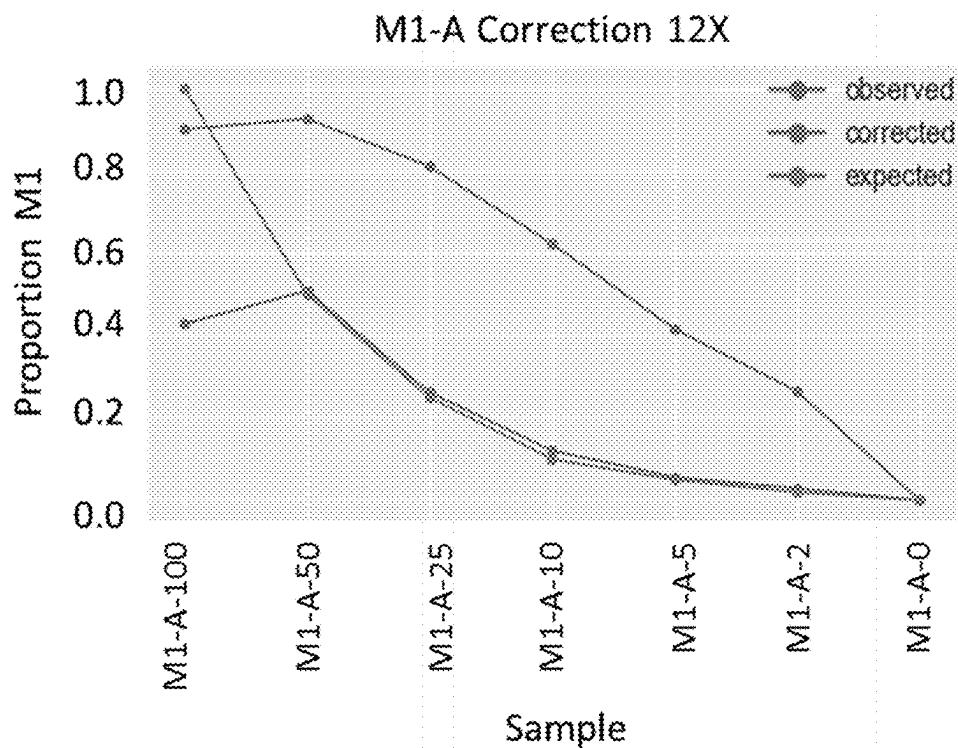


FIG. 10A

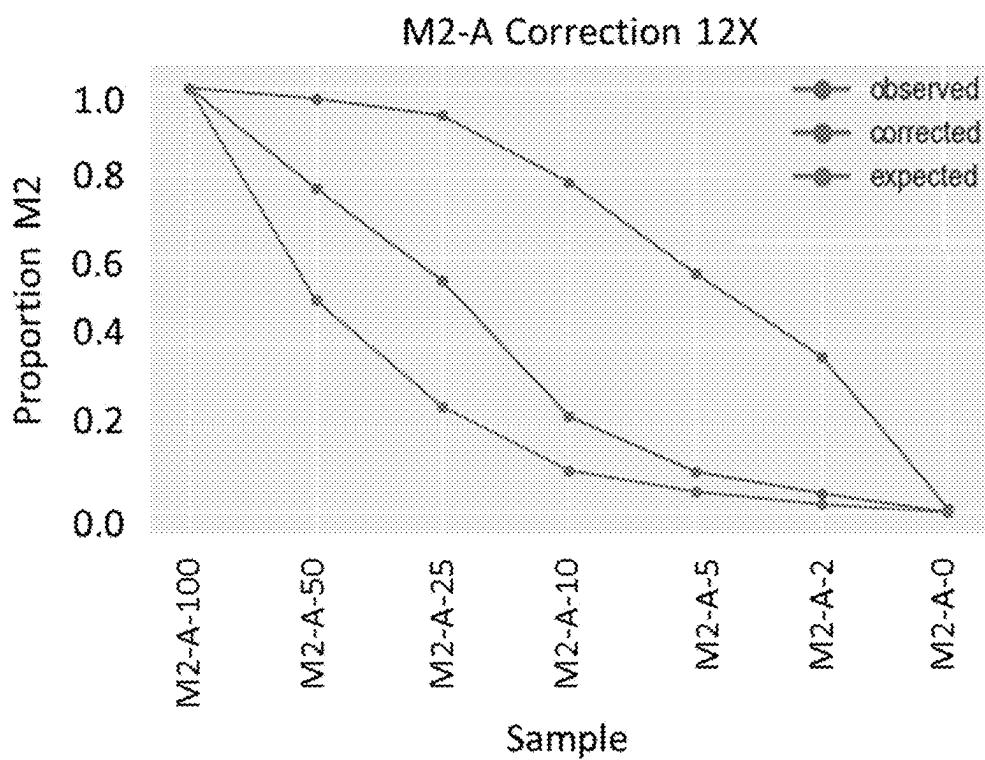


FIG. 10B

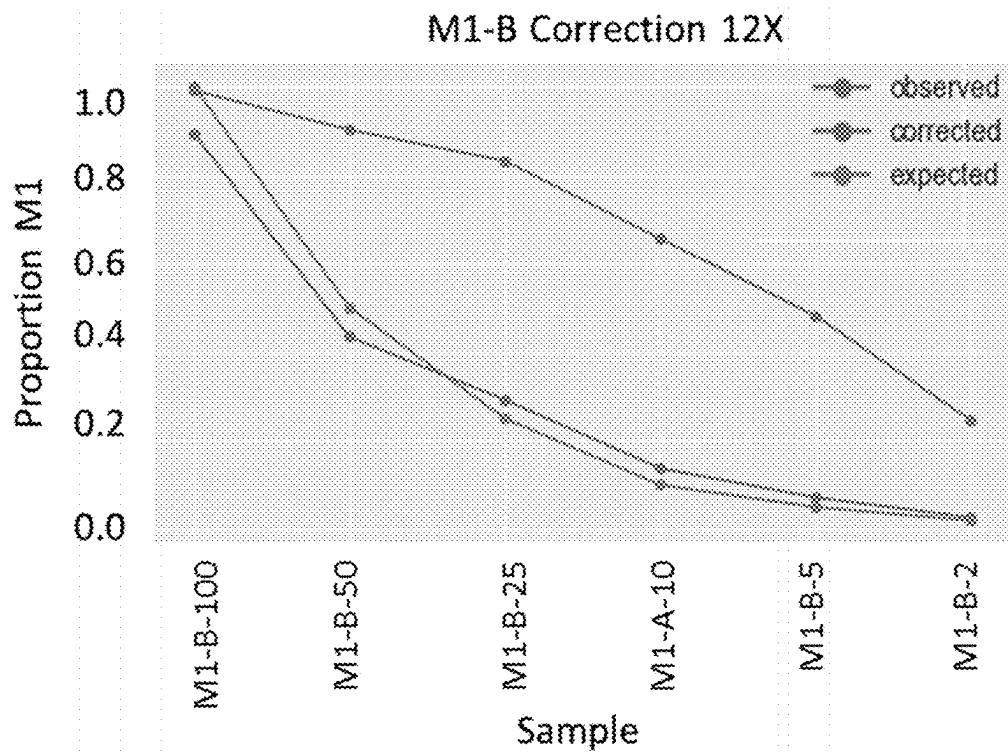


FIG. 10C

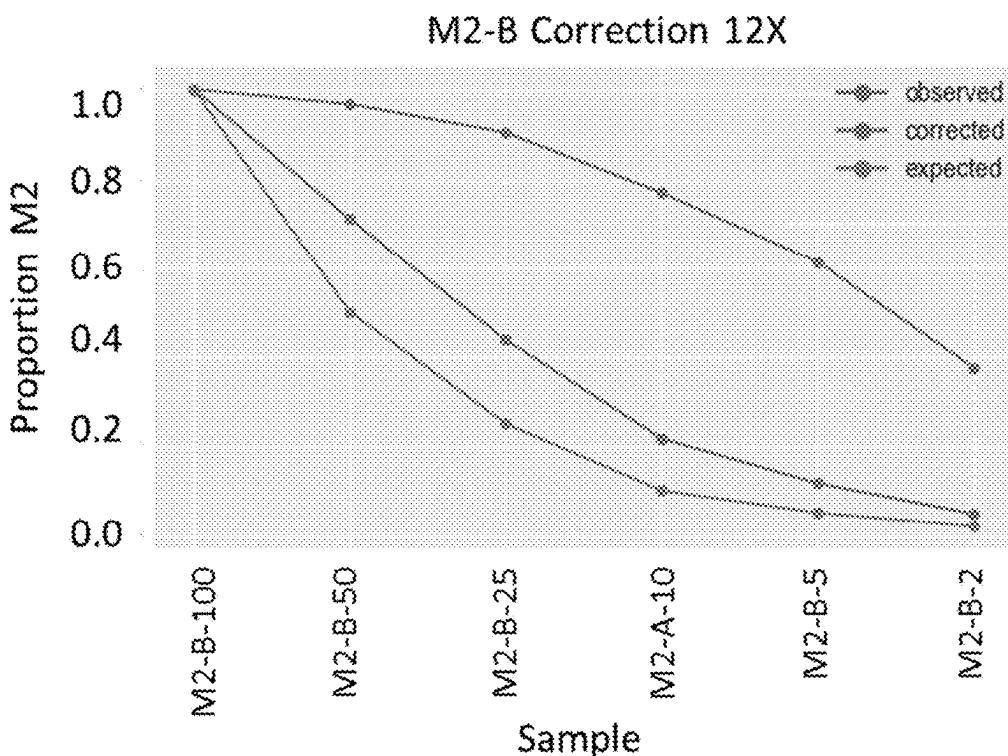


FIG. 10D

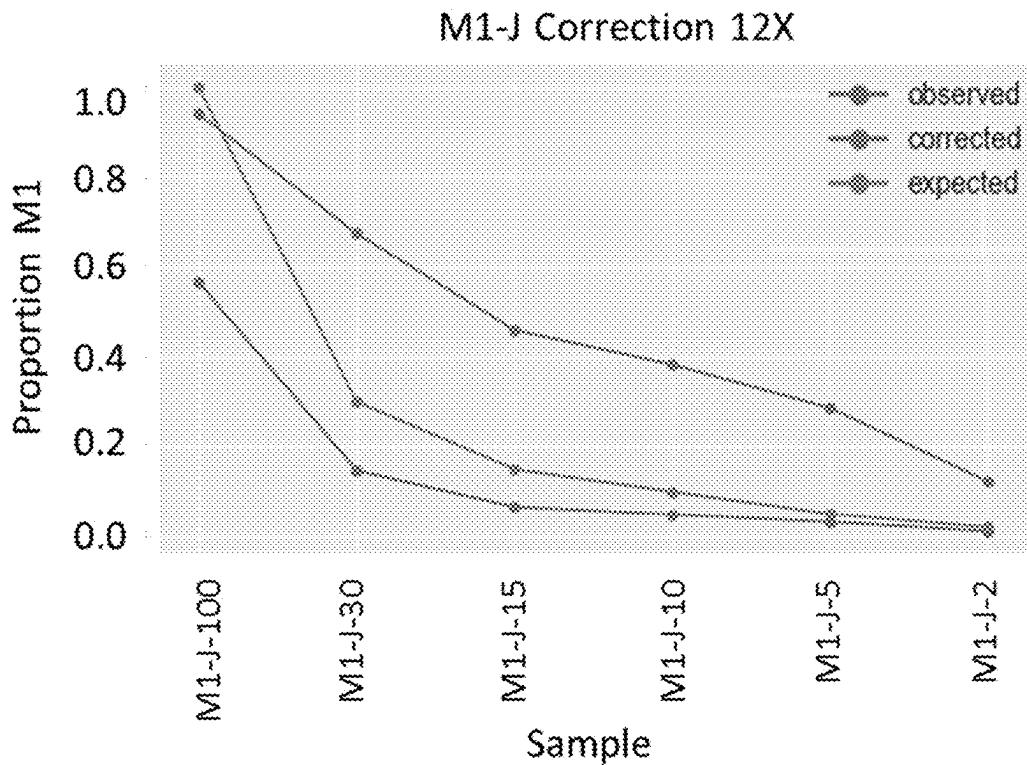


FIG. 10E

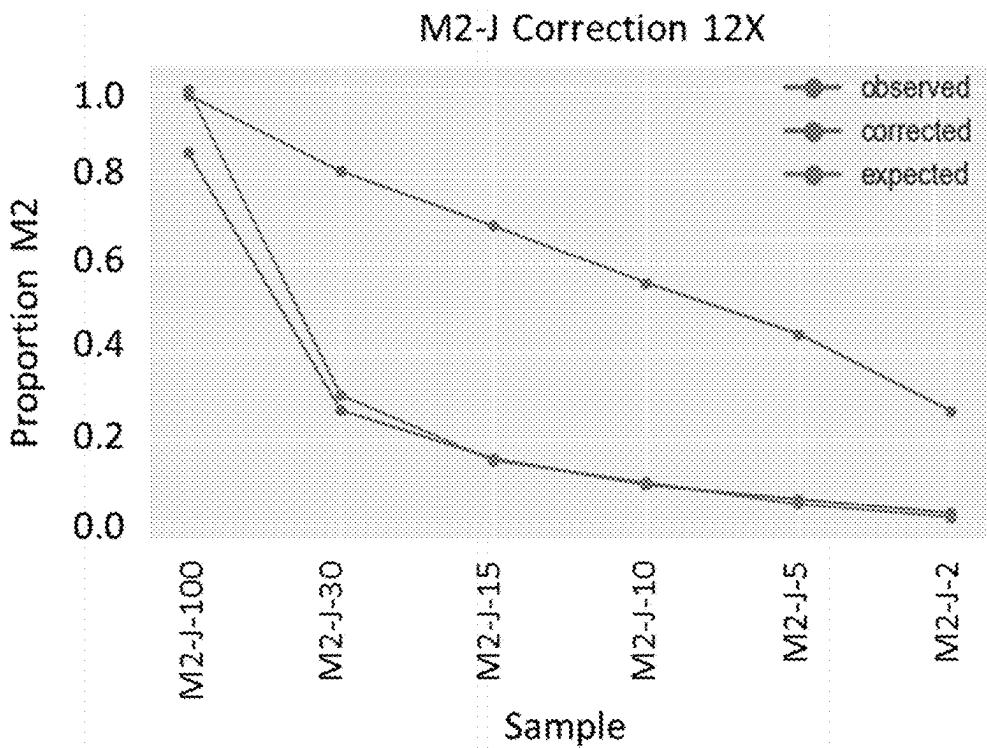
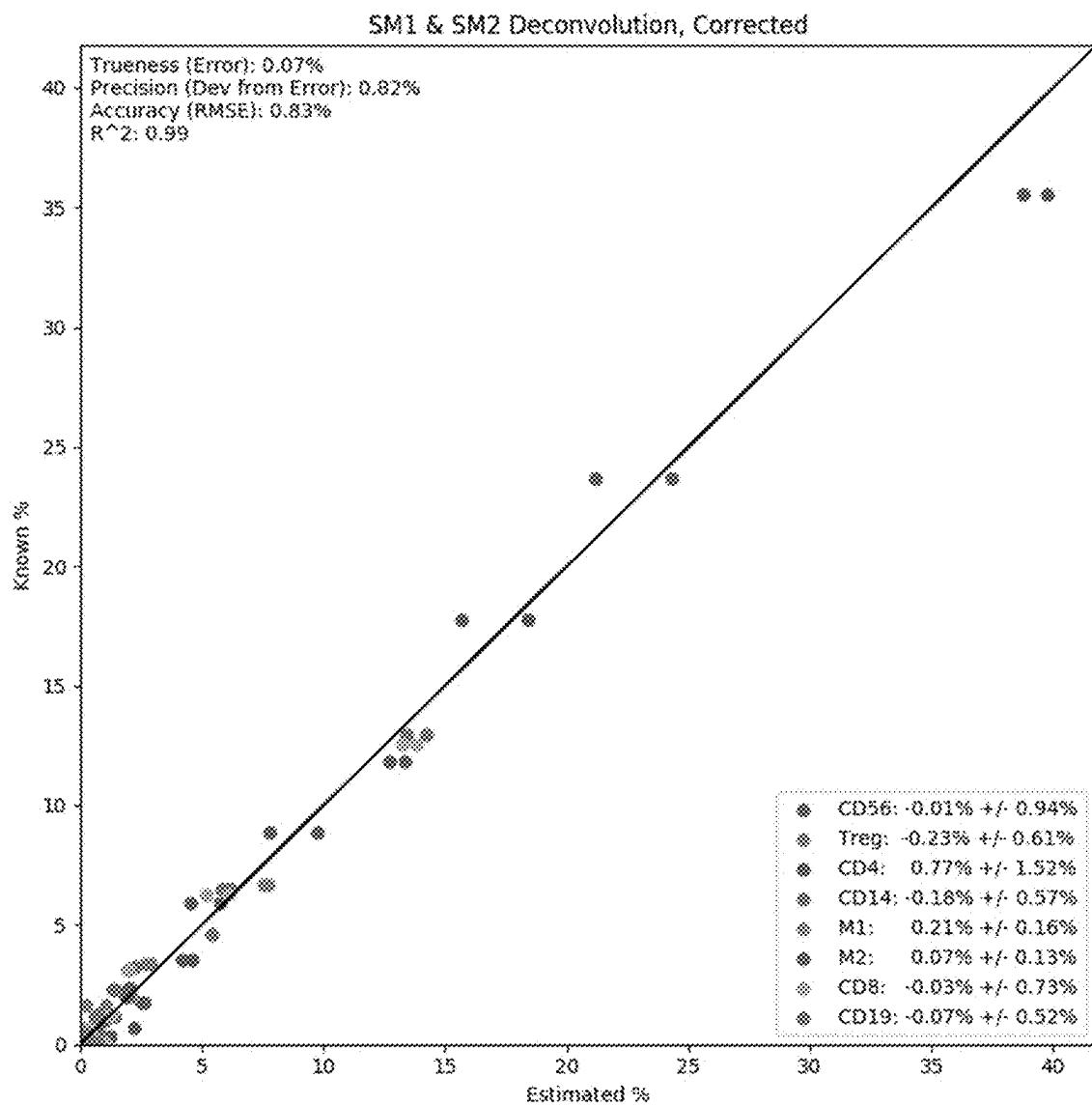


FIG. 10F



**FIG. 11**

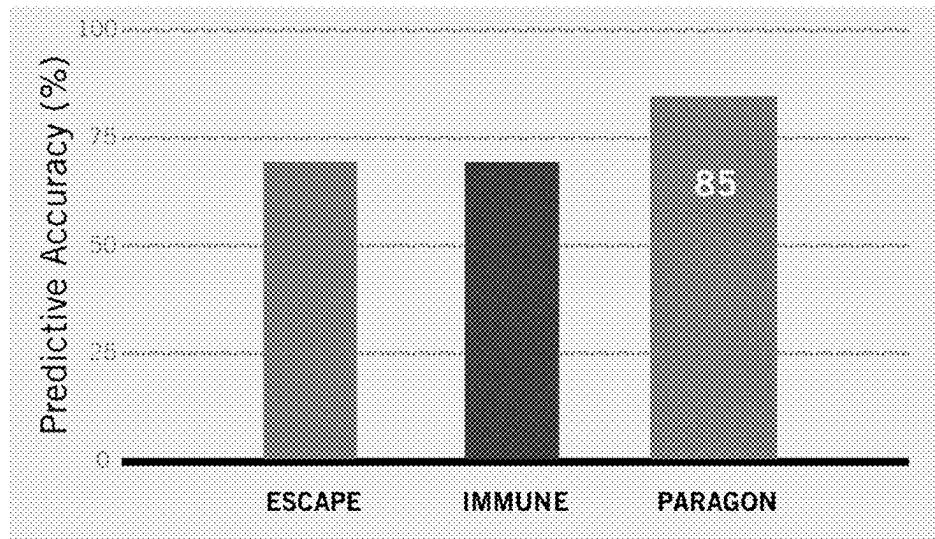


FIG. 12A

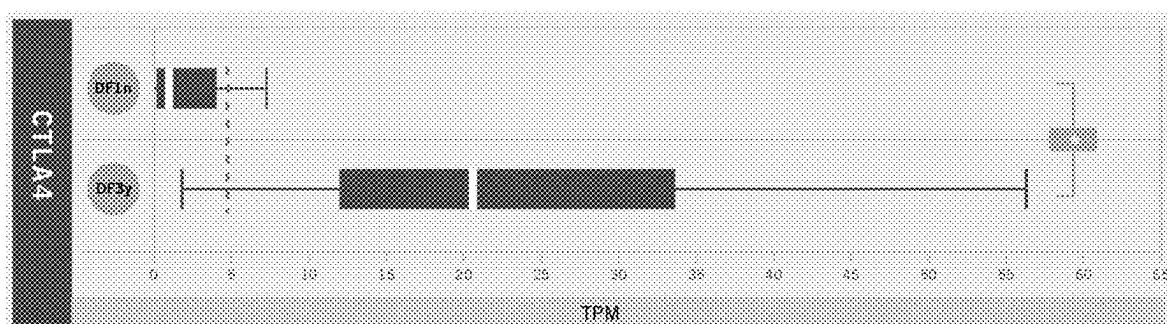


FIG. 12B

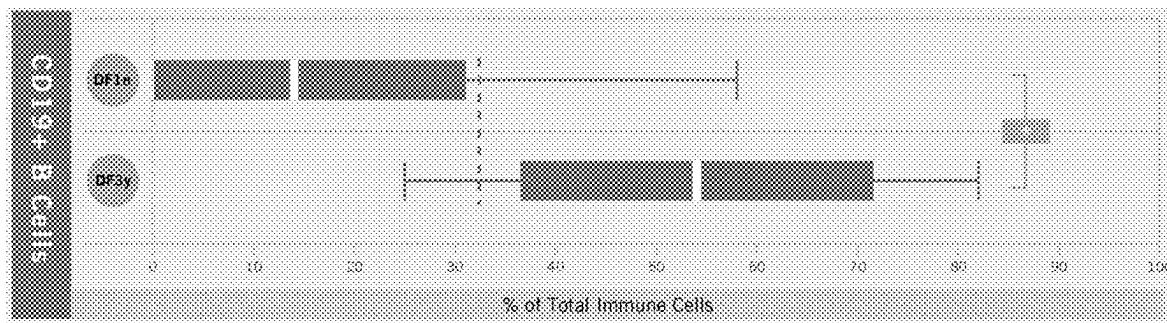


FIG. 12C

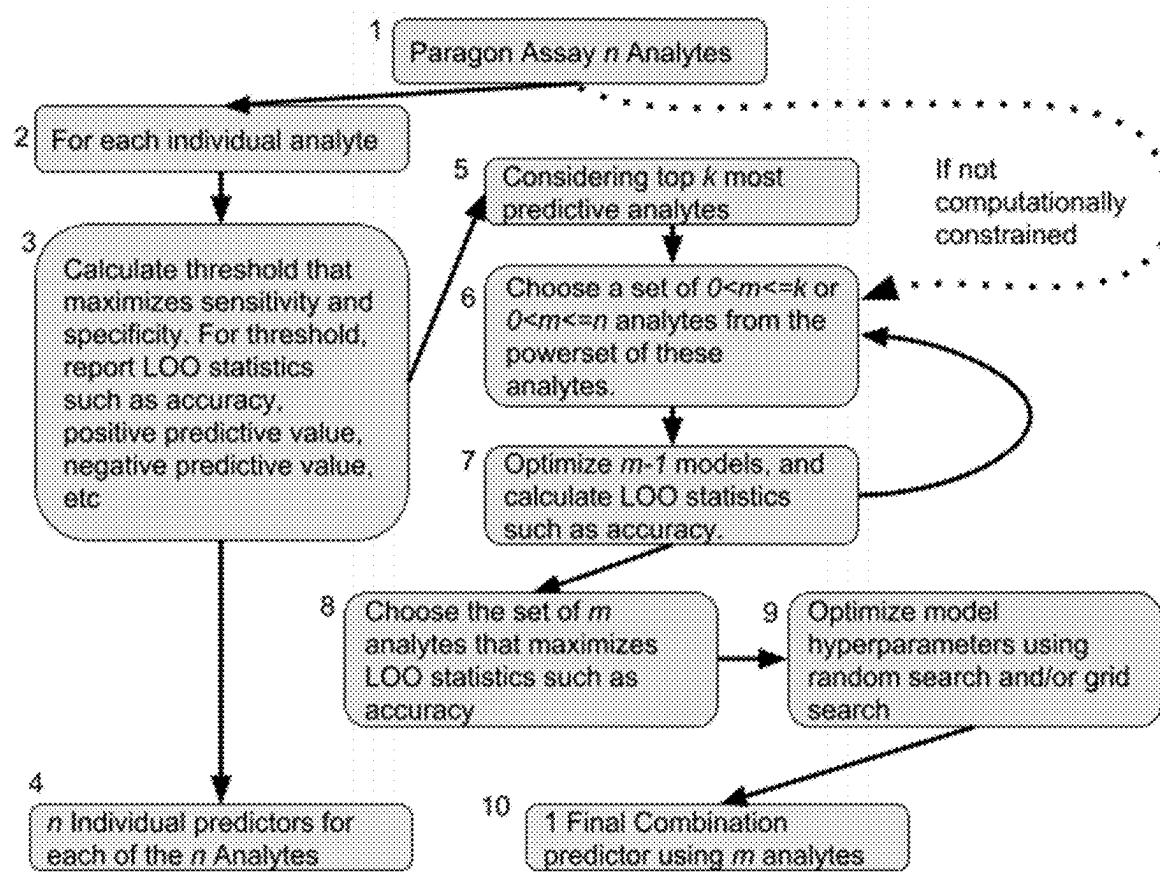


FIG. 13

## IMMUNO-ONCOLOGY APPLICATIONS USING NEXT GENERATION SEQUENCING

### CROSS-REFERENCE

[0001] This application is a continuation of U.S. application Ser. No. 16/823,195, filed Mar. 18, 2020, which is a continuation of U.S. application Ser. No. 16/056,406, filed Aug. 6, 2018, which is a continuation of International Application No. PCT/US2018/042176, filed Jul. 13, 2018, which claims the benefit of U.S. Provisional Patent Application No. 62/532,921, filed Jul. 14, 2017, and U.S. Provisional Patent Application No. 62/658,418, filed Apr. 16, 2018, each of which are incorporated herein by reference in their entireties.

### BACKGROUND

[0002] Cancer is a complex group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Millions of new cases of cancer occur globally each year. Understanding the immune and tumor profile may help with diagnosis and treatment.

### SUMMARY

[0003] In an aspect, disclosed herein are methods for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; and (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some instances, the plurality of expression signature genes comprises at least one gene from each of Tables 1A, 1B, 1C, 1D, and 1E. In some instances, the plurality of expression signature genes comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 genes from Table 2, 3, or 4. In some instances, the immune-oncology profile comprises a predicted response to a therapeutic intervention. In some instances, the predicted response is a positive response or a negative response to the therapeutic intervention. In some instances, the positive response comprises tumor regression, slowing tumor progression, or halt of tumor progression. In some instances, the negative response comprises tumor progression, lack of response to the therapeutic intervention, or a combination thereof. In some instances, the therapeutic intervention comprises an immune-therapy. In some instances, the therapeutic intervention further comprises radiation, chemotherapy, surgery, or a combination thereof. In some instances, the method further comprises providing a recommendation based on the immune-oncology profile. In some instances, the recommendation is to start, stop, change, or continue a therapeutic intervention. In some instances, the method further comprises using the profile to provide a therapeutic intervention to the subject. In some instances, the therapeutic intervention is an active immuno-

therapy, a passive immunotherapy, or a combination thereof. In some instances, the therapeutic intervention is a cancer vaccine, cytokine therapy, immune cell therapy, antibody therapy, or a combination thereof. In some instances, the antibody therapy comprises introducing tumor-targeting monoclonal antibodies, immune cell activating antibodies, or a combination thereof. In some instances, the immune-oncology profile comprises a predicted level of resistance to one or more therapeutic agents based on one or more cancer gene expression signatures identified in (d). In some instances, in (c), the deconvolution algorithm applies a deconvolution matrix to the RNA sequencing data to quantify the one or more cell types that are present in the sample. In some instances, the deconvolution matrix comprises a plurality of immune cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of tumor cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression counts for each expression signature gene is normalized across the plurality of cell types. In some instances, the deconvolution algorithm identifies and quantifies the one or more cell types that are present in the sample using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, in (c) the mutational burden is calculated across at least 500 genes. In some instances, in (c) the mutational burden is calculated across at least 1000 genes. In some instances, in (c) the mutational burden is calculated across at least 2000 genes. In some instances, in (c) the plurality of genes includes at least 500 genes. In some instances, in (c) the plurality of genes includes at least 1000 genes. In some instances, in (d) the plurality of expression signature genes includes at least 100 cell expression signature genes. In some instances, in (d) the plurality of expression signature genes includes at least 200 cell expression signature genes. In some instances, in (d) the plurality of expression signature genes includes genes having a bimodal expression signature between at least two cell types with no more than a 50% overlap between modes. In some instances, in (d) the deconvolution algorithm requires no more than 200 cell expression signature genes to identify and quantify the one or more cell types with at least 90% accuracy for 100 independent samples. In some instances, in (d) the one or more cell types includes at least one leukocyte cell type, stromal cell type, tumor cell type, or a combination thereof. In some instances, in (d) the one or more cell types comprise at least 10 leukocyte types. In some instances, in (d) the one or more cell types comprise at least 20 leukocyte types. In some instances, in (d) the one or more cell types comprise at least 1 tumor cell type. In some instances, (a) comprises obtaining RNA molecules from the sample and measuring the level of gene expression on the RNA molecules. In some instances, (a) comprises obtaining RNA molecules from the sample and performing reverse transcription polymerase chain reaction on the RNA molecules to generate complementary deoxyribonucleic acid (cDNA) molecules, and sequencing the cDNA molecules. In some instances, the cDNA molecules are tagged with unique molecular identifiers and amplified by polymerase chain reaction prior to sequencing. In some instances, (a) com-

prises performing next generation RNA sequencing on a cDNA library generated from the sample. In some instances, the at least one immune modulatory molecule is CTLA-4, PD-L1, LAG-3, KIR, TIM-3, CECAM1, VISTA, TIGIT, CD73, or a combination thereof. In some instances, the sample is a tumor biopsy. In some instances, the sample is at least one formalin-fixed paraffin-embedded (FFPE) curl. In some instances, the sample has an RNA integrity number (RIN) of no more than 6.0. In some instances, the sample has an RNA integrity number (RIN) of no more than 2.0. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 90% of total RNA in the sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 60% of total RNA in the sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 30% of total RNA in the sample. In some instances, the sample is obtained from skin, blood, brain, bladder, bone, bone marrow, breast, colon, stomach, esophagus, ovary, uterus, gallbladder, fallopian tube, testicle, kidney, liver, pancreas, adrenal gland, cervix, endometrium, head or neck, lung, prostate, thymus, thyroid, lymph node, or urinary bladder. In some instances, the subject has cancer. In some instances, the method further comprises presenting the immune-oncology profile as a report with graphical elements representing the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types identified and quantified in (d). In some instances, the level of gene expression determined in (b) is displayed on the report in combination with a reference expression level. In some instances, the mutational burden calculated in (c) is displayed along a single axis having a range between low and high mutational burden. In some instances, the one or more cell types identified and quantified in (d) are displayed in a pie chart indicating a percentage of each cell type in the sample.

**[0004]** In another aspect, disclosed herein are methods for recommending a therapeutic intervention using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d); and (f) recommending a therapeutic intervention based on the immune-oncology profile.

**[0005]** In another aspect, disclosed herein are methods for predicting a clinical outcome using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (d) applying a deconvolution algorithm

to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d); and (f) making a prediction of a clinical outcome to a therapeutic intervention based on the immune-oncology profile, the prediction having a positive predictive value of at least 90% for at least 100 independent samples.

**[0006]** In another aspect, disclosed herein are methods for providing a therapeutic intervention based on ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d); and (f) providing a therapeutic intervention based on the immune-oncology profile.

**[0007]** In another aspect, disclosed herein are systems for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: a database comprising the RNA sequencing data from a sample obtained from a subject; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) evaluate at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (b) analyze at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (c) apply a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; and (d) generate an immune-oncology profile based on the level of gene expression determined in (a), the mutational burden calculated in (b), and the one or more cell types quantified in (c). In some instances, the plurality of expression signature genes comprises at least one gene from each of Tables 1A, 1B, 1C, 1D, and 1E. In some instances, the plurality of expression signature genes comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 genes from Table 2, 3, or 4. In some instances, the immune-oncology profile comprises a predicted response to a therapeutic intervention. In some instances, the predicted response is a positive response or a negative response to the therapeutic intervention. In some instances, the positive response comprises tumor regression, slowing tumor progression, or halt of tumor progression. In some instances, the negative response comprises tumor progression, lack of response to the therapeutic intervention, or a combination thereof. In some instances, the therapeutic intervention comprises an immune-therapy. In some instances, the therapeutic intervention further comprises

radiation, chemotherapy, surgery, or a combination thereof. In some instances, the one or more computer processors are programmed to provide a recommendation based on the immune-oncology profile. In some instances, the recommendation is to start, stop, change, or continue a therapeutic intervention. In some instances, the profile is used to provide a therapeutic intervention to the subject. In some instances, the therapeutic intervention is an active immunotherapy, a passive immunotherapy, or a combination thereof. In some instances, the therapeutic intervention is a cancer vaccine, cytokine therapy, immune cell therapy, antibody therapy, or a combination thereof. In some instances, the antibody therapy comprises introducing tumor-targeting monoclonal antibodies, immune cell activating antibodies, or a combination thereof. In some instances, the immune-oncology profile comprises a predicted level of resistance to one or more therapeutic agents based on one or more cancer gene expression signatures identified in (d). In some instances, in (c), the deconvolution algorithm applies a deconvolution matrix to the RNA sequencing data to quantify the one or more cell types that are present in the sample. In some instances, the deconvolution matrix comprises a plurality of immune cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of tumor cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression counts for each expression signature gene is normalized across the plurality of cell types. In some instances, the deconvolution algorithm identifies and quantifies the one or more cell types that are present in the sample using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression (SVR). In some instances, in (c), the mutational burden is calculated across at least 500 genes. In some instances, in (c), the mutational burden is calculated across at least 1000 genes. In some instances, in (c), the mutational burden is calculated across at least 2000 genes. In some instances, in (c) the plurality of genes includes at least 500 genes. In some instances, in (c) the plurality of genes includes at least 1000 genes. In some instances, in (d) the plurality of expression signature genes includes at least 100 cell expression signature genes. In some instances, in (d) the plurality of expression signature genes includes at least 200 cell expression signature genes. In some instances, in (d) the plurality of expression signature genes includes genes having a bimodal expression signature between at least two cell types with no more than a 50% overlap between modes. In some instances, in (d) the deconvolution algorithm requires no more than 200 cell expression signature genes to identify and quantify the one or more cell types with at least 90% accuracy for at least 100 independent samples. In some instances, in (d) the one or more cell types includes at least one leukocyte cell type, stromal cell type, tumor cell type, or a combination thereof. In some instances, in (d) the one or more cell types comprise at least 10 leukocyte types. In some instances, in (d) the one or more cell types comprise at least 20 leukocyte types. In some instances, in (d) the one or more cell types comprise at least 1 tumor cell type. In some instances, (a) comprises obtaining RNA molecules from the sample and measuring the level of gene expression

on the RNA molecules. In some instances, (a) comprises obtaining RNA molecules from the sample and performing reverse transcription polymerase chain reaction on the RNA molecules to generate complementary deoxyribonucleic acid (cDNA) molecules, and sequencing the cDNA molecules. In some instances, the cDNA molecules are tagged with unique molecular identifiers and amplified by polymerase chain reaction prior to sequencing. In some instances, (a) comprises performing next generation RNA sequencing on a cDNA library generated from the sample. In some instances, the at least one immune modulatory molecule is CTLA-4, PD-L1, LAG-3, KIR, TIM-3, CECAM1, VISTA, TIGIT, CD73, or a combination thereof. In some instances, the sample is a tumor biopsy. In some instances, the sample is at least one formalin-fixed paraffin-embedded (FFPE) curl. In some instances, the sample has an RNA integrity number (RIN) of no more than 6.0. In some instances, the sample has an RNA integrity number (RIN) of no more than 2.0. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 90% of total RNA in the sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 60% of total RNA in the sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 30% of total RNA in the sample. In some instances, the sample is obtained from skin, blood, brain, bladder, bone, bone marrow, breast, colon, stomach, esophagus, ovary, uterus, gallbladder, fallopian tube, testicle, kidney, liver, pancreas, adrenal gland, cervix, endometrium, head or neck, lung, prostate, thymus, thyroid, lymph node, or urinary bladder. In some instances, the subject has cancer. In some instances, the immune-oncology profile is presented as a report with graphical elements representing the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some instances, the level of gene expression determined in (b) is displayed on the report in combination with a reference expression level. In some instances, the mutational burden calculated in (c) is displayed along a single axis having a range between low and high mutational burden. In some instances, the one or more cell types identified and quantified in (d) are displayed in a pie chart indicating a percentage of each cell type in the sample.

**[0008]** In another aspect, disclosed herein are systems for recommending a therapeutic intervention using ribonucleic acid (RNA) sequencing data, comprising: a database comprising the RNA sequencing data from a sample obtained from a subject; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) evaluate at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (b) analyze at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (c) apply a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; (d) generate an immune-oncology profile based on the level of gene expression determined in (a), the mutational burden calculated in (b), and the one or

more cell types quantified in (c); and (e) recommend a therapeutic intervention based on the immune-oncology profile.

[0009] In another aspect, disclosed herein are systems for predicting a clinical outcome using ribonucleic acid (RNA) sequencing data, comprising: a database comprising the RNA sequencing data from a sample obtained from a subject; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) evaluate at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (b) analyze at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes from Table 5; (c) apply a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes from Tables 1A-1E; (d) generate an immune-oncology profile based on the level of gene expression determined in (a), the mutational burden calculated in (b), and the one or more cell types quantified in (c); and (e) make a prediction of a clinical outcome to a therapeutic intervention based on the immune-oncology profile, the prediction having a positive predictive value of at least 90% for at least 100 independent samples.

[0010] In another aspect, disclosed herein are methods of analyzing ribonucleic acid (RNA) sequencing data, the method comprising: (a) obtaining a library comprising a plurality of RNA molecules from a sample of a subject; (b) contacting the library with a bait set configured to enrich for RNA molecules corresponding to target genes, the target genes comprising a plurality of genes selected from Tables 1A-1E and Table 5; (c) performing RNA sequencing on the target genes to generate RNA sequencing data; and (d) analyzing the RNA sequencing data to generate an immune-oncology profile comprising: gene expression for at least one immune modulatory gene, mutational burden, and cell type quantification. In some instances, the immune-oncology profile comprises a predicted response to a therapeutic intervention. In some instances, the predicted response is a positive response or a negative response to the therapeutic intervention. In some instances, the positive response comprises tumor regression, slowing tumor progression, or halt of tumor progression. In some instances, the negative response comprises tumor progression, lack of response to the therapeutic intervention, or a combination thereof. In some instances, the therapeutic intervention comprises an immune-therapy. In some instances, the therapeutic intervention further comprises radiation, chemotherapy, surgery, or a combination thereof. In some instances, the methods further comprise providing a recommendation based on the immune-oncology profile. In some instances, the recommendation is to start, stop, change, or continue a therapeutic intervention. In some instances, the methods further comprise using the profile to provide a therapeutic intervention to the subject. In some instances, the therapeutic intervention is an active immunotherapy, a passive immunotherapy, or a combination thereof. In some instances, the therapeutic intervention is a cancer vaccine, cytokine therapy, immune cell therapy, antibody therapy, or a combination thereof. In some instances, the antibody therapy comprises introducing tumor-targeting monoclonal antibodies, immune cell activating antibodies, or a combination thereof. In some

instances, the immune-oncology profile comprises a predicted level of resistance to one or more therapeutic agents based on one or more cancer gene expression signatures identified in (d). In some instances, in (d), a deconvolution algorithm applies a deconvolution matrix to the RNA sequencing data to quantify one or more cell types that are present in the sample. In some instances, the deconvolution matrix comprises a plurality of immune cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of tumor cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression counts each expression signature gene is normalized across the plurality of cell types. In some instances, the deconvolution algorithm identifies and quantifies the one or more cell types that are present in the sample using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, the plurality of expression signature genes includes at least 200 cell expression signature genes. In some instances, the plurality of expression signature genes includes genes having a bimodal expression signature between at least two cell types with no more than a 50% overlap between modes. In some instances, the deconvolution algorithm requires no more than 200 cell expression signature genes to identify and quantify the one or more cell types with at least 90% accuracy for 100 independent samples. In some instances, the one or more cell types include at least one leukocyte cell type, stromal cell type, tumor cell type, or a combination thereof. In some instances, the one or more cell types comprise at least 10 leukocyte types. In some instances, the one or more cell types comprise at least 20 leukocyte types. In some instances, the one or more cell types comprise at least 1 tumor cell type. In some instances, in (d), the mutational burden is calculated across at least 500 genes. In some instances, in (d), the mutational burden is calculated across at least 1000 genes. In some instances, in (d), the mutational burden is calculated across at least 2000 genes. In some instances, in (d) the plurality of genes includes at least 500 genes. In some instances, in (d) the plurality of genes includes at least 1000 genes. In some instances, in (d) the plurality of expression signature genes includes at least 100 cell expression signature genes. In some instances, (c) comprises measuring level of gene expression for the target genes. In some instances, (a) comprises obtaining RNA molecules from the sample and performing reverse transcription polymerase chain reaction on the RNA molecules to generate complementary deoxyribonucleic acid (cDNA) molecules, and sequencing the cDNA molecules. In some instances, the at least one immune modulatory molecule is CTLA-4, PD-L1, LAG-3, KIR, TIM-3, CECAM1, VISTA, TIGIT, CD73, or a combination thereof. In some instances, the sample is a tumor biopsy. In some instances, the sample is at least one formalin-fixed paraffin-embedded (FFPE) curl. In some instances, the sample has an RNA integrity number (RIN) of no more than 6.0. In some instances, the sample has an RNA integrity number (RIN) of no more than 2.0. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 90% of total RNA in the

sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 60% of total RNA in the sample. In some instances, the sample comprises RNA molecules at least 200 nucleotides in size that constitute no more than 30% of total RNA in the sample. In some instances, the sample is obtained from skin, blood, brain, bladder, bone, bone marrow, breast, colon, stomach, esophagus, ovary, uterus, gall-bladder, fallopian tube, testicle, kidney, liver, pancreas, adrenal gland, cervix, endometrium, head or neck, lung, prostate, thymus, thyroid, lymph node, or urinary bladder. In some instances, the subject has cancer. In some instances, the methods further comprise presenting the immune-oncology profile as a report with graphical elements representing the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some instances, the level of gene expression determined in (b) is displayed on the report in combination with a reference expression level. In some instances, the mutational burden calculated in (c) is displayed along a single axis having a range between low and high mutational burden. In some instances, the one or more cell types identified and quantified in (d) are displayed in a pie chart indicating a percentage of each cell type in the sample.

[0011] In another aspect, disclosed herein are methods of analyzing a data set comprising information from a plurality of components from a single source, the method comprising: (a) obtaining the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluating at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyzing at least a subset of the data set to calculate a variation indicator based on the sequence information; (d) applying a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; and (e) generating an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d). In some instances, the output profile comprises a predicted response to a procedure. In some instances, the predicted response is a positive response or a negative response to the procedure. In some instances, the methods further comprise providing a recommendation based on the output profile. In some instances, the recommendation is to start, stop, change, or continue a procedure. In some instances, the methods further comprise using the profile to provide a procedure. In some instances, in (d), the deconvolution algorithm applies a deconvolution matrix to the subset of the data set to identify and quantify the one or more components that constitute the plurality of components. In some instances, the deconvolution matrix comprises a plurality of data member signatures for a plurality of components. In some instances, the deconvolution matrix comprises a plurality of components, each component comprising a plurality of data member signatures, wherein each data member signature is normalized across the plurality of components. In some instances, the deconvolution algorithm identifies and quantifies the plurality of components using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray

microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, in (d), the variation indicator is calculated across at least 500 data members. In some instances, in (d), the variation indicator is calculated across at least 1000 data members. In some instances, in (d), the variation indicator is calculated across at least 2000 data members. In some instances, in (d) the plurality of data members includes at least 500 data members. In some instances, in (d) the plurality of data members includes at least 1000 data members. In some instances, in (d) the plurality of data member signatures includes at least 100 data member signatures. In some instances, the plurality of data member signatures includes at least 200 data member signatures. In some instances, the plurality of data member signatures includes data member signatures having a bimodal expression signature between at least two components with no more than a 50% overlap between modes. In some instances, the deconvolution algorithm requires no more than 200 data member signatures to identify and quantify the one or more components with at least 90% accuracy for 100 independent data sets. In some instances, the methods further comprise presenting the output profile as a report with graphical elements representing the numerical quantifier for at least one data member determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d). In some instances, numerical quantifier for at least one data member determined in (b) is displayed on the report in combination with a reference numerical quantifier. In some instances, the variation indicator calculated in (c) is displayed along a single axis having a range between low and high variation. In some instances, the one or more components identified and quantified in (d) are displayed in a pie chart indicating a percentage of each component.

[0012] In another aspect, disclosed herein are methods of making a recommendation based on a data set comprising information from a plurality of components from a single source, the method comprising: (a) obtaining the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluating at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyzing at least a subset of the data set to calculate a variation indicator based on the sequence information; (d) applying a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; (e) generating an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d); and (f) recommending a procedure based on the output profile.

[0013] In another aspect, disclosed herein are methods of making a recommendation based on a data set comprising information from a plurality of components from a single source, the method comprising: (a) obtaining the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluating at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyzing at least a subset of the data set to calculate a variation indicator based on the sequence infor-

mation; (d) applying a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; (e) generating an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d); and (f) making a prediction of an outcome to a procedure based on the output profile, the prediction having a positive predictive value of at least 90% for at least 100 independent data sets.

[0014] In another aspect, disclosed herein are systems for analyzing a data set comprising information from a plurality of components from a single source, comprising: a database comprising the data set; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) obtain the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluate at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyze at least a subset of the data set to calculate a variation indicator based on the sequence information; (d) apply a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; and (e) generate an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d). In some instances, the output profile comprises a predicted response to a procedure. In some instances, the predicted response is a positive response or a negative response to the procedure. In some instances, the output profile is used to provide a recommendation. In some instances, the recommendation is to start, stop, change, or continue a procedure. In some instances, the output profile is used to provide a procedure. In some instances, in (d), the deconvolution algorithm applies a deconvolution matrix to the subset of the data set to identify and quantify the one or more components that constitute the plurality of components. In some instances, the deconvolution matrix comprises a plurality of data member signatures for a plurality of components. In some instances, the deconvolution matrix comprises a plurality of component signatures, each component signature comprising a plurality of data member signatures, wherein each data member signature is normalized across the plurality of component signatures. In some instances, the deconvolution algorithm identifies and quantifies the plurality of components using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, in (d), the variation indicator is calculated across at least 500 data members. In some instances, in (d), the variation indicator is calculated across at least 1000 data members. In some instances, in (d), the variation indicator is calculated across at least 2000 data members. In some instances, in (d) the plurality of data members includes at least 500 data members. In some instances, in (d) the plurality of data members includes at least 1000 data members. In some instances, in (d) the plurality of data member

signatures includes at least 100 data member signatures. In some instances, the plurality of data member signatures includes at least 200 data member signatures. In some instances, the plurality of data member signatures includes data member signatures having a bimodal expression signature between at least two component signatures with no more than a 50% overlap between modes. In some instances, the deconvolution algorithm requires no more than 200 data member signatures to identify and quantify the one or more components with at least 90% accuracy for 100 independent data sets. In some instances, the output profile is presented as a report with graphical elements representing the numerical quantifier for at least one data member determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d). In some instances, the numerical quantifier for at least one data member determined in (b) is displayed on the report in combination with a reference numerical quantifier. In some instances, the variation indicator calculated in (c) is displayed along a single axis having a range between low and high variation. In some instances, the one or more components identified and quantified in (d) are displayed in a pie chart indicating a percentage of each component.

[0015] In another aspect, disclosed herein are systems for analyzing a data set comprising information from a plurality of components from a single source, comprising: a database comprising the data set; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) obtain the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluate at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyze at least a subset of the data set to calculate a variation indicator based on the sequence information; (d) apply a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; (e) generate an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d); and (f) recommend a procedure based on the output profile.

[0016] In another aspect, disclosed herein are systems for analyzing a data set comprising information from a plurality of components from a single source, comprising: a database comprising the data set; and one or more computer processors that are coupled to the database, wherein the one or more computer processors are individually or collectively programmed to: (a) obtain the data set, the data set comprising sequence and quantity information for a plurality of data members across the plurality of components; (b) evaluate at least a subset of the data set to determine a numerical quantifier for at least one data member; (c) analyze at least a subset of the data set to calculate a variation indicator based on the sequence information; (d) apply a deconvolution algorithm to at least a subset of the data set to identify and quantify one or more components that constitute the plurality of components based on a plurality of data member signatures; (e) generate an output profile based on the numerical quantifier determined in (b), the variation indicator calculated in (c), and the one or more components identified and quantified in (d); and (f) make a prediction of

an outcome to a procedure based on the output profile, the prediction having a positive predictive value of at least 90% for at least 100 independent data sets.

[0017] In another aspect, disclosed herein are methods for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes, wherein the mutation burden is calculated with a correlation of at least about 80% with a mutational burden Gold Standard; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes, wherein the one or more cell types are identified and quantified with a correlation of at least about 80% with a deconvolution Gold Standard; and (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some instances, the mutational burden is calculated with a correlation of at least about 90% with the mutational burden Gold Standard. Sometimes, the one or more cell types are identified and quantified with a correlation of at least about 90% with the deconvolution Gold Standard.

[0018] In another aspect, disclosed herein are methods for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes, wherein the mutation burden is calculated at an accuracy of at least about 80%; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes, wherein the one or more cell types are identified and quantified at an accuracy of at least about 80%; and (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some instances, the mutational burden is calculated at a specificity of at least about 90%. Sometimes, the one or more cell types are identified and quantified at a specificity of at least about 90%.

[0019] In another aspect, disclosed herein are methods for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes, wherein the mutation burden is calculated at a specificity of at least about 80%; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes, wherein the one or more cell

types are identified and quantified at a specificity of at least about 80%; and (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). In some cases, the mutational burden is calculated at a specificity of at least about 90%. Sometimes, the one or more cell types are identified and quantified at a specificity of at least about 90%.

[0020] In another aspect, disclosed herein are methods for generating an immune-oncology profile using ribonucleic acid (RNA) sequencing data, comprising: (a) obtaining RNA sequencing data from a sample obtained from a subject; (b) evaluating at least a subset of the RNA sequencing data to determine level of gene expression for at least one immune modulatory gene; (c) analyzing at least a subset of the RNA sequencing data to calculate a mutational burden based on a plurality of genes, wherein the mutation burden is calculated at a sensitivity of at least about 80%; (d) applying a deconvolution algorithm to at least a subset of the RNA sequencing data to identify and quantify one or more cell types that are present in the sample based on a plurality of expression signature genes, wherein the one or more cell types are identified and quantified at a sensitivity of at least about 80%; and (e) generating an immune-oncology profile based on the level of gene expression determined in (b), the mutational burden calculated in (c), and the one or more cell types quantified in (d). Sometimes, the mutational burden is calculated at a sensitivity of at least about 90%. In certain instances, the one or more cell types are identified and quantified at a sensitivity of at least about 90%.

[0021] In another aspect, disclosed herein are methods for identifying at least one cell type in a biological sample comprising a plurality of cell types, comprising: (a) obtaining the biological sample from a subject and enriching for ribonucleic acids (RNA) corresponding to a plurality of expression signature genes present in a deconvolution matrix, the deconvolution matrix tailored to identify cell types present in the biological sample based on sample type; (b) sequencing the RNA to obtain RNA sequencing data; (c) determining a level of gene expression for the RNA enriched from the biological sample; (d) applying the deconvolution matrix to evaluate at least a subset of the RNA sequencing data and the level of gene expression to identify a plurality of cell types and proportions of the plurality of cell types at an accuracy of at least 90%. In some instances, the plurality of cell types includes at least two cell types. Sometimes, the plurality of cell types includes at least three cell types. In various aspects, the biological sample is a non-cancer sample. In certain cases, the biological sample is a cancer sample. The biological sample is oftentimes a non-cancer sample. In some instances, the plurality of expression signature genes comprises at least one gene from at least two of Tables 1A, 1B, 1C, 1D, and 1E. Sometimes, the plurality of expression signature genes comprises at least one gene from at least three of Tables 1A, 1B, 1C, 1D, and 1E. In various aspects, the plurality of expression signature genes comprises at least one gene from at least four of Tables 1A, 1B, 1C, 1D, and 1E. In certain cases, the plurality of expression signature genes comprises at least one gene from each of Tables 1A, 1B, 1C, 1D, and 1E. In some instances, the plurality of expression signature genes comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 genes from Table 2, 3, or 4. Oftentimes, the method further comprises predicting a disease diagnosis based on the plurality of cell

types and proportions of the plurality of cell types. In certain instances, sequencing the RNA comprises performing reverse transcription on the RNA to generate complementary DNA (cDNA). Sometimes, the method further comprises sequencing the cDNA or a derivative thereof to obtain the RNA sequencing data.

**[0022]** In some aspects, disclosed herein are methods for identifying at least one cell type in a biological sample comprising a plurality of cell types, comprising: (a) obtaining the biological sample from a subject and enriching for ribonucleic acids (RNA) corresponding to a plurality of expression signature genes present in a deconvolution matrix, the deconvolution matrix tailored to identify cell types present in the biological sample based on sample type; (b) sequencing the RNA to obtain RNA sequencing data; (c) determining a level of gene expression for the RNA enriched from the biological sample; (d) applying the deconvolution matrix to evaluate at least a subset of the RNA sequencing data and the level of gene expression to identify a plurality of cell types and proportions of the plurality of cell types at a specificity of at least 90%. In some instances, the plurality of cell types includes at least two cell types. Sometimes, the plurality of cell types includes at least three cell types. In various aspects, the biological sample is a non-cancer sample. In certain cases, the biological sample is a cancer sample. The biological sample is oftentimes a non-cancer sample. In some instances, the plurality of expression signature genes comprises at least one gene from at least two of Tables 1A, 1B, 1C, 1D, and 1E. Sometimes, the plurality of expression signature genes comprises at least one gene from at least three of Tables 1A, 1B, 1C, 1D, and 1E. In various aspects, the plurality of expression signature genes comprises at least one gene from at least four of Tables 1A, 1B, 1C, 1D, and 1E. In certain cases, the plurality of expression signature genes comprises at least one gene from each of Tables 1A, 1B, 1C, 1D, and 1E. In some instances, the plurality of expression signature genes comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 genes from Table 2, 3, or 4. Oftentimes, the method further comprises predicting a disease diagnosis based on the plurality of cell types and proportions of the plurality of cell types. In certain instances, sequencing the RNA comprises performing reverse transcription on the RNA to generate complementary DNA (cDNA). Sometimes, the method further comprises sequencing the cDNA or a derivative thereof to obtain the RNA sequencing data.

**[0023]** In another aspect, disclosed herein are methods for identifying at least one cell type in a biological sample comprising a plurality of cell types, comprising: (a) obtaining the biological sample from a subject and enriching for ribonucleic acids (RNA) corresponding to a plurality of expression signature genes present in a deconvolution matrix, the deconvolution matrix tailored to identify cell types present in the biological sample based on sample type; (b) sequencing the RNA to obtain RNA sequencing data; (c) determining a level of gene expression for the RNA enriched from the biological sample; (d) applying the deconvolution matrix to evaluate at least a subset of the RNA sequencing data and the level of gene expression to identify a plurality of cell types and proportions of the plurality of cell types at a sensitivity of at least 90%. In some instances, the plurality of cell types includes at least two cell types. Sometimes, the plurality of cell types includes at least three cell types. In various aspects, the biological sample is a non-cancer

sample. In certain cases, the biological sample is a cancer sample. The biological sample is oftentimes a non-cancer sample. In some instances, the plurality of expression signature genes comprises at least one gene from at least two of Tables 1A, 1B, 1C, 1D, and 1E. Sometimes, the plurality of expression signature genes comprises at least one gene from at least three of Tables 1A, 1B, 1C, 1D, and 1E. In various aspects, the plurality of expression signature genes comprises at least one gene from at least four of Tables 1A, 1B, 1C, 1D, and 1E. In certain cases, the plurality of expression signature genes comprises at least one gene from each of Tables 1A, 1B, 1C, 1D, and 1E. In some instances, the plurality of expression signature genes comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 genes from Table 2, 3, or 4. Oftentimes, the method further comprises predicting a disease diagnosis based on the plurality of cell types and proportions of the plurality of cell types. In certain instances, sequencing the RNA comprises performing reverse transcription on the RNA to generate complementary DNA (cDNA). Sometimes, the method further comprises sequencing the cDNA or a derivative thereof to obtain the RNA sequencing data.

**[0024]** The present disclosure also provides computer-implemented methods for analyzing a biological sample obtained from a subject having a disease or condition, comprising: (a) obtaining gene expression data comprising the expression of at least one immune modulatory gene from the biological sample; (b) identifying and quantifying a percentage of at least one cell type that is present in the biological sample based on a plurality of expression signature genes; and (c) using a classifier to analyze the expression of the at least one immune modulatory gene and the percentage of the at least one cell type in order to classify the sample. In some instances, the sample is classified into one of at least two groups. In some instances, the at least two groups comprise a group having a positive response to a therapeutic intervention to the disease or condition and a group having a negative response to the therapeutic intervention. In some instances, the positive response comprises tumor regression, slowing tumor progression, or halt of tumor progression. In some instances, the negative response comprises tumor progression, lack of response to the therapeutic intervention, or a combination thereof. In some instances, the therapeutic intervention comprises immunotherapy. In some instances, the immunotherapy comprises a cancer vaccine, cytokine therapy, immune cell therapy, antibody therapy, or any combination thereof. In some instances, the therapeutic intervention comprises radiation, chemotherapy, surgery, or a combination thereof. In some instances, the method further comprises providing instructions to start, stop, change, or continue the therapeutic intervention. In some instances, the disease or condition is cancer. In some instances, the at least one cell type comprises at least one immune cell type. In some instances, the at least one immune cell type is selected from the group consisting of CD4+ memory T-cells, CD4+ naive T-cells, CD4+ T-cells, central memory T (Tcm) cells, effector memory T (Tem) cells, CD4+ Tcm, CD4+ Tem, CD8+ T-cells, CD8+ naive T-cells, CD8+ Tcm, CD8+ Tem, regulatory T cells (Tregs), T helper (Th) 1 cells, Th2 cells, gamma delta T (Tgd) cells, natural killer (NK) cells, natural killer T (NKT) cells, B-cells, naive B-cells, memory B-cells, class-switched memory B-cells, pro B-cells, and plasma cells. In some instances, the at least one immune cell type is

selected from the group consisting of M1 macrophages, M2 macrophages, CD19+ B cells, CD14+ monocytes, CD56+ NK cells, CD8+ T cells, Treg cells, and CD4+ T cells. In some instances, the at least one immune modulatory gene is selected from the group consisting of CTLA4, OX40, PD-1, IDO1, CD47, PD-L1, TIM-3, BTLA, ICOS, and ARG1. In some instances, the at least one cell type comprises at least 2, 3, 4, 5, 6, 7, or 8 cell types. In some instances, the at least immune modulatory gene comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 genes. In some instances, the classifier is generated using a machine learning algorithm. In some instances, the machine learning algorithm is a random forest algorithm. In some instances, the classifier is trained using data from no more than 50 samples. In some instances, the biological sample comprises cancer or pre-cancer tissue. In some instances, the prediction of an outcome for the subject is a positive response or a negative response to a therapeutic intervention. In some instances, the prediction of an outcome for the subject is a prognosis of the disease or condition. In some instances, the percentage of the at least one cell type is generated using a deconvolution algorithm that applies a deconvolution matrix to RNA sequencing data for the biological sample. In some instances, the deconvolution algorithm applies a deconvolution matrix to the gene expression data to identify and quantify the percentage of the at least one cell type. In some instances, the deconvolution matrix comprises a plurality of immune cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of tumor cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression counts for each expression signature gene is normalized across the plurality of cell types. In some instances, the deconvolution algorithm identifies and quantifies the at least one cell type that is present in the biological sample using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, the deconvolution algorithm performs an RNA normalization step to compensate for variation in RNA quantity amongst the at least one cell type in order to improve accuracy of the quantified percentage. In some instances, the deconvolution algorithm is a machine learning algorithm trained using comparison data comprising an actual percentage of the at least one cell type. In some instances, the actual percentage is generated using flow cytometry. In some instances, the method further comprises performing next generation RNA sequencing on the biological sample to obtain the RNA expression data and the plurality of expression signature genes. In some instances, the method further comprises obtaining mutational burden data for the biological sample and inputting the mutational burden data into the classifier for analysis in order to enhance classification of the biological sample. In some instances, the classifier is trained on data from no more than 15, 20, 25, 30, 35, 40, 45, or 50 samples and provides an accuracy of at least 70%, 75%, 80%, 85%, 90%, or 95%. In some instances, the accuracy is calculated using a leave-one-out cross-validation method. In some instances, the

gene expression data is RNA sequencing data. In some instances, the gene expression data is obtained using next generation sequencing.

[0025] The present disclosure also discloses systems comprising for analyzing a biological sample obtained from a subject having a disease or condition, comprising: a database comprising the data set; and at least one computer processor that is coupled to the database, wherein the at least one computer processor is programmed to perform steps comprising: (a) obtaining gene expression data for at least one immune modulatory gene from the biological sample; (b) identifying and quantifying a percentage of at least one cell type that is present in the biological sample based on a plurality of expression signature genes; and (c) using a classifier to analyze the expression of the at least one immune modulatory gene and the percentage of the at least one cell type in order to classify the sample. In some instances, the sample is classified into one of at least two groups. In some instances, the at least two groups comprise a group having a positive response to a therapeutic intervention to the disease or condition and a group having a negative response to the therapeutic intervention. In some instances, the positive response comprises tumor regression, slowing tumor progression, or halt of tumor progression. In some instances, the negative response comprises tumor progression, lack of response to the therapeutic intervention, or a combination thereof. In some instances, the therapeutic intervention comprises immunotherapy. In some instances, the immunotherapy comprises a cancer vaccine, cytokine therapy, immune cell therapy, antibody therapy, or any combination thereof. In some instances, the therapeutic intervention comprises radiation, chemotherapy, surgery, or a combination thereof. In some instances, the at least one processor is further programmed to provide instructions to start, stop, change, or continue the therapeutic intervention. In some instances, the disease or condition is cancer. In some instances, the at least one cell type comprises at least one immune cell type. In some instances, the at least one immune cell type is selected from the group consisting of CD4+ memory T-cells, CD4+ naive T-cells, CD4+ T-cells, central memory T (Tcm) cells, effector memory T (Tem) cells, CD4+ Tcm, CD4+ Tem, CD8+ T-cells, CD8+ naive T-cells, CD8+ Tcm, CD8+ Tem, regulatory T cells (Tregs), T helper (Th) 1 cells, Th2 cells, gamma delta T (Tgd) cells, natural killer (NK) cells, natural killer T (NKT) cells, B-cells, naive B-cells, memory B-cells, class-switched memory B-cells, pro B-cells, and plasma cells. In some instances, the at least one immune cell type is selected from the group consisting of M1 macrophages, M2 macrophages, CD19+ B cells, CD14+ monocytes, CD56+ NK cells, CD8+ T cells, Treg cells, and CD4+ T cells. In some instances, the at least one immune modulatory gene is selected from the group consisting of CTLA4, OX40, PD-1, IDO1, CD47, PD-L1, TIM-3, BTLA, ICOS, and ARG1. In some instances, the at least one cell type comprises at least 2, 3, 4, 5, 6, 7, or 8 cell types. In some instances, the at least immune modulatory gene comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 genes. In some instances, the classifier is generated using a machine learning algorithm. In some instances, the machine learning algorithm is a random forest algorithm. In some instances, the classifier is trained using data from no more than 50 samples. In some instances, the biological sample comprises cancer or pre-cancer tissue. In some instances, the prediction of an outcome for the subject is a

positive response or a negative response to a therapeutic intervention. In some instances, the prediction of an outcome for the subject is a prognosis of the disease or condition. In some instances, the percentage of the at least one cell type is generated using a deconvolution algorithm that applies a deconvolution matrix to RNA sequencing data for the biological sample. In some instances, the deconvolution algorithm applies a deconvolution matrix to the gene expression data to identify and quantify the percentage of the at least one cell type. In some instances, the deconvolution matrix comprises a plurality of immune cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of tumor cell expression signature genes. In some instances, the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression counts for each expression signature gene is normalized across the plurality of cell types. In some instances, the deconvolution algorithm identifies and quantifies the at least one cell type that is present in the biological sample using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression. In some instances, the deconvolution algorithm performs an RNA normalization step to compensate for variation in RNA quantity amongst the at least one cell type in order to improve accuracy of the quantified percentage. In some instances, the deconvolution algorithm is a machine learning algorithm trained using comparison data comprising an actual percentage of the at least one cell type. In some instances, the actual percentage is generated using flow cytometry. In some instances, the RNA expression data and the plurality of expression signature genes are obtained from the biological sample using next generation RNA sequencing. In some instances, the at least one processor is further programmed to obtain mutational burden data for the biological sample and inputting the mutational burden data into the classifier for analysis in order to enhance classification of the biological sample. In some instances, the classifier is trained on data from no more than 15, 20, 25, 30, 35, 40, 45, or 50 samples and provides an accuracy of at least 70%, 75%, 80%, 85%, 90%, or 95%. In some instances, the accuracy is calculated using a leave-one-out cross-validation method. In some instances, the gene expression data is RNA sequencing data. In some instances, the gene expression data is obtained using next generation sequencing.

[0026] Another aspect of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0027] Another aspect of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0028] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present

disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

#### INCORPORATION BY REFERENCE

[0029] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0031] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0032] FIG. 1 depicts an example workflow for characterization of a tumor microenvironment.

[0033] FIG. 2 depicts an example workflow for cell type and ratio deconvolution.

[0034] FIG. 3 depicts a schematic for normalization.

[0035] FIG. 4A, FIG. 4B, FIG. 4C, and FIG. 4D depict methods for identifying tumor mutational burden.

[0036] FIG. 5A and FIG. 5B depict gene expression levels in glioblastoma.

[0037] FIG. 6 depicts gene expression levels in colorectal and blood cancers.

[0038] FIG. 7 depicts a report showing immune modulatory molecule expression, mutational burden, and cell deconvolution results.

[0039] FIG. 8 schematically illustrates a computer system that is programmed or otherwise configured to implement methods provided herein.

[0040] FIG. 9 shows the average amount of total RNA per cell for each immune cell type.

[0041] FIG. 10A shows a graph of the observed, expected, and corrected number of M1 macrophages calculated at different dilution points through deconvolution.

[0042] FIG. 10B shows a graph of the observed, expected, and corrected number of M2 macrophages calculated at different dilution points through deconvolution.

[0043] FIG. 10C shows another graph of the observed, expected, and corrected number of M1 macrophages calculated at different dilution points through deconvolution.

[0044] FIG. 10D shows another graph of the observed, expected, and corrected number of M2 macrophages calculated at different dilution points through deconvolution.

[0045] FIG. 10E shows another graph of the observed, expected, and corrected number of M1 macrophages calculated at different dilution points through deconvolution.

[0046] FIG. 10F shows another graph of the observed, expected, and corrected number of M2 macrophages calculated at different dilution points through deconvolution.

[0047] FIG. 11 shows a graph plotting a comparison of known cell percentages to estimated cell percentages obtained using the deconvolution methods described herein.

[0048] FIG. 12A shows a bar plot indicating the predictive accuracy of an “escape” biomarker, an “immune” biomarker, and a multi-analyte “paragon” classifier.

[0049] FIG. 12B shows box-and-whisker plots that visualize the statistics for groups of samples for the most predictive “escape” biomarker.

[0050] FIG. 12C shows box-and-whisker plots that visualize the statistics for groups of samples for the most predictive “immune” biomarker.

[0051] FIG. 13 depicts an example flow chart illustrating methods of generating single-analyte and multi-analyte classifiers as described in Example 7.

#### DETAILED DESCRIPTION

[0052] The present disclosure employs, unless otherwise indicated, conventional molecular biology techniques, which are within the skill of the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art.

[0053] Throughout this disclosure, various embodiments are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of any embodiments. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range to the tenth of the unit of the lower limit unless the context clearly dictates otherwise. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual values within that range, for example, 1.1, 2, 2.3, 5, and 5.9. This applies regardless of the breadth of the range. The upper and lower limits of these intervening ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure, unless the context clearly dictates otherwise.

[0054] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of any embodiment. As used herein, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms “comprises” and/or “comprising,” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0055] The term “ribonucleic acid” or “RNA,” as used herein refers to a molecule comprising at least one ribo-

nucleotide residue. RNA may include transcripts. By “ribonucleotide” is meant a nucleotide with a hydroxyl group at the 2' position of a beta-D-ribo-furanose moiety. The term RNA includes, but not limited to, mRNA, ribosomal RNA, tRNA, non-protein-coding RNA (npcRNA), non-messenger RNA, functional RNA (lRNA), long non-coding RNA (lncRNA), pre-mRNAs, and primary miRNAs (pri-miRNAs). The term RNA includes, for example, double-stranded (ds) RNAs; single-stranded RNAs; and isolated RNAs such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinant RNA, as well as altered RNA that differ from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules described herein can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxy-nucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[0056] Unless specifically stated or obvious from context, as used herein, the term “about” in reference to a number or range of numbers is understood to mean the stated number and numbers +/−10% thereof, or 10% below the lower listed limit and 10% above the higher listed limit for the values listed for a range.

[0057] The term “sample,” as used herein, generally refers to a biological sample of a subject. The biological sample may be a tissue or fluid of the subject, such as blood (e.g., whole blood), plasma, serum, urine, saliva, mucosal excretions, sputum, stool and tears. The biological sample may be derived from a tissue or fluid of the subject. The biological sample may be a tumor sample or heterogeneous tissue sample. The biological sample may have or be suspected of having disease tissue. The tissue may be processed to obtain the biological sample. The biological sample may be a cellular sample. The biological sample may be a cell-free (or cell free) sample, such as cell-free DNA or RNA. The biological sample may comprise cancer cells, non-cancer cells, immune cells, non-immune cells, or any combination thereof. The biological sample may be a tissue sample. The biological sample may be a liquid sample. The liquid sample can be a cancer or non-cancer sample. Non-limiting examples of liquid biological samples include synovial fluid, whole blood, blood plasma, lymph, bone marrow, cerebrospinal fluid, serum, seminal fluid, urine, and amniotic fluid.

[0058] The term “variant,” as used herein, generally refers to a genetic variant, such as an alteration, variant or polymorphism in a nucleic acid sample or genome of a subject. Such alteration, variant or polymorphism can be with respect to a reference genome, which may be a reference genome of the subject or other individual. Single nucleotide polymorphisms (SNPs) are a form of polymorphisms. In some examples, one or more polymorphisms comprise one or more single nucleotide variations (SNVs), insertions, deletions, repeats, small insertions, small deletions, small repeats, structural variant junctions, variable length tandem repeats, and/or flanking sequences. Copy number variants (CNVs), transversions and other rearrangements are also forms of genetic variation. A genomic alternation may be a base change, insertion, deletion, repeat, copy number variation, or transversion.

**[0059]** The term “subject,” as used herein, generally refers to an animal, such as a mammalian species (e.g., human) or avian (e.g., bird) species, or other organism, such as a plant. More specifically, the subject can be a vertebrate, a mammal, a mouse, a primate, a simian or a human. Animals include, but are not limited to, farm animals, sport animals, and pets. The subject can be a healthy individual, an individual that has or is suspected of having a disease or a pre-disposition to the disease, or an individual that is in need of therapy or suspected of needing therapy. The subject can be a patient. The subject may have or be suspected of having a disease.

#### Generation of Immune-Oncology Profile

**[0060]** Provided herein are systems and methods for generating an immune-oncology profile using sequencing data. The immune-oncology profile often comprises at least one of immune modulatory molecule expression, cell type and ratio, and mutational burden for a given sample. In some cases, sequencing data is used to determine at least one of immune modulatory molecule expression, cell type and ratio, and mutational burden. Systems and methods for determining cell type and ratio may comprise deconvolution methods. An immune-oncology profile comprising immune modulatory molecule expression, cell type and ratio, and mutational burden may be used for therapeutic applications. For example, following determination of immune modulatory molecule expression, cell type and ratio, and mutational burden may provide information for diagnosis or treatment.

**[0061]** A first example workflow process is depicted in FIG. 1. In a first step a formalin-fixed paraffin-embedded (FFPE) sample **101** is provided. RNA extraction **103** is performed followed by sequencing library generation **105**. Target gene enrichment **107** is then performed followed by sequencing. Raw sequencing data **109** is generated, which is then used to determine immune modulatory molecule expression **111**, cell type and ratio deconvolution **113**, and perform mutational burden calculation **115**. The immune modulatory molecule expression **111**, cell type and ratio deconvolution **113**, and mutational burden calculation **115** is then used to generate a report **117** of an immune-oncology profile. An example of a report is illustrated in FIG. 7, showing immune modulatory molecule expression, mutational burden, and cell type and ratio deconvolution results. Expression of immune modulatory or immune escape genes are shown at the top of the report in FIG. 7, which include PD-1, PD-L1, CTLA-4, OX-40, TIM-3, BTLA, ICOS, CD47, IDO1, and ARG1. The “immune deconvolution” section of the report includes a pie chart showing the percentages of general cell types identified in the sample, including 30% immune, 60% tumor, and 5% stromal. More specific breakdowns of the immune cell types and their percentages are shown at the left. The cell types are further divided into categories including T cells (CD4+, CD8+, naïve CD4+, naïve CD8+, Central Memory CD4+, Effector Memory CD4+, Central Memory CD8+, Effector Memory CD8+, and Tregs), CD4+ subtypes (Th1, Th2, Th17), Myeloid cells (monocytic cells, granulocytic cells, macrophages, dendritic cells, MDSC, M1 macrophages, M2 macrophages), NK cells, and B cells (naïve B cells, memory B cells, activated B cells, and plasma B cells). At the bottom of the report is a mutational burden readout. In this case, the mutational burden is provided as a graphic showing a scale between lower mutational burden to the left (left endpoint=0 MutMB) and increasing mutational burden to the right (right

endpoint=2000 MutMB), with the circle indicating the sample's mutational burden position on this scale.

**[0062]** Provided herein are systems and methods for generating an immune-oncology profile from a sample of a subject. In some instances, the subject is has or is suspected of having a disease or disorder. In some instances, the immune-oncology profile is used for diagnosing the subject with a disease or disorder. Alternatively or in combination, the immune-oncology profile is used for determining or predicting a response to a therapeutic intervention in the subject.

**[0063]** Generation of an immune-oncology profile as described herein comprises first obtaining a sample from a subject. In some instances, the sample is any fluid or other material derived from the body of a normal or disease subject including, but not limited to, blood, serum, plasma, lymph, urine, saliva, tears, cerebrospinal fluid, milk, amniotic fluid, bile, ascites fluid, organ or tissue extract, and culture fluid in which any cells or tissue preparation from a subject has been incubated. In some instances, the sample is obtained from skin, blood, brain, bladder, bone, bone marrow, breast, colon, stomach, esophagus, ovary, uterus, gallbladder, fallopian tube, testicle, kidney, liver, pancreas, adrenal gland, cervix, endometrium, head or neck, lung, prostate, thymus, thyroid, lymph node, or urinary bladder. In some instances, the sample is a cancer sample. The cancer sample is typically a solid tumor sample or a liquid tumor sample. For example, the cancer sample is obtained from excised tissue. In some instances, the samples, is fresh, frozen, or fixed. In some instances, a fixed sample comprises paraffin-embedded or fixation by formalin, formaldehyde, or gluteraldehyde. In some instances, the sample is formalin-fixed paraffin-embedded.

**[0064]** In some instances, the sample is stored after it has been collected, but before additional steps are to be performed. In some instances, the sample is stored at less than 8° C. In some instances, the sample is stored at less than 4° C. In some instances, the sample is stored at less than 0° C. In some instances, the sample is stored at less than -20° C. In some instances, the sample is stored at less than -70° C. In some instances, the sample is stored a solution comprising glycerol, glycol, dimethyl sulfoxide, growth media, nutrient broth or any combination thereof. The sample may be stored for any suitable period of time. In some instances the sample is stored for any period of time and remains suitable for downstream applications. For example, the sample is stored for any period of time before nucleic acid (e.g., ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) extraction. In some instances, the sample is stored for at least or about 1 day, 2 day, 3 days, 4 days, 5 days, 6 days, 7 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or more than 12 months. In some instances, the sample is stored for at least 1 year, 2 years, 3, years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, or more than 12 years.

**[0065]** Methods and systems as described herein comprise generating an immune-oncology profile from a sample of a subject, wherein the sample comprises a nucleic acid molecule. In some instances, the nucleic acid molecule is RNA, DNA, fragments, or combinations thereof. In some instances, after a sample is obtained, the sample is processed further before analysis. In some instances, the sample is

processed to extract the nucleic acid molecule from the sample. In some instances, no extraction or processing procedures are performed on the sample. In some instances, the nucleic acid is extracted using any technique that does not interfere with subsequent analysis. Extraction techniques include, for example, alcohol precipitation using ethanol, methanol or isopropyl alcohol. In some instances, extraction techniques use phenol, chloroform, or any combination thereof. In some instances, extraction techniques use a column or resin based nucleic acid purification scheme such as those commonly sold commercially. In some instances, following extractions, the nucleic acid molecule is purified. In some instances, the nucleic acid molecule is further processed. For example, following extraction and purification, RNA is further reverse transcribed to cDNA. In some instances, processing of the nucleic acid comprises amplification. Following extraction or processing, in some instances, the nucleic acid is stored in water, Tris buffer, or Tris-EDTA buffer before subsequent analysis. In some instances, the sample is stored at less than 8° C. In some instances, the sample is stored at less than 4° C. In some instances, the sample is stored at less than 0° C. In some instances, the sample is stored at less than -20° C. In some instances, the sample is stored at less than -70° C. In some instances, the sample is stored for at least or about 1 day, 2 day, 3 days, 4 days, 5 days, 6 days, 7 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or more than 12 months.

**[0066]** A nucleic acid molecule obtained from a sample comprises may be characterized by factors such as integrity of the nucleic acid molecule or size of the nucleic acid molecule. In some instances, the nucleic acid molecule is DNA. In some instances, the nucleic acid molecule is RNA. In some instances, the RNA or DNA comprises a specific integrity. For example, the RNA integrity number (RIN) of the RNA is no more than about 2. In some instances, the RNA molecules in a sample have a RIN of about 2 to about 10. In some instances, the RNA molecules in a sample have a RIN of at least about 2. In some instances, the RNA molecules in a sample have a RIN of at most about 10. In some instances, the RNA molecules in a sample have a RIN of about 2 to about 3, about 2 to about 4, about 2 to about 5, about 2 to about 6, about 2 to about 7, about 2 to about 8, about 2 to about 9, about 2 to about 10, about 3 to about 4, about 3 to about 5, about 3 to about 6, about 3 to about 7, about 3 to about 8, about 3 to about 9, about 3 to about 10, about 4 to about 5, about 4 to about 6, about 4 to about 7, about 4 to about 8, about 4 to about 9, about 4 to about 10, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 5 to about 9, about 5 to about 10, about 6 to about 7, about 6 to about 8, about 6 to about 9, about 6 to about 10, about 7 to about 8, about 7 to about 9, about 7 to about 10, about 8 to about 9, about 8 to about 10, or about 9 to about 10. The RNA molecule in a sample may be characterized by size. In some instances, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, or more of the RNA molecules in a sample are at least 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, or more than 400 nucleotides in size. In some instances, the RNA molecules in the sample are at least 200 nucleotides in size. In some instances, the RNA molecules of at least 200 nucleotides in size comprise a percentage of the sample (DV200). For example, the percentage is at least or about 40%, 45%, 50%, 55%, 60%,

65%, 70%, 75%, 80%, 85%, 90%, 95%, or more than 95%. In some instances, the RNA molecules in a sample have a DV200 value of about 10% to about 90%. In some instances, the RNA molecules in a sample have a DV200 value of at least about 10%. In some instances, the RNA molecules in a sample have a DV200 value of at most about 90%. In some instances, the RNA molecules in a sample have a DV200 value of about 10% to about 20%, about 10% to about 30%, about 10% to about 40%, about 10% to about 50%, about 10% to about 60%, about 10% to about 70%, about 10% to about 80%, about 10% to about 90%, about 20% to about 30%, about 20% to about 40%, about 20% to about 50%, about 20% to about 60%, about 20% to about 70%, about 20% to about 80%, about 20% to about 90%, about 30% to about 40%, about 30% to about 50%, about 30% to about 60%, about 30% to about 70%, about 30% to about 80%, about 30% to about 90%, about 40% to about 50%, about 40% to about 60%, about 40% to about 70%, about 40% to about 80%, about 40% to about 90%, about 50% to about 60%, about 50% to about 70%, about 50% to about 80%, about 50% to about 90%, about 60% to about 80%, about 60% to about 90%, about 70% to about 80%, about 70% to about 90%, or about 80% to about 90%.

**[0067]** In some instances, after the samples have been obtained and nucleic acid molecule isolated, the nucleic acid molecule is prepared for sequencing. In some instances, a sequencing library is prepared. Numerous library generation methods have been described. In some instances, methods for library generation comprise addition of a sequencing adapter. Sequencing adapters may be added to the nucleic acid molecule by ligation. In some instances, library generation comprises an end-repair reaction.

**[0068]** Sometimes, library generation for sequencing comprises an enrichment step. For example, coding regions of the mRNA are enriched. In some instances, the enrichment step is for a subset of genes. In some instances, the enrichment step comprises using a bait set. The bait set may be used to enrich for genes used for specific downstream applications. A bait set generally refers to a set of baits targeted toward a selected set of genomic regions of interest. For example, a bait set may be selected for genomic regions relating to at least one of immune modulatory molecule expression, cell type and ratio, or mutational burden. In some instances, one bait set is used for determining immune modulatory molecule expression, a second bait set is used for determining cell type and ratio, and a third bait set is used for determining mutational burden. In some instances, the same bait set is used for determining immune modulatory molecule expression, cell type and ratio, mutational burden, or combinations thereof. In some instances, a bait set comprises at least one unique molecular identifier (UMI). The term "unique molecular identifier (UMI)" or "UMI" as used herein refers to nucleic acid having a sequence which can be used to identify and/or distinguish one or more first molecules to which the UMI is conjugated from one or more second molecules. In some instances, the UMI is conjugated to one or more target molecules of interest or amplification products thereof. UMIs may be single or double stranded.

**[0069]** The systems and methods disclosed herein provide for the sequencing for a number of genes. In some instances, the number of genes is at least about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500,

9000, 9500, 10000, or more than 10000 genes. In some instances, the number of genes to be sequenced is in a range of about 500 to about 1000 genes. In some instances, the number of genes to be sequenced is in a range of about at least 200. In some instances, the number of genes to be sequenced is in a range of about at most 10,000. In some instances, the number of genes to be sequenced is in a range of about 200 to 500, 200 to 1,000, 200 to 2,000, 200 to 4,000, 200 to 6,000, 200 to 8,000, 200 to 10,000, 500 to 1,000, 500 to 2,000, 500 to 4,000, 500 to 6,000, 500 to 8,000, 500 to 10,000, 1,000 to 2,000, 1,000 to 4,000, 1,000 to 6,000, 1,000 to 8,000, 1,000 to 10,000, 2,000 to 4,000, 2,000 to 6,000, 2,000 to 8,000, 2,000 to 10,000, 4,000 to 6,000, 4,000 to 8,000, 4,000 to 10,000, 6,000 to 8,000, 6,000 to 10,000, or 8,000 to 10,000. Examples of genes to be sequenced are seen in Tables 1A-1E or Table 5.

[0070] Sequencing may be performed with any appropriate sequencing technology. Examples of sequencing methods include, but are not limited to single molecule real-time sequencing, Polony sequencing, sequencing by ligation, reversible terminator sequencing, proton detection sequencing, ion semiconductor sequencing, nanopore sequencing, electronic sequencing, pyrosequencing, Maxam-Gilbert sequencing, chain termination (e.g., Sanger) sequencing, +S sequencing, or sequencing by synthesis.

[0071] Sequencing methods may include, but are not limited to, one or more of: high-throughput sequencing, pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, RNA-Seq (Illumina), Digital Gene Expression (Helicos), Next generation sequencing, Single Molecule Sequencing by Synthesis (SMSS) (Helicos), massively-parallel sequencing, Clonal Single Molecule Array (Solexa), shotgun sequencing, Maxim-Gilbert sequencing, and primer walking. Sequencing may generate sequencing reads ("reads"), which may be processed (e.g., alignment) to yield longer sequences, such as consensus sequences. Such sequences may be compared to references (e.g., a reference genome or control) to identify variants, for example.

[0072] An average read length from sequencing may vary. In some instances, the average read length is at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 20000, 30000, 40000, 50000, 60000, 70000, 80000, or more than 80000 base pairs. In some instances, the average read length is in a range of about 100 to 80,000. In some instances, the average read length is in a range of about at least 100. In some instances, the average read length is in a range of about at most 80,000. In some instances, the average read length is in a range of about 100 to 200, 100 to 300, 100 to 500, 100 to 1,000, 100 to 2,000, 100 to 4,000, 100 to 8,000, 100 to 10,000, 100 to 20,000, 100 to 40,000, 100 to 80,000, 200 to 300, 200 to 500, 200 to 1,000, 200 to 2,000, 200 to 4,000, 200 to 8,000, 200 to 10,000, 200 to 20,000, 200 to 40,000, 200 to 80,000, 300 to 500, 300 to 1,000, 300 to 2,000, 300 to 4,000, 300 to 8,000, 300 to 10,000, 300 to 20,000, 300 to 40,000, 300 to 80,000, 500 to 1,000, 500 to 2,000, 500 to 4,000, 500 to 8,000, 500 to 10,000, 500 to 20,000, 500 to 40,000, 500 to 80,000, 1,000 to 2,000, 1,000 to 4,000, 1,000 to 8,000, 1,000 to 10,000, 1,000 to 20,000, 1,000 to 40,000, 1,000 to 80,000, 2,000 to 4,000, 2,000 to 8,000, 2,000 to 10,000, 2,000 to 20,000, 2,000 to 40,000, 2,000 to 80,000, 4,000 to 8,000, 4,000 to 10,000, 4,000 to 20,000, 4,000 to

40,000, 4,000 to 80,000, 8,000 to 10,000, 8,000 to 20,000, 8,000 to 40,000, 8,000 to 80,000, 10,000 to 20,000, 10,000 to 40,000, 10,000 to 80,000, 20,000 to 40,000, 20,000 to 80,000, or 40,000 to 80,000.

[0073] In some instances, a number of nucleotides that are sequenced are at least or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 300, 400, 500, 2000, 2500, 3000, or more than 3000 nucleotides. In some instances, the number of nucleotides that are sequenced are about 5 to about 3,000 nucleotides. In some instances, the number of that are sequenced are at least 5 nucleotides. In some instances, the number of nucleotides that are sequenced are at most 3,000 nucleotides. In some instances, the number of nucleotides that are sequenced are 5 to 50, 5 to 100, 5 to 200, 5 to 400, 5 to 600, 5 to 800, 5 to 1,000, 5 to 1,500, 5 to 2,000, 5 to 2,500, 5 to 3,000, 50 to 100, 50 to 200, 50 to 400, 50 to 600, 50 to 800, 50 to 1,000, 50 to 1,500, 50 to 2,000, 50 to 2,500, 50 to 3,000, 100 to 200, 100 to 400, 100 to 600, 100 to 800, 100 to 1,000, 100 to 1,500, 100 to 2,000, 100 to 2,500, 100 to 3,000, 200 to 400, 200 to 600, 200 to 800, 200 to 1,000, 200 to 1,500, 200 to 2,000, 200 to 2,500, 200 to 3,000, 400 to 600, 400 to 800, 400 to 1,000, 400 to 1,500, 400 to 2,000, 400 to 2,500, 400 to 3,000, 600 to 800, 600 to 1,000, 600 to 1,500, 600 to 2,000, 600 to 2,500, 600 to 3,000, 800 to 1,000, 800 to 1,500, 800 to 2,000, 800 to 2,500, 800 to 3,000, 1,000 to 1,500, 1,000 to 2,000, 1,000 to 2,500, 1,000 to 3,000, 1,500 to 2,000, 1,500 to 2,500, 1,500 to 3,000, 2,000 to 2,500, 2,000 to 3,000, or 2,500 to 3,000 nucleotides.

[0074] Sequencing methods may include a barcoding or "tagging" step. In some instances barcoding (or "tagging") can allow for generation of a population of samples of nucleic acids, wherein each nucleic acid can be identified from which sample the nucleic acid originated. In some instances, the barcode comprises oligonucleotides that are ligated to the nucleic acids. In some instances, the barcode is ligated using an enzyme, including but not limited to, *E. coli* ligase, T4 ligase, mammalian ligases (e.g., DNA ligase I, DNA ligase II, DNA ligase III, DNA ligase IV), thermostable ligases, and fast ligases.

[0075] Barcoding or tagging may occur using various types of barcodes or tags. Examples of barcodes or tags include, but are not limited to, a radioactive barcode or tag, a fluorescent barcode or tag, an enzyme, a chemiluminescent barcode or tag, and a colorimetric barcode or tag. In some instances, the barcode or tag is a fluorescent barcode or tag. In some instances, the fluorescent barcode or tag comprises a fluorophore. In some instances, the fluorophore is an aromatic or heteroaromatic compound. In some instances, the fluorophore is a pyrene, anthracene, naphthalene, acridine, stilbene, benzoazazole, indole, benzindole, oxazole, thiazole, benzothiazole, canine, carbocyanine, salicylate, anthranilate, xanthenes dye, coumarin. Examples of xanthene dyes include, e.g., fluorescein and rhodamine dyes. Fluorescein and rhodamine dyes include, but are not limited to 6-carboxyfluorescein (FAM), 2'7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), tetrachlorofluorescein (TET), 6-carboxyrhodamine (R6G), N,N,N';N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX). In some instances, the fluorescent barcode or tag also includes the naphthylamine dyes that have an amino group in the alpha or beta position. For example, naphthylamino compounds include 1-dimethylaminonaphthyl-5-sulfonate, 1-anilino-8-naphthalene sulfonate and 2-p-toluidinyl-6-naphthalene sulfonate, 5-(2'-aminoethyl)

aminonaphthalene-1-sulfonic acid (EDANS). Examples of coumarins include, e.g., 3-phenyl-7-isocyanatocoumarin; acridines, such as 9-isothiocyanatoacridine and acridine orange; N-(p-(2-benzoxazolyl)phenyl) maleimide; cyanines, such as, e.g., indodicarbocyanine 3 (Cy3), indodicarbocyanine 5 (Cy5), indodicarbocyanine 5.5 (Cy5.5), 3-(carboxy-pentyl)-3'-ethyl-5,5'-dimethyloxacarbocyanine (CyA); 1H, 5H, 11H, 15H-Xantheno[2,3, 4-ij: 5,6, 7-i'j']diquinolizin-18-iun, 9-[2 (or 4)-[[6-[2,5-dioxo-1-pyrrolidinyl]oxy]-6-oxohexyl]amino]sulfonyl]-4 (or 2)-sulfophenyl]-2,3, 6,7, 12,13, 16,17-octahydro-immer salt (TR or Texas Red); or BODIPY™ dyes.

**[0076]** In some instances, a different barcode or tag is supplied a sample comprising nucleic acids. Examples of barcode lengths include barcode sequences comprising, without limitation, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more bases in length. Examples of barcode lengths include barcode sequences comprising, without limitation, from 1-5, 1-10, 5-20, or 1-25 bases in length. Barcode systems may be in base 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or a similar coding scheme. In some instances, a number of barcodes is at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, 18000, 20000, 25000, 30000, 40000, 50000, 100000, 500000, 1000000, or more than 1000000 barcodes. In some instances, a number of barcodes is in a range of 1-1000000 barcodes. In some instances, the number of barcodes is in a range of about 1-10 1-50 1-100 1-500 1-1000 1-5,000 1-10000 1-50000 1-100000 1-500000 1-1000000 10-50 10-100 10-500 10-1000 10-5,000 10-10000 10-50000 10-100000 10-500000 10-1000000 50-100 50-500 50-1000 50-5,000 50-10000 50-50000 50-100000 50-500000 50-1000000 100-500 100-1000 100-5,000 100-10000 100-50000 100-100000 100-500000 100-1000000 500-1000 500-5,000 500-10000 500-50000 500-100000 500-500000 500-1000000 1000-5,000 1000-10000 1000-500000 1000-1000000 5,000-10000 5,000-50000 5,000-100000 5,000-500000 5,000-1000000 10000-50000 10000-100000 10000-500000 10000-1000000 50000-100000 50000-500000 50000-1000000 100000-500000 100000-1000000 100000-1000000 or 500000-1000000 barcodes.

**[0077]** Following sequencing of a sample, sequencing data as described herein can be used for at least one of determining immune modulatory molecule expression, performing cell type and ratio deconvolution, and calculating mutational burden. An example of a workflow is seen in FIG. 2. Referring to FIG. 2, sequencing data is used to determine tumor expression counts **201** and immune cell expression signatures **203**. The tumor expression counts **201** and immune cell expression signatures **203** are then subjected to a deconvolution algorithm **205** to calculate immune cell percentage **207**.

**[0078]** Sequencing data as provided herein are used to determine gene expression. In some instances, the sequencing data is obtained from sequencing RNA from a sample. In some instances, the gene expression is of an immune modulatory molecule such as an immune checkpoint molecule or immune inhibitory molecule. Examples of immune modulatory molecules include, but are not limited to, one or more of 2B4 (CD244), A2aR, B7H3 (CD276), B7H4 (VTCN1), B7H6, B7RP1, BTLA (CD272), butyrophilins, CD103, CD122, CD137 (4-1BB), CD137L, CD160, CD2,

CD200R, CD226, CD26, CD27, CD28, CD30, CD39, CD40, CD48, CD70, CD73, CD80 (B7.1), CD86 (B7.2), CEACAM1, CGEN-15049, CTLA-4, DR3, GAL9, GITR, GITRL, HVEM, ICOS, ICOSL (B7H2), IDO1, IDO2, ILT-2 (LILRB1), ILT-4 (LILRB2), KIR, KLRG1, LAG3, LAIR1 (CD305), LIGHT (TNFSF14), MARCO, NKG2A, NKG2D, OX-40, OX-40L, PD-1, PDL-1 (B7-H1, CD 274), PDL-2 (B7-DC, CD 273), PS, SIRPalpha (CD47), SLAM, TGFR, TIGIT, TIM1, TIM3 (HAVCR2), TIM4, or VISTA.

**[0079]** In some instances, the gene expression of a sample is compared to a reference sample. Sometimes, the systems and methods disclosed herein generate an immune-oncology profile comprising a visual representation of immune modulatory molecule gene expression. In some cases, the visual representation presents the gene expression of one or more immune modulatory molecules relative to a reference expression level. In some instances, the reference expression level is obtained from a reference sample. Sometimes, the reference sample comprises the same cell or tissue type as the sample being evaluated for gene expression. Sometimes, the gene expression of a sample is compared to an averaged or plurality of reference samples. As an example, a cancer sample being evaluated for gene expression is compared to average gene expression for reference samples of the same cancer type as the cancer sample in a reference database (e.g., TCGA database).

**[0080]** Provided herein are systems and methods for generating an immune-oncology profile comprising determining cell type and ratio in a sample using sequencing data. The sample often comprises a heterogeneous composition of different cell types and/or subtypes. Sometimes, the sample is a tumor sample. The cell types and/or subtypes that make up the sample includes one or more of cancer cells, non-cancer cells, and/or immune cells. Examples of non-immune cells include salivary gland cells, mammary gland cells, lacrimal gland cells, ceruminous gland cells, eccrine sweat gland cells, apocrine sweat gland cells, sebaceous gland cells, Bowman's gland cells, Brunner's gland cells, prostate gland cells, seminal vesicle cells, bulbourethral gland cells, keratinizing epithelial cells, hair shaft cells, epithelial cells, exocrine secretory epithelial cells, uterus endometrium cells, isolated goblet cells of respiratory and digestive tracts, stomach lining mucous cells, hormone secreting cells, pituitary cells, gut and respiratory tract cells, thyroid gland cells, adrenal gland cells, chromaffin cells, Leydig cells, theca interna cells, macula densa cells of kidney, peripolar cells of kidney, mesangial cells of kidney, hepatocytes, white fat cells, brown fat cells, liver lipocytes, kidney cells, kidney glomerulus parietal cells, kidney glomerulus podocytes, kidney proximal tubule brush border cells, loop of Henle thin segment cells, kidney distal tubule cells, endothelial fenestrated cells, vascular endothelial continuous cells, synovial cells, serosal cells, squamous cells, columnar cells of endolymphatic sac with microvilli, columnar cells of endolymphatic sac without microvilli, vestibular membrane cells, stria vascularis basal cells, stria vascularis marginal cells, choroid plexus cells, respiratory tract ciliated cells, oviduct ciliated cells, uterine endometrial ciliated cells, rete testis ciliated cells, ductulus efferens ciliated cells, ciliated ependymal cells of central nervous system, organ of Corti interdental epithelial cells, loose connective tissue fibroblasts, corneal fibroblasts, tendon fibroblasts, bone marrow reticular tissue fibroblasts, other nonepithelial fibroblasts, pericytes, skeletal muscle cells, red skeletal muscle cells,

white skeletal muscle cells, intermediate skeletal muscle cells, nuclear bag cells of muscle spindle, nuclear chain cells of muscle spindle, satellite cells, cardiac muscle cells, ordinary cardiac muscle cells, nodal cardiac muscle cells, purkinje fiber cells, smooth muscle cells, myoepithelial cells of iris, myoepithelial cells of exocrine glands, erythrocytes, megakaryocytes, monocytes, epidermal Langerhans cells, osteoclasts, sensory neurons, olfactory receptor neurons, pain-sensitive primary sensory neurons, photoreceptor cells of retina in eye, photoreceptor rod cells, proprioceptive primary sensory neurons (various types), touch-sensitive primary sensory neurons, taste bud cells, autonomic neuron cells, Schwann cells, satellite cells, glial cells, astrocytes, oligodendrocytes, melanocytes, germ cells, nurse cells, interstitial cells, and pancreatic duct cells. Various cell types may be determined for the sample using methods as described herein including, but not limited to, lymphoid cells, stromal cells, stem cells, and myeloid cells. Examples of lymphoid cells include, but are not limited to, CD4+ memory T-cells, CD4+ naive T-cells, CD4+ T-cells, central memory T (Tcm) cells, effector memory T (Tem) cells, CD4+ Tcm, CD4+ Tem, CD8+ T-cells, CD8+ naive T-cells, CD8+ Tem, CD8+ Tem, regulatory T cells (Tregs), T helper (Th) 1 cells, Th2 cells, gamma delta T (Tgd) cells, natural killer (NK) cells, natural killer T (NKT) cells, B-cells, naive B-cells, memory B-cells, class-switched memory B-cells, pro B-cells, and plasma cells. In some instances, the cells are stromal cells, for example, mesenchymal stem cells, adipocytes, preadipocytes, stromal cells, fibroblasts, pericytes, endothelial cells, microvascular endothelial cells, lymphatic endothelial cells, smooth muscle cells, chondrocytes, osteoblasts, skeletal muscle cells, myocytes. Examples of stem cells include, but are not limited to, hematopoietic stem cells, common lymphoid progenitor cells, common myeloid progenitor cells, granulocyte-macrophage progenitor cells, megakaryocyte-erythroid progenitor cells, multipotent progenitor cells, megakaryocytes, erythrocytes, and platelets. Examples of myeloid cells include, but are not limited to, monocytes, macrophages, macrophages M1, macrophages M2, dendritic cells, conventional dendritic cells, plasmacytoid dendritic cells, immature dendritic cells, neutrophils, eosinophils, mast cells, and basophils. Other cell types may be determined using methods as described herein, for example, epithelial cells, sebocytes, keratinocytes, mesangial cells, hepatocytes, melanocytes, keratocytes, astrocytes, and neurons.

**[0081]** In some instances, the sequencing data is used to determine immune cell expression. Examples of immune cells to be detected by methods described herein include, but are not limited to, CD4+ memory T-cells, CD4+ naive T-cells, CD4+ T-cells, central memory T (Tcm) cells, effector memory T (Tem) cells, CD4+ Tcm, CD4+ Tem, CD8+ T-cells, CD8+ naive T-cells, CD8+ Tcm, CD8+ Tem, regulatory T cells (Tregs), T helper (Th) 1 cells, Th2 cells, gamma delta T (Tgd) cells, natural killer (NK) cells, natural killer T (NKT) cells, B-cells, naive B-cells, memory B-cells, class-switched memory B-cells, pro B-cells, and plasma cells. In some instances, the sequencing data is used to determine expression of non-immune cells including, but not limited to, stromal cells, stem cells, or tumor cells.

**[0082]** Methods and systems for determining cell type and ratio may comprise determining gene expression. In some instances, determining cell type and ratio may further comprise methods relating to deconvolution. In some instances,

a deconvolution matrix is used. The deconvolution matrix typically comprises gene expression for one or more cell types. In some instances, the matrix is used for a complex data set of RNA sequencing gene expression data to allow for identification of cell types in the data and the relative proportions of each cell type. See FIG. 3. In some instances, individual cell types/subtypes and the relative proportion of these individual cell types/subtypes are determined from sequencing data using a deconvolution matrix. In some cases, the relative proportion of at least 2 cell types/subtypes, at least 3 cell types/subtypes, at least 4 cell types/subtypes, at least 5 cell types/subtypes, at least 6 cell types/subtypes, at least 7 cell types/subtypes, at least 8 cell types/subtypes, at least 9 cell types/subtypes, at least 10 cell types/subtypes, at least 11 cell types/subtypes, at least 12 cell types/subtypes, at least 13 cell types/subtypes, at least 14 cell types/subtypes, at least 15 cell types/subtypes, at least 16 cell types/subtypes, at least 17 cell types/subtypes, at least 18 cell types/subtypes, at least 19 cell types/subtypes, at least 20 cell types/subtypes, at least 21 cell types/subtypes, at least 22 cell types/subtypes, at least 23 cell types/subtypes, or at least 24 cell types are determined from sequencing data using a deconvolution matrix. A matrix equation illustrates the mathematical relationship between a matrix comprising expression signatures of individual cell types, the percentage of each cell type, and the bulk expression counts. In some instances, the matrix equation is  $Ax=b$ , where A is the cell expression fingerprints (i.e., deconvolution matrix), x is the cell percentages, and b is the bulk expression counts. In some instances, the matrix equation is solved by methods such as matrix algebra, regression analysis, and/or machine learning. Alternately or in combination, deconvolution methods comprise linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression (SVR). In some instances, deconvolution comprises a normalization step. Referring to FIG. 3, normalization may occur across a row or down a column. For example, normalization occurs across a row, wherein the row includes distinct cell types or down a column, wherein the column includes gene expression of cells for a specific cell type. In some instances, normalization occurs across a row. In some instances, cell fractions are considered in determining gene expression (FIG. 3). In some instances, a deconvolution matrix is generated for each type of sample analyzed. For example, certain cell types have a different gene expression signature depending on the local tissue environment. As a result, a one-size-fits-all deconvolution matrix is sometimes less accurate than a deconvolution matrix "tailored" to a specific sample type. In some instances, the deconvolution algorithm maintains a database comprising a plurality of deconvolution matrices. In some instances, the deconvolution algorithm selects a deconvolution matrix for analyzing the gene expression data of a sample based on the sample type. The use of a tailored deconvolution matrix enables the use of a narrower set of genes for deconvolution of the sample. The narrower set of genes can increase speed of analysis and the number of samples that are processed at one time. In some instances, a smaller capture or bait set is used to enrich for the narrower set of genes for downstream analysis (e.g., RNA-Seq).

**[0083]** Methods and systems for determining cell type and ratio comprising methods relating to deconvolution may further comprise normalizing RNA content. In some instances, the RNA content is normalized or corrected based on cell type. For example, RNA content is normalized based on the amount of RNA in an individual cell type. In some instances, normalizing RNA content comprises determining a number of cells used to generate the RNA. In some instances, the number of cells is determined by flow cytometry, manual cell counting, automated cell counting, microscopy, or spectrophotometry. In some instances, the number of cells is at least or about 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1 million, 2 million, 3 million, 4 million, or more than 4 million cells.

**[0084]** Following determination of RNA content for an individual cell type, a correction value may be determined. In some instances, the cell is an immune cell. Examples of immune cells include, but are not limited to, a CD4+ T cell, a CD8+ T cell, a monocyte, a B-cell, a natural killer cell (NK), a M1 macrophage, or a M2 macrophage. In some instances, the immune cell is a CD4+ T cell. In some instances, a correction value for each individual cell type is determined. For example, the cell correction value for CD4+ T cell is about 1.00. Sometimes, the cell correction value for CD4+ T cell is from 0.9 to 1.1. In some instances, the cell correction value for CD8+ T cell is about 1.03. Sometimes, the cell correction value for CD8+ T cell is from 0.93 to 1.13. In some instances, the cell correction value for a monocyte is about 1.35. Sometimes, the cell correction value for a monocyte is from 1.25 to 1.45. In some instances, the cell correction value for a B-cell is about 0.53. Sometimes, the cell correction value for a B-cell is from 0.43 to 0.63. In some instances, the cell correction value for a natural killer cell (NK) is about 0.47. Sometimes, the cell correction value for a NK cell is from 0.37 to 0.57. In some instances, the cell correction value for a M1 macrophage is about 7.59. Sometimes, the cell correction value for a M1 macrophage is from 6.59 to 8.59. In some instances, the cell correction value for a M2 macrophage is about 12.26. Sometimes, the cell correction value for a M2 macrophage is from 11.26 to 13.26.

**[0085]** The correction value may be used to identify cell percentages of individual cell types. In some instances, the correction value is used in combination with deconvolution methods to determine cell percentages of individual cell types. In some instances, the correction value is applied prior to deconvolution methods. For example, the correction value is applied prior to support vector regression of RNA sequence data. In some instances, the correction value is applied following support vector regression and the cell types have been deconvoluted.

**[0086]** Methods and systems for determining cell type and ratio comprising methods relating to deconvolution and normalizing RNA content may result in an accurate determination of immune cell type percentages in a sample. In some instances, the accuracy is at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% improved using methods and systems as described herein as compared to methods and systems where RNA content is not normalized.

**[0087]** An immune-oncology profile may comprise the cell types and ratios present in a sample using deconvolution

of expression data for a plurality of genes. The genes typically exhibit differential expression in at least two cell types that are evaluated using deconvolution. In some cases, the genes exhibit differential expression between cancer and non-cancer cells, between different types of cancer cells, between immune and non-immune cells, between different types of immune cells, between different types of non-cancer cells, or any combination thereof. Examples of genes for inclusion in a deconvolution matrix include those listed in Tables 1A-1E. In some instances, a deconvolution matrix comprises at least about 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, or more than 3000 genes. In some instances, a deconvolution matrix comprises no more than about 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, or about 3000 genes. In some instances, a deconvolution matrix comprises a number of genes in a range of about 50 to 100, 50 to 200, 50 to 300, 50 to 400, 50 to 500, 50 to 600, 50 to 700, 50 to 800, 50 to 900, 50 to 1,000, 50 to 1,500, 100 to 200, 100 to 300, 100 to 400, 100 to 500, 100 to 600, 100 to 700, 100 to 800, 100 to 900, 100 to 1,000, 100 to 1,500, 200 to 300, 200 to 400, 200 to 500, 200 to 600, 200 to 700, 200 to 800, 200 to 900, 200 to 1,000, 200 to 1,500, 300 to 400, 300 to 500, 300 to 600, 300 to 700, 300 to 800, 300 to 900, 300 to 1,000, 300 to 1,500, 400 to 500, 400 to 600, 400 to 700, 400 to 800, 400 to 900, 400 to 1,000, 400 to 1,500, 500 to 600, 500 to 700, 500 to 800, 500 to 900, 500 to 1,000, 500 to 1,500, 600 to 700, 600 to 800, 600 to 900, 600 to 1,000, 600 to 1,500, 700 to 800, 700 to 900, 700 to 1,000, 700 to 1,500, 800 to 900, 800 to 1,000, 800 to 1,500, 900 to 1,000, 900 to 1,500, or 1,000 to 1,500 genes. In some instances, a deconvolution matrix comprises at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or about 120 genes from Tables 1A-1E. In some instances, a deconvolution matrix comprises no more than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or about 120 genes from Tables 1A-1E.

TABLE 1A

Deconvolution Genes for CD4+ T-cells			
ALS2CL	ANKRD55	ZNF483	TRAV13-1
ST6GALNAC1	SEMA3A	TRBV5-4	DNAH8
IL2RA	TRBV11-2	TRAV8-2	KRT72
EPPK1	FAM153B	TRAV12-2	TRAV8-6
TRBV6-5	TRAV10	IGKV5-2	IGLV6-57
TRAV12-1	CTLA4	TSHZ2	FOXP3
IGHV4-28	TRAV2	SORCS3	TRAV5
MDS2	NTN4	IGLV10-54	DACT1
TRBV5-5	THEM5	HPCAL4	CD4

TABLE 1B

Deconvolution Genes for CD8+ T-cells			
FLT4	TRBV4-2	TRBV6-4	SPRY2
S100B	TNIP3	CD248	ROBO1
CD8B	TRBV2	CYP4F22	PZP
LAG3	KLRC4-KLRK1	CRTAM	SHANK1
ANAPC1P1	NRCAM	JAKMIP1	KLRC2
KLRC3	CD8A	TRAV4	FBLN2

TABLE 1C

Deconvolution Genes for Monocytes			
DES	HLX	FPR3	FCGR1B
LOXHD1	EPHB2	LPL	LIPN
AQP9	MILR1	RETN	GPNMB
CYP2S1	PDK4	LILRA6	SEPT10
PLA2G4A	FOLR2	FOLR3	C1QB
SLC6A12	SLC22A16	DOCK1	NRG1
RXFP2	RIN2	ARHGEF10L	
LPAR1	CES1	FPR2	

TABLE 1D

Deconvolution Genes for NK cells			
IGFBP7	LDB2	GUCY1A3	KLRF1
DTHD1	AKR1C3	FASLG	KLRC1
XCL1	DAB2	FAT4	CD160
BNC2	CXCR1	SIGLEC17P	SH2D1B
DGKK	ZMAT4	LGALS9B	NMUR1
LGALS9C	MLC1	LIM2	
NCR1	CCNJL	PCDH1	

TABLE 1E

Deconvolution Genes for B-cells			
UGT8	IGKV1QR2-108	IGHE	SCN3A
IGLV2-8	IGKV1D-16	MYO5B	ENAM
RP11-148O21.2	IGLC7	IGHV1-2	IGKJ5
SOX5	TNFRSF13B	IGKV2D-29	IGKV1-17
IGLV2-18	IGHV2-70	CHL1	
IGKV3D-20	IGLV8-61	IGKV6-21	

[0088] There are potentially around 19,700 possible gene identifiers that can be used from the transcriptome for

generating a basis or deconvolution matrix. In some instances, genes are selected for deconvolution if the genes are differentially expressed in pairwise cell type differential expression analysis. In some instances, genes are selected for deconvolution if the genes are expressed at a consistent level within a cell type across samples. The present disclosure has identified a small subset of the transcriptome as being useful for carrying out deconvolution of immune cell types. Table 2 shows a list of 293 total genes and corresponding Ensembl gene identifiers in a 15 differentially expressed gene list. The genes in Table 2 are generated by performing pairwise comparisons for each cell type and tallying up the top 15 differentially expressed genes in each comparison. Tables 3 and 4 show genes generated using this same approach with Table 3 showing a 10 differentially expressed gene list (232 total genes) and Table 4 showing a 5 differentially expressed gene list (134 total genes). Table 2 has the longest list since it includes the top 15 differentially expressed genes. Table 3 has a gene list that is a subset of Table 2. Likewise, Table 4 has a gene list that is a subset of Table 3. In some instances, a deconvolution matrix comprises at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or about 250 genes from Table 2. In some instances, a deconvolution matrix comprises no more than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or about 250 genes from Table 2. In some instances, a deconvolution matrix comprises at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, or about 200 genes from Table 3. In some instances, a deconvolution matrix comprises no more than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, or about 200 genes from Table 3. In some instances, a deconvolution matrix comprises at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, or about 130 genes from Table 4. In some instances, a deconvolution matrix comprises no more than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, or about 130 genes from Table 4.

TABLE 2

Top 15 Differentially Expressed Genes			
gene_id	gene_name	gene_id	gene_name
ENSG00000128203.6	ASPHD2	ENSG00000105369.8	CD79A
ENSG00000171777.14	RASGRP4	ENSG00000146373.15	RNF217
ENSG00000186469.7	GNG2	ENSG00000152969.15	JAKMIP1
ENSG00000186806.5	VSIG10L	ENSG00000146776.13	ATXN7L1
ENSG00000198894.6	CIPC	ENSG00000068831.17	RASGRP2
ENSG00000156475.17	PPP2R2B	ENSG00000186891.12	TNFRSF18
ENSG00000178199.12	ZC3H12D	ENSG00000155307.16	SAMSN1
ENSG00000206190.10	ATP10A	ENSG00000183023.17	SLC8A1
ENSG00000117090.13	SLAMF1	ENSG00000240891.5	PLCXD2
ENSG00000263528.6	IKBKE	ENSG00000175857.7	GAPT
ENSG00000198851.8	CD3E	ENSG00000103313.10	MEFV
ENSG00000100351.15	GRAP2	ENSG00000100365.13	NCF4
ENSG00000146285.12	SCML4	ENSG00000164483.15	SAMD3
ENSG00000197208.5	SLC22A4	ENSG00000125810.9	CD93
ENSG00000126217.19	MCF2L	ENSG00000178562.16	CD28
ENSG00000186827.9	TNFRSF4	ENSG00000151948.10	GLT1D1
ENSG00000111913.14	FAM65B	ENSG00000153563.14	CD8A
ENSG00000182183.13	FAM159A	ENSG00000134460.14	IL2RA
ENSG00000175489.9	LRRC25	ENSG00000132185.15	FCRLA
ENSG00000170962.11	PDGFD	ENSG00000152582.11	SPEF2
ENSG00000104974.9	LILRA1	ENSG00000101842.12	VSIG1
ENSG00000185883.9	ATP6V0C	ENSG00000168229.3	PTGDR
ENSG00000151490.12	PTPRO	ENSG00000203747.8	FCGR3A
ENSG00000157445.13	CACNA2D3	ENSG00000011600.10	TYROBP
ENSG00000184060.9	ADAP2	ENSG00000085514.14	PILRA
ENSG00000172243.16	CLEC7A	ENSG00000104972.13	LILRB1
ENSG00000158869.9	FCER1G	ENSG00000065413.15	ANKRD44

TABLE 2-continued

Top 15 Differentially Expressed Genes			
gene_id	gene_name	gene_id	gene_name
ENSG00000100427.14	MLC1	ENSG00000196220.14	SRGAP3
ENSG00000150045.10	KLRF1	ENSG00000162415.6	ZSWIM5
ENSG0000018280.15	SLC11A1	ENSG00000167984.15	NLRC3
ENSG00000122223.11	CD244	ENSG00000178573.6	MAF
ENSG00000176928.5	GCNT4	ENSG00000173258.11	ZNF483
ENSG00000162599.14	NFIA	ENSG00000187554.10	TLR5
ENSG00000131042.12	LILRB2	ENSG00000069020.17	MAST4
ENSG00000164398.11	ACSL6	ENSG00000181036.12	FCRL6
ENSG00000160683.4	CXCR5	ENSG00000172456.15	FGGY
ENSG00000102445.17	KIAA0226L	ENSG0000010671.14	BTK
ENSG00000160883.9	HK3	ENSG00000114013.14	CD86
ENSG00000198816.5	ZNF358	ENSG00000144218.17	AFF3
ENSG00000179041.3	RRS1	ENSG00000104043.13	ATP8B4
ENSG00000053524.10	MCF2L2	ENSG00000129450.7	SIGLEC9
ENSG00000102245.6	CD40LG	ENSG00000082074.14	FYB
ENSG00000124203.5	ZNF831	ENSG00000153064.10	BANK1
ENSG00000137441.7	FGFBP2	ENSG00000164867.9	NOS3
ENSG00000109944.9	C11orf63	ENSG00000143226.12	FCGR2A
ENSG00000183813.6	CCR4	ENSG0000011590.12	ZBTB32
ENSG00000198879.10	SFMBT2	ENSG00000160185.12	UBASH3A
ENSG00000173208.3	ABCD2	ENSG00000163393.11	SLC22A15
ENSG00000144843.10	ADPRH	ENSG00000133574.8	GIMAP4
ENSG00000183621.14	ZNF438	ENSG00000196218.10	RYR1
ENSG00000174946.6	GPR171	ENSG00000128218.7	VPREB3
ENSG00000066056.12	TIE1	ENSG00000181847.10	TIGIT
ENSG00000176438.11	SYNE3	ENSG00000155849.14	ELMO1
ENSG00000153283.11	CD96	ENSG00000182621.15	PLCB1
ENSG00000167286.8	CD3D	ENSG00000148655.13	C10orf11
ENSG00000179934.6	CCR8	ENSG00000128815.16	WDFY4
ENSG00000127507.16	EMR2	ENSG00000188404.7	SELL
ENSG00000167850.3	CD300C	ENSG00000100368.12	CSF2RB
ENSG00000197629.5	MPEG1	ENSG00000141293.14	SKAP1
ENSG00000100385.12	IL2RB	ENSG00000213047.10	DENND1B
ENSG00000133561.14	GIMAP6	ENSG00000196418.11	ZNF124
ENSG00000179921.13	GPBAR1	ENSG00000113319.10	RASGRF2
ENSG00000263264.1	CTB-133G6.1	ENSG00000140968.9	IRF8
ENSG00000152213.3	ARL11	ENSG00000066294.13	CD84
ENSG00000077420.14	APBB1IP	ENSG00000188848.14	BEND4
ENSG00000145416.12	1-Mar	ENSG00000183918.13	SH2D1A
ENSG00000095585.15	BLNK	ENSG00000236609.3	ZNF853
ENSG00000158714.9	SLAMF8	ENSG00000165521.14	EML5
ENSG00000188822.7	CNR2	ENSG00000013725.13	CD6
ENSG00000030419.15	IKZF2	ENSG00000110002.14	VWA5A
ENSG00000151366.11	NDUFCA2	ENSG00000134539.15	KLRD1
ENSG00000121964.13	GTDC1	ENSG00000170006.10	TMEM154
ENSG00000126264.8	H CST	ENSG00000042980.11	ADAM28
ENSG0000010030.12	ETV7	ENSG00000142303.12	ADAMTS10
ENSG00000186265.8	BTLA	ENSG00000162881.6	OXER1
ENSG00000187796.12	CARD9	ENSG00000150681.8	RGS18
ENSG00000182866.15	LCK	ENSG00000103569.8	AQP9
ENSG00000100450.11	GZMH	ENSG00000186074.17	CD300LF
ENSG00000158473.6	CD1D	ENSG00000172116.20	CD8B
ENSG00000149970.13	CNKS R2	ENSG00000100055.19	CYTH4
ENSG00000104490.16	NCALD	ENSG00000170909.12	OSCAR
ENSG00000107954.9	NEURL1	ENSG00000035720.6	STAP1
ENSG00000155846.15	PPARGC1B	ENSG00000139193.3	CD27
ENSG0000003400.13	CASP10	ENSG00000066336.10	SPII
ENSG00000115956.9	PLEK	ENSG00000110448.9	CD5
ENSG00000175556.15	LONRF3	ENSG00000184221.11	OLIG1
ENSG00000187116.12	LILRA5	ENSG0000005471.14	ABCB4
ENSG00000165591.6	FAAH2	ENSG00000105227.13	PRX
ENSG00000140090.16	SLC24A4	ENSG00000145990.9	GFO D1
ENSG0000010319.5	SEMA3G	ENSG00000159339.12	PADI4
ENSG00000136573.11	BLK	ENSG00000105374.8	NKG7
ENSG00000155629.13	PIK3AP1	ENSG00000235568.5	NFAM1
ENSG00000177455.10	CD19	ENSG00000110777.10	POU2AF1
ENSG00000152495.9	CAMK4	ENSG00000154655.13	L3MBTL4
ENSG00000117091.8	CD48	ENSG00000158481.11	CD1C
ENSG00000170819.4	BFSP2	ENSG00000140678.15	ITGAX
ENSG00000198821.9	CD247	ENSG00000146094.12	DOK3
ENSG00000173762.6	CD7	ENSG00000117009.10	KMO
ENSG00000120278.13	PLEKHG1	ENSG00000164124.9	TMEM144
ENSG000000119866.19	BCL11A	ENSG00000247077.5	PGAM5

TABLE 2-continued

Top 15 Differentially Expressed Genes			
gene_id	gene_name	gene_id	gene_name
ENSG00000120594.15	PLXDC2	ENSG00000132704.14	FCRL2
ENSG00000145649.7	GZMA	ENSG00000107242.16	PIP5K1B
ENSG00000158517.12	NCF1	ENSG00000142235.7	LMTK3
ENSG00000180061.8	TMEM150B	ENSG00000186854.9	TRABD2A
ENSG00000127152.16	BCL11B	ENSG00000196159.10	FAT4
ENSG00000116824.4	CD2	ENSG00000106034.16	CPED1
ENSG00000170458.12	CD14	ENSG00000154451.13	GBP5
ENSG00000090376.7	IRAK3	ENSG00000167995.14	BEST1
ENSG0000000938.11	FGR	ENSG00000151623.13	NR3C2
ENSG00000143184.4	XCL1	ENSG00000112182.13	BACH2
ENSG00000180739.13	S1PR5	ENSG00000124772.10	CPNE5
ENSG0000012124.13	CD22	ENSG00000221926.10	TRIM16
ENSG00000177272.8	KCNA3	ENSG00000130810.18	PPAN
ENSG00000172673.9	THEMIS	ENSG00000049768.13	FOXP3
ENSG00000273749.3	CYFIP1	ENSG00000198223.13	CSF2RA
ENSG00000278540.3	ACACA	ENSG00000271383.5	NBPF19
ENSG00000136404.14	TM6SF1	ENSG00000079263.17	SP140
ENSG00000086730.15	LAT2	ENSG00000073861.2	TBX21
ENSG00000255587.6	RAB44	ENSG00000105383.13	CD33
ENSG00000163519.12	TRAT1	ENSG00000111052.6	LIN7A
ENSG00000198734.9	F5	ENSG00000196092.11	PAX5
ENSG00000117322.15	CR2	ENSG000001711051.7	FPR1
ENSG00000065675.13	PRKCQ	ENSG00000162654.8	GBP4
ENSG00000198574.5	SH2D1B	ENSG00000159958.4	TNFRSF13C
ENSG00000187912.10	CLEC17A	ENSG00000010610.8	CD4
ENSG00000267534.2	S1PR2	ENSG00000126759.11	CFP
ENSG00000119535.16	CSF3R	ENSG00000104921.13	FCER2
ENSG00000166523.6	CLEC4E	ENSG00000160856.19	FCRL3
ENSG00000164330.15	EBF1	ENSG00000080493.12	SLC4A4
ENSG00000163563.7	MNDA	ENSG00000186462.8	NAP1L2
ENSG00000179088.13	C12orf42	ENSG00000261371.4	PECAM1
ENSG00000145687.14	SSBP2	ENSG00000085265.9	FCN1
ENSG00000205544.3	TMEM256	ENSG00000205730.6	ITPR1PL2
ENSG00000172543.6	CTSW	ENSG00000266412.4	NCOA4
ENSG00000124406.15	ATPA8A1	ENSG00000087903.11	RFX2
ENSG00000136867.9	SLC31A2	ENSG00000161405.15	IKZF3
ENSG00000113263.11	ITK	ENSG00000144152.11	FBLN7
ENSG00000172578.10	KLHL6	ENSG00000165071.13	TMEM71
ENSG00000119457.7	SLC46A2	ENSG00000265808.3	SEC22B
ENSG00000153485.5	TMEM251	ENSG00000162804.12	SNED1
ENSG00000203710.9	CR1	ENSG00000105967.14	TFEC
ENSG00000175294.5	CATSPER1	ENSG00000197540.6	GZMM
ENSG00000111452.11	GPR133	ENSG00000090612.19	ZNF268
ENSG00000160654.8	CD3G	ENSG00000171596.6	NMUR1
ENSG00000189430.11	NCRI		
ENSG00000197705.8	KLHL14		
ENSG00000089012.13	SIRPG		
ENSG00000181409.10	AATK		
ENSG00000112394.15	SLC16A10		

**[0089]** Provided herein are systems and methods for determining an immune-oncology profile comprising determining cell type and ratio using deconvolution methods, wherein following deconvolution, percentages of immune cells may be determined. In some instances, immune cells may be further grouped based on shared lineage and percentages of immune cells based on lineage is determined. For example, immune cells are divided into T cells, CD4+ subtypes, myeloid cells, and natural killer cells. In some instances, percentages of non-immune cells are determined. In some instances, percentages of immune cells and percentages of non-immune cells are determined. Sometimes, an immune-oncology profile comprises determining a percentage of immune cells and non-immune cells such as tumor cells and/or stromal cells.

**[0090]** Following deconvolution, a number of cell types of various immune and non-immune cell types may be determined. In some instances, deconvolution identifies at least or

about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 immune cell types. In some instances, deconvolution identifies a range of about 5 to about 20 immune cell types. In some instances, deconvolution identifies at least or about 5 to 10, 5 to 15, 5 to 20, 10 to 15, 10 to 20, or 15 to 20 immune cell types. Deconvolution may be used to identify non-immune cell types. In some instances, deconvolution identifies at least or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 non-immune cell types. In some instances, deconvolution identifies a range of about 5 to about 20 non-immune cell types. In some instances, deconvolution identifies at least or about 5 to 10, 5 to 15, 5 to 20, 10 to 15, 10 to 20, or 15 to 20 non-immune cell types. In some cases, deconvolution results are evaluated by comparing to the Gold Standard. Sometimes, the Gold Standard is generated by sorting the samples evaluated by deconvolution. For example, a sample is split into two portions with one portion

evaluated by nucleic acid sequencing and deconvolution and the other portion evaluated by sorting (e.g., flow cytometry or FACS) to obtain the Gold Standard. The results of the deconvolution are then compared to the Gold Standard to evaluate for accuracy, specificity, sensitivity, correlation to the Gold Standard, or any combination thereof.

**[0091]** Provided herein are systems and methods for generating an immune-oncology profile comprising mutational burden determined using sequencing data. In some instances, mutational burden is calculated for somatic mutations. In some instances, mutational burden is calculated by excluding germline variations. Germline variations may be excluded based on frequency. In some instances, exclusion is based on a frequency of at least 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25% or more than 25%. In some instances, the germline variations are determined using germline variation databases.

**[0092]** Mutational burden may be determined using a plurality of genes. Examples of genes used to determine mutational burden are seen in Table 5. In some instances, a number of genes for determining mutational burden is at least or about 250 to 5,000 genes. In some instances, a number of genes for determining mutational burden is at least or about 250 genes. In some instances, a number of genes for determining mutational burden is at most 5,000 genes. In some instances, a number of genes for determining mutational burden is at least or about 250 to 500, 250 to 750, 250 to 1,000, 250 to 1,500, 250 to 2,000, 250 to 2,500, 250 to 3,000, 250 to 3,500, 250 to 4,000, 250 to 4,500, 250 to 5,000, 500 to 750, 500 to 1,000, 500 to 1,500, 500 to 2,000, 500 to 2,500, 500 to 3,000, 500 to 3,500, 500 to 4,000, 500 to 4,500, 500 to 5,000, 750 to 1,000, 750 to 1,500, 750 to 2,000, 750 to 2,500, 750 to 3,000, 750 to 3,500, 750 to 4,000, 750 to 4,500, 750 to 5,000, 1,000 to 1,500, 1,000 to 2,000, 1,000 to 2,500, 1,000 to 3,000, 1,000 to 3,500, 1,000 to 4,000, 1,000 to 4,500, 1,000 to 5,000, 1,500 to 2,000, 1,500 to 2,500, 1,500 to 3,000, 1,500 to 3,500, 1,500 to 4,000, 1,500 to 4,500, 1,500 to 5,000, 2,000 to 2,500, 2,000 to 3,000, 2,000 to 3,500, 2,000 to 4,000, 2,000 to 4,500, 2,000 to 5,000, 2,500 to 3,000, 2,500 to 3,500, 2,500 to 4,000, 2,500 to 4,500, 2,500 to 5,000, 3,000 to 3,500, 3,000 to 4,000, 3,000 to 4,500, 3,000 to 5,000, 3,500 to 4,000, 3,500 to 4,500, 3,500 to 5,000, 4,000 to 4,500, 4,000 to 5,000, or 4,500 to 5,000 genes.

**[0093]** Mutational burden as determined herein may be determined as a range between low and high mutational burden. In some instances, mutational burden is determined as low, medium, or high mutational burden. Sometimes, mutational burden is determined as the number of nonsynonymous somatic mutations per megabase in the exome. In some cases, mutational burden is compared to the Gold Standard mutational burden calculated using paired normal analysis of DNA. Specifically, the Gold Standard mutational burden measures somatic mutations using DNA sequence data obtained from the sample by comparing allele frequencies in normal and tumor sample alignments, annotating the identified mutations, and aggregating the mutations.

TABLE 5

Mutational Burden Genes						
TC2N	TRAF6	PRMT2	STIM2	DDX59	EYA3	DPF2
LIPA	TRAF5	PRMT3	STIM1	DDX58	AGAP3	UBQLN4
TBL3	GDPD5	PRMT6	N4BP1	DDX54	AGAP6	UBR1
LIMA1	GEM	SMARCA2	N4BP2	ATP6V0A2	AEBP1	ARID1B
LINS4	GKAP1	SMAP1	N4BP2L2	DDX51	ADSS	ARRDC1
LIMK1	TPRKB	SMAD7	NDUFA3	DDX50	ADPRH	ARRB2
TCF4	TPR	PRKRIP1	NHLRC2	DDX5	FADS1	UBR2
TCF3	TPP2	SMARCC2	NHLRC3	ZNF438	TUBG1	DNAJB14
LINT7B	TPP1	PRKAG2	NID1	ATP5SL	ADNP	DPH1
TCF25	TPMT	SMARCC1	NID2	DENNND2D	ADNP2	DPH2
TCF20	GLA	PRKAR1A	NGDN	DENNND1A	TUBGCP2	DPP7
TCF12	GLCE	PRKAR1B	NIN	ATP5G2	ADSL	ARID2
TCERG1	GIT2	PRKAR2A	NIPAL2	UPF3B	FAAH	DPP3
TBL1X	GIPC1	PRKCA	STAB1	UQCC2	ZNF707	ARID4A
TBKBP1	GIGYF2	PRKCD	STAG1	UQCRCB	F8	ARID4B
LMO7	GIMAP4	PRKCE	NDUFA4	BM1I	F3	ARID5B
LMTK2	GIMAP6	SMARCB1	NFKBIE	USP35	ADRM1	ARRDC3
TBK1	GIMAP7	PRKC1	NFIX	USP38	EVI2B	EFNB1
LNK2	GOLGA5	PRKCZ	NFKBIA	ZNF384	AGFG1	ANO10
LONP1	TP53	SMAD4	NFYC	USP40	AGRН	EFTUD2
TBC1D8	TOX4	SMAD3	NFRKB	BET1	AGTPBP1	DHX34
LOXL1	TNKS	SLMAP	NFX1	BFAR	ZNF691	ZNF574
TBC1D5	TNK2	PRR14	NFYA	USP47	EXOC2	DHX32
LMNB1	TNIP1	PRR14L	NIPBL	ZNF37A	EXOC1	DHX36
TBCK	GRK5	SLTM	NIPSNAP1	USP34	ZNF689	DHX37
TBCEL	TNFRSF21	SLP1	NLRX1	BDH1	AHCTF1	ANP32B
TBCE	TNFRSF1B	PROSER1	NMD3	BHLHE40	EXD3	DHX57
LLGL2	TNKS1BP1	PRR4	NISCH	USP24	EXD2	ATG16L2
LLPH	GRB10	PRRC2B	NME6	CYP51A1	TUT1	DHX30
TBCD	GPS2	PRR12	NME7	ZNF397	AHCYL1	EHMT1
LMAN2L	TNPO2	PRPSAP2	NLRP1	BCL2L11	AHCYL2	EHHADH
LMBRD1	GPRC5C	SMAD2	NLRC5	BCL2L12	AHDC1	ZNF576
LMBRD2	TNKS2	PRPF18	NLN	BCL2L13	AHI1	ANKS1A
LMF1	GRAMD1A	PRPF3	ST7L	BCL2L2	AHNAK	EHD1
LIMD1	GRN	PRPF38B	NKAP	BCL6	AHR	EHBP1L1
TCF7	GSTO1	PRPF4	NKTR	BCL7B	EVL	EHBP1
TECPR1	GTDC1	PRPF40A	ST6GALNAC4	USP25	EVISL	EGR2
LCMT2	GTF2E2	PRPF8	ST3GAL4	USP28	EVIS5	EGR1

TABLE 5-continued

Mutational Burden Genes						
TECR	GRWD1	SMAD1	NFIA	BCL9	AGPS	ZNF568
LCOR	GSAP	PPP4C	NFE2L1	BCL9L	EXT2	EFCAB2
LCORL	TNFRSF10B	PPP4R1	NDUFB8	BCLAF1	AGFG2	DIEXF
LCP1	GSPT1	SMG6	NDUFB9	USP3	EXT1	ANXA6
LDB1	GSPT2	PPP6C	NDUFC2	BCOR	AGGF1	ATF7IP2
LDHA	GSR	PPP6R1	NDUFS2	USP30	AGK	AOAH
LDLR	TNRC18	PPP6R3	NDUFS7	BCR	AGL	UBE2E2
LDLRAD4	TNRC6A	PPRC1	NDUFSV1	USP31	AGO1	DIP2A
TDRKH	TNRC6B	PPWD1	NECAP1	USP32	EXOSC7	DIP2B
LCLAT1	GOSR1	SMG7	NDUFA8	USP48	AGO2	ATF6
TELO2	GOSR2	PPP1R7	NDUFA9	CYP20A1	EXOSC2	ATF5
LATS1	TOPBP1	PPP2R5A	STAM	BLMH	EXOSC10	ATF4
TET2	TOP3B	PPP2R5B	NEO1	CYB561A3	AGO4	DIDO1
LBR	TOP2B	PPP2R5C	NEU3	CYB561	EXOG	DHX8
TESK2	GPALPP1	PPP2R5E	NEURL4	USPL1	EXOC7	DIAPH1
TESK1	GPAM	PQLC1	STAMB P	CXorf40A	EPS8L2	ANTXR2
TESC	GPATCH2	PQLC3	NEDD1	BLZF1	ZNF615	ANXA1
TERF1	GPATCH2L	PRKAA1	NF1	BIVM	EML4	ZNF571
TEP1	GOPC	PREPL	NFATC1	USP5	EML3	ANXA2
TDRD7	TOR2A	PRDX5	NFATC2IP	ZNF362	UBA7	ANXA3
TCP11L2	TOR1AIP1	PRDX6	NFATC3	USP53	ANKAR	DICER1
TCP11L1	TOPORS	PREB	NENF	USP54	ANKDD1A	EFCAB7
TCOF1	GOLGB1	SMG1	NEDD9	ZNF358	ZNF609	ATG2A
LHPP	GOLIM4	SMARCD1	NEK1	USP6NL	ZNF608	EIF4A2
TCHP	GOLM1	PRIMPOL	NEK3	USP7	ANKHD1	EIF3G
LIG3	GON4L	PRADC1	NEK4	CYLD	ANKIB1	ATG9A
LGALSL	GPATCH8	SMCHD1	NEK6	BIN1	ZNF606	ANKRD36
LGALS9	TNRC6C	SMC4	NEK7	USP9X	UBA3	DHRS3
LEPR	GPR155	PRC1	SPATA7	CYHR1	ANAPC1	ANKRD39
LETMD1	TOM1L2	PRCC	PLEKHA5	CYFIP2	ENOSF1	DHRS12
TDP2	GPBP1	PRDM10	PLEKHB2	CYCS	UAP1	EIF4G3
LFNG	TNS3	PRDM11	PLEKHG1	CYBRD1	ENO1	EIF4G2
TDP1	TNS1	PRDM15	PLEKHG2	CYBB	ANAPC7	DHCR7
TDG	GPR137	PRDM2	PLEC	BIRC3	ENKD1	EIF4G1
LGALS3BP	TOP1MT	PRDM4	SNIP1	BIRC6	ENGASE	UNK
TCTN3	GPD2	PRRC2C	PLEKHJ1	CYB5R4	ENG	ATHL1
TCTN2	TOP1	QKI	PLEKHM2	B3GNTL1	ANAPC2	ZNF592
TBC1D4	GPR107	PTPRE	PLEKHM3	DBN1	ANKLE2	ATG7
LTA4H	GPR108	PTPN18	PLEK	DBF4B	ANKRD13C	ATG4B
LTBP1	FLYWCH1	PTPN2	PLCD1	DBF4	ELK4	EIF2AK3
LTBP3	FMNL1	PTPN23	PLAGL2	DAZAP1	ELK3	ANKRD50
LTBR	FMO5	PTPN6	PLAUR	USP12	ANKRD16	EIF2AK2
LTN1	FMR1	PTPRA	PLBD1	USP13	ANKRD17	ATG4A
LTV1	FN1	PTPRC	PLCB2	USP14	UBAP1	ANKRD42
LUM	FLNA	PTPRF	PLCB3	USP15	ZNF597	ATG2B
LUZP1	FNBp4	PTPRJ	SNRK	BACE2	ELF1	DHTKD1
TAMM41	FNDC3A	PTPRM	PLCG1	BACH1	UBAP2	DHX15
LSG1	FNDC3B	PTPRS	PLCL2	B4GALT2	UBAP2L	EIF2AK4
TAOK2	FLNB	PTRHD1	SNRNP200	DCAF4	ANKRD26	ANKRD44
TAOK1	FKBP7	SLFN13	PLD2	DCAF17	ANKRD27	ZNF585A
TANGO6	FKBP15	PTEN	PML	DCAF16	ELAVL1	EIF3E
TANGO2	FKBP1C	PTGES2	PLXNB1	DCAF13	EIF5B	EIF3D
LRSAM1	FKBP2	PTK7	PLXNB2	BAG3	ANKRD28	ANKRD46
LRWD1	FKBP4	PTOV1	PLXND1	BBS2	ANKRD12	EIF3C
LSM6	FKBP5	PXMP2	SNAPC4	DAB2	ELL2	ATG4D
LSM4	FKBP8	PXN	PMPCA	BBS9	ANKRA2	EIF2S3L
LYSMD4	FLCN	SLC4A7	PMPCB	DAAM2	ANKMY1	DHRS4
LYST	FLII	PYGB	PMS1	CYYR1	EMC10	EIF2D
TAF2	FNIP1	PYGL	PMS2	CYTIP	EMB	EIF2B3
TAF1C	FNIP2	PYGO2	PLXNA3	CYTH4	ELP6	DNAJC24
LZTR1	FNTA	PYROXD2	PLXDC2	CYTH3	ELP4	E4F1
LZTS2	TRMT10A	QARS	PLXDC1	BCAS3	ANKMY2	ATAD3B
MACF1	FOXN2	PXK	PLK3	CYTH1	ELOVL5	DLAT
LYAR	FOXO4	PXDN	SND1	USP21	ELL3	APEX2
TAF3	FOXP1	PWWP2B	PLOD1	BCCIP	ANKRD10	DLG5
LYPLAL1	TRIP6	PUM1	PLOD2	BCKDHA	ELOF1	EDC3
LRRK2	TRIP4	PUM2	SNAPIN	BCKDHB	ELMSAN1	AP3B1
LRRK1	FOXJ2	PURB	PLRG1	USP20	ELMO2	EDF1
LRPAP1	TRNAU1AP	PUS7	PLAC9	BAG4	ELMO1	EDEM3
LRP1	TRMU	PUS7L	PIK3R4	BBS1	ZNF619	ULK3
LRP12	FOSB	PWP1	PIK3R5	BAG5	ATL3	EDEM2
TBC1D22B	FOSL2	PWP2	PIKFYVE	DAPK1	ENOX2	APIP
TBC1D22A	TRMT6	PTDSS1	PIM2	BANP	ALDH5A1	UBE2I
LRP5	TRMT5	SLC7A6	PISD	BAP1	ALDH9A1	ATAD2B
LRPPRC	TRMT2B	PSMA2	PIN4	USP19	TYW1	ECHDC2

TABLE 5-continued

Mutational Burden Genes						
LRRC14	FDPS	PSMA4	PINX1	ZNF408	ALG11	APAF1
TBC1D15	FCGR3A	PSMB5	PIP5K1A	ZNF407	ALG12	APBA3
LRMP	FCHO2	PSMB7	PIK3R1	BASP1	ALG13	ECH1
TBC1D23	FCHSD2	SLC8B1	PIK3CB	DAGLB	ALG14	ATAD2
TBC1D24	TSHZ1	SLC9A1	PIGO	BAZ1A	ALG3	APBB1IP
LRIG2	FES	PRSS23	PIGQ	BAZ1B	ALG6	APBB2
LPCAT2	FDXR	SLFN11	PIGT	BAZ2A	TYW5	DLG1
LPCAT4	FECH	SLCO3A1	PIGX	DAG1	EPG5	APC
LPIN1	FCGR2A	SLCO2B1	SNW1	BAZ2B	EPDR1	ATAD1
LPIN2	FCER1G	SLC9B2	PIH1D1	CEP95	EPC2	ECI1
LPP	FBXO6	SLC9A8	PIK3C2A	CEP85L	ALG8	AP4B1
LPXN	FBXO8	PRUNE2	PIK3C2B	CEP78	EPC1	DLD
LRBA	FBXW11	SLC9A3R1	PIK3C3	CEP70	ALDH4A1	ZNF565
LRCH1	FBXW9	PSD4	PIK3CA	CEP57L1	EPHB4	DLG4
LRCH3	TRPM7	PSEN2	PKM	CEP57	AKT3	ECD
LRCH4	FGFRL1	PSTPIP1	PKN1	CEP41	EPS15L1	ECE1
TBC1D2B	FGGY	PSMF1	PKNOX1	CEP350	EPKS	DLG3
LRIF1	TRPT1	PSMG1	PKP4	WWP2	TYK2	UMPS
LRRC28	FGR	PSMG4	PLA2G4A	CEP250	ZNF668	APMAP
TARSL2	FHL2	PSPC1	PLA2G6	CEP192	EPOR	AP1B1
TARS2	FHOD1	PSPH	PITPNB	CEP170	EPN2	EEF1G
TARDBP	FICD	PTBP1	PITPNCl	CEP135	EPHB6	EEF1A1
TARBP1	FIG4	PTBP2	PITPNM1	CBWD3	ALCAM	EEF2K
TAPT1	FIP1L1	PTCD1	SNTB1	CEP89	ALDH16A1	DIS3L2
LRRC8A	FGRFR1	PTCD3	SNRPN	CBFA2T2	EPN1	AP1M1
LRRC8B	TTRAP	PTCH1	PKD1	CBL	EPM2A	DZIP3
LRRC11	FEZ2	PSME4	PKDCC	CBLB	ALDH1A1	EEF1D
LRRFIP1	FGD2	PSME2	PNISR	CBLL1	ALKBH1	EEF2
LRRFIP2	FGD3	PSMC6	PPAN	WWC3	U2SURP	UNC45A
LRRC61	FGD6	PSMD12	PPARA	CES4A	ZNF638	DIRC2
LRRC32	TSC22D1	PSMD3	PPARG	ZBTB5	ENTPD6	ZNF559
LRRC37B	TSC2	PSMD5	PPFIA1	CES2	ALS2	UNC13D
TBC1D10B	TSC1	RIC8A	PPFIBP1	CES1	ENTPD1	ULK1
TBC1D10A	FFGT	SAV1	PPFIBP2	CERS6	ZNF627	AP2M1
TBC1D1	GALM	SART1	PPIA	WWOX	AMBRA1	ULK2
TAX1BP1	GALNS	SATB1	POU6F1	WWP1	ZNF626	DIS3
TATDN2	GALNT10	SHOC2	SMPD4	WRNIP1	AMFR	ASUN
LARS2	GALNT2	SBF1	POP4	XAF1	ZNF623	EED
TIMELESS	GALT	SBF2	POR	ZBTB43	ENPP4	AP2A2
KHNYN	GANAB	SHKBP1	SMOX	CCDC146	AMN1	EEA1
KHSRP	GANC	SBNO1	POU2F1	XIAP	AMPD2	DLGAP4
KIAA0100	TRIP12	SAFB	PPP1CC	CECR5	AMPD3	AP1AR
KIAA0141	TRIM22	SAFB2	PPP1R12A	CCDC149	ENTPD7	CCDC68
KIAA0196	TRIM16	SHPRH	PPP1R13B	CECR1	ALKBH3	ASS1
KDM6B	TRIM14	SAMD9L	PPP1R15A	ZBTB40	EP400NL	ZNF813
KDM5C	TRIM11	SAMHD1	PPP1CB	ZBTB4	ALKBH4	ZSCAN12
KIF3A	GAK	SAMM50	PPIL2	ZBTB39	ALKBH5	PRICKLE3
KCTD9	TRAPPC9	SAP130	PPIP5K1	ZBTB38	EPB41L3	ZNF471
KDM1A	TRAPPC3	SBNO2	PPIP5K2	CEP104	EPB41L2	NOSTRIN
KDM2A	GAPVD1	SH3RF1	PPM1B	CBX5	ZNF655	A4GALT
KDM5B	GCC1	SCMH1	PPM1L	CBX6	ALMS1	ZRANB3
KDM2B	GBP2	SCML1	PPM1M	CENPQ	EPB41	PPP1R26
KDM3B	TRAPPC12	SH3TC1	POMT2	CBX7	EPAS1	ABHD6
KDM4A	TRAPPC10	SCN1B	POMT1	CBY1	ALOX5	CBS
KDM4B	GCAT	SH3RF3	POMP	CC2D1A	EP400	CD248
KDM4C	GCC2	SCAF1	POGZ	CC2D1B	EP300	NFASC
KDM5A	TRAP1	SH3KBP1	POLA1	ZBTB48	ALOX5AP	SEMA5A
KIAA0232	TRANK1	SCO1	POLD1	CCAR1	EOGT	CD300A
KIAA0355	GBF1	SCP2	POLK	CCAR2	ALPK1	ABI3BP
TIE1	GBE1	SCPEP1	POLE	CENPC	ZNF641	NES
KIAA1715	GART	SHB	POLH	CCDC109B	FADS2	CD2
KIDINS220	GAS2L1	SCAF4	POC5	CCDC115	FADS3	NOTCH3
KIF13A	GAS6	SCAF8	SMYD3	XAB2	AC138035.2	STAG3
KIF13B	GATAD2A	SCAP	POC1B	WSB1	AC009403.2	SLC7A5
KIAA0368	GATAD2B	SCAPER	SNAP29	WRN	ZRANB2	ACSM3
KIF1B	GATM	SCARB2	SMYD5	CAPS	AC013461.1	ZAP70
KIF1C	GBA	SCD	PNPLA6	CHKA	ZNFX1	PTPRU
KIF22	TRIM24	SCFD2	PNPLA8	CHID1	ZNF91	ABLIM3
TICAM1	FTSJ3	SAAL1	POLI	WHSC1L1	FAM73B	CCDC80
TIAM2	TRIM5	S1PR3	POLL	ZC3HAV1	FAM73A	SEMA3C
TIAM1	TRIM44	RSBN1L	POLR2M	CAPZA2	ZNF880	SEMA4A
KIAA1586	FTO	RSF1	POLR3A	ZC3H7B	ZNF862	AC005943.2
KIAA1551	FUBP1	SIK3	POLR3B	CARD16	ZSCAN21	PPP1R13L
KIAA1468	FUK	SIK1	POLR3C	CHFR	ABLIM1	SGCE
KIAA0391	FURIN	RSPRY1	POLR3D	ZC3H7A	FANCC	AB13

TABLE 5-continued

Mutational Burden Genes						
KIAA0430	TRIM52	RRP9	POLR3E	ZC3H6	ABI2	NFIB
KIAA0556	TRIM56	RRP8	POLM	CHD9	ABL1	NCKAP1L
KIAA0586	FRA10AC1	RRP36	POLRMT	CHD8	FANCA	SEMA6D
KIAA0753	TRIP11	RRBP1	POM121	CHD7	FAN1	SFRP1
KIAA0922	FRG1	RREB1	SMURF1	CHD6	TSPAN7	SASH1
KIAA1109	TRIOBP	RRM2B	SMU1	CARD6	ABL2	PPP1R16B
KIAA1191	FRMD4B	RRP1B	SMTN	CHD4	FAM98C	SASH3
KIAA1324L	TRIO	S1PR1	SMYD2	CHD3	FAM98B	PTPRG
KIAA1328	FRY	RXRA	POLR1B	CHD1L	FAM98A	NCEH1
KAT6B	FRYL	RYBP	POLR2A	CHM	ZSCAN25	SH3BP4
KANSL2	TRIM68	SIGIRR	POLR2B	CAPRIN2	ABR	PTPRB
KANSL3	FUS	S100A10	POLR2E	CAND1	ZSCAN30	AXL
KAT2B	TRIM34	SIAH2	POLR2F	CHMP4B	FAM84B	PTN
KAT6A	GAA	S100A8	POLR2G	CAPG	ABRACL	SLC9A3R2
KAT7	TRIM41	RTEL1	PCF11	ZCCHC11	ABT1	ACE
KBTBD6	G3BP1	RTF1	SOS1	CAPN15	ABTB1	SH3PXD2B
KAT8	G2E3	RTN3	SORT1	CAPN3	TSR1	NMNAT3
KATNB1	FUT8	RTTN	SORL1	CAPN5	ZNF844	PRDM1
KATNBL1	FYB	RUFY1	SORBS3	CAPN7	ACER3	PTAFR
KBTBD3	FYCO1	RUFY2	SORBS1	ZC3H1	ACADVL	CCDC122
KANSL1	FYN	RUND1	SON	CHML	FAM47E	PSTPIP2
KANK2	FZD1	RUSC1	PCGF5	CARD8	TTBK2	ZNF441
JMJD6	GTF2IRD2B	RUSC2	PCF1	CASP10	ZNF841	CCDC121
TM9SF4	TMEM173	RUVBL1	SOS2	ZC3H13	TTC13	NEDD4
JUNB	TMEM168	RUVBL2	SOCS2	WLS	ACAP1	B3GALNT1
JUND	TMEM167B	SCRIB	PBRM1	CFLAR	ACAP2	ZBTB45
JUP	IGHMBP2	SGPL1	PBXIP1	CFL2	ACAP3	ATP8B4
KANK1	IGF2R	SGTA	PC	CFL1	FAM46A	SERAC1
KBTBD7	TMEM165	SGSM2	PCBP2	CASP4	ACAT1	ZNF429
TIMM50	TMEM161A	SGSH	SP1	WNK1	ACAT2	NINL
TIMP1	TMEM175	SERPINB6	PCCA	CASP6	ACBD3	CCND1
KCTD10	TMEM176A	SERPINB8	PCCB	CASP9	ACBD4	SLC9A9
KCMF1	ILVBL	SGPP1	PCDH1	ZC3H11A	ACBD5	SERINC2
KCTD13	IFRD2	SERPING1	SP2	ZC3H10	FAM35A	ATXN7L2
KCTD18	IFT122	SERPINH1	SP140L	CAST	TTC17	PTGER4
KCNAB2	IFT140	SERPINI1	SP110	CAT	ACCS	SERPINA3
TLR4	IFT172	SERTAD1	SP100	CATSPER2	ACD	PRKD1
TLR1	TMEM176B	SESN1	PDCD6IP	ZBTB7B	FAM49A	ZNF431
TLN1	IFT88	SEPT2	SOCS5	WRAP73	ACAD11	ASAP3
TLK2	IGF1R	SEPT5	PDE2A	WIZ	AC138969.4	PTGFRN
TLK1	IKBKAP	SEPT7	PDE4DIP	ZC3H14	TSSC1	AVIL
TLE4	IKZF1	SEPT9	PDE5A	CARF	TSSC4	PTGR2
TLE3	TMEM131	SH2D3C	PDE7A	CARHSP1	ACAA1	PTGS1
TIP2	IL2RG	SERINC3	PDE8A	WIPF2	ACAA2	ABCC9
KRT8	IL32	SERPIN1A	PDGFC	CHAMP1	TST	PPP4R1L
TFPI	IL4R	SF3B4	PCNT	CHAF1A	TSTA3	SMARCA1
TFEB	ILF3	SFI1	PCOLCE2	ZC3H4	ACACB	SHC2
TFDP1	IL1R1	SFMBT1	PCSK7	CARS	ACAD10	ABC46
TFCP2	IL10RA	SFPQ	PCYT1A	CARS2	FAM65B	PROCR
TFAP4	IL13RA1	SFSWAP	SOCS6	ZC3H3	ACAD8	CC2D2A
TIA1	IL15RA	SFT2D2	PDCD11	ZC3H18	ACAD9	SLIT2
KPNA3	IL16	SET	PAXIP1	ZC3H15	ACADM	SCUBE2
KPNA4	IL17RA	SGK3	PAXBP1	CASC4	TSTD1	PROS1
TGIF2	IL17RC	SGK1	PAFAH1B2	CASK	FAM53C	SGK494
KPTN	TMEM144	SFXN2	SPATA13	ZBTB34	ACADS	SCARA3
KRCC1	IFNGR2	SFXN5	PAN2	CHMP7	ACADSB	AASS
TGIF1	IFNGR1	SF3B3	PAK2	CDC34	TSTD2	SLIT3
KRIT1	IARS	SF3B2	SPAG9	CDC27	ABI1	PPP1R3D
TGFBRAP1	HUWE1	SF3B1	PALB2	YBX1	ABHD8	PPP1R3C
KRT10	HYOU1	SF3A3	PAM	CCNK	ABHD5	SEPT10
TGFBR3	IARS2	SETD1A	P4HA1	CDC25B	ABHD4	ACOX2
KSR1	IBA57	SETD1B	P4HA2	CCP110	AARS	SLC8A1
KTN1	ICA1	SETD2	PABPC1L	CDAN1	AARS2	ZNF827
LARP1	TMEM184B	SETD3	PACS1	CDC40	AARSD1	ABC47
LARP7	HTT	SETD5	PACSIN2	CDCA7L	FBXL20	SHF
LAMC1	HTRA2	SETD6	PAN3	YTHDF2	AASDH	ATP8B1
TEX10	HSPG2	SETD7	PARP12	YTHDC2	AASDHPP7	CCR1
LAMB2	HTATSF1	SETDB1	PARP14	CDC5L	ABAT	ZNF845
LAMB1	TMEM206	SETDB2	PARP3	CCNB1	ABCA1	PSD3
L2HGDH	TMEM2	SETX	PARP4	CDC42EP4	ABCA2	ZNF852
L3MBTL2	TMEM192	SGMS1	PARP6	CDC42EP3	ABCA5	ZNF443
LACTB	IFNAR2	SF3A1	PARP8	CCND2	FBXL19	SETBP1
LAIR1	IFIH1	SF3A2	PARVB	CDC42BPB	ABCB1	SLC7A2
TEX2	IFI27L1	SH3BGRL2	PARVG	CDC42BPA	ABCB10	ZBTB46
LAMA5	IFI30	SDPR	SPAG16	CD99L2	FBXL14	SCD5

TABLE 5-continued

Mutational Burden Genes						
KPNA1	IFIT2	SH3BP5L	PASK	CD55	FBXL12	STARD8
KNTC1	IFITM3	SEC11A	PATL1	CCZ1B	FBRS	STARD13
KLHDC3	IFNAR1	SEC14L1	PAWR	CD47	ZW10	CCDC3
KLF6	IDS	SEC24B	PARP10	CD44	ABCB6	CCDC102B
KLF7	HSPBAP1	SH3BP5	PANK4	CD14	ABCB7	NDN
KLF9	TMCO3	SH3BP2	PAPD5	CD40	ABCB8	SH3D19
KLHL18	ISOC1	SEC16A	PAPLN	CD4	FBXL6	ABCA8
THOC5	TMCC3	SEC23A	PAPOLG	CD163	FBXO25	SPAG1
THOC2	ISY1	SEC23B	PAPSS2	YIPF3	A2M	BTNL9
KIF9	ITFG1	SEC23IP	PARL	CD36	FBXO38	PARVA
KIFAP3	ITFG2	SCYL1	PHC1	YIF1A	ZZEF1	ALDH7A1
KIFC2	ITGA5	SDAD1	PHC2	CD302	AAAS	PARD3B
THUMPD3	ITGA7	SDCBP	PHF10	CD2AP	FBXO28	PARD3
KLC1	ITGAE	SDCCAG8	PHF12	YES1	AACS	RBM47
KLC2	ITGAL	SEC24C	PHB	CD93	ZYX	C3
KLC4	ITGAM	SEMA3F	PHF2	CD9	TSPAN14	C3AR1
THRAP3	TMCO4	SEMA6A	PHF20	YLPM1	ZXDB	ROBO1
KLHL2	IRF2BPL	SEMA6C	PHF20L1	CD86	FBXO22	C3orf33
KNSTRN	IRF3	SENP1	PHF23	CD83	FBXO18	PIK3AP1
TGS1	IRF8	SEC24D	SNX14	CD82	AAED1	PID1
THAP4	TMED4	SENP5	PGM1	YBX3	FBXO11	PCDHGC3
THADA	TMED1	SENP7	PGD	CD74	AAGAB	ZNF283
KMT2A	ISG15	SEPP1	PGGT1B	CCSER2	AAK1	SORBS2
KMT2C	ITGAX	SEC31A	SNX17	CCT2	AAMDC	SLC38A5
KMT2D	ITGB1	SEC31B	PGRMC2	CCT3	AAMP	C10orf128
KMT2E	JAG1	SEC61G	PHACTR2	CCT4	ZXDC	ZNF14
TGOLN2	IWS1	SECISBP2	PHF3	CD63	AAR2	SOX13
THBD	JAK2	SEL1L3	PI4K2A	YIPF6	ABCC1	SOX7
KLHL21	TMA16	RIC8B	PI4KA	ZBTB14	TSPAN3	RBP1
KLHL22	JARID2	RNF8	PIAS4	CDKN2AIPNL	FBN1	ZNF135
THEM4	IVD	RNGTT	PICALM	CDKN2AIP	FASN	PBLD
KLHL5	ITSN2	RNH1	PICK1	CCDC28A	FARSB	SLC39A14
KLHL7	ITSN1	RNMT	PIGC	XPR1	ABHD13	PALMD
THBS1	ITGB2	RNPC3	PIGG	XRCC1	FARSA	NPAS2
MAD1L1	ITGB4	ROBO3	PHYKPL	ZBTB11	ABHD14A	SLC25A23
MRPL1	ITIH4	ROCK2	PHYHD1	CDKAL1	FARS2	ZNF555
STYXL1	ITM2A	SLC25A39	PHYH	CDK8	ZSCAN31	RASAL2
MRPL10	ITPKB	RNF214	PHGDH	ZBTB10	ABHD14B	P2RX7
SUCLG2	ITPR1	RNF217	SNX13	ZBTB1	ABHD11	ZNF554
MPP5	ITPR2	RNF34	PHIP	CDK5RAP3	ABHD15	RARRES2
MPP6	ITPRP1	RNF38	PHKA2	CDK5RAP2	ABHD16A	SPDYE3
MPP7	IRF2	RNF4	PHKB	CDK5RAP1	ABHD17A	RARG
SUCO	INPP5A	RNF40	PHLDB2	CCDC22	ABHD17B	OTUD3
MPST	INO80	RP11-231C14.4	PHLPP1	CCDC57	ABHD17C	PLCB1
MPZL1	INO80C	RP11-166B2.1	PHLPP2	ZBTB25	ABHD18	SLC22A17
MR1	INO80D	SLC24A1	PHOSPHO2	ZBTB24	ABHD2	SLC1A3
MRAS	INPP5B	SLC22A23	PHRF1	XPNPEP1	ABHD3	PALLD
MRC2	INPP5D	SLC22A18	PGBD2	ZBTB21	ABHD12	PALD1
MRE11A	INPPL1	SLC25A36	PGAP3	XPO1	ABHD10	APCDD1
MRPL54	TMEM109	SLC1A5	PDXDC1	XPO5	ABCC10	ZNF322
MRPL33	IMP4	SLC25A13	PDZD8	XPO6	ABCD4	SPARCL1
MRPL35	IMP4A	SLC25A14	PEAK1	XPO7	FBLN5	SLC25A33
MRPL38	IMPACT	SLC25A32	PEAR1	ZBTB17	ZSWIM8	PKN3
MRPL39	INCENP	SLC25A26	PEPB1	CDR2	ZSWIM6	RBFOX2
MPHOSPH6	INF2	SLC25A16	PECR	ZBTB16	ABCC3	RAVER2
MLLT3	ING3	RNF213	PELP1	CCDC71	ABCC5	RCAN2
MLLT4	ING4	RLF	PDS5B	CCDC88B	ABCD1	AOC3
SUN1	IPO8	RLIM	PDS5A	CCDC88C	ABCD3	PGM5
MLXIP	IPO9	SLC29A3	PDIA4	CCDC9	ZSCAN9	ZNF248
MMAA	IQCB1	RMDN3	PDIK1L	CCDC91	ABCE1	RHBDF1
MLLT1	IQCE	RMI1	PDK4	CCDC92	ABCF1	ZNF205
MMD	IQCG	SLC29A1	PDLIM5	CCDC93	ABCF2	PHACTR1
SUMO3	IQCK	SLC27A4	PDPR	CCDC94	ABCF3	C1orf112
SUMF2	IQGAP1	SLC2A11	PEMT	CCDC97	ABCG1	PDGFRB
SUMF1	IQGAP2	RICTOR	PER1	XRN1	FASTKD3	ANK3
SUPT20H	IQSEC1	RIF1	SNX3	CDK4	FASTKD2	PDGFRA
MKL1	IRAK1	RIMKLB	PFKFB2	CCDC69	ZSWIM4	ZNF582
MKL2	IРЕB2	SLC2A3	PFKFB3	CDK2AP2	FASTKD1	PDE9A
MKLN1	IPO7	RIN2	PFKP	CCDC61	ZSWIM1	RGS5
MKS1	IPO5	RIN3	PER2	CDK19	TTC21B	RFX3
SUOX	IPO4	RIOK1	SNX29	CDK18	TUBA1B	ZNF570
SUN2	IPO13	RNF2	SNX25	CDK14	ADAR	RGL1
MLF1	INSR	SLC26A2	PFAS	ZBED5	ADARB1	RGL3

TABLE 5-continued

Mutational Burden Genes						
MLH1	INTS1	SLC26A11	SNX30	CDK13	ADAT1	PEX11A
MMS19	INTS10	RNASEL	PEX7	CDK12	ADCK1	ZNF223
SUGP2	INTS12	RNF185	PES1	CCDC66	FAM13A	ZNF232
MORC2	INTS3	RNF14	PET117	CDK11A	TUBA1C	ZNF235
MORF4L1	INTS4	RNASET2	PEX1	CDIPT	FAM135A	PER3
MOV10	INTS7	RNF103	PEX11B	CAMTA2	ADCK3	ZNF599
SUFU	IP6K1	SLC27A3	PEX19	CAMTA1	FAM134A	ZNF600
MON2	IP6K2	RNF111	PEX5	CNTROB	FAM133B	PELI3
MOK	IPO11	RNF13	PEX6	WASF2	ADCY3	PDZRN3
MNAT1	HSPBP1	RNF130	FBXO42	C3orf58	ZNF765	ANK2
MNDA	HEATR5B	RNF135	HIF1AN	ZMIZ2	ZNF777	SLC30A4
MOB1B	HDHD3	RNF138	BTBD7	ZMIZ1	ADAM15	ZNF273
MOC52	HDLBP	SLC18B1	CTC1	WASH4P	TTLL4	AMOTL2
SULF2	HEATR5A	RPL8	BTD	ZMAT1	TTLL5	ZNF169
MOGS	HEATR6	RPN1	ZNF302	ZKSCAN8	ZNF778	SLC38A1
MTMR12	HECA	RPN2	VAV2	C4orf3	TTYH3	ANPEP
MTMR14	HECTD1	RPL7A	CTBP2	C4orf33	ADAM28	RNASE4
MTMR4	HECTD3	RPP38	BTN3A1	WBP11	FAM160B1	PHLDB1
MTMR6	HECTD4	RPRD1B	VCAM1	CNST	ADAM9	RCSD1
MTO1	HEG1	RPRD2	ZNF304	ZKSCAN5	FAM160A2	ALS2CL
MTMR10	HDAC9	SIN3B	ZNF317	CNTRL	ADCY4	SNX10
MTOR	HIPK1	SIN3A	BROX	ZMYM4	ACIN1	ALPL
MTPAP	HCF1	RPL7L1	BRPF1	WARS2	FAM129B	RNF144A
MTRR	HCFC2	SLC18A2	BRPF3	WAS	ZNF721	ANGPTL2
MTMR11	HCK	SIPA1	BRWD1	COIL	FAM107B	PDE4B
MTMR1	HCLS1	RPL5	BRWD3	ZMYM3	ADH5	PDE4A
MTF2	HDAC8	RPS10	ZNF319	COG4	FAM105A	PDE1B
MTFP1	HDAC4	RPS6KA1	BTAF1	COG3	ADHFE1	SLC35F2
MTFR1	HDAC6	RPS6KA2	ZNF318	ZMYM2	FAM102A	RERG
MTM1	HDAC7	RPS6KA3	CTAGE5	COASY	TUBB6	REPS2
MTHFD2L	HELZ	RPS6KA4	ZNF28	ZMPSTE24	ADIPOR1	AN08
MTHFR	TMEM67	RPS6KB1	CSTF1	ZKSCAN1	FAHD2A	ANKS6
MTIF2	TMEM69	RPS6KC1	VIM	WDFY1	ADIPOR2	SLC35G1
MTSS1L	HIBADH	SIMC1	C11orf49	ZFYVE26	ZNF746	ZNF618
MVK	HID1	RPTOR	ZNF275	C6orf120	ADD3	REEP6
MVP	HIGD1A	RPS3	CSR1P	WDFY3	FAM129A	SLC2A10
MX1	HELZ2	RPS27L	CSRNP2	ZFYVE16	ADCY7	SLC41A2
MYADM	HIGD2A	RPS15	ZNF280D	CMTR2	FAM126A	C7
MTX1	HINFP	RPS15A	VCP	CMTR1	ADD1	RPS6KL1
STRA13	HINT2	SLAIN1	BUD13	C6orf203	FAM120B	NTN4
MYBBP1A	HIP1	SLA	BZW2	ZFYVE1	FAM120AOS	CAMSAP2
MYCBP2	HEMK1	SIRT3	C10orf10	C6orf226	FAM118B	BCAT1
STOML2	HERC1	SKI	ZNF292	ZFX	FAM118A	ZNF521
MUTYH	HERC2	RP2	ZNF286A	CMTM3	FAM117A	ZNF763
MTX3	HERC3	SIRT7	VGLL4	WDPCP	FAM111A	ZC4H2
STUB1	HERC5	SLC16A3	C10orf54	C5orf42	ZNF821	BCAR1
STT3A	HERC6	SLC12A9	ZNF282	CNOT6L	TTC3	PORCN
STRN4	HERPUD2	SLC12A7	BRIX1	CNOT6	ZNF816	ADAMTSL4
STRN3	TMEM99	SLC12A6	CUTC	CNOT4	ACSF2	ADAMTSL3
STRIP1	GUF1	SLC12A4	CUL9	CNOT3	ACSF3	ADAMTS9
STRBP	GUSB	SLC12A2	CUL7	ZHX3	FAM216A	RSAD2
STRADB	GXYLT1	SLC10A7	CUL5	ZHX2	ACSL1	ADAMTS2
MUS81	TMX2	SLC10A3	CUL4B	CNOT10	FAM214B	NRSN2
MUT	TMX1	SIRT1	CUL4A	CNOT1	FAM214A	BCL6B
MS4A6A	GYS1	RPL22	CUL3	CNN2	ACSL3	PODXL
STXBP5	GZF1	SIPA1L1	CUL1	ZMYND11	FAM210B	NUDT13
STXBP4	TMX4	RPL10A	CUEDC2	COPS7A	FAM210A	ZDHHC11
STXBP3	GTF3C1	RPA1	UTP6	COPS4	ACSL4	ZDHHC1
STXBP2	GTF3C3	RPA3	UTRN	COPRS	ACSL5	CALCR
STX8	GTF3C4	RPA1P1	BPGM	COPG2	FAM208B	ARHGEF19
STX4	GTPBP1	RPA2P2	UVRAG	COPA	FAM208A	ARHGEF25
MSH2	GTPBP10	RPE	BPHL	COQ2	FAM206A	ZNF74
MSH3	GTPBP2	RPF1	ZNF33B	COMM38	ZNF808	RAB11FIP5
MRPS9	GTPBP3	RPGR	ZNF33A	ZNF175	ZNF823	BCO2
MRPS23	TNFAIP3	SIPA1L2	CUX1	C1orf35	ACP6	ZNF77
MRPS27	TNFAIP2	RPH3AL	ZNF335	COTL1	FAM222B	NRP2
MRPS31	TNFAIP1	RPIA	BMP2K	C1orf50	ACKR3	ADAMTS10
MRPS5	H2AFY	PPP1R3B	ZNF350	CORO1C	ACLY	NPR2
MSH6	HAUS1	NRIP1	UTP14C	CORO1A	ACO1	NPNT
MTDH	HAUS3	NRP1	ZNF35	ZNF17	ACO2	ZC3H12A
MT-ND6	HAUS4	NSDHL	UTP18	ZNF160	ACOT1	ZNF786
MTA1	HAUS6	NSFL1C	BMS1	COQ5	FAM227B	ZBTB8A
MTA2	HBB	NSMAF	CXCL12	C1QA	ACOT13	NPHP4
MT-ND3	TMPO	NRDE2	BNIP2	C1QB	ACOT2	ARMCX2
MSL1	HARS2	NR1D2	BNIP3L	COL18A1	ACOT7	SAMD4A

TABLE 5-continued

Mutational Burden Genes						
MSL3	HARS	NR1H3	BOD1L1	C21orf59	ACP5	SAMD9
MSRB1	H2AFZ	SRRM2	ZNF343	ZNF124	ACOT8	ACTG2
MST1	H6PD	SRRM1	UTP2O	ZNF121	ACOT9	ZNF783
MSTO1	HACE1	NR2C1	CWF19L2	ZNF117	TTC27	SMOC2
MT-ATP6	HAACL1	NR3C1	CWF19L1	C2CD3	ACOX1	SMO
MT-ATP8	HADHB	NR4A1	CWC27	ZNF106	FAM220A	CARD10
MT-CO3	HIP1R	NR4A2	CWC22	ZNF101	FAM21C	RTP4
MT-CYB	HIPK2	SRR	ZNF337	VWA9	FAM21A	ZNF780A
MAST4	TMEM254	NRAS	CTNNBL1	C2orf47	ACOX3	ZNF780B
MARK2	HP1BP3	NRBP1	CTNNB1	ZNF10	ACP1	ACVRL1
MARK3	TMEM256-	NSMCE4A	BRD4	C2orf49	ACP2	ZNF41
	PLSCR3					
MARK4	HPRT1	NUDC	VAC14	COL1A2	ACSS1	NR3C2
MARS	HPS3	SRGN	CTNNA1	COL1A1	FAM200B	PPIC
MAST3	HPS4	NUB1	CTIF	C21orf3	ZNF785	CADPS2
MASTL	HRAS	NUBP2	BRE	COMM3D7	TTF1	PODN
MAT2A	HIPK3	NUBPL	BRF1	COMM3D3	TTF2	PLEKH2
MATN2	HNRNPUL2	NUCB2	BRF2	COLGALT1	TTI1	C8orf58
MAVS	HOOK3	NSRP1	CTDSPL	ZNF142	FAM179B	RAII4
MAPKAPK2	TMEM39B	SRI	CTDP1	C1R	FAM175B	ARAP2
MAPK7	TMEM248	NTPCR	VAMP2	C1RL	ACVR1B	SPIN3
MAPK8IP3	HS1BP3	NSUN2	CTNND1	C1S	FAM173B	SNED1
MAPKAPK3	HSP90B1	NSUN4	ZNF333	VWA5A	ACSS2	SPIRE1
MARCH7	TMEM222	SRP54	ZNF331	VWA8	ZNF789	BMPR2
MAPKB1	TMEM219	SRP19	ZNF329	COL6A2	ACTA2	PLLP
MARCH2	HSP90AB1	NR1D1	BRAF	ZNF134	ACTB	PLS3
MARCH6	HSPA13	SRRT	CTSF	ZNF133	ACTG1	SPRED1
MBD1	HSPA5	NONO	BRAP	CHN2	TTC37	ZFHX3
MCM2	HSDL2	NOP56	CTSC	ZDHHC20	ACTL6A	OLFML3
MCCC2	TMEM241	NOMO1	CTSB	ZDHHC17	FAM199X	RAI2
SYNCRIP	HSD17B4	NOS3	CTSA	ZDHHC16	ZNF791	NUP210
SYMPK	HNRNPK	NOTCH1	BRAT1	ZDHHC13	TTC39B	C8orf44
SYK	HIVEP2	NOTCH2	BRCA1	WDR77	TTC39C	OSCP1
MCM6	HK1	NOMO2	UXS1	CKAP5	FAM193B	AIM1
MCM8	HLTF	SSRP1	BRD1	CACTIN	FAM193A	APOL4
MCM9	HLA-DRB1	NNT	CTR9	CACUL1	FAM189B	RAPGEF5
MCMBP	HIVEP1	NOA1	CSRNP1	CAD	FAM188A	OSBPL7
MCAM	HIRA	NOB1	CSPP1	WDR81	TTC7A	OSBPL3
SYNE1	HIST1H1C	NOC2L	ZNF217	CALCOCO2	TTC7B	PLEKHA4
SYNJ1	TMEM63B	NOC4L	C19orf47	ZCWPW1	EIF5	PLEKHA7
MBD4	TMEM62	NOD1	CPVL	CLASP1	CD2BP2	RAP1GAP2
MBD5	HLCS	NOLC1	CPT1B	CA5B	ZNF500	SLAMF8
MBIP	HMOX2	NOL10	CPT1A	ZDHHC8	ZNF529	SLAMF7
SYNGR2	TMEM45A	NOL6	CPSF7	WDR7	ARHGAP1	RANBP17
MBNL1	TMEM44	NOL8	CPSF1	WDR70	UGCG	C8orf4
MBOAT2	HM13	NOL9	CRADD	CLCN7	ARHGAP10	PLEKHG4
MBTPS1	HNRNPC	SSH1	CPQ	CLCN6	ARHGAP12	AQP3
MBTPS2	HNRNPD	SSBP4	CREBPF	CAB39L	ZNF502	SPRY4
SYTL4	HNRNPDL	NPM3	CREBBP	CABIN1	ARHGAP17	PLVAP
SYTL2	HMCES	SRSF9	ZNF224	CLCN2	ARHGAP19	OLF2M
MAGED2	HMG20A	SRSF5	CREB3L2	CABLES1	ARHGAP21	BIN2
MAN2A1	HMGB1	NP1PB3	C17orf75	WDR73	ARHGAP26	CACHD1
MALT1	STOML1	NPC1	ZNF222	WDR74	ARFIP1	RAB3D
MAML1	RAN	NPDC1	C18orf25	WDR75	ARFGEF1	BICC1
MAML3	RANBP10	NPHP3	CRAT	CALD1	APOBEC3G	ZNF708
MAN1A1	RANBP2	NPIPA2	ZNF211	CHRNB1	AREL1	NUTM2D
MAN1B1	RANBP6	ORC3	CPPED1	CAMK1	ARFGAP3	CACNB1
SYVN1	RANBP9	SPICE1	VTA1	CAMK1D	ZNF493	CACNB3
SZT2	RAP1A	SPHK2	COX6A1P2	ZCCHC4	DTX3	ADORA2A
TAFA1	RALY	ORMDL2	ZNF180	WHAMM	ZNF496	PNPLA7
TADA3	RAP1GDS1	OSBPL10	C1orf216	ZCCHC2	DMXL1	BHLHB9
TADA1	RAPGEF1	SPNS1	ZNF207	CHPF2	DMXL2	ADIRF
TACC1	RAPGEF2	SPPL3	ZNF197	ZCCHC14	ARHGAP9	BGN
MAN2A2	RAPGEF3	SPRY1	VPS54	WHSC1	UBE2V2	ZNF704
MAP3K8	SLC43A2	SPRED2	CPNE8	WDR82	ARHGEF10	NYNRIN
MAP3K13	RAD52	OPA1	C1GALT1	CHST11	DNAH1	PLXNA1
MAP3K2	RAD54L2	SPNS2	VPS72	CIC	ARHGEF10L	OGN
MAP3K4	RAE1	SPOPL	C1GALT1C1	CALM2	ARHGEF11	PLXNA2
MAP3K5	SLC43A3	SPOP	ZNF202	ZCRB1	ARHGEF12	RAD51AP1
MAP3K7	RAF1	SPON2	ZNF200	WDR90	ZNF507	SIPA1L3
MAP4	RAI1	SPOCK2	CPNE2	ZCCHC8	EIF4H	PMEPA1
MAN2B1	RALBP1	OTUD5	VPS8	CHST15	DROSHA	ARHGEF25
MAP4K2	RALGAPA1	OTUD6B	CPEB3	WDR91	UBE2V1	ZNF354C
MAP4K4	RALGAPA2	SPDL1	CPEB2	ZDHHC9	UFSP2	ZNF701
MAP7	RALGAPB	OSBPL5	CPD	ZEB1	ASAP1	BLNK

TABLE 5-continued

Mutational Burden Genes						
MAP7D1	RALGDS	OXLD1	VRK3	C6orf89	ARHGAP30	CA2
MAP3K12	RAPGEF6	OXSM	VSIG10	ZFP64	ZNF503	ARHGAP29
MAP3K1	RBBP8	OXSR1	VPS41	CLPTM1L	DST	OBSCN
MAN2C1	RB1	SPATS2L	CRELD1	CLPTM1	UFL1	SPTLC3
MANBA	RB1CC1	SPEC1L	CSK	WDR18	ARHGAP32	ARHGAP31
MANSC1	RBBP5	OSBPL8	VOPP1	C8orf33	ARHGAP33	TLDC1
MAP1S	RBBP6	OSBPL9	C12orf73	CLNS1A	UBE2R2	L3MBTL1
MAP2K1	RBBP9	OSMR	VPS13A	CLN6	ARHGAP35	CX3CL1
MAP2K2	RARA	SPG21	ZNF251	CLN3	ZNF528	UST
MAP2K3	SLC39A9	SPG20	CSF3R	CLK4	ARVCF	TGM2
MAP2K4	RBL1	SPG11	VPS13B	CLK2	ARHGAP4	CYB561D1
MAP2K6	RBL2	SPEN	VPS13C	CLK1	ZNF527	KLRK1
MCOLN1	RBM10	SPEF2	ZNF250	CLIP2	ARHGAP5	THBS2
MFSD6	RBM12	NUP62	CSF1R	CLSTN1	ARCN1	THNSL1
MFSD9	RARS	NUP88	CSF1	C7orf26	ARAP3	THNSL2
MFSD1	RASA2	NUP93	CSE1L	C7orf43	UBE2Q2	KLHL26
MGA	RASA4	NUP98	VPS13D	CLYBL	DYM	KLHL23
MGAT4A	SLC40A1	SRF	CRYZ	CLUH	DVL3	KLHL17
MGAT4B	RASGRP3	SRD5A1	ZNF253	CLUAP1	ASNS	CYP2U1
MFNG	RASL11A	SREK1	CSNK2A1	CLU	DUSP22	CYP4F12
METTL25	RAD51D	SREBF2	CSNK1E	CLTC	DUSP6	KIRREL
METTL2A	RAD51B	SREBF1	CSNK1D	CLSTN3	APOL2	CYR61
METTL4	SLC4A1AP	NUP35	ZNF264	ZFP91	APOL3	LAMA4
METTL6	RAB18	NUP214	C12orf4	ZFP90	UBE2J2	CTTNBP2NL
METTL7A	RAD50	NUDTS	C12orf43	CLIP4	ASPH	LAMB3
SUV39H2	QSER1	NUFIP1	ZNF254	CLIP1	DYRK4	LIMS2
MFAP4	QSOX1	NUMA1	C14orf159	WDR61	DLST	LIMCH1
MFF	R3HDM1	NUMBL	VPS16	ZFAND4	DYRK1B	LILRB4
MFHAS1	R3HDM2	NUP153	C16orf58	CLEC16A	UHMK1	LILRB2
MGME1	RAB11FIP1	NUP155	C16orf62	ZFAND3	DYNLT3	LIFR
MGMT	RAB11FIP2	NUP160	ZNF236	WDR47	ZNF548	LG14
MIER2	RAB11FIP3	NUP188	C16orf72	C9orf78	DYNC1LI2	VCAN
MIER3	RAB3GAP1	NUP205	VPS37A	ZFAND1	DYNC1LI1	VAV3
SURF1	RAB3GAP2	SRD5A3	VPS37B	ZER1	DMAP1	VAV1
MIIP	RAC1	OClAD2	C16orf87	WDR59	ASPSCR1	LEF1
MINA	RAB3IP	OCRL	ZNF23	ZEB2	DUSP23	LDOC1
MINK1	RACGAP1	ODC1	CRNKL1	WDR6	ASCC2	TEAD2
SUPV3L1	RADI	ODF2	ZNF25	WDR60	AQP1	LDB2
SUPT5H	RAD17	SPTAN1	CRYBG3	WDR44	UGGT2	TEF
SUPT3H	RABGAP1L	OGDH	C14orf28	WDR43	ASH1L	TEK
MIA3	RABGAP1	OGFOD1	CRY2	CLIC4	ZNF480	CTH
MIB2	RABEPK	OBSL1	CRTC2	CLIC2	ASF1A	LARP6
MICAL1	RABEP2	SRCAP	CRTAP	CLHC1	UGGT1	KIF21A
MICAL2	SLC46A3	NXPE3	C15orf39	WDR26	ASCC3	DENND5B
MICAL3	RAB40C	SQLE	CROT	CLEC7A	ASCC1	DDR2
MICALL1	SLC45A4	SPTY2D1	CROCC	WDR27	ASB8	KAZN
METTL23	RABEP1	OAS1	BMP1	WDR3	AQR	KATNAL1
MED10	RBM12B	OAS3	CXCR4	WDR33	ARAP1	KALRN
MED13	RBM14	OAZ2	DDOST	C9orf142	ASB7	JAM2
MED13L	RFTN1	NMT1	ZNF436	WDR35	UGDH	TMC4
MED14	RFWD2	NAT9	ATP6V1H	ZFC3H1	APPL2	ITPR1L2
MED15	RFWD3	NAV1	ATP7A	WDR37	APPL1	ITPR3
MED17	RFX1	NAV2	ATP8A1	WDR4	DUSP2	ITIH5
MED27	RFX5	NBAS	ATP9A	ZFAT	DMKN	ITGB7
MED18	RFX7	NBEAL1	DDI2	DDX18	DUSP16	ITGA9
MED19	SLC35E1	NBEAL2	ATP9B	ERN1	APOL6	TLR3
MED21	RGP1	STAU1	ATR	AK9	APOO	ITGA4
MED22	SLC35B3	STAT5B	ATRN	AKAP1	UBE2O	ITGA1
MED23	SLC35B2	NCAM1	ATRX	AKAP11	ZNF473	ISLR
MED24	SLC35E2	STAT3	DDHD1	ESF1	ZNF48	DHFRL1
MECP2	REST	NCAPD2	ATXN1	AKAP12	APP	IQSEC2
MCTP1	SLC35F5	NCAPD3	ATP6V0D1	AKAP13	DUS4L	INTU
MCUR1	RETSAT	STAT6	DDX3X	ESCO1	DUS3L	TM4SF18
MDH1	REV3L	NAPEPLD	DDX24	ESAM	UBE3C	KCNE3
MDM1	REXO1	NANP	DDX21	ZNF680	DOCK5	KDEL1
MDN1	RFC1	NAP1L4	DDX19A	AKAP2	DOCK4	KIAA1958
MDP1	RFC2	NAPA	DDX11	ERV3-1	UBR4	DAPK2
ME2	RFC3	NAPB	DDX10	ZNF678	DOCK2	TIGD6
ME3	RFNG	NARF	ATXN2	AKAP7	ARL8B	KIAA1217
METAP2	RGPD8	NARS	DDX41	ERP44	ZNF518B	TIGD7
SVIL	RHOBTB2	NARS2	B3GALNT2	TWISTNB	DNTTIP2	KIAA0513
MESDC2	SLC30A6	NAT10	DCTPP1	AK1	UBR5	KDR
METAP1	RHOB	NCAPG2	ZNF420	AIP	DNPH1	DCHS1
METTL10	RHOBTB1	NCAPH2	B3GAT3	AIFM1	DOCK7	TLR2
METTL14	RHOBTB3	NCBP1	URB2	ZNF688	DOCK8	TIMP3

TABLE 5-continued

Mutational Burden Genes						
METTL17	SLC35B1	NCSTN	DCP1B	ZNF687	DOPEY1	KCNQ1
MEN1	RHOG	NDC1	DCN	ETV6	DNAJB6	KCNMA1
MED7	RHOT1	NDOR1	DCLRE1C	ZNF684	ARL4A	KCNJ8
MEF2A	RHOT2	STARD5	DCLRE1A	ETS1	UBXN2B	TLE2
MEF2D	SLC2A9	NDUFA10	URI1	AIFM2	UBR3	TLN2
SWT1	RHBDF2	NCOR2	DCK	ETFA	DOK4	TCEA3
MEGF6	RGS12	NCOR1	ZNF426	ETF1	DOCK9	INTS2
MEGF8	RGS14	NCOA6	B2M	TWF2	ZNF516	TBXAS1
MEGF9	RGS3	STAT2	ATXN2L	ESYT2	DNMT3A	SYBU
JMJD1C	RBMX2	NCDN	DDB1	ESYT1	ARNTL	CLIP3
GMEB1	SLC39A4	NCF1	ZNF432	AKAP9	ARMCX4	MECOM
TPDS2	RBMS	NCF2	ATXN7	TXND5	DNMT1	ME1
TPCN2	RBMX	NCF4	ATXN7L1	ERCC8	DNAJC3	CMKLR1
GMDS	RBMXL1	NCK2	ATXN7L3	ERCC6L2	UBXN2A	CMPK2
GMEB2	RBPJ	NCKAP5L	AUP1	ERCC6	DNAJC21	MCF2L
GMIP	SLC39A3	NCOA1	AUTS2	ERCC5	DNMBP	CNKS3
GMNN	SLC39A10	NCOA2	AVL9	ERCC4	ZNF514	CNN1
GLTSCR2	SLC38A9	NCOA3	AXIN1	ERCC2	DNAJC13	SYNM
GLRX5	SLC39A6	NAIP	UROD	TXNL1	ARMCS5	SYNPO
GLS	RBM43	STK24	ATP13A1	ERC1	DNHD1	MARCH3
GNA11	RBM42	MYO18A	ATP13A3	ZNF671	ARPC3	SYNPO2
TP53I11	RBM19	STK3	DERA	ERBB2	ARMC9	MARCH1
TP53BP2	RBM23	MYO1D	DEPD5C	ERAP2	ARPC2	CNTLN
GNL2	RBM27	MYO1F	DENND6A	ERAL1	ZNF512B	MAPK13
TP53BP1	RBM28	STK11P	ATP2A2	TXNRD1	DOPEY2	MAPK10
GNPTAB	RBM3	MYO5A	ATP2A3	EPT1	UCHL5	MAP3K10
GNPTG	RBM33	MYO5C	ATP2B1	ERG	ARHGEF6	CLEC3B
GOLGA4	RBM38	MYO9A	ZNF440	ERGIC1	UBLCP1	MEIS2
GNG7	RBM4	MYO9B	ATP2C1	AKNA	DPYD	MMRN2
TP53I13	RECQL5	MYOF	DENND4C	ERMP1	ARHGEF40	MMP2
GNAI2	RDH5	MYOM2	ZNF44	ERMARD	DPY19L1	CHN1
GNAI3	RDX	MYO10	ATP13A2	ERMAP	ARHGEF7	MMACHC
GNAS	RECK	MYNN	DENND4A	ERLIN1	DPP9	CHPF
GNB5	RECQL	MYH10	ATP11C	TXND11	ARHGEF9	CHRD
GNG2	REEP4	STK4	ATM	TXND12	ARID1A	WFS1
TPM2	SLC36A4	STK39	ATMIN	ERGIC2	ARSB	MID2
GFP1	SLC36A1	MYH9	ATN1	TXND16	UBN1	MERTK
GET4	REL	STK38L	DGKE	ERICH1	UBN2	CKAP2
TRAF3IP3	RELA	MYLK	UPF1	ERI3	UBQLN1	SUSD1
GFM1	SLC38A10	STK11	DGKA	ERI2	UBXN7	SUSD2
GFM2	RC3H1	NACC1	DGCR8	AKR1C1	DPYSL2	WDR76
TRAF3IP1	RC3H2	NAB2	DGCR2	AKR1C3	UBE4A	CKS2
GFOD2	RCBTB1	NABP1	ATP10D	EVA1C	ARSG	WDR5B
GGA1	RCCD1	NACA	ATP11B	EXTL3	UBFD1	CLEC14A
GGA2	RCN1	NACC2	UPF2	F2R	ARHGEF18	WASF1
GGCX	RCN2	STK10	DENND4B	TUBGCP6	UBL3	COL12A1
TRAF3	RCOR1	NADK	DENND3	F13A1	ARHGEF2	COL14A1
TRAF7	RCOR3	NADSYN1	DEF8	F11R	UBL4A	TANC2
GDAP1	PRKX	NAF1	DEC1	AFF4	UBL7	CPE
TRAK2	SMARCAL1	NAGLU	DDX60L	EZH1	DNAJB1	CPED1
TRAFD1	SMARCAD1	STIP1	DDX60	AFMID	DPP8	LRRC8C
GEMIN5	SMARCA4	MYSM1	DDX6	AGA	DOT1L	

**[0094]** Provided herein are systems and methods for calculating a mutational burden and/or deconvolution of the identities and proportions of cell types in a sample. In some instances, the mutational burden and/or deconvolved cell types are calculated at or above a statistical threshold. The accuracy, specificity, sensitivity, positive predictive value, negative predictive value, or any combination thereof may be determined for an assay for mutational burden and/or deconvolution, for example, by running the assay against a set of independent samples. True positive is a positive test result that detects the condition when the condition is present. True negative is a negative test result that does not detect the condition when the condition is absent. False positive is a test result that detects the condition when the condition is absent. False negative is a test result that does not detect the condition when the condition is present. Accuracy is defined as the sum of true positive and true

negative divided by the sum of true positive, true negative, false positive, and false negative. Specificity is defined as true negative divided by the sum of true negative and false positive. Sensitivity is defined as true positive divided by the sum of true positive and false negative. Positive predictive value is defined as true positive divided by true positive and false positive. Negative predictive value is defined as true negative divided by the sum of true negative and false negative.

**[0095]** In some instances, deconvolved cell identities and proportions (of the identities) in a sample are calculated at an accuracy of at least 5000, 6000, 7000, 8000, 9000, 9500, 9900 or more. In some instances, deconvolution is calculated at an accuracy of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, deconvolved cell identities and proportions (of the identities) in a sample

are calculated at a sensitivity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more. In some instances, deconvolution is calculated at a sensitivity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, deconvolved cell identities and proportions (of the identities) in a sample are calculated at a specificity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more. In some instances, deconvolution is calculated at a specificity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, deconvolution has a correlation with the Gold Standard of at least 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, 0.99 or more. In some instances, deconvolution has a correlation with the Gold Standard of at least 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, 0.99 or more for at least 100, 200, 300, 400, or 500 or more independent samples.

[0096] In some instances, the mutational burden in a sample is calculated at an accuracy of at least at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more. In some instances, the mutational burden is calculated at an accuracy of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, the mutational burden in a sample is calculated at a sensitivity of at least at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more. In some instances, the mutational burden is calculated at a sensitivity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, the mutational burden in a sample is calculated at a specificity of at least at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more. In some instances, the mutational burden is calculated at a specificity of at least 50%, 60%, 70%, 80%, 90%, 95%, 99% or more for at least 100, 200, 300, 400, or 500 or more independent samples. In some instances, the mutational burden has a correlation with the Gold Standard of at least 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, or 0.99 or more. In some instances, the mutational burden has a correlation with the Gold Standard of at least 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, or 0.99 or more for at least 100, 200, 300, 400, or 500 or more independent samples.

#### Therapeutic Applications

[0097] Provided herein are methods and systems for determining an immune-oncology profile using sequencing data, wherein the profile may be used for therapeutic applications. In some instances, the profile comprises immune modulatory molecule expression, cell type and ratio, and mutational burden. In some instances, the profile is determined for diagnosis of a disease or disorder. In some instances, the profile is determined for treatment purposes. For example, the profile is used to determine efficacy of a treatment regimen. In some instances, the profile is used to recommend a therapeutic intervention.

[0098] In some instances, determination of the immune-oncology profile occurs prior to a treatment, during a treatment, or after a treatment. In some instances, determination of the immune-oncology profile occurs one or more time points prior to a treatment, during a treatment, or after a treatment. Time points for the monitoring and response-to-treatment methods provided herein, include any interval of time. In some instances, the time points are 1 day, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3, weeks, 4

weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years or longer apart. In some instances, samples are obtained at any number of time points, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more time points.

[0099] In some instances, the immune-oncology profile is used to determine a specific treatment for a disease or disorder subject. In some instances, a sample is a first sample obtained from a subject at a first time point. In some instances, the method further comprises determining the immune-oncology profile by determining the immune modulatory molecule expression, cell type and ratio, and mutational burden from a second sample obtained from the subject having the related disease or disorder at a second time point; and comparing the immune-oncology profile from the first time point to the second time point. Sometimes, immune-oncology profiles are generated for a subject at multiple time points, wherein the profiles are compared to evaluate the progression of a disease or disorder and/or a response to treatment.

[0100] In some cases, the methods and systems described herein are used for diagnosing or treating a disease or disorder, wherein the disease or disorder is cancer. In some instances, the cancer is a solid cancer or a hematopoietic cancer. Sometimes, a cancer targeted herein is a recurrent and/or a refractory cancer. In some instances, the cancer is an acute cancer or a chronic cancer. In some instances, the cancer is an accelerated refractory cancer. In some instances, the cancer is in remission. In some instances, the cancer is a stage I, stage II, stage III, or stage IV cancer. In some instances, the cancer is a juvenile cancer or adult cancer. Examples of cancers include, but are not limited to, breast cancer such as a ductal carcinoma, medullary carcinomas, colloid carcinomas, tubular carcinomas, and inflammatory breast cancer; ovarian cancer, including epithelial ovarian tumors and adenocarcinoma in the ovary; uterine cancer; cervical cancer such as adenocarcinoma in the cervix epithelial, squamous cell carcinoma and adenocarcinomas; prostate cancer, including adenocarcinoma; pancreatic cancer, including epithelioid carcinoma in the pancreatic duct tissue and adenocarcinoma in the pancreatic duct; bladder cancer, including transitional cell carcinoma, urothelial carcinomas, tumors in the urothelial cells, squamous cell carcinomas, adenocarcinomas, and small cell cancers; leukemia, including acute myeloid leukemia (AML), acute lymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, myelodysplasia, myeloproliferative disorders, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), mastocytosis, chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and myelodysplastic syndrome (MDS); bone cancer; lung cancer, including non-small cell lung cancer (NSCLC) such as squamous cell carcinomas, adenocarcinomas, and large cell undifferentiated carcinomas, and small cell lung cancer; skin cancer, including basal cell carcinoma, melanoma, and squamous cell carcinoma; eye retinoblastoma; cutaneous or intraocular melanoma; primary liver cancer; kidney cancer; autoimmune deficiency syndrome related lymphoma, including diffuse large B-cell lymphoma, B-cell immunoblastic lymphoma and small non-cleaved cell lymphoma; Kaposi's Sarcoma; viral-induced cancers including hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatocellular carcinoma; human lymphotropic virus-type 1 (HTLV-1) and adult T-cell leukemia/lymphoma;

and human papilloma virus (HPV) and cervical cancer; central nervous system (CNS) cancers, including primary brain tumors such as astrocytoma, anaplastic astrocytoma, or glioblastoma multiforme, oligodendrogiomas, ependymomas, meningiomas, lymphomas, schwannomas, and medulloblastomas; peripheral nervous system (PNS) cancers, including acoustic neuromas and malignant peripheral nerve sheath tumors (MPNST) such as neurofibromas and schwannomas, malignant fibrous cytomas, malignant fibrous histiocytomas, malignant meningiomas, malignant mesotheliomas, and malignant mixed Müllerian tumors; oral cavity and oropharyngeal cancer such as hypopharyngeal cancer, laryngeal cancer, nasopharyngeal cancer, and oropharyngeal cancer; stomach cancer, including lymphomas, gastric stromal tumors, and carcinoid tumors; testicular cancer such as germ cell tumors (GCTs), which include seminomas and nonseminomas, and gonadal stromal tumors, which include Leydig cell tumors and Sertoli cell tumors; thymus cancer, including thymomas, thymic carcinomas, Hodgkin disease, non-Hodgkin lymphomas carcinoids or carcinoid tumors; rectal cancer; and colon cancer.

**[0101]** In some cases, the methods and systems disclosed herein for determining immune modulatory molecule expression, cell type and ratio, and mutational burden are used for treating cancer. For example, at least one of immune modulatory molecule expression, cell type and ratio, and mutational burden is determined prior to cancer treatment. In some cases, at least one of immune modulatory molecule expression, cell type and ratio, and mutational burden is measured in a sample. In some instances, the sample is obtained from tumor tissues. In some cases, the sample is obtained from non-tumor tissues. In some cases, the sample is obtained from a subject who has cancer or has been diagnosed with cancer. In some cases, the sample is obtained from subjects who have not been diagnosed with cancer. In some cases, the sample is obtained from subjects who are in remission. Following determination of an immune-oncology profile based on at least one of immune modulatory molecule expression, cell type and ratio, and mutational burden, a cancer treatment may be applied. Examples of treatments for cancer include, but are not limited to, chemotherapy, radiation, surgery, or immunotherapy.

**[0102]** In some instances, determination of the immune-oncology profile occurs in conjunction with surgery. For example, determination of the immune-oncology profile occurs prior to tumor surgery and/or following tumor surgery. In some instances, the immune-oncology profile is indicative of the efficacy of the surgery. The immune-oncology profile may be determined any time following surgery. In some instances, the immune-oncology profile is determined 1 day, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3, weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, or more than 2 years following surgery. In some instances, the immune-oncology profile is determined at any number of time points, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more time points.

**[0103]** In some instances, determination of the immune-oncology profile occurs in conjunction with chemotherapy. For example, determination of the immune-oncology profile occurs prior to chemotherapy and following chemotherapy. In some instances, determination of the immune-oncology profile indicates the efficacy of the chemotherapy. Examples

of chemotherapy includes, but are not limited to, cyclophosphamide, paclitaxel, 5-fluorouracil, 5-aza-2'-deoxycytidine, mitomycin, doxorubicin, and mitoxantrone. The immune-oncology profile may be determined any time following chemotherapy. In some instances, the immune-oncology profile is determined 1 day, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3, weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, or more than 2 years following chemotherapy. In some instances, the immune-oncology profile is determined at any number of time points, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more time points.

**[0104]** In some instances, determination of the immune-oncology profile occurs in conjunction with radiation treatment. For example, determination of the immune-oncology profile occurs prior to radiation treatment and/or following radiation treatment. In some instances, the immune-oncology profile indicates the efficacy of the radiation treatment. The immune-oncology profile may be determined any time following radiation treatment. In some instances, the immune-oncology profile is determined 1 day, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3, weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, or more than 2 years following radiation treatment. In some instances, the immune-oncology profile is determined at any number of time points, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more time points.

**[0105]** Alternately or in combination with surgery, chemotherapy, or radiation, determination of the immune-oncology profile occurs in conjunction with immune therapy. In some instances, the immune therapy comprises administration of a modulatory agent for an immune checkpoint. Examples of immune checkpoint targets include, but are not limited to, 2B4 (CD244), A2aR, B7H3 (CD276), B7H4 (VTCN1), B7H6, B7RP1, BTLA (CD272), butyrophilins, CD103, CD122, CD137 (4-1B), CD137L, CD160, CD2, CD200R, CD226, CD26, CD27, CD28, CD30, CD39, CD40, CD48, CD70, CD73, CD80 (B7.1), CD86 (B7.2), CEACAM1, CGEN-15049, CTLA-4, DR3, GAL9, GITR, GITRL, HVEM, ICOS, ICOSL (B7H2), IDO1, IDO2, ILT-2 (LILRB1), ILT-4 (LILRB2), KIR, KLRG1, LAG3, LAIR1 (CD305), LIGHT (TNFSF14), MARCO, NKG2A, NKG2D, OX-40, OX-40L, PD-1, PDL-1 (B7-H1, CD 274), PDL-2 (B7-DC, CD 273), PS, SIRPalpha (CD47), SLAM, TGFR, TIGIT, TIM1, TIM3 (HAVCR2), TIM4, or VISTA. An immune checkpoint modulatory agent in some cases is at least one of a small molecule, an antibody, a nucleic acid encoding an antibody, an antigen binding fragment, a RNA interfering agent, a peptide, a peptidomimetic, a synthetic ligand, and an aptamer. In some instances, an immune checkpoint inhibitor is administered. Examples of immune checkpoint inhibitors are Enoblituzumab (e.g., MGA271), Ipilimumab (e.g., BMS-734016, MDX-010), Tremelimumab (e.g., CP-675, CP-675,206), Lirilumab (e.g., BMS-986015, IPH2102), BMS986016, Pembrolizumab (e.g., MK-3475, SCH 900475), Nivolumab (e.g., BMS-936558, MDX-1106, ONO-4538), Pidilizumab (e.g., CT-011, MDV9300), Atezolizumab (e.g., MPDL3280A, RG7446, R05541267), BMS-936559 (e.g., MDX-1105), Durvalumab, Avelumab, and Bavituximab. In some instances, the immune therapy is CAR T cell or T cell receptor therapy.

**[0106]** Methods and systems provided herein for determination of an immune-oncology profile may be used for prediction of a clinical outcome in response to a therapy. In some instances, the therapy is surgery, radiation, chemotherapy, or immune therapy. In some instances, the immune-oncology profile is used to predict a level of resistance to one or more chemotherapeutic agents. In some instances, the prediction of a clinical outcome based on the immune-oncology profile has an accuracy, specificity, sensitivity, positive predictive value (PPV), a negative predictive value (NPV), or a combination thereof for a type of response. In some instances, the type of response is a positive response. In some instances, a positive response is partial remission (e.g., cancer/tumor has gotten smaller) or complete remission (e.g., all signs of cancer are gone) of the tumor. In some instances, a positive response is the cancer has stopped growing or expanding. In some instances, a positive response is a statistically higher survival rate for a treated subject population compared to an untreated subject population. In some instances, the survival rate is a 1 year, 2 year, 3 year, 4 year, 5 year, 6 year, 7 year, 8 year, 9 year, or 10 year survival rate. In some instances, the type of response is a negative response. In some instances, a negative response is the absence of a positive response. In some instances, a negative response is continued cancer progression or growth. In some instances, a negative response is the continued presence of the cancer. In some instances, a positive response is continued cancer progression or growth at the predicted rate for an untreated subject population. In some instances, a prediction of a clinical outcome (e.g., a positive or negative response) has a positive predictive value for a set of independent samples. In some instances, the PPV for a response to a therapy is at least or about 90% for at least 100 independent samples. A positive predictive value may be accurately determined in at least about 50%, 60%, 70%, 80%, 90%, 95%, or 99% of at least 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 independent samples.

**[0107]** In some instances, a prediction is generated using a classifier. In some instances, the proportions of cell types/subtypes determined by deconvolution, mutational burden, immune modulatory molecule expression, or any combination thereof are associated with an outcome such as, for example, a clinical outcome, a diagnosis of disease, and/or a response to therapy. In some instances, the classifier is trained using data comprising one or more of cell type/subtype proportions, mutational burden, and immune modulatory molecule expression along with associated outcomes. In some instances, the classifier comprises a panel of cell type/subtype proportions that are predictive of an outcome. In some instances, the classifier comprises a panel of immune modulatory molecules predictive of an outcome. In some instances, the classifier comprises a panel of mutational burden predictive of an outcome.

**[0108]** Disclosed herein, in some instances, are systems and methods for generating and/or using a classifier to make a prediction of an outcome. The classifier can be a machine learning algorithm or model trained using data from the immune-oncology profile. The data utilized from the immune-oncology profile can include the cell type/subtype proportions or percentages (e.g., immune cell types and percentages in a tumor sample). Examples of the cell types or subtypes include M1 macrophages, M2 macrophages, CD19+ B cells, CD14+ monocytes, CD56+NK cells, CD8+

T cells, Treg cells, CD4+ T cells, or any combination thereof. Additional examples of cell types or subtypes are found throughout the present disclosure. In some cases, the data includes expression of immune-inhibitory genes or immune escape genes which can include, for example, CTLA4, OX40, PD-1, IDO1, CD47, PD-L1, TIM-3, BTLA, ICOS, ARG1, or any combination thereof. The data can also, in certain cases, include mutational burden information relating to the sample.

**[0109]** The classifier or trained algorithm of the present disclosure may be used to make a prediction. The prediction can be based on information from an immune-oncology profile of a sample such as at least one of percentage(s) of cell type(s)/subtype(s), level(s) of immune inhibitory or escape gene(s), or mutational burden. The prediction can comprise stratifying a sample into two or more categories. The prediction can relate to diagnosis and/or prognosis. The prediction can also be based on monitoring the success of treatment of disease. Predictions can also be based on quality of life or symptomatic response. As an example, the prediction for a tumor sample obtained from a subject includes a positive identification of the sample as pancreatic ductal adenocarcinoma (PDA). The prediction optionally also includes a corresponding prediction classifying the sample as having poor survival based on immune-oncology profile data including high PD-L1 expression level and high Treg cell percentage infiltrating the tumor sample. The categories or groups can correspond to various predicted outcomes such as predicted treatment outcome or responsiveness to treatment.

**[0110]** The classifier used to generate predictions includes one or more selected feature spaces such as cell type/subtype proportion/percentage, immune inhibitory gene expression level, and mutational burden. The values for these features obtained from a sample can be fed into the classifier or trained algorithm to generate one or more predictions. In some cases, the methods disclosed herein select for the variables that are of predictive value, for example, by culling the features to generate a feature subset used for generating predictions in the final classifier or model. Methods that reduce the number of variables or features can be selected from a non-limiting group of algorithms including principal component analysis (PCA), partial least squares (PLS) regression, and independent component analysis (ICA). In some cases, the methods disclosed herein analyze numerous variables directly and are selected from a non-limiting group of algorithms including methods based on machine learning processes. Machine learning processes can include random forest algorithms, bagging techniques, boosting methods, or any combination thereof. Methods may be statistical methods. Statistical methods can include penalized logistic regression, prediction analysis of microarrays, methods based on shrunken centroids, support vector machine analysis, or regularized linear discriminant analysis.

**[0111]** The classifier or trained algorithm of the present disclosure as described herein can comprise one feature space. The classifier or trained algorithm of the present disclosure as described herein can comprise two or more feature spaces. The two or more feature spaces may be distinct from one another. Each feature space can comprise types of information about a sample, such as cell type/subtype percentage, expression of immune inhibitory molecules or genes, or mutational burden. The accuracy of the classification may be improved by combining two or more

feature spaces in a classifier rather than using a single feature space. In some cases, combining both cell type/subtype percentage and immune inhibitory gene expression results in superior accuracy than using those features individually. Sometimes, accuracy is further improved by incorporating mutational burden. Individual feature spaces may have different dynamic ranges. The difference in the dynamic ranges between feature spaces may be at least 1, 2, 3, 4, or 5 orders of magnitude. As a non-limiting example, the cell subtype percentage feature space may have a dynamic range between 0 and 100, and the immune inhibitory gene expression feature space may have a dynamic range between 0 and about 20.

[0112] A feature space can comprise a panel of cell types/subtypes and their percentage or proportion within a sample. A feature space can comprise a panel of immune inhibitory genes and their expression level. A feature space can comprise one or more representations of mutational burden. A panel of an individual feature space may be associated with an outcome such as, for example, responsiveness to treatment. For example, a positive response to an immunotherapy may be associated with certain immune cell types exceeding a threshold percentage within a tumor sample. As another example, a negative response to an immunotherapy may be associated with an immune-inhibitory gene such as PD-L1 exceeding a threshold expression level within a tumor sample. In some cases, the classifier or trained algorithm comprises a panel of cell type/subtype percentages comprising at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 20 cell types/subtypes. The classifier can comprise a panel of immune-inhibitory genes comprising at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 genes.

[0113] The classifier of the present disclosure may be trained with a set of samples obtained from subjects. A set of samples can comprise samples from at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, or more subjects. In some cases, the classifier is trained on a limited sample set with no more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 samples. The classifier may be trained on a limited sample set with no more than 15-20 samples or no more than 20-30 samples. The accuracy of the classifier takes on even greater importance when the sample size is small. A significant factor in the accuracy of the classifier is the quality of the data parameters input into the classifier to generate the prediction or classification. Likewise, the quality of the data input used to train the classifier is important to its predictive ability. For example, a classifier trained on a training data set having cell subtype percentages that were inaccurately determined will incorporate this inaccuracy during the training, which compromises its predictive ability with regards to new samples. When the sample size is large, a few poor data points will not have a significant impact on the resulting classifier. However, in the case when sample size is small such as around 15-25 samples, a few poor data points can negatively impact the classifier's predictive abil-

ity to a significant degree. Accordingly, the methods disclosed herein utilizing RNA normalization techniques that account for quantitative differences in RNA content amongst different cell types help generate highly accurate cell type/ subtype percentages, which in turn allow for the generation of classifiers that effectively generate predictions despite being trained on small data sets such as, for example, no more than 15, 20, 25, 30, 35, 40, 45, 50, or 60 samples. This capability is critical for small-scale studies such as, for example, Phase I/II clinical trials which often entail small sample sizes. Indeed, larger Phase II trials may have 60 subjects, but the experimental group may still be only 15-20 when accounting for controls (e.g., 20 negative placebo controls, 20 receiving traditional treatment, and 20 receiving experimental treatment).

[0114] Moreover, in certain instances, the methods disclosed herein utilize end-to-end sample processing and analysis for quality control. As an example, FFPE curls obtained from tumor tissues are obtained, processed, and sequenced via next generation sequencing in a continuous workflow. In this example, the features utilized by the classifier are all mined from the sequencing data. For instance, RNA expression data (RNASeq) is fed into a deconvolution algorithm to determine cell type/subtype percentages. Likewise, the expression levels of immune inhibitory genes are also obtained from the sequencing data. Mutational burden can also be determined from the sequencing data.

[0115] A classifier may generate a different prediction each time it is given new sample data. Using different samples on the same classifier can generate a different or unique output each time the classifier is run. Using the same samples on the same classifier can generate a different or unique output each time the classifier is run. The classifier may analyze a sample by comparing it against the panel of features predictive of an outcome or response. In some cases, the classifier carries out the comparing, statistical analysis, downstream analyses, or any combination thereof.

[0116] In some cases, the features (e.g., cell type percentages, immune escape gene expression, and mutational burden) are analyzed using feature selection techniques. Feature selection techniques can include filters for evaluating feature relevance by examining the data properties, wrappers that embed the model hypothesis within a feature subset search, or embedded protocols that build the search for an optimal feature set is built into a classifier algorithm. In some cases, the methods described herein comprise a feature selection step in which relevant features are selected for inclusion in the final classifier and/or irrelevant or low relevance features are culled or removed from the final classifier.

[0117] Examples of filters that can be beneficial for use in the methods of the present disclosure include parametric methods such as two sample t-tests, analysis of variance (ANOVA) analyses, Gamma distribution models, or Bayesian models. Filters can include model free methods such as Wilcoxon rank sum tests, rank products methods, random permutation methods, between-within class sum of squares tests, or threshold number of misclassification. In some cases, filters include multivariate methods such as bivariate analysis, correlation based feature selection methods, minimum redundancy maximum relevance, Markov blanket filter, and uncorrelated shrunken centroid methods.

[0118] Wrappers that may be beneficial for use in the methods of the present disclosure can include sequential

search methods, estimation of distribution algorithms, or genetic algorithms. Embedded protocols that may be beneficial for use in the methods of the present disclosure can include random forest algorithms, weights of logistic regression algorithms, or weight vector of support vector machine algorithms.

[0119] The statistical results obtained from the methods described herein can provide the likelihood the prediction is accurate. In some cases, the prediction is presented as a diagnosis along with a likelihood of accuracy such as, for example, a prediction of a positive response to a therapeutic cancer treatment with at least a 70%, 75%, 80%, 85%, 90%, or 95% estimated accuracy. The predictions may be analyzed using statistical tools including students T test, two sided T test, Pearson rank sum analysis, hidden Markov model analysis, analysis of q-q plots, principal component analysis, one way analysis of variance (ANOVA), two way ANOVA, and other statistical methods.

#### Computer Systems

[0120] The present disclosure provides computer systems that are programmed to implement methods of the disclosure. FIG. 8 shows a computer system 801 that is programmed or otherwise configured to carry out executable instructions. The computer system may be programmed to process nucleic acid sequencing information to generate a classifier comprising a panel of genetic variations predictive of adverse response to chemotherapy, by associating the nucleic acid sequencing information with adverse response to chemotherapy. The computer system may be programmed with a classifier for analyzing genetic information to generate a prediction of an adverse response to one or more chemotherapeutic agents. The computer system 801 can regulate various aspects of the methods of the present disclosure, such as, for example, training the algorithm with the nucleic acid sequencing information of a set of samples to generate a trained algorithm or classifier. The computer system 801 may determine the positive predictive value of a classifier by analyzing a set of independent samples with the classifier and comparing the actual incidents of adverse response to the predicted risk of adverse response. The computer system 801 can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device.

[0121] The computer system 801 includes a central processing unit (CPU, also "processor" and "computer processor" herein) 805, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system 801 also includes memory or memory location 810 (e.g., random-access memory, read-only memory, flash memory), electronic storage unit 815 (e.g., hard disk), communication interface 820 (e.g., network adapter) for communicating with one or more other systems, and peripheral devices 825, such as cache, other memory, data storage and/or electronic display adapters. The memory 810, storage unit 815, interface 820 and peripheral devices 825 are in communication with the CPU 805 through a communication bus (solid lines), such as a motherboard. The storage unit 815 can be a data storage unit (or data repository) for storing data. The computer system 801 can be operatively coupled to a computer network ("network") 830 with the aid of the communication interface 820. The network 830 can be the Internet, an internet and/or extranet,

or an intranet and/or extranet that is in communication with the Internet. The network 830 in some cases is a telecommunication and/or data network. The network 830 can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network 830, in some cases with the aid of the computer system 801, can implement a peer-to-peer network, which may enable devices coupled to the computer system 801 to behave as a client or a server.

[0122] The CPU 805 can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory 810. The instructions can be directed to the CPU 805, which can subsequently program or otherwise configure the CPU 805 to implement methods of the present disclosure. Examples of operations performed by the CPU 805 can include fetch, decode, execute, and writeback.

[0123] The CPU 805 can be part of a circuit, such as an integrated circuit. One or more other components of the system 801 can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[0124] The storage unit 815 can store files, such as drivers, libraries and saved programs. The storage unit 815 can store user data, e.g., user preferences and user programs. The computer system 801 in some cases can include one or more additional data storage units that are external to the computer system 801, such as located on a remote server that is in communication with the computer system 801 through an intranet or the Internet.

[0125] The computer system 801 can communicate with one or more remote computer systems through the network 830. For instance, the computer system 801 can communicate with a remote computer system of a user (e.g., a laptop or a smart phone). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system 801 via the network 830.

[0126] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system 801, such as, for example, on the memory 810 or electronic storage unit 815. The machine executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor 805. In some cases, the code can be retrieved from the storage unit 815 and stored on the memory 810 for ready access by the processor 805. In some situations, the electronic storage unit 815 can be precluded, and machine-executable instructions are stored on memory 810.

[0127] The code can be pre-compiled and configured for use with a machine having a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[0128] Aspects of the systems and methods provided herein, such as the computer system 801, can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code

and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage" media, terms such as computer or machine "readable medium" refer to any medium that participates in providing instructions to a processor for execution.

[0129] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases or other components shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[0130] The computer system 801 can include or be in communication with an electronic display 835 that comprises a user interface (UI) 840 for providing, for example, reports or results of risk stratification analysis of the nucleic acid sequencing information of a sample. Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

[0131] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit 805. The algorithm can, for example, analyze the nucleic acid sequencing information obtained from a sample to stratify a risk of adverse response to chemotherapy (e.g., one or more chemotherapeutic agents) for the subject from whom the sample was obtained.

[0132] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

## EXAMPLES

[0133] The following examples are given for the purpose of illustrating various embodiments of the disclosure and are not meant to limit the present disclosure in any fashion. The present examples, along with the methods described herein are presently representative of certain embodiments, and are not intended as limitations on the scope of the disclosure. Changes therein and other uses which are encompassed within the spirit of the disclosure as defined by the scope of the claims will occur to those skilled in the art.

### Example 1: Classification of a Tumor Microenvironment

[0134] A tumor microenvironment was characterized by determining immune modulatory molecule expression levels, cell type and ratio, and tumor mutational burden.

#### RNA Extraction

[0135] RNA was extracted from formalin fixed paraffin embedded (FFPE) samples using the RNeasy kit (Qiagen) according to manufacturer's instructions.

#### Generation of Sequencing Library

[0136] Following RNA extraction, a sequencing library for next generation sequencing was generated according to manufacturer's instructions (Illumina). Coding regions were captured using Illumina Access kits.

### Next Generation Sequencing

**[0137]** The enriched genes were sequenced on an Illumina's NextSeq sequencing machine to generate sequencing data and expression data.

### Inhibitory Molecule Expression

**[0138]** The sequencing and expression data is used to determine the expression level of selected immune modulatory molecules including PD-1, PD-L1, CTLA-4, OX40, TIM-3, BTLA, ICOS, CD47, IDO1, and ARG1.

### Cell Type and Ratio Deconvolution

**[0139]** Following next generation sequencing, sequencing data was analyzed for cell type and by ratio deconvolution. The use of next generation sequencing to generate sequencing and expression data for use in deconvolving cell types/subtypes as described herein is a new approach that provides superior performance compared to, for example, conventional gene expression systems such as those using microarrays. In addition, routine studies looking at immune cell percentages in tissue use a conventional approach of utilizing public databases of microarray expression data, which may be able to identify changes in cell proportions between different samples, but are ineffective for accurately determining the true percentage of a cell type/subtype in a given sample.

**[0140]** Ratio deconvolution was carried out using a deconvolution matrix comprising a set of cell expression signatures or "fingerprints." The cell expression signatures or "fingerprints" were generated using sequencing data obtained from samples substantially composed of specific cell types (e.g., a CD4+ naive cell fingerprint obtained from a purified population of CD4+ naive cells). The cell-specific fingerprints were then placed into the deconvolution matrix. This matrix was then applied to the complex data set of RNA sequencing and gene expression data to allow for identification of cell types in the data and the relative proportions of each cell type. Included in the cell expression signatures or fingerprints were genes that were significantly differentially expressed in pairwise cell type differential expression analysis as well as those genes that were expressed at a consistent level within cell type across biological replicates.

**[0141]** Ratio deconvolution was also performed. The process of determining individual components from bulk sequencing and expression profiles was accomplished by solving the matrix equation:  $Ax=b$  where A was the cell expression fingerprints, x was the cell percentages, and b was the bulk expression counts. A vector regression method with data normalization was performed. See FIG. 3. Briefly, to deconvolve a mixture with N cell types using M genes, the problem is set up according to FIG. 3. Cell fractions were determined and normalization across rows was performed. The expression counts of each gene were normalized to be in the range of 0 to 1 across each cell type and the sample in question such as a mixture of cells. All genes were weighted equally regardless of their absolute expression value.

### Tumor Mutational Burden Calculation

**[0142]** Tumor mutational burden was calculated. Following RNA sequencing, all the variants including somatic and germline were determined as shown in FIG. 4A with the

variants indicated by the dark squares along the representative gene sequences. Total somatic mutational burden was thus determined without use of a paired normal. A panel of genes (~4000) was then used for subsequent analysis since they were determined to correlate with mutational burden across the genome. This concept is illustrated in FIG. 4B in which the correlated genes A, C, and F are circled. Several germline variation databases were used to identify the initial variant calls that were germline variants based on a frequency of greater than 0.01%, and the identified germline variants were then excluded from mutational burden analysis. As shown in FIG. 4C, the excluded germline variants are crossed out with an "X". From the remaining variants (which are circled in FIG. 4D) on the panel of genes, the total somatic mutational burden was extrapolated.

### Tumor Microenvironment Summary Report

**[0143]** Data from the immune modulatory molecule expression, cell type and ratio deconvolution, and tumor mutational burden calculation was then compiled to generate a summary of these metrics in the tumor microenvironment. The summary was presented in an immune-oncology profile that displayed the information in a graphical output. The immune modulatory molecule expression was displayed in a bar graph alongside a reference or control expression level. A general breakdown of the cell types (immune, tumor, and stromal cells) and their relative percentages/proportions of the overall cell population in the sequenced sample were displayed in a pie chart. A more specific breakdown based on the percentages of specific cell types (T cells, CD4+ cells, myeloid cells, NK cells, and B cells) and cell sub-types (e.g., CD4+ and CD8+ T cells) in the sample was graphically displayed. Finally, the mutational burden was displayed as an indicator or marker on an axis ranging from low mutational burden (0 mutations per Megabase) to high mutational burden (2000 mutations per Megabase).

### Example 2: Mutational Burden Analysis

**[0144]** Genes that provide improved resolution and accuracy for mutational burden analysis were determined. All the human genes were ranked in order using a weighted average of their likelihood to be mutated using data from public databases as well as by their consistency of expression across multiple tissues. Three different cancer types were analyzed with about 50 samples of each cancer. The accuracy was determined with an increasing number of genes from Table 5.

**[0145]** From Table 6, correlation with a Gold Standard mutational burden increased with about 2000 genes and then started to plateau. Mutational burden was compared to the Gold Standard mutational burden calculated using paired normal analysis of DNA. Specifically, the Gold Standard mutational burden measures somatic mutations using DNA sequence data obtained from the sample by comparing allele frequencies in normal and tumor sample alignments, annotating the identified mutations, and aggregating the mutations.

TABLE 6

Genes	Correlation with Gold Standard		
	HNSC	Lung	Breast
100	0.65	0.32	0.46
250	0.75	0.54	0.82
500	0.84	0.58	0.84
1000	0.87	0.7	0.9
2000	0.9	0.85	0.91
4000	0.93	0.91	0.91

**Example 3: Characterization of a Glioblastoma Tumor Microenvironment**

[0146] Using similar methods as described in Examples 1-2, a glioblastoma tumor microenvironment was characterized. As seen in FIG. 5A, different cell types were on the y-axis, while genes were on the x-axis. Gene expression level was represented by color with a darker purple color indicating relatively higher expression. In black and white, the darker shade indicates higher expression, while the lighter shade indicates lower expression. Gene 406 was identified as distinguishing a glioblastoma cancer cell type and immune cell types.

[0147] A similar gene matrix was also generated using about 800 genes (FIG. 5B). Referring to FIG. 5B, expression levels of multiple genes was determined and used to characterize the glioblastoma tumor microenvironment. The genes listed on the y-axis in FIG. 5B from top down include gbm, gMDSC, DCs, mMDS, B-cell, Th2, Th17, Th1, Naïve-CD4-pos, Naïve-CD8-pos, CM-CD4-pos, Tregs, CM-CD8-pos, EM-CD8-pos, Stromal, M1, M2a, M2b, and M2c. The legend in FIG. 5B shows decreased expression indicated by a blue color, increased expression indicated by a red/orange color, and white being neutral or no change in expression. Most of the visible expression data indicates no change or increased expression.

**Example 4: Characterization of Tumor Microenvironment for Various Types of Cancers**

[0148] A tumor microenvironment was determined for various cancer types. Using similar methods as described in Examples 1-2, expression levels of multiple genes was identified in colorectal and blood cancers (FIG. 6). Gene expression levels were used to characterize the colorectal and blood cancer microenvironment. The genes listed on the y-axis in FIG. 6 for colorectal cancer from top down include B-cell, CD4-pos, CD8-pos, gMDSC, Macrophage, DCs, mMDS, and stromal. The genes listed on the y-axis in FIG. 6 for blood from top down include gbm, gMDSC, DCs, mMDS, B-cell, Th2, Th17, Th1, Naïve-CD4-pos, Naïve-CD8-pos, CM-CD4-pos, Tregs, CM-CD8-pos, EM-CD8-pos, Stromal, M1, M2a, M2b, and M2c. The legend in FIG. 6 shows decreased expression indicated by a blue color, increased expression indicated by a red/orange color, and white being neutral or no change in expression. Most of the visible expression data indicates no change or increased expression.

**Example 5: Transformation of RNA Sequencing Data**

[0149] The amount of RNA per immune cell type was determined.

**RNA Per Cell Calculations**

[0150] Immune cell types (CD4+ T cells, CD8+ T cells, B-cells, Monocytes, Treg and natural killer cells) were purified by flow cytometry from multiple peripheral blood mononuclear cell (PBMC) donors. Macrophages M1 and M2 were differentiated in cell culture from monocyte cell donors and purified using fluorescent activated cell sorting (FACS) to obtain pure M1 and M2 populations. RNA was extracted from the purified cells and sequenced. The number of cells obtained from FACS was recorded with the amount of RNA extracted from each cell enabling calculations for the amount of RNA per cell for each cell type. FIG. 9 shows the average amount of total RNA per cell for each immune cell type.

**Cell Correction Methods**

[0151] The effect on cell percentages and ratios calculated from deconvolution using SVM (support vector machines) when the different cell types contained distinct amounts of total RNA were determined. Cell mixtures were generated in duplicate from Macrophages M1 and M2 spiked into peripheral blood mononuclear cell (PBMC) samples in decreasing ratios. These cell mixtures represent a “gold standard” or sample where the true answer (percentages of cell types in the mixture) was known before deconvolution. The cell mixture ratios are shown for PBMC samples comprising 500,000 cells in Table 7.

TABLE 7

Sample Name	% Macrophage	% PBMC
M1-100	100	0
M1-50	50	50
M1-25	25	75
M1-10	10	90
M1-5	5	95
M1-2	2	98
M1-0	0	100
M2-100	100	0
M2-50	50	50
M2-25	25	75
M2-10	10	90
M2-5	5	95
M2-2	2	98
M2-0	0	100
*M1M2-100	100	0
M1M2-50	50	50
M1M2-25	25	75
M1M2-10	10	90
IvM2-5	5	95
M1M2-2	2	98
M1M2-0	0	100

\*M1M2 = 50% M1 + 50% M2 mix

[0152] Total RNA was extracted from the cell mixtures and sequenced. Following sequencing, the resulting data was analyzed for M1 and M2 cell percentages, using SVM-based deconvolution as described herein, and the computed cell type percentages were compared to the known percentages (ground truth). As seen in FIGS. 10A-10F, since the total RNA amount contained in macrophages is much higher than observed in other immune cells, the deconvolution method resulted in the calculation of a higher percentage of macrophage cells than as compared to truth. When observing FIG. 10A as a non-color drawing, at the leftmost data points on the chart, the M1 proportion data is shown in order from top to bottom in order as “expected”, “observed”, and

“corrected”. In FIG. 10B, the highest M2 proportions are “observed” followed by “corrected” and then “expected”. In FIG. 10C, at the leftmost data points on the chart, the highest data point corresponds to expected followed by observed and then corrected. In FIG. 10D, the highest M2 proportions are “observed” followed by “corrected” and then “expected”. In FIG. 10E, at the leftmost data points on the chart, the highest data point corresponds to “expected” followed by “observed” and then “corrected”. In FIG. 10F, at the leftmost data points on the chart, the highest data point corresponds to “expected” followed by “observed” and then “corrected”. Based on these results, a method for correcting cell percentages for any cell type as required based on cellular RNA amount was developed. The equation below is one method for correcting cell percentages following deconvolution:

$$(1 - o_k)i_k m_k - \sum_{j \neq k} o_k m_j i_j = 0 \quad \text{Equation 1}$$

$$\sum i_k = 1 \quad \text{Equation 2}$$

[0153] Equation 1 was used for each of k cell types. Referring to the equation,  $o_k$  is the observed output  $i_k$  is the actuation output, and  $m_k$  is the multiplier. For each cell type equation, the sum subtracted is over the  $o$ ,  $m$ ,  $i$  values for the “other” cell types. All k equations of equation 1 were set to 1. The second equation is the constraint that all inputs add to 1.

#### Correction of Macrophages M1 and M2 Percentages

[0154] Because the amount of RNA in macrophage cells was much higher than that seen in other immune cell types, the analysis algorithms compute this increased amount of RNA as a greater percentage of cells than expected. In order to bring the percentage of macrophage cells in line with the expected percent, Equation 1 and Equation 2 were used. This correction translated the percentages from “transcript space” to “cell space” by applying a correction based on amount of RNA (transcripts) per cell. Once the equation is applied to the M1 and M2 cell percentage cells, the percentages highly resemble the expected percentages. The factor of correction used here was 12×. Results using methods as described herein are seen in Table 8.

TABLE 8

Cell Type	Amount of RNA per Cell (pg/cell)	Cell correction value for deconvolution (normalized to CD4+ T cell)
CD4+ T cell	0.52	1.00
CD8+ T cell	0.54	1.03
Monocyte	0.71	1.35
B-cell	0.28	0.53
NK	0.24	0.47
M1 Macro	3.98	7.59
M2 Macro	6.44	12.26

#### Example 6—Cell Percentage Accuracy

[0155] The estimated cell percentages generated according to the methods disclosed herein were compared to known cell percentages calculated using flow cytometry to

determine the accuracy of the deconvolution algorithm, as shown in FIG. 11. The percentage of immune cells in complex mixtures was measured using flow cytometry (y-axis=Known %) and compared to the estimated percentage of immune cell types from RNA sequencing data of the complex mixtures (x-axis=Estimated %). As shown in the graph, the cell percent estimates exhibit high trueness, precision, accuracy and correlation in comparison to the known percent.

[0156] This experiment estimating cell percentages and calculating true cell percentages using flow cytometry and was performed in triplicate, and one of the replicates was used to train for cell corrections in comparison to the known percent from flow. The data shown in FIG. 11 represents an assessment of the trained model using testing data (using the training corrections). Thus, this method corrected for deviations from the true cell percentages by training the model as opposed to using cell corrections based on RNA content/quantity per cell. The high accuracy of the model is indicated by the deviations indicated in FIG. 11, which are also shown in Table 9 below.

TABLE 9

Cell type/subtype	estimated cell percentage deviation from true cell percentage	
	% Deviation of Estimated Percentage from Known Percentage	
CD56	-0.01%	+/- 0.94%
Treg	-0.23%	+/- 0.61%
CD4	0.77%	+/- 1.52%
CD14	-0.18%	+/- 0.57%
M1	0.21%	+/- 0.16%
M2	0.07%	+/- 0.13%
CD8	-0.03%	+/- 0.73%
CD19	-0.07%	+/- 0.52%

#### Example 7—Clinical Sample Classification for Diagnosis and Prognosis

[0157] An immune-oncology profile that includes the relative quantities of 8 cell types and expression level for 10 immune-inhibitory genes (e.g., “escape genes”) was determined for 15-20 biological samples obtained from human subjects according to the methods described herein. The samples were previously categorized into two groups: subjects who were responsive to a stated therapy and subjects who were not responsive to the therapy.

[0158] The immune-oncology profile was used to understand differences across two groups of samples based on single or a combination of analytes used as predictive biomarker(s). Specifically, the immune-oncology profile analyte information was used to train a machine learning algorithm for classifying samples into the two groups.

[0159] The machine learning algorithm was used to generate classifiers based on individual analytes and multiple analytes. Single analyte biomarkers were used to solve for a threshold that maximizes the sensitivity and specificity simultaneously. With a large enough (normal) distribution of samples, this criteria may maximize the accuracy. Based on the small sample set (~10 in each group), which is typical in many studies such as early phase clinical trials, this optimization metric best approximates where the maximal accuracy may be if more samples were available.

**[0160]** Multi analyte biomarkers were found by optimizing the same statistics. Instead of using a linear threshold for single analytes, a random forest model was optimized to maximize the above predictive statistics. The algorithms chose the random forest model with the best set of individual analytes and the best set of hyper-parameters (e.g., the tuning knobs of the random forest technique).

**[0161]** The predictive accuracies calculated for the most predictive escape gene ("escape") and the most predictive immune cell type ("immune"). Also shown is the predictive accuracy of using one or more analytes in the Paragon Assay ("PARAGON"). This "marker" is created by learning a machine learning model that incorporates information from one or more analytes. These results are shown in the predictive accuracy bar plot in FIG. 12A. The "escape" biomarker and "immune" biomarker both generated a predictive accuracy of just under 75%. Meanwhile, the "paragon" multi-analyte classifier resulted in an 85% predictive accuracy, demonstrating how the multi-analyte approach produced superior accuracy in the context of small sample sizes.

**[0162]** FIG. 12B shows box-and-whisker plots that visualize the statistics for groups of samples for the 2 most predictive analytes. The left and right sides of the box indicate the 1st and 3rd quartiles of the respective data set. The median is indicated by the white line inside the box. The minimum and maximum inlier data points are denoted by the ends of the whiskers, while outliers are shown as empty circles. The optimal threshold for a given analyte is shown as a vertical dotted line. Wilcoxon rank-sum testing is used to test the null hypothesis that the two groups are sampled from the same distribution. Significance of rejecting this hypothesis is denoted for p-values of <0.05, <0.01, and <0.001 by 1, 2, and 3 stars, respectively.

**[0163]** In this case, the "escape" biomarker identified as being the most predictive of the list of 10 immune-inhibitory genes assessed by the algorithm was CTLA4, which produced the just below 75% predictive accuracy when used to group a sample based on a threshold CTLA4 expression level. As shown in FIG. 12B, the dotted line indicates a threshold value of about 5 TPM (transcripts per kilobase million) separating the two groups or categories of samples. Likewise, the cell type identified as the most predictive "immune" biomarker was CD19+ B cells. As shown in FIG. 12C, a threshold value just above 30% separates the two sample categories. A flow chart illustrating the generation of a single-analyte classifier and a multi-analyte classifier is shown in FIG. 13. In single-analyte/biomarker analysis, the individual analyte is assessed by determining the threshold that maximizes sensitivity and specificity (steps 1-4 in FIG. 13). Separate predictors or classifiers are generated for each analyte. In multi-analyte analysis, the most predictive analytes are combined to generate a combination predictor or classifier based on multiple analytes (e.g., multiple immune escape gene(s) and/or immune cell(s) percentages infiltrating the tumor sample) (steps 5-10).

**[0164]** The statistical performance of these models was tested using the leave-one-out cross validation to calculate the accuracy, positive predictive value (ppv), and negative predictive value (npv) for each analyte. For a dataset limited in size, leave-one-out cross validation gives the best approximation to how an estimator will generalize to future, independent samples. The process works by iterating n times (where there are n data points), each time learning a thresh-

old considering n-1 points and testing the prediction of the nth, left out, point. Then, all n predictions were considered to calculate prediction statistics. Thresholds were determined by optimizing equally for sensitivity and specificity using all samples. For data points that are normally distributed, this threshold may be the same threshold optimized for accuracy. The results of the statistical assessment of the model and/or analyte performance are shown below in Table 10.

TABLE 10

Analyte	ppv (%)	DF1n Median (TPM)	DF3y Median (TPM)	npv (%)	accuracy (%)	thresh-old (TPM)	p-value
CTLA4	71.42	0.97	20.59	66.66	69.23	4.78	0.0151
OX40	71.42	3.65	7.79	66.66	69.23	5.56	0.0864
PD-1	66.66	1.12	2.52	57.14	61.53	1.35	0.253
IDO1	57.14	7.22	3.13	50	53.84	5.46	0.475
CD47	57.14	71.77	51.14	50	53.84	65	0.475
PD-L1	57.14	3.64	3.89	50	53.84	3.77	0.475
TIM-3	57.14	15.8	13.83	50	53.84	13.71	0.5677
BTLA	57.14	0.29	0.28	50	53.84	0.33	0.775
ICOS	57.14	2.23	1.56	50	53.84	1.58	0.8303
ARG1	57.14	12.23	17.78	50	53.84	14.57	0.8864
M2	71.42	16.5	2	66.66	69.23	11.11	0.0455
Macrophages							
CD19+	71.42	14	54	66.66	69.23	32.39	0.0455
B Cells							
CD14+	71.42	39.5	12	66.66	69.23	28.87	0.2246
Monocytes							
CD56+	80	0	7	62.5	69.23	0	0.3531
NK Cells							
CD+	100	0	0	54.54	61.53	0	0.3913
T Cells							
Treg	100	0	0	50	53.84	0	0.6682
Cells							
CD4+	57.14	5.5	7	50	53.84	7.24	0.7209
T Cells							
M1	50	3.5	3	42.85	46.15	3.01	0.775
Macrophages							
Paragon	100	N/A	N/A	75	84.61	N/A	N/A

**[0165]** While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

What is claimed is:

1. A computer-implemented method for analyzing a biological sample obtained from a subject having or suspected of having a disease or condition, comprising:
  - (a) obtaining gene expression data comprising (i) an expression level of at least one immune modulatory gene and (ii) expression levels of a plurality of expression signature genes from the biological sample;
  - (b) using a deconvolution algorithm to process said expression levels of said plurality of expression signa-

- ture genes to identify and quantify a percentage of at least one cell type that is present in the biological sample; and
- (c) using a classifier to analyze the expression level of the at least one immune modulatory gene and the percentage of the at least one cell type from (b) to determine a likelihood that said subject will be responsive or non-responsive to therapy.
2. The method of claim 1, wherein the therapy comprises immunotherapy.
3. The method of claim 1, further comprising providing instructions to start, stop, change, or continue the therapy.
4. The method of claim 1, wherein the disease or condition is cancer, and wherein (c) comprises determining that said likelihood that said subject will be responsive or non-responsive to said therapy for said cancer.
5. The method of claim 1, wherein the at least one cell type comprises at least one immune cell type.
6. The method of claim 5, wherein the at least one immune cell type comprises M1 macrophages, M2 macrophages, CD19+ B cells, CD14+ monocytes, CD56+NK cells, CD8+ T cells, Treg cells, CD4+ T cells, or any combination thereof.
7. The method of claim 1, wherein the at least one immune modulatory gene comprises CTLA4, OX40, PD-1, IDO1, CD47, PD-L1, TIM-3, BTLA, ICOS, ARG1, or any combination thereof.
8. The method of claim 1, wherein the classifier is generated using a machine learning algorithm.
9. The method of claim 8, wherein the machine learning algorithm is a random forest algorithm.
10. The method of claim 1, wherein the deconvolution algorithm in (b) applies a deconvolution matrix to process said expression levels of said plurality of expression signature genes to identify and quantify the percentage of the at least one cell type.
11. The method of claim 10, wherein the deconvolution matrix comprises a plurality of immune cell expression signature genes.
12. The method of claim 10, wherein the deconvolution matrix comprises a plurality of tumor cell expression signature genes.
13. The method of claim 10, wherein the deconvolution matrix comprises a plurality of cell types, each cell type comprising a plurality of expression signature genes, wherein expression count for each expression signature gene is normalized across the plurality of cell types.
14. The method of claim 1, wherein the deconvolution algorithm processes said expression levels of said plurality of expression signature genes using linear least-squares regression (LLSR), quadratic programming (QP), perturbation model for gene expression deconvolution (PERT), robust linear regression (RLR), microarray microdissection with analysis of differences (MMAD), digital sorting algorithm (DSA), or support vector regression.
15. The method of claim 14, wherein the deconvolution algorithm performs an RNA normalization step to compensate for variation in RNA quantity amongst the at least one cell type in order to improve accuracy of the percentage of the at least one cell type.
16. The method of claim 15, wherein the deconvolution algorithm is a machine learning algorithm trained using comparison data comprising an actual percentage of the at least one cell type.
17. The method of claim 1, wherein the gene expression data and the plurality of expression signature genes are obtained from the biological sample using next generation RNA sequencing.
18. The method of claim 1, further comprising processing the gene expression data to determine mutational burden for the biological sample and inputting the mutational burden into the classifier for analysis in order to enhance classification of the biological sample.
19. The method of claim 1, wherein the classifier is trained on data from no more than 50 samples and provides an accuracy of at least 85%.
20. A system comprising for analyzing a biological sample obtained from a subject having or suspected of having a disease or condition, comprising:
- a database comprising gene expression data comprising
    - (i) an expression level of at least one immune modulatory gene and (ii) expression levels of a plurality of expression signature genes from the biological sample; and
  - at least one computer processor that is coupled to said database, wherein said at least one computer processor is programmed to:
- (a) use a deconvolution algorithm to process said expression levels of said plurality of expression signature genes to identify and quantify a percentage of at least one cell type that is present in the biological sample;
- (b) use a classifier to analyze the expression level of the at least one immune modulatory gene and the percentage of the at least one cell type from (b) to determine a likelihood that said subject will be responsive or non-responsive to therapy.
21. The system of claim 20, wherein the at least one cell type comprises M1 macrophages, M2 macrophages, CD19+ B cells, CD14+ monocytes, CD56+NK cells, CD8+ T cells, Treg cells, CD4+ T cells, or any combination thereof.
22. The system of claim 20, wherein the at least one immune modulatory gene comprises CTLA4, OX40, PD-1, IDO1, CD47, PD-L1, TIM-3, BTLA, ICOS, ARG1, or any combination thereof.
23. The system of claim 20, wherein the classifier is generated using a machine learning algorithm.
24. The system of claim 23, wherein the machine learning algorithm is a random forest algorithm.
25. The system of claim 20, wherein the deconvolution algorithm in (a) applies a deconvolution matrix to process said expression levels of said plurality of expression signature genes to identify and quantify the percentage of the at least one cell type.
26. The system of claim 25, wherein the deconvolution algorithm performs an RNA normalization step to compensate for variation in RNA quantity amongst the at least one cell type in order to improve accuracy of the percentage of the at least one cell type.
27. The system of claim 20, wherein the gene expression data and the plurality of expression signature genes are obtained from the biological sample using next generation RNA sequencing.
28. The system of claim 27, wherein the at least one processor is further programmed to obtain mutational burden data for the biological sample and inputting the mutational burden data into the classifier for analysis in order to enhance classification of the biological sample.

**29.** The system of any one of claim **20**, wherein the classifier is trained on data from no more than 50 samples and provides an accuracy of at least 85%.

**30.** Non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, performs a method comprising:

- (a) obtaining gene expression data comprising (i) an expression level of at least one immune modulatory gene and (ii) expression levels of a plurality of expression signature genes from the biological sample;
- (b) using a deconvolution algorithm to process said expression levels of said plurality of expression signature genes to identify and quantify a percentage of at least one cell type that is present in the biological sample; and
- (c) using a classifier to analyze the expression level of the at least one immune modulatory gene and the percentage of the at least one cell type from (b) to determine a likelihood that said subject will be responsive or non-responsive to therapy.

\* \* \* \* \*