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(54) Title: T CELL GENE EXPRESSION ANALYSIS FOR USE IN T CELL THERAPIES

(57) Abstract: The application provides T cell gene expression signatures that can be used to predict T cell therapy outcomes.

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T CELL GENE EXPRESSION ANALYSIS FOR USE IN T CELL THERAPIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/842,260, filed May 2, 2019, the disclosure of which is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant number AI114442 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 2, 2020, is named 243734_000132_SL.txt and is 763 bytes in size.

FIELD

[0004] The application relates to T cell gene expression signatures that can be used to predict T cell therapy outcomes.

BACKGROUND

[0005] Cellular immunotherapy with adoptively transferred chimeric antigen receptor (CAR) modified T cells is an attractive approach to improve the outcomes for patients with cancer. However, even for the most successful CAR T cell therapy, CD19-CAR T cell therapy for CD19+ acute lymphoblastic leukemia (ALL), only 50% of patients have responses that last more than one year (Maude et al., NEJM 2018). Complete responses are much lower for CD19+ chronic lymphatic leukemia (Fraietta et al., Nature Med 2018), and only few long term survivors have been reported for CAR T cell therapies targeting solid tumor antigens such as HER2 (Ahmed et al., JCO 2015). Thus, there is a great need in the art to develop methods for predicting individual patient's responsiveness to CAR T cell therapies prior to the use of such therapies, so that an appropriate individual treatment plan can be developed.

[0006] The need to develop predictive markers does not only apply to CAR T cell therapies, but also to all forms of T cell therapies, which include therapies with i) T cells that express an endogenous $\alpha\beta$ TCR, which is specific for a peptide derived from viral or tumor-associated antigens (including neoantigens); ii) T cells that transgenically express an $\alpha\beta$ TCR, which is specific for a peptide derived from viral or tumor-associated antigens (including neoantigens); iii) T cells that transgenically express bispecific antibodies, which recognize viral or tumor-associated antigens (including neoantigens)/or a peptide derived from them and an activating molecule expressed on T cells such as CD3; and/or iv) T cells that are generated via stimulation with for examples but not limited to peptides, antigen presenting and/or artificial antigen presenting cells (*in vitro* sensitized [IVS] T cell therapy). Lastly, T cell therapies in which the therapeutic genes are delivered *in vivo* are included (*in vivo* T cell therapy).

SUMMARY OF THE INVENTION

[0007] As specified in the Background section above, there is a great need in the art for developing methods for predicting individual patient's responsiveness to CAR T cell therapies and other T cell therapies prior to the use of such therapies. The present application addresses these and other needs.

[0008] In one aspect provided herein is a method for predicting a subject's responsiveness to an autologous T cell therapy. The method comprises: a) determining gene expression level of one or more genes in a T cell sample isolated from the subject, wherein one or more of said genes are methylation targets of DNA (cytosine-5)-methyltransferase 3A (DNMT3A), b) generating a Diagnostic Expression Score for the T cell sample isolated from the subject by calculating and summing absolute or weighted gene expression level(s) determined in step (a), or by calculating and summing relative gene expression level(s) relative to reference expression level(s) obtained using responders and non-responders in a reference dataset, and c) (i) determining that the subject is not likely to respond to an autologous T cell therapy if the Diagnostic Expression Score generated in step (b) is less than a threshold score; (ii) determining that the subject is likely to respond to an autologous T cell therapy if the Diagnostic Expression Score generated in step (b) is greater than the threshold score. In some embodiments, the Diagnostic Expression Score is generated by Z-score summation and the threshold score is 0.

[0009] In some embodiments, the subject has a cancer, an infectious disease, an inflammatory disorder, or an autoimmune disease.

[0010] In some embodiments, the method further comprises improving the subject's T cell functioning in T cell therapies. In some embodiments, improving the subject's T cell functioning in T cell therapies comprises inhibiting DNMT3A-mediated *de novo* DNA methylation and/or activating STAT5 signaling pathway in the subject's T cells.

[0011] In some embodiments, inhibiting DNMT3A-mediated *de novo* DNA methylation in the subject's T cells is achieved by inhibiting enzymatic activity of DNMT3A protein or making DNMT3A gene deleted or defective. In some embodiments, the enzymatic activity of the DNMT3A protein is inhibited by exposing the cell to a DNMT3A active site inhibitor. In some embodiments, the DNMT3A gene is mutated in DNMT3A catalytic domain so that the enzymatic activity of the DNMT3A protein is inhibited. In some embodiments, the level of functional DNMT3A protein in the cell is decreased by 50% or more.

[0012] In some embodiments, the STAT5 signaling pathway is activated by either stimulating the T cell with a signaling molecule or genetically modifying the T cell to express a signaling molecule. In some embodiments, the signaling molecule is a common gamma chain cytokine. In some embodiments, the cytokine is IL-15, IL-7, IL-2, IL-4, IL-9, or IL-21. In some embodiments, the STAT5 signaling pathway is activated by modifying the T cell to express a constitutively active cytokine receptor or a switch receptor. In some embodiments, the constitutively active cytokine receptor is a constitutively active IL7 receptor (C7R). In some embodiments, the switch receptor is an IL-4/IL-7 receptor or an IL-4/IL-2 receptor.

[0013] In various embodiments, improving the subject's T cell functioning as described herein is conducted *ex vivo* or *in vitro*.

[0014] In some embodiments, the method further comprises repeating the method described to predict a subject's responsiveness to an autologous T cell therapy on the subject's T cells which were treated to improve the subject's T cell functioning.

[0015] In some embodiments, if the subject is determined in step (c) as not likely to respond to an autologous T cell therapy, the method further comprises administering to the subject an alternative therapy which is not a T cell therapy. The alternative therapy may be selected from antiviral therapies, bone marrow transplant, chemotherapies, checkpoint blockade, and any combinations thereof.

[0016] In some embodiments, the subject is determined in step (c) as likely to respond to an autologous T cell therapy, the method further comprises using the subject's T cells for an autologous T cell therapy.

[0017] In another aspect provided herein is a method for determining if T cells of a subject can be used for an allogeneic T cell therapy. The method comprises a) determining gene

expression level of one or more genes in a T cell sample isolated from the subject, wherein one or more of said genes are methylation targets of DNA (cytosine-5)-methyltransferase 3A (DNMT3A), b) generating a Diagnostic Expression Score for the T cell sample isolated from the subject by calculating and summing absolute or weighted gene expression level(s) determined in step (a), or by calculating and summing relative gene expression level(s) relative to reference expression level(s) obtained using responders and non-responders in a reference dataset, and c) (i) determining that the T cells of the subject cannot be used for an allogeneic T cell therapy if the Diagnostic Expression Score generated in step (b) is less than a threshold score; (ii) determining that the T cells of the subject can be used for an allogeneic T cell therapy if the Diagnostic Expression Score generated in step (b) is greater than the threshold score. In some embodiments, the Diagnostic Expression Score is generated by Z-score summation and the threshold score is 0.

[0018] In some embodiments, the method further comprises improving the subject's T cell functioning in T cell therapies. In some embodiments, improving the subject's T cell functioning in T cell therapies comprises inhibiting DNMT3A-mediated *de novo* DNA methylation and/or activating STAT5 signaling pathway in the subject's T cells.

[0019] In some embodiments, inhibiting DNMT3A-mediated *de novo* DNA methylation in the subject's T cells is achieved by inhibiting enzymatic activity of DNMT3A protein or making DNMT3A gene deleted or defective. In some embodiments, the enzymatic activity of the DNMT3A protein is inhibited by exposing the cell to a DNMT3A active site inhibitor. In some embodiments, the DNMT3A gene is mutated in DNMT3A catalytic domain so that the enzymatic activity of the DNMT3A protein is inhibited. In some embodiments, the level of functional DNMT3A protein in the cell is decreased by 50% or more.

[0020] In some embodiments, the STAT5 signaling pathway is activated by either stimulating the T cell with a signaling molecule or genetically modifying the T cell to express a signaling molecule. In some embodiments, the signaling molecule is a common gamma chain cytokine. In some embodiments, the cytokine is IL-15, IL-7, IL-2, IL-4, IL-9, or IL-21. In some embodiments, the STAT5 signaling pathway is activated by modifying the T cell to express a constitutively active cytokine receptor or a switch receptor. In some embodiments, the constitutively active cytokine receptor is a constitutively active IL7 receptor (C7R). In some embodiments, the switch receptor is an IL-4/IL-7 receptor or an IL-4/IL-2 receptor.

[0021] In various embodiments, improving the subject's T cell functioning as described herein is conducted *in vitro*.

[0022] In some embodiments, the method further comprises repeating the method described to determine if T cells can be used for an allogeneic T cell therapy on the subject's T cells which were treated to improve the subject's T cell functioning.

[0023] In some embodiments, if it is determined in step (c) that the T cells of the subject can be used for an allogeneic T cell therapy, the method further comprises using the subject's T cells for an allogeneic T cell therapy.

[0024] In various embodiments, methods described herein comprise obtaining a sample of T cells from the subject prior to step (a). In some embodiments, the sample of T cells is derived from blood, marrow, or tissue of the subject. In some embodiments, the subject has cancer and the sample of T cells is derived from a tumor of the subject.

[0025] In various embodiments, methods described herein comprise stimulating the T cells *in vitro* or *ex vivo* prior to step (a). In some embodiments, the T cells are stimulated using anti-CD3 and anti-CD28 stimulation.

[0026] In various embodiments, determining the gene expression level in step (a) comprises isolating mRNA from the T cells. In some embodiments, determining the gene expression level in step (a) is performed using mRNA sequencing, microarray gene expression profiling, or qPCR.

[0027] In various embodiments, methods described herein further comprise banking the subject's T cells.

[0028] In various embodiments, the DNMT3A target gene(s) is selected from the genes recited in Table 1.

[0029] In various embodiments, the DNMT3A target gene(s) is selected from the genes recited in Table 2.

[0030] In various embodiments, the DNMT3A target gene(s) is selected from the genes recited in Table 3.

[0031] In various embodiments, methods described herein comprise determining the expression level of 10 or more DNMT3A target genes in step (a). In some embodiments, the method comprises determining the expression level of RORA, EOMES, STAT1, EGR2, ASCL1, BACH2, E2F5, ZBTB16, IRF4, HIC1, BCL3, CBFA2T3, TRPS1, NFKBIA, EGR3, KLF7, TCF7, NR4A3, SETBP1, EGR1, MYB, TFAP2A, BCL6, LEF1, and NRIP1 genes in step (a).

[0032] In various embodiments, the T cell is selected from a CD8⁺ T cell, a CD4⁺ T cell, a cytotoxic T cell, an $\alpha\beta$ T cell receptor (TCR) T cell, a natural killer T (NKT) cell, a $\gamma\delta$ T cell, a memory T cell, a T-helper cell, and a regulatory T cell (Treg).

[0033] In various embodiments, the subject is human.

[0034] In various embodiments, the T cell therapy is a CAR T cell therapy. In various embodiments, the T cell therapy is an $\alpha\beta$ TCR therapy. In various embodiments, the T cell therapy is a $\gamma\delta$ TCR therapy. In various embodiments, the T cell therapy is an iNKT therapy. In various embodiments, the T cell therapy is a tumor-infiltrating lymphocyte (TIL) therapy. In various embodiments, the T cell therapy is an *in vitro* sensitized (IVS) T cell therapy. In various embodiments, the T cell therapy is an *in vivo* T cell therapy.

[0035] These and other aspects of the present invention will be apparent to those of ordinary skill in the art in the following description, claims and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] **Figure 1** is a plot showing the comparison of the expression score of DNMT3A target genes in the CAR T-cell products prior to infusion between patients with a Complete Response (CR), Partial Response (PR), Partial Response with Relapse (PRtd), and No Response (NR).

[0037] **Figure 2** is a plot showing the comparison of the expression score of DNMT3A target genes in the CAR T-cell products prior to infusion between patients who exhibited any type of response and no response.

[0038] **Figure 3** shows an outlier in the data from a patient with a Partial Response (PR).

[0039] **Figure 4** is a plot showing the comparison of expression score of DNMT3A target genes in the CAR T-cell products prior to infusion between patients with a Complete Response (CR), Partial Response (PR), Partial Response with Relapse (PRtd), and No Response (NR) with the “outlier” data point excluded. p value between NR and PRtd <0.05; p value between NR and CR <0.01; p value between NR and PR <0.01.

[0040] **Figure 5** is a plot showing the comparison of expression score of a limited list of target genes in the CAR T-cell products prior to infusion between patients who exhibited no response and any type of response.

[0041] **Figures 6A-6L** show the relative expression (Z-score) of a limited list of target genes in the CAR T-cell products prior to infusion between patients who exhibited no response and any type of response.

[0042] **Figures 7A-7L** show the absolute expression (log2 value) of a limited list of target genes in the CAR T-cell products prior to infusion between patients who exhibited no response and any type of response.

[0043] **Figure 8** is a plot showing the comparison of expression score of a limited list of target genes in the CAR T-cell products prior to infusion between patients with a Complete

Response (CR), Partial Response (PR), Partial Response with transformation to aggressive B-cell lymphoma (PRtd), and No Response (NR).

[0044] **Figure 9** is a plot showing the results of the Principal Component Analysis (PCA).

[0045] **Figure 10** is a plot showing the comparison of expression score of genes that were significantly upregulated in either Fourth Stimulation or Fifth Stimulation (or both) DNMT3A knockout CAR lines in the CAR T-cell products prior to infusion between patients with a Complete Response (CR), Partial Response (PR), Partial Response with transformation to aggressive B-cell lymphoma (PRtd), and No Response (NR).

[0046] **Figure 11** is a plot showing the comparison of expression score of genes that were significantly upregulated in either Fourth Stimulation or Fifth Stimulation (or both) DNMT3A knockout CAR lines and exhibited a significant methylation difference in the CAR T-cell products prior to infusion between patients with a Complete Response (CR), Partial Response (PR), Partial Response with transformation to aggressive B-cell lymphoma (PRtd), and No Response (NR). p value between PR and CR <0.05; p value between NR and CR = 0.057.

[0047] **Figure 12** shows the guide RNAs used to knockout DNMT3A. Guide 2 and guide 3 comprise the nucleotide sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

DETAILED DESCRIPTION

[0048] The present invention is based on an unexpected discovery that relatively higher levels of expression of certain genes such as genes which are methylation targets of DNA (cytosine-5)-methyltransferase 3A (DNMT3A) in a patient's T-cell products correlate with increased likelihood of such patient's responsiveness to T cell therapies. In some embodiments, the T cell gene expression signature comprises one or more genes which are methylation targets of DNMT3A. In one specific embodiment, such target genes of DNMT3A are selected from the genes provided in Table 1. In another specific embodiment, such target genes of DNMT3A are selected from the genes provided in Table 2. In yet another specific embodiment, such target genes of DNMT3A are selected from the genes provided in Table 3. In some embodiments, the T cell gene expression signature comprises at least 10 genes. In one specific embodiment, the T cell gene expression signature comprises the 25 genes listed in Table 3: namely RORA, EOMES, STAT1, EGR2, ASCL1, BACH2, E2F5, ZBTB16, IRF4, HIC1, BCL3, CBFA2T3, TRPS1, NFKBIA, EGR3, KLF7, TCF7, NR4A3, SETBP1, EGR1, MYB, TFAP2A, BCL6, LEF1, and NR1P1.

[0049] The gene expression signatures of the present invention may be useful for, for example but not limited to, (1) predicting individual patient's responsiveness to an autologous

CAR T cell therapy prior to initiation of such therapy; (2) determining if a given subject can be used as a T cell donor for allogeneic CAR T cell therapies (e.g., HaploCAR T cell therapy, using T cells obtained from a close relative [e.g., parents, siblings]; universal CAR T cell therapy, using T cells from a donor unrelated to the patient also known as “off-the-shelf” CAR T cell therapy); (3) determining if patient’s or donor’s T cells should be subject to additional treatment(s) to improve their functioning in CAR T cell therapies (such as but not limited to, inhibition of DNMT3A-mediated *de novo* DNA methylation [e.g., by inhibiting enzymatic activity of DNMT3A protein or making DNMT3A gene deleted or defective] and/or activation of STAT5 signaling pathway in the T cells); (4) determining if a CAR T cell therapy should be combined with other therapeutic agents or therapies (such as but not limited to, checkpoint blockade, enhanced expression of genes such as IL15, antiviral therapies, bone marrow transplant, chemotherapies, and any combinations thereof).

[0050] In certain embodiments, methods of the present invention include obtaining T cells and testing for potential utility in CAR T cell therapy before beginning any other therapies. The T cells may be banked even if they are not planned for use in CAR T cell therapy immediately.

[0051] While the DNMT3A score has been developed to predict efficacy of CAR T cells, it can be applicable to all other forms of T cell therapy in which T cells are obtained from a donor and manipulated for therapeutic intent *ex vivo*. It is applicable, since DNMT3A regulates transcriptional programs that prevent exhaustion in all T cells (Youngblood et al., Nature 2017; Abdelsamed et al., JEM 2017; Ghoneim et al., Cell 2017; each of which is hereby incorporated by reference in its entirety) and not only CAR T cells. Thus the score can be applicable to, for example but not limited to, cell therapies with conventional or genetically-modified $\alpha\beta$ TCR T cells, $\gamma\delta$ T cells, iNKT cells, or tumor infiltrating lymphocytes (TILs).

[0052] In some embodiments, the methods of the present disclosure may be carried out using one or more steps from the process described below.

[0053] (a) Obtaining T cells

[0054] A sample of the T cells being proposed for use in T cell product generation may be obtained from a subject. This could be obtained from peripheral blood (e.g. standard blood draw, leukapheresis, sorting of antigen-specific T cells [e.g. tetramer, pentamer, or streptamer sorting, IFN γ capture assay]) or a tumor biopsy (e.g. tumor infiltrating lymphocytes [TIL]). In addition, T cells could be generated from induced pluripotent stem (IPS) cells. T cells may be isolated using standard procedures that match those for T cell product preparation. T cells could

also be obtained during T cell product generation. Unstimulated T cells could be used for mRNA extraction (see step (c)) or stimulated prior to mRNA extraction as described in step (b).

[0055] (b) Stimulation of T cells

[0056] T cells may be stimulated *ex vivo* or *in vitro* using standard procedures known in the art, such as, e.g., anti-CD3 and anti-CD28 stimulation (e.g., using Gibco™ Dynabeads™ Human T-Activator CD3/CD28), PMA/Ionomycin stimulation, or stimulation with polyclonal stimulators such as Concanavalin A with or without cytokines such as IL2, IL7, and/or IL15. In addition, antigen presenting cells (APCs) such as dendritic cells or monocytes, or artificial APCs such as K562, genetically-modified to express HLA molecules, antigens, or immune stimulatory molecules may be used for T cell stimulation. Further, tumor cells or subcellular fractions of cells such as exomes may be used for T cell stimulation. As needed T cells may be expanded by adding additional cytokines such as IL2, IL7, and/or IL15 and/or repeating the entire stimulation procedure.

[0057] (c) mRNA extraction

[0058] mRNA may be extracted from the stimulated T cells for gene expression analysis. Methods of extraction of RNA are well known in the art and are described, for example, in Sambrook J., et al., “Molecular Cloning: A Laboratory Manual”, Second Ed. (Coldspring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, Volume 1, Chapter 7), which is incorporated herein by reference in its entirety.

[0059] (d) Gene expression analysis

[0060] Extracted mRNA may be subjected to gene expression analysis. Non-limiting examples of techniques that can be used for gene expression analysis include mRNA sequencing, microarray gene expression profiling, and qPCR.

[0061] (e) Evaluation of target genes

[0062] The expression of one or more target genes may be analyzed. In some embodiments, the target genes may be selected from the list of DNMT3A target genes provided in Table 1, Table 2 or Table 3.

[0063] In some embodiments, the T cell gene expression signature comprises at least about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 110, about 115, about 120, about 125, about 130, about 140, about 150, about 160, about 170, about 180, about 190, or about 200 genes selected from the genes provided in Table 1. In some embodiments, the T cell gene expression signature comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,

42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 115, 120, 125, 130, 135, 140, 145, 150, 160, 170, 180, 190, 200 or more genes selected from the genes provided in Table 1.

[0064] In some embodiments, the T cell gene expression signature comprises at least about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, or about 100 genes selected from the genes provided in Table 2. In some embodiments, the T cell gene expression signature comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, or 107 genes selected from the genes provided in Table 2.

[0065] In some embodiments, the T cell gene expression signature comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 genes selected from the genes provided in Table 3.

[0066] (f) Generation of Expression Score

[0067] Expression levels of the target genes may be compared to known responders and non-responders in a reference dataset to generate an Expression Score. The “Expression Score” can refer to a summation of absolute, weighted, or relative gene expression values that is calculated and interpreted relative to the reference dataset. For example, the reference dataset may comprise known responders and non-responders from publicly available expression data (e.g., Fraietta et al., Nature Medicine 2018 May;24(5):563-57, which is incorporated herein by reference in its entirety); this reference dataset may be expanded to include data from additional trials or may be changed entirely to provide disease-specific points of comparison or to further refine the predictive value of the Expression Score. Using one or more of the target genes, a set of Reference Expression Scores can be generated from the reference dataset, for example by summing the Z-scores of the expression of those genes, which provides a range of scores that overlaps known responders and known non-responders. A Diagnostic Expression Score can then be generated for a sample of interest by calculating and summing absolute or weighted gene expression values for comparison to the Reference Expression Scores, or by calculating and summing relative gene expression values relative to the variation in expression observed within the reference dataset. This process thereby provides a diagnostic score based on known

patterns of diagnostic outcomes with regard to specific genes (which are identified herein) underlying the mechanisms associated with those outcomes.

[0068] (g) Data interpretation

[0069] The expression score may be interpreted in relative terms: e.g., higher is better, lower is worse. Higher means overall more expression of genes (for expression scores based on Z-score summations specifically, higher than average across the reference dataset) whereas lower means overall lower expression of genes (for expression scores based on Z-score summations specifically, lower than average across the reference dataset).

[0070] Thresholds for clinical recommendations can be created. One exemplary set of thresholds for Z-score based summations may be: i) expression score less than zero indicates low chance of clinical response; and ii) expression score greater than zero indicates high chance of clinical response or poised T cells. In this example, when the score equals zero it indicates that the cumulative expression of the genes is “average” among the reference dataset. In the case that the expression score is based on absolute or weighted summations of expression values, the threshold can be set based on the observed delineations between known responders and non-responders. It is to be understood that the thresholds may be adjusted as additional comparison data become available.

[0071] (h) Clinical recommendations

[0072] General and/or specific clinical recommendations can be made based on the patient's expression score relative to the thresholds outlined above in the context of other patient-specific information. Some of these clinical suggestions may be predictive of a time in the future when T cell therapy is integrated to standard clinical practice as opposed to a last resort.

[0073] When a low chance of clinical response is indicated (e.g., by a relative diagnostic expression score less than zero): (1) If the patient has not previously received other therapies, T cells may be banked but alternative therapies before T cell therapy should be attempted. New T cell samples may be obtained and banked intermittently to re-assess for any changes. The complete or partial effectiveness of alternative therapies may make room for the return of appropriately poised T cells, which can be assessed and banked in the event of a future relapse (e.g., antiviral therapies, bone marrow transplant, or chemotherapy may allow for T cell recuperation). (2) If the patient has experienced repeated failures of alternative therapies, alternative approaches should be considered which include but are not limited to: i) T cell therapy with additional genomic engineering (e.g., DNMT3A knockout, transgenic expression of IL15, or other known or as-of-yet unknown alterations that can increase long-lived effector potential of engineered T cells); ii) combination therapy (e.g., T cell therapy with the addition

of checkpoint blockade); iii) HaploCAR therapy, using expression-score tested T cells obtained from a close relative (e.g., parent, sibling); and iv) “off-the-shelf” CAR T cell therapy (e.g., using T cells from a donor unrelated to the patient).

[0074] When a high chance of clinical response is indicated (e.g., by a relative diagnostic expression score greater than zero): (1) T cells may be banked for future production of the therapeutic T cell product even if T cell therapy is not considered as initial therapy, because alternative therapies may impact the potential utility of the patient’s T cells in the future when the generation of a therapeutic T cell therapy may become necessary; (2) Use of these T cells for T cell therapy can be considered in place of other therapies (e.g., chemotherapies, antiviral therapies) in order to reduce treatment-based side effects.

[0075] In additional embodiments, methods of the present invention involve determining the methylation status at the promoter of the target genes. Promoter methylation may be indicative of the gene expression levels.

Definitions

[0076] The term “immune effector cell” as used herein refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Non-limiting examples of immune effector cells include T cells (e.g., $\alpha\beta$ T cells and $\gamma\delta$ T cells), B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes. Stem cells, such induced pluripotent stem cells (iPSCs), that are capable of differentiating into immune cells are also included here.

[0077] The terms “T cell” and “T lymphocyte” are interchangeable and used synonymously herein. As used herein, T cell includes thymocytes, naive T lymphocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a CD8⁺ T cell, a CD4⁺ T cell, a helper T cell or T-helper cell (HTL; CD4⁺ T cell), a cytotoxic T cell (CTL; CD8⁺ T cell), a tumor infiltrating cytotoxic T cell (TIL; CD8⁺ T cell), CD4⁺CD8⁺ T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naive T cells and memory T cells. Also included are “ $\alpha\beta$ T cell receptor (TCR) T cells”, which refer to a population of T cells that possess a TCR composed of α - and β -TCR chains. Also included are “NKT cells”, which refer to a specialized population of T cells that express a semi-invariant $\alpha\beta$ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include NK1.1⁺ and NK1.1⁻, as well as CD4⁺, CD4⁻, CD8⁺ and CD8⁻ cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the

MHC I-like molecule CD Id. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance. Also included are “gamma-delta T cells ($\gamma\delta$ T cells),” which refer to a specialized population that to a small subset of T cells possessing a distinct TCR on their surface, and unlike the majority of T cells in which the TCR is composed of two glycoprotein chains designated α - and β -TCR chains, the TCR in $\gamma\delta$ T cells is made up of a γ -chain and a δ -chain. $\gamma\delta$ T cells can play a role in immunosurveillance and immunoregulation, and were found to be an important source of IL-17 and to induce robust CD8⁺ cytotoxic T cell response. Also included are “regulatory T cells” or “Tregs”, which refer to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. Tregs cells are typically transcription factor Foxp3-positive CD4⁺T cells and can also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing CD4⁺ T cells.

[0078] The terms “natural killer cell” and “NK cell” are used interchangeable and used synonymously herein. As used herein, NK cell refers to a differentiated lymphocyte with a CD16⁺ CD56⁺ and/or CD57⁺ TCR- phenotype. NKs are characterized by their ability to bind to and kill cells that fail to express “self” MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

[0079] The term “signaling molecule” as used herein, refers to any molecule that is capable of inducing a direct or indirect response in at least one cellular signaling pathway. The response may be stimulatory or inhibitory. One of the cellular signaling pathways may be the STAT5 signaling pathway.

[0080] The term “switch receptor” used herein refers to a receptor that is capable of converting a potentially inhibitory signal into a positive signal. Switch receptors are also known as inverted cytokine receptors.

[0081] The term “chimeric antigen receptor” or “CAR” as used herein is defined as a cell-surface receptor comprising an extracellular target-binding domain, a transmembrane domain and a cytoplasmic domain, comprising a lymphocyte activation domain and optionally at least one co-stimulatory signaling domain, all in a combination that is not naturally found together on a single protein. This particularly includes receptors wherein the extracellular domain and the cytoplasmic domain are not naturally found together on a single receptor protein. The chimeric antigen receptors of the present invention are intended primarily for use with lymphocyte such as T cells and natural killer (NK) cells.

[0082] As used herein, the term “antigen” refers to any agent (e.g., protein, peptide, polysaccharide, glycoprotein, glycolipid, nucleic acid, portions thereof, or combinations thereof) molecule capable of being bound by a T-cell receptor. An antigen is also able to provoke an immune response. An example of an immune response may involve, without limitation, antibody production, or the activation of specific immunologically competent cells, or both. A skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components, organisms, subunits of proteins/antigens, killed or inactivated whole cells or lysates.

[0083] The term “antigen-binding moiety” refers to a target-specific binding element that may be any ligand that binds to the antigen of interest or a polypeptide or fragment thereof, wherein the ligand is either naturally derived or synthetic. Examples of antigen-binding moieties include, but are not limited to, antibodies; polypeptides derived from antibodies, such as, for example, single chain variable fragments (scFv), Fab, Fab', F(ab')₂, and Fv fragments; polypeptides derived from T Cell receptors, such as, for example, TCR variable domains; secreted factors (e.g., cytokines, growth factors) that can be artificially fused to signaling domains (e.g., “zytokines”); and any ligand or receptor fragment (e.g., CD27, NKG2D) that binds to the antigen of interest. Combinatorial libraries could also be used to identify peptides binding with high affinity to the therapeutic target.

[0084] The terms “antibody” and “antibodies” refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), intrabodies, minibodies, diabodies and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antigen-specific TCR), and epitope-binding fragments of any of the above. The terms “antibody” and “antibodies” also refer to covalent diabodies such as those disclosed in U.S. Pat. Appl. Pub. 2007/0004909 and Ig-DARTS such as those disclosed in U.S. Pat. Appl. Pub. 2009/0060910, each of which are incorporated by reference in their entirety for all purposes. Antibodies useful as a TCR-binding molecule include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1 and IgA2) or

subclass. Also included are “bispecific antibodies”, which refer to antibodies that are capable of binding to two different antigens or different epitopes of the same antigen.

[0085] The term “host cell” means any cell that contains a heterologous nucleic acid. The heterologous nucleic acid can be a vector (*e.g.*, an expression vector). For example, a host cell can be a cell from any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme. An appropriate host may be determined. For example, the host cell may be selected based on the vector backbone and the desired result. By way of example, a plasmid or cosmid can be introduced into a prokaryote host cell for replication of several types of vectors. Bacterial cells such as, but not limited to DH5 α , JM109, and KCB, SURE® Competent Cells, and SOLOPACK Gold Cells, can be used as host cells for vector replication and/or expression. Additionally, bacterial cells such as *E. coli* LE392 could be used as host cells for phage viruses. Eukaryotic cells that can be used as host cells include, but are not limited to yeast (*e.g.*, YPH499, YPH500 and YPH501), insects and mammals. Examples of mammalian eukaryotic host cells for replication and/or expression of a vector include, but are not limited to, HeLa, NIH3T3, Jurkat, 293, COS, CHO, Saos, and PC12.

[0086] Host cells of the present invention include T cells and natural killer cells that contain the DNA or RNA sequences encoding the CAR and express the CAR on the cell surface. Host cells may be used for enhancing T cell activity, natural killer cell activity, treatment of cancer, and treatment of autoimmune disease.

[0087] The terms “activation” or “stimulation” means to induce a change in their biologic state by which the cells (*e.g.*, T cells and NK cells) express activation markers, produce cytokines, proliferate and/or become cytotoxic to target cells. All these changes can be produced by primary stimulatory signals. Co-stimulatory signals can amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity. A “co-stimulatory signal” refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell and/or NK cell proliferation and/or upregulation or downregulation of key molecules.

[0088] The term “proliferation” refers to an increase in cell division, either symmetric or asymmetric division of cells. The term “expansion” refers to the outcome of cell division and cell death.

[0089] The term “differentiation” refers to a method of decreasing the potency or proliferation of a cell or moving the cell to a more developmentally restricted state.

[0090] The terms “express” and “expression” mean allowing or causing the information in a gene or DNA sequence to become produced, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an “expression product” such as a protein. The expression product itself, e.g., the resulting protein, may also be said to be “expressed” by the cell. An expression product can be characterized as intracellular, extracellular or transmembrane.

[0091] The term “transfection” means the introduction of a “foreign” (*i.e.*, extrinsic or extracellular) nucleic acid into a cell using recombinant DNA technology. The term “genetic modification” means the introduction of a “foreign” (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a “cloned” or “foreign” gene or sequence, may include regulatory or control sequences operably linked to polynucleotide encoding the chimeric antigen receptor, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been “genetically engineered.” The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or from a different genus or species.

[0092] The term “transduction” means the introduction of a foreign nucleic acid into a cell using a viral vector.

[0093] The terms “genetically modified” or “genetically engineered” refers to the addition of extra genetic material in the form of DNA or RNA into a cell.

[0094] As used herein, the term “derivative” in the context of proteins or polypeptides (e.g., CAR constructs or domains thereof) refer to: (a) a polypeptide that has at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to the polypeptide it is a derivative of; (b) a polypeptide encoded by a nucleotide sequence that has at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to a nucleotide sequence encoding the polypeptide it is a derivative of; (c) a polypeptide that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid mutations (*i.e.*, additions, deletions and/or substitutions) relative to the polypeptide it is a derivative of; (d) a polypeptide encoded by nucleic acids can hybridize under high, moderate or typical stringency hybridization conditions to nucleic acids encoding the

polypeptide it is a derivative of; (e) a polypeptide encoded by a nucleotide sequence that can hybridize under high, moderate or typical stringency hybridization conditions to a nucleotide sequence encoding a fragment of the polypeptide, it is a derivative of, of at least 20 contiguous amino acids, at least 30 contiguous amino acids, at least 40 contiguous amino acids, at least 50 contiguous amino acids, at least 75 contiguous amino acids, at least 100 contiguous amino acids, at least 125 contiguous amino acids, or at least 150 contiguous amino acids; or (f) a fragment of the polypeptide it is a derivative of.

[0095] Percent sequence identity can be determined using any method known to one of skill in the art. In a specific embodiment, the percent identity is determined using the “Best Fit” or “Gap” program of the Sequence Analysis Software Package (Version 10; Genetics Computer Group, Inc., University of Wisconsin Biotechnology Center, Madison, Wisconsin). Information regarding hybridization conditions (*e.g.*, high, moderate, and typical stringency conditions) have been described, see, *e.g.*, U.S. Patent Application Publication No. US 2005/0048549 (*e.g.*, paragraphs 72-73).

[0096] The terms “vector”, “cloning vector” and “expression vector” mean the vehicle by which a DNA or RNA sequence (*e.g.*, a foreign gene) can be introduced into a host cell, so as to genetically modify the host and promote expression (*e.g.*, transcription and translation) of the introduced sequence. Vectors include plasmids, synthesized RNA and DNA molecules, phages, viruses, etc. In some embodiments, the vector is a viral vector such as, but not limited to, viral vector is an adenoviral, adeno-associated, alphaviral, herpes, lentiviral, retroviral, baculoviral, or vaccinia vector.

[0097] The term “regulatory element” refers to any *cis*-acting genetic element that controls some aspect of the expression of nucleic acid sequences. In some embodiments, the term “promoter” comprises essentially the minimal sequences required to initiate transcription. In some embodiments, the term “promoter” includes the sequences to start transcription, and in addition, also include sequences that can upregulate or downregulate transcription, commonly termed “enhancer elements” and “repressor elements”, respectively.

[0098] As used herein, the term “operatively linked,” and similar phrases, when used in reference to nucleic acids or amino acids, refer to the operational linkage of nucleic acid sequences or amino acid sequence, respectively, placed in functional relationships with each other. For example, an operatively linked promoter, enhancer elements, open reading frame, 5' and 3' UTR, and terminator sequences result in the accurate production of a nucleic acid molecule (*e.g.*, RNA). In some embodiments, operatively linked nucleic acid elements result in the transcription of an open reading frame and ultimately the production of a polypeptide

(i.e., expression of the open reading frame). As another example, an operatively linked peptide is one in which the functional domains are placed with appropriate distance from each other to impart the intended function of each domain.

[0099] By “enhance” or “promote,” or “increase” or “expand” or “improve” refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a greater physiological response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A measurable physiological response may include an increase in T cell expansion, activation, effector function, persistence, and/or an increase in antitumor activity (e.g., cancer cell death killing ability), among others apparent from the understanding in the art and the description herein. In some embodiments, an “increased” or “enhanced” amount can be a “statistically significant” amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response produced by vehicle or a control composition.

[00100] By “decrease” or “lower,” or “lessen,” or “reduce,” or “abate” refers generally to the ability of composition contemplated herein to produce, elicit, or cause a lesser physiological response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. In some embodiments, a “decrease” or “reduced” amount can be a “statistically significant” amount, and may include a decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response (reference response) produced by vehicle, a control composition, or the response in a particular cell lineage.

[00101] The terms “inhibit” or “inhibition” as used herein refer to reducing a function or activity to an extent sufficient to achieve a desired biological or physiological effect. Inhibition may be complete or partial.

[00102] The terms “treat” or “treatment” of a state, disorder or condition include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition, but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least

one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[00103] The term “effective” applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular drug or drugs employed, the mode of administration, and the like.

[00104] The phrase “pharmaceutically acceptable”, as used in connection with compositions described herein, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, a human). Preferably, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[00105] The term “protein” is used herein encompasses all kinds of naturally occurring and synthetic proteins, including protein fragments of all lengths, fusion proteins and modified proteins, including without limitation, glycoproteins, as well as all other types of modified proteins (*e.g.*, proteins resulting from phosphorylation, acetylation, myristoylation, palmitoylation, glycosylation, oxidation, formylation, amidation, polyglutamylation, ADP-ribosylation, pegylation, biotinylation, etc.).

[00106] The terms “nucleic acid”, “nucleotide”, and “polynucleotide” encompass both DNA and RNA unless specified otherwise. By a “nucleic acid sequence” or “nucleotide sequence” is meant the nucleic acid sequence encoding an amino acid, the term may also refer to the nucleic acid sequence including the portion coding for any amino acids added as an artifact of cloning, including any amino acids coded for by linkers

[00107] The terms “patient”, “individual”, “subject”, and “animal” are used interchangeably herein and refer to mammals, including, without limitation, human and veterinary animals (*e.g.*, cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models. In a preferred embodiment, the subject is a human.

[00108] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water

and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[00109] Singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[00110] The term "about" or "approximately" includes being within a statistically meaningful range of a value. Such a range can be within an order of magnitude, preferably within 50%, more preferably within 20%, still more preferably within 10%, and even more preferably within 5% of a given value or range. The allowable variation encompassed by the term "about" or "approximately" depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art.

[00111] The practice of the present invention employs, unless otherwise indicated, conventional techniques of statistical analysis, molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such tools and techniques are described in detail in e.g., Sambrook et al. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York; Ausubel et al. eds. (2005) *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc.: Hoboken, NJ; Bonifacino et al. eds. (2005) *Current Protocols in Cell Biology*. John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) *Current Protocols in Immunology*, John Wiley and Sons, Inc.: Hoboken, NJ; Coico et al. eds. (2005) *Current Protocols in Microbiology*, John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) *Current Protocols in Protein Science*, John Wiley and Sons, Inc.: Hoboken, NJ; and Enna et al. eds. (2005) *Current Protocols in Pharmacology*, John Wiley and Sons, Inc.: Hoboken, NJ. Additional techniques are explained, e.g., in U.S. Patent No. 7,912,698 and U.S. Patent Appl. Pub. Nos. 2011/0202322 and 2011/0307437.

[00112] The technology illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein.

[00113] The terms and expressions which have been employed are used as terms of description and not of limitation, and use of such terms and expressions do not exclude any equivalents of the features shown and described or portions thereof, and various modifications are possible within the scope of the technology claimed.

EXAMPLES

[00114] The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing from the invention in spirit or in scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

EXAMPLE 1. Gene expression signature with complete list of DNMT3A targets

[00115] To demonstrate the usefulness of the developed gene expression signature, a publicly available gene expression dataset was analyzed which was collected from CD19-CAR T-cell products that were used in a clinical study of 41 patients with chronic lymphocytic leukemia (CLL). See Fraietta et al., Nature Medicine 2018 May;24(5):563-57, which is incorporated herein by reference in its entirety. For 34 out of 41 patients gene expression data were available.

[00116] The dataset analyzed from the Fraietta et al. publication consists of RNAseq data from stimulated CTL019 infusion products for patients who either exhibited a Complete Response to therapy (CR, n = 5), exhibited a Partial Response (PR, n = 5), exhibited a Partial Response followed by a relapse that had transformed into aggressive B cell lymphoma (PRtd, n = 3), or exhibited No Response (NR, n = 21). The median peak expansion (MPE) of CAR T cells in these 4 groups of patients was 58,570 (CR), 13,257 (PR), 130,258 (PRtd), and 205 (NR).

[00117] Transcript counts were obtained from Fraietta et al. 2018 “Supplemental Table 5b: Transcriptomic profiling of CAR-stimulated CTL019 infusion products” and filtered, normalized, and analyzed using the R packages ‘edgeR’ (Robinson MD et al., Bioinformatics. 2010;26(1):139-40) and ‘limma’ (Ritchie ME et al., Nucleic Acids Res. 2015;43(7):e47).

[00118] The target genes identified from DNMT3A-knockout CAR T cells (herein referred to as “DNMT3A targets”, listed in Table 1) were assessed. The DNMT3A targets were identified

using whole genome DNA methylation profiling. Whole genome DNA methylation profiling was performed using CD8 T cells from two independent wild-type (WT) vs DNMT3A knockout CAR T cell co-culture experiments. During these experiments, CAR T cells were continually re-cultured with fresh tumor cells every week. After the WT T cells became terminally differentiated, whole genome methylation profiling was performed to identify DNMT3A-associated differences in methylation profiles. The two experiments had different receptors, further ensuring that the differentially methylated regions (DMRs) identified were related to T cell biology and not the receptors. From these two datasets DMRs that were exactly shared (the same genomic coordinates) between the two experiments were selected. DMRs were then assigned to the nearest genes. This list of genes was then used for the analyses to assess for an association between responder and non-responder CAR T cell gene expression data. The selection criteria for the list was considered very stringent as only DMRs that were exactly shared among the two experiments were used. 1,033 gene identifiers matched the 1,298 previously identified DNMT3A targets and were used to calculate a relative DNMT3A-target expression score.

[00119] A relative DNMT3A-target expression score was calculated, and each gene's log₂-expression was standardized to represent its mean-centered variation in order to equalize the weights of genes that were relatively highly or lowly expressed across the dataset. The expression score was then calculated as the sum of those normalized expression values. In subsequent Examples this score was also calculated using a limited gene set that either included only those differentially expressed genes (DGEs) between *in vitro* DNMT3A knockout and wildtype cells (as assayed by Affymetrix Clariom S Human microarray; WT N = 3, Knockout N = 8) or the intersection between these DGEs and the previously identified DNMT3A targets. The nonparametric Kruskal Wallis test and Mann-Whitney U test were used to assess significant variation across the patient outcomes defined by the originating study, and plots were generated with ggplot2 (Wickham H., Springer; 2016).

Table 1. Human DNMT3A target genes

AAK1	DOK3	LOC100130298	PRTFDC1	TREML2
ABHD2	DPEP2	LOC100132735	PSMG1	TRERF1
ABLIM1	DPF3	LOC100288798	PTCD3	TRIB1
ACOXL	DPP6	LOC100288911	PTEN	TRIM46
ACSF3	DPYD	LOC100289473	PTGER4	TRIO
ACSL1	DTNB	LOC100505478	PTK2B	TRPC6
ACSL5	DTX2	LOC100505530	PTPN18	TRPM8
ACSL6	DUSP14	LOC100505658	PTPN6	TRPS1

ACTB	DYRK1A	LOC100506178	PTPRC	TSC22D2
ACTL9	E2F5	LOC100996263	PTPRJ	TSHR
ACTN1	ECE1	LOC100996286	PTPRK	TSHZ2
ACTR2	EFCAB11	LOC100996291	PTTG1IP	TSNAX-DISC1
ACVR2A	EGR1	LOC101060498	PUDP	TSPAN14
ADAM19	EGR2	LOC101926941	PUM3	TSPAN17
ADAM6	EGR3	LOC101927539	PVRL3	TSPAN2
ADAMTS10	ELK3	LOC101927543	PVT1	TSPAN5
ADAP1	ELMO1	LOC101927630	PWP2	TSSC1
ADARB1	ELMSAN1	LOC101927637	PXN	TTC34
ADCY7	EMC8	LOC101927817	PYGB	TTC39C
ADD3	EMX2OS	LOC101927851	R3HDM1	TTC7A
ADGRE5	ENPP7P13	LOC101927865	RAB11FIP4	TTC9
ADGRG1	EOMES	LOC101928100	RAB28	TUSC5
ADORA2A	EPB41	LOC101928794	RAB37	TXK
ADRA2B	EPHB1	LOC101929076	RAB8B	UBAC2
AEBP2	EPS15L1	LOC101929241	RAD51B	UBAP2L
AGFG1	ERGIC1	LOC101929331	RAI1	UBE2B
AGO2	ERI1	LOC101929378	RALGDS	UBE2E1
AGPAT3	ERI3	LOC101929406	RALGPS1	UBE2E1-AS1
AGTPBP1	ERICH1	LOC101929452	RAMP1	UBE2G2
AGTRAP	ESYT2	LOC101929551	RANBP3	UBE2H
AHNAK	ETS1	LOC101929567	RAPGEF1	UCP2
AHRR	ETV6	LOC101929698	RAPGEF6	UHRF1BP1
AIM1	EVL	LOC102546299	RARA-AS1	ULK4
AKAP11	EXOC2	LOC102723854	RARG	UPF2
AKAP13	EXOC4	LOC102724511	RASA3	URGCP-MRPS24
AKAP2	EYA2	LOC102724539	RASGRF2	USP10
AMFR	EZH1	LOC102724699	RASGRP2	USP12
AMZ1	EZR	LOC103091866	RASGRP3	USP20
ANAPC1	F11R	LOC152225	RASSF3	USP3
ANAPC16	FAM102A	LOC220729	RB1	USP35
ANKRD11	FAM107B	LOC285847	RBM33	UTRN
ANKRD33B	FAM110A	LOC389033	RBM38	VAC14
ANKRD44	FAM134B	LOC442497	RBMS1	VAMP4
ANKRD53	FAM13A	LPGAT1	RBPJ	VAMP5
ANXA2P3	FAM150B	LPIN1	RCAN3	VAV3
ANXA6	FAM178B	LPIN2	RCSD1	VAV3-AS1
ANXA9	FAM193A	LPP	RDH10-AS1	VGLL4
AOAH	FAM47A	LPP-AS2	REC8	VOPP1
AP1M1	FAM53B	LRCH1	REEP3	VPS37B
AP1S3	FAM65B	LRIG1	RERE	VPS45
AP2A2	FAM71A	LRMP	REV1	VPS53
AP2B1	FAM72A	LRRC41	RFC2	VWF
APBA2	FAM73A	LRRC8C	RFC3	WASF2
APBB1	FAM76B	LRRC8D	RFFL	WBP1L
APBB1IP	FBLN5	LRRFIP1	RGCC	WDFY2
APH1B	FBXL14	LRRK1	RGPD3	WDR1
APLP2	FBXW11	LRRN2	RGS1	WDR72
AQP3	FBXW7	LSP1	RGS10	WIPF1
ARFGEF2	FCGBP	LTBP3	RGS3	WISP3
ARHGAP10	FCMR	LTC4S	RGS6	WT1
ARHGAP15	FCN3	LUZP1	RHBDF2	WWOX

ARHGAP22	FDFT1	LY86	RHOH	XBP1
ARHGAP31	FES	LY86-AS1	RHOT1	XYLT1
ARHGEF1	FFAR2	LY9	RILPL1	YWHAE
ARHGEF12	FGD3	LYN	RIN1	ZAP70
ARHGEF18	FGF17	LZTFL1	RIN3	ZBP1
ARHGEF2	FGGY	LZTS1	RMI2	ZBTB16
ARID1B	FIP1L1	LZTS1-AS1	RNF157	ZBTB34
ARID3A	FIRRE	MAB21L3	RNF216	ZC3H3
ARID5A	FKBP5	MACROD2	RNF4	ZCCHC2
ARID5B	FLI1	MAD1L1	RNF44	ZDHHC14
ARL3	FLJ21408	MAEA	RORA	ZEB2
ARL4C	FLJ22447	MAF	RPL34	ZFH3
ARRB1	FLJ45079	MALT1	RPL34-AS1	ZFP36L2
ARX	FLOT1	MAML2	RPS6KA1	ZFX
ASAP1	FNBP1	MAML3	RPS9	ZFYVE21
ASCC1	FOSL2	MAN1C1	RPTOR	ZFYVE28
ASCL1	FOXB1	MANEA-AS1	RREB1	ZHX2
ASIC2	FOXD2-AS1	MAP3K1	RRN3P2	ZHX3
ASPM	FOKK1	MAP3K4	RSBN1L	ZIC3
ASXL1	FOXN3	MAP3K7	RSPH9	ZMAT4
ATG5	FOXO1	MAP4K4	RTN4	ZMIZ1
ATM	FOXO3	MAPK14	RTN4RL1	ZMPSTE24
ATP10A	FOXP1	MAPKAP1	RUNX1	ZNF124
ATP1A1	FOXP1-AS1	MAPKBP1	RUNX2	ZNF217
ATP7A	FOXR1	MAPRE1	RUNX3	ZNF318
ATP8A1	FUNDC2P2	MAPRE2	S1PR1	ZNF335
ATP8B1	FUT7	MARK2	SAE1	ZNF395
ATP8B2	FXYD2	MATN1	SALRNA3	ZNF414
ATXN1	FYB	MBOAT1	SAMHD1	ZNF438
ATXN7	FYN	MBP	SAR1A	ZNF445
AUTS2	G3BP2	MBTD1	SARAF	ZNF496
B3GNT2	GALM	MBTPS1	SART3	ZNF609
B3GNTL1	GALNT10	MCTP2	SATB1	ZNF683
B4GALT4	GALNT2	MDM4	SATB1-AS1	ZNF775
B9D2	GALNT6	MDS2	SCARB1	ZNF831
BACH2	GAS7	MEAT6	SCML4	ZNF862
BAHD1	GATA3	MED12L	SDHA	ZNRF1
BANP	GATAD2A	MED15	SDK1	
BATF	GFOD1	MED7	SDK2	
BATF3	GIMAP4	MEF2A	SEC14L1	
BBC3	GIT2	MELK	SELL	
BCAR3	GLB1	METTL7A	SEMA3E	
BCAS4	GLRX	MEX3C	SEMA4B	
BCAT1	GLTSCR1	MFHAS1	SEMA4D	
BCKDHB	GLTSCR1L	MFSD2A	SERINC5	
BCL11B	GNAI3	MGAT4A	SERP2	
BCL2	GNAQ	MGAT5	SERPINE2	
BCL2L13	GOLGA5	MGLL	SERTAD2	
BCL2L14	GPD2	MICAL2	SESN2	
BCL3	GPR132	MIEN1	SETBP1	
BCL6	GPR55	MINK1	SETD2	
BCL9	GPR65	MIR10A	SFMBT2	
BCL9L	GRAMD4	MIR1208	SFSWAP	

BCOR	GRAP2	MIR1254-2	SFXN1	
BCR	GRB2	MIR133A2	SGCA	
BFSP2	GRIK3	MIR138-2	SGK1	
BID	GRK5	MIR181A1HG	SGK223	
BIN1	GRK6	MIR202	SGMS1	
BIN3	GSE1	MIR24-2	SGSM3	
BIRC2	H3F3C	MIR3134	SH2B3	
BMPRI1A	HADH	MIR31HG	SH3BP2	
BORCS5	HDAC4	MIR3201	SH3BP5	
BRD4	HDAC7	MIR4276	SH3PXD2A	
BRDTP1	HDGFRP3	MIR4425	SH3RF2	
BRF1	HECA	MIR4426	SH3TC1	
BRINP3	HERC1	MIR4433A	SIK1	
BTBD11	HERC2P9	MIR4435-2	SIK3	
BTLA	HGSNAT	MIR4435-2HG	SIL1	
C10orf128	HIC1	MIR4471	SKI	
C10orf54	HIF1A	MIR4487	SKIDA1	
C10orf55	HIPK1	MIR4492	SLC11A2	
C11orf63	HIPK2	MIR4493	SLC12A7	
C12orf65	HIVEP2	MIR4494	SLC12A8	
C12orf80	HIVEP3	MIR4632	SLC1A2	
C14orf177	HK1	MIR466	SLC20A1	
C14orf180	HMGB1	MIR4680	SLC24A2	
C15orf39	HMGXB4	MIR4708	SLC25A12	
C15orf53	HOXB-AS3	MIR4779	SLC25A25	
C15orf62	HOXB3	MIR5093	SLC25A33	
C18orf25	HPCAL1	MIR5095	SLC25A44	
C18orf42	HRASLS2	MIR5189	SLC30A7	
C19orf33	HS3ST4	MIR548AN	SLC37A1	
C1orf162	HSBP1L1	MIR650	SLC38A1	
C1QTNF3-AMACR	HTRA4	MIR6764	SLC3A1	
C1QTNF4	ICA1	MIR6785	SLC7A5P1	
C1QTNF6	ICAM2	MIR8086	SLC7A6	
C20orf27	ICOS	MKI67	SLCO3A1	
C2orf48	ID2	MKL1	SLCO4C1	
C3orf17	ID2-AS1	MKLN1	SLFN12	
C3orf18	IFFO2	MLANA	SLFN12L	
C4orf22	IFITM1	MLLT3	SMAD3	
C4orf47	IFITM3	MLXIP	SMARCA2	
C7orf72	IFITM5	MMP14	SMARCB1	
CA6	IFNAR2	MPZL3	SMG1P2	
CABIN1	IFNGR1	MRPS5	SMPD1	
CABLES1	IGSF9B	MRPS6	SMPD3	
CACNA2D3	IKBKE	MSH3	SMU1	
CAMK2G	IKZF1	MSI2	SNAP47	
CAMK4	IKZF3	MSL3	SND1	
CAMKK2	IL10	MSN	SNHG5	
CAPZB	IL18RAP	MTA1	SNRK	
CARD11	IL1RAPL2	MTDH	SNX9	
CARNS1	IL21R	MTM1	SOCs1	
CBFA2T3	IL2RA	MTSS1	SOCs2	
CBL	IL3	MUT	SORCS2	

CBLB	IL31RA	MVB12B	SORL1	
CBLN4	IL6	MVP	SPAG4	
CBX5	IL6R	MYB	SPAG9	
CCDC109B	IL6ST	MYEOV	SPANXN3	
CCDC150	IL7R	MYH9	SPATA13	
CCDC57	INF2	MYO18A	SPATA3	
CCDC66	INPP5A	MYO1A	SPATA5	
CCDC69	IQCD	MYO3B	SPECC1L- ADORA2A	
CCDC88C	IQCE	N4BP2	SPEF2	
CCND2-AS1	IQGAP2	NAA60	SPEN	
CCND3	IQSEC1	NABP1	SPOCD1	
CCR4	IRF4	NARF	SPOCK2	
CCR7	IRF5	NAV2	SPPL3	
CD101	IRX3	NBPF8	SPRED2	
CD200	ISG20	NCK2	SPRY1	
CD226	ISL2	NCOA2	SPTBN1	
CD244	ISM1	NCOR2	SPTLC2	
CD247	ITGA4	NDFIP1	SREBF2	
CD27	ITGA6	NDRG1	SRGN	
CD28	ITGAE	NEDD9	SRP14	
CD300A	ITGB1	NEK6	SRP14-AS1	
CD34	ITK	NELL2	SSBP3	
CD47	ITM2B	NET1	SSBP4	
CD48	ITM2C	NEURL3	SSC4D	
CD5	ITPK1	NFATC1	SSSCA1	
CD6	ITPKB	NFATC2	ST3GAL1	
CD70	ITPKB-IT1	NFKBIA	ST3GAL2	
CD79A	ITPR1	NIN	ST3GAL3	
CD8A	JADE2	NLK	ST3GAL5	
CD8B	JAK1	NLRC5	ST6GAL1	
CDC45	JAKMIP1	NME4	ST7	
CDH19	JAML	NMRK1	ST8SIA4	
CDH23	JARID2	NMT1	ST8SIA6	
CDHR3	JAZF1	NOL4L	STAG3	
CDK17	JHDM1D-AS1	NOMO2	STAMBPL1	
CDK2AP1	JMJD6	NOSIP	STAT1	
CDKL4	KANSL1	NOTCH1	STAT5A	
CDKN2A	KAT6B	NR4A2	STAT5B	
CDKN2A-AS1	KAT7	NR4A3	STK17A	
CDKN2B	KCNC4	NRIP1	STK17B	
CDKN2B-AS1	KCNIP1	NRP1	STK24	
CEACAM21	KCNN1	NSL1	STK31	
CELF1	KCNN3	NSMCE1	STK32C	
CELF2	KCNQ4	NT5E	STK38	
CELP	KDM4B	NTPCR	STK39	
CEP170	KIAA0319L	NUAK2	STK4	
CEP41	KIAA0922	NUCB2	STK40	
CEP83	KIAA1671	NUMA1	STRADA	
CEP85L	KIAA2012	NUP210	STX10	
CFAP77	KIDINS220	NXPH1	SUMO1P1	
CFDP1	KIF24	NXPH4	SUSD3	
CHD7	KIR3DL3	OAT	SVIL	

CHFR	KLF12	OLIG2	SYK	
CHKA	KLF13	OR4N3P	SYNJ2	
CHMP4B	KLF6	OR5B21	SYPL1	
CHMP7	KLF7	OSR2	SYTL2	
CHST11	KLHDC7B	OXNAD1	TAB2	
CHSY1	KLHL2	PACSIN2	TAF1B	
CLASP2	KLHL3	PAG1	TAF4	
CLCN4	KRTAP12-4	PALD1	TAGAP	
CLEC16A	L3MBTL3	PALLD	TARBP1	
CLIC3	LAMA3	PAPD7	TBC1D1	
CLIC5	LANCL2	PAQR8	TBC1D14	
CLK1	LAPTM5	PARP11	TBC1D5	
CMIP	LASP1	PARVB	TBL1X	
CMTM2	LBH	PASK	TBL1XR1	
CMTM3	LBR	PATZ1	TCF20	
CMTM7	LCK	PCAT29	TCF7	
CNKSRI	LCLAT1	PCBP1-AS1	TEC	
CNTN3	LCP2	PCCA	TERT	
CNTNAP5	LDLRAD4	PCDHGB3	TESPA1	
COA1	LDLRAP1	PCNX	TET3	
COG1	LEF1	PDCD6IP	TFAP2A	
COL6A3	LEPROTL1	PDE4A	TG	
COLQ	LIG4	PDE7B	TGFBR1	
COPB1	LILRA4	PDE9A	TGFBR2	
CORO1C	LIME1	PDIA5	TGFBR3	
COTL1	LIMK2	PDK1	TGIF2LX	
COX10	LINC-PINT	PDPK1	TH2LCRR	
CPPED1	LINC00158	PDXK	THRA	
CPXCR1	LINC00282	PEBP4	TIAM1	
CRADD	LINC00365	PFKFB2	TIMM23B	
CREB1	LINC00381	PFKFB4	TIMP2	
CREBBP	LINC00426	PGLYRP2	TKTL1	
CRIM1	LINC00470	PGS1	TLDC1	
CRLF3	LINC00540	PHF19	TLE3	
CRTC1	LINC00578	PHLDA1	TLE4	
CRTC3	LINC00593	PIAS1	TLK1	
CSGALNACT1	LINC00599	PIGV	TLR9	
CSK	LINC00645	PIK3C2B	TMC6	
CSNK1D	LINC00702	PIK3CD	TMCC1	
CSNK1G2	LINC00707	PIK3CG	TMCC2	
CTAGE1	LINC00708	PIK3IP1	TMCO5A	
CTCFL	LINC00856	PIK3R5	TMEM110	
CTDP1	LINC00861	PIM3	TMEM123	
CTDSP2	LINC00887	PIP4K2A	TMEM161B	
CTDSPL	LINC00911	PITPNC1	TMEM163	
CTNNA1	LINC00936	PLAC8	TMEM261	
CTS2	LINC00963	PLCG1	TMEM263	
CUBN	LINC00971	PLCL1	TMEM30B	
CX3CR1	LINC01011	PLCL2	TMEM63A	
CXCR4	LINC01108	PLEKHA2	TMEM65	
CXCR6	LINC01117	PLEKHO1	TMEM92-AS1	
CXXC5	LINC01119	PLOD2	TMIGD3	

CYFIP2	LINC01126	PLXNA4	TMPRSS13	
CYSLTR2	LINC01128	PLXNB2	TNFAIP8	
CYTH1	LINC01132	PNRC1	TNFRSF10A	
DAB1	LINC01136	POLR2E	TNFRSF8	
DAOA-AS1	LINC01160	POM121	TNFSF4	
DAPK2	LINC01197	PPCDC	TNP2	
DDAH2	LINC01237	PPP1R16B	TNPO1	
DEF6	LINC01271	PPP1R37	TNRC6B	
DENND2D	LINC01304	PPP2R5C	TNRC6C	
DENND3	LINC01307	PQLC1	TNS4	
DENND5A	LINC01359	PRDM1	TOM1L2	
DGKA	LINC01366	PRDM11	TOMM20	
DGKD	LINC01381	PRDM13	TOP2B	
DGKZ	LINC01412	PRDM8	TOX	
DHRS3	LINC01420	PREP	TOX2	
DIDO1	LINC01435	PREX1	TP53INP1	
DIP2A	LINC01503	PRKAR1B	TPCN1	
DIP2B	LINC01550	PRKCA	TPM4	
DIS3L2	LINC01554	PRKCB	TPST2	
DLEU1	LINC01578	PRKCH	TPTE	
DMD	LINC01599	PRKCI	TRA2A	
DMXL1	LINC01619	PRKCQ	TRABD2A	
DNAJB12	LINC01629	PRMT2	TRAF1	
DNAJC6	LINS1	PROSER3	TRAF3IP2	
DNMT1	LIPC	PRR3	TRAF3IP2-AS1	
DNMT3A	LITAF	PRR34	TRAF3IP3	
DOC2GP	LMNA	PRR5	TRAF5	
DOCK10	LMO7	PRR7-AS1	TRAK1	
DOCK5	LMTK2	PRRC2B	TRAPPC10	
DOCK9	LOC100129345	PRRX2	TRAPPC9	

[00120] As shown in Figure 1, patients with a Complete Response (CR), Partial Response (PR), and Partial Response with Relapse (PRtd) on average have higher relative expression of DNMT3A targets in comparison to patients who exhibited No Response (NR). This indicates that, cumulatively, the target genes identified as DNMT3A methylation targets in the CAR experiments are more highly expressed in patients who responded to CAR T cell therapy. Based on the explanation of previous methylation experiments that led to this list of targets, these genes are also expected to be more highly expressed in DNMT3A-knockout CAR T cells. Importantly, the differences visualized in Figure 1 are statistically significant both when considering all conditions simultaneously (Kruskal Wallis non-parametric ANOVA, $p = 0.00988$) or when specifically comparing CR to NR (Mann-Whitney U Test, $p = 0.009851$). Since responders also had a 65- to 635-fold greater expansion in comparison to non-responders, these data highlight that the expansion potential of T cells is closely linked to expression of DNMT3A-targeted genes.

[00121] Because Complete Response (CR) was not significantly different from either of the Partial Response (PR) groups (CR-vs-PR: $p = 0.3095$; CR-vs-PRtd: $p = 0.7857$), all patients who exhibited any type of response were also pooled and compared to No Response (NR) (see Figure 2). Unsurprisingly, the difference between these two groups was also statistically significant ($p = 0.001021$).

[00122] Notably, there is an extreme outlier from a patient with a Partial Response (PR) (see Figure 3, the point in the upper-right corner). However, the comparisons presented above are statistically significant even with the inclusion of this outlier. Figure 4 shows the comparison with the “outlier” data point excluded. All of the comparisons remain significant.

EXAMPLE 2. Gene expression signature with limited list of DNMT3A targets

[00123] A limited list of 107 genes (listed in Table 2) were selected from the list of DNMT3A targets. The selected genes showed $\log(\text{fold change}) > 0.5$ in the expected direction. The limited list allows improved predictive power of the test by excluding excess noise.

Table 2. Selected subset of target genes

ACOXL	EGR2	LY9	SATB1	WISP3
ADAMTS10	EGR3	LZTS1	SCML4	XYLT1
ADRA2B	EOMES	MACROD2	SDK1	ZBP1
ANKRD53	EPHB1	MAML3	SDK2	ZBTB16
APBA2	FAM134B	MATN1	SEMA3E	ZDHHC14
ATP10A	FES	MCTP2	SETBP1	ZMAT4
AUTS2	FLJ21408	MDS2	SFMBT2	ZNF683
BACH2	FOXP1	MGAT4A	SIK1	
BATF3	FOXR1	MIR31HG	SLC12A7	
BCL3	GIMAP4	MYEOV	SLC37A1	
BCL6	GPR55	NELL2	SPRY1	
C1QTNF4	GRIK3	NR4A3	SSBP3	
CA6	HTRA4	NT5E	STK31	
CACNA2D3	IFITM5	PEBP4	SVIL	
CAMK4	IGSF9B	PFKFB2	TCF7	
CBLB	IL10	PLAC8	TEC	
CD244	IL18RAP	PLCL1	TGFBR3	
CD27	IL2RA	PLXNA4	TPTE	
CDKL4	IL3	PRR5	TRIO	
CNTNAP5	INPP5A	PRRX2	TRPM8	
COL6A3	IRX3	RASA3	TTC34	
CRIM1	ITM2C	RGS6	TTC39C	
DGKD	LAMA3	RIN3	TXK	
DPF3	LINC00470	RNF157	VAV3	
DPP6	LOC152225	RTN4RL1	VWF	

[00124] As shown in Figure 5, the developed gene expression signature correlates well with the outcome. The relative expression (Z-score) of the 107 target genes in the *No Response* and *Response* groups are shown in Figures 6A-6L, and the absolute expression (log2 expression value) of the 107 target genes in No Response and Response groups are shown in Figures 7A-7L. The comparison of expression score of the 107 targets in Figure 8 shows 100% of patients in the current reference dataset with a score less than or equal to zero have failed to respond to CAR T cell therapy. In this example, a diagnostic expression score greater than zero is indicative of a 70% chance of clinical response to CAR therapy.

[00125] Next, the inventors focused on a specific type of genes (transcription factors) within the list of target genes and used multinomial logistic regression to predict the response and to weight the relative importance of those transcription factors in determining if a sample will produce a good or bad clinical outcome. The analysis was expanded outside of the context of “Response” vs “No Response” to include “Partial Response” and “Complete Response”. The PRtd data were combined with PR data, yielding 5 CR, 21 NR, and 7 PR. The top 25 most variable genes were first selected based on the median absolute deviation across the samples. The importance of these 25 genes were identified based on mean decrease in prediction accuracy (listed in Table 3, below). Ten-fold cross validation (training on 9/10 data set and testing on 1/10 data set) was used to assess the prediction accuracy using these 25 genes as the features. The average accuracy in this context was 0.58. However, for the two-group comparison (responder vs. non-responder), the accuracy increased to 0.83 for the same 25 genes. Importantly, in this analysis the gene selection was unbiased, i.e. no sample information (responder vs. non-responder) was used. Given the small training size and unbalanced group size, the result was considered reasonable.

Table 3. Top 25 genes ranked by importance

Gene name	Importance
RORA	50.35547409
EOMES	36.70115203
STAT1	35.8282896
EGR2	35.00431056
ASCL1	34.29389072
BACH2	31.89637543
E2F5	31.01251769
ZBTB16	26.14435488
IRF4	25.99010816
HIC1	25.72321649
BCL3	25.22608155
CBFA2T3	24.71426408
TRPS1	24.35677209
NFKBIA	22.88194743

Gene name	Importance
EGR3	21.76960602
KLF7	19.79639324
TCF7	19.69848553
NR4A3	19.04712791
SETBP1	18.53614676
EGR1	18.35355323
MYB	18.26125122
TFAP2A	17.3860791
BCL6	15.99984695
LEF1	13.20699353
NRIP1	4.064724136

[00126] A full model was then built using the entire dataset based on the expression value of the 25 featured genes. The prediction result is presented in Table 4. In the table, each value represents the probability of the patient sample falling in the corresponding group based on the overall model. The sum of each row is 1.

Table 4. Prediction result using 25 featured genes

Sample	No Response (NR)	Complete Response (CR)	Partial Response (PR)
NR.1	1	2.58E-29	7.41E-26
NR.5	1	8.24E-11	5.80E-16
NR.6	1	3.15E-17	1.34E-23
NR.7	1	7.45E-41	6.84E-25
NR.8	1	3.44E-12	8.99E-26
NR.9	1	3.32E-48	1.28E-33
NR.11	1	2.95E-19	2.13E-17
NR.13	1	4.65E-45	2.14E-17
NR.15	1	9.23E-56	5.61E-92
NR.16	1	3.12E-28	1.65E-66
NR.17	1	3.03E-56	3.35E-15
NR.18	1	1.87E-37	7.50E-62
NR.20	1	4.33E-39	3.86E-37
NR.21	1	9.88E-29	6.57E-10
NR.22	1	1.26E-44	1.22E-21
NR.23	1	8.63E-10	1.06E-09
NR.24	1	9.86E-23	1.40E-22
NR.29	1	5.87E-18	1.88E-31
NR.30	0.999951	4.92E-05	8.40E-09
NR.31	1	5.85E-14	1.05E-38
NR.33	1	6.54E-25	1.33E-60
PR.10	9.05E-16	3.36E-42	1
PR.19	1.71E-09	3.39E-28	0.999999998
PR.26	6.14E-16	3.39E-21	1
PR.28	2.25E-17	1.62E-29	1
PRtd.12	1.98E-14	1.91E-27	1
PRtd.14	9.66E-31	9.97E-33	1
PRtd.32	7.24E-06	7.51E-34	0.999992756
CR.2	5.12E-35	1	1.02E-25
CR.3	1.06E-14	0.999999856	1.44E-07

Sample	No Response (NR)	Complete Response (CR)	Partial Response (PR)
CR.4	2.86E-14	1	7.18E-28
CR.25	3.18E-14	1	5.12E-14
CR.27	1.80E-32	1	6.48E-35

EXAMPLE 3. Microarray analysis

[00127] Multiple DNMT3A-knockout and a "control" knockout CAR T cell lines were generated and stimulated with IL-15 multiple times. The DNMT3A knockout and control knockout CAR T cells were generated as follows: Peripheral blood mononuclear cells (PBMC) were isolated from consented healthy donors (IRB XPD15-086) via density gradient separation using Lymphoprep (StemCell Technologies, Vancouver, BC). Cells were then plated in 24 well non tissue culture-treated plates pre-coated with 250 ng each of anti-CD3 and anti-CD28 monoclonal antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). Culture medium for initial stimulation was RPMI 1640 supplemented with 10% fetal bovine serum and 2 mmol/L GlutaMAX (Thermo Fisher, Waltham, MA). IL-7 and IL-15 were added at 10 ng/mL and 5 ng/mL, respectively, 24 hours later. The following day, cells were transduced on RetroNectin (Takara Bio, Mountain View, CA)-coated plates and after 24 hours electroporated with *S. pyogenes* Cas9-single guide RNA RNP complexes targeting DNMT3A or mCherry (Control; MC19). Guide RNAs were purchased from Synthego (Menlo Park, CA) and recombinant Cas9 was purchased from the Macro Lab at the University of California, Berkeley. Two DNMT3A-specific sgRNA sequences (guide 2 and guide 3) were used which target the catalytic domain (exon 19) (Liao J et al., Nat Genet. 2015;47(5):469-78) of DNMT3A (see Figure 12). Electroporation was performed using the Neon Transfection System (1600V, 3 pulses, 10 ms) according to the manufacturer's protocol (Thermo Fisher, Waltham, MA). Electroporated T-cells were left to recover in RPMI 1640 supplemented with 20% FBS, Glutamax, 10 ng/mL IL-7, and 5 ng/mL IL-15 for 72 hours. Following recovery, the media was switched to RPMI 1640 containing 10% FBS and GlutaMAX. The cells were then expanded for 10-12 days with IL-7 and IL-15 added every 2-3 days at the same concentrations indicated above. A repeat stimulation assay was performed (Krenciute G et al., Cancer immunology research. 2017; 5(7):571-81; Mata M et al., Cancer discovery. 2017; 7(11):1306-19) in which CAR T cells were cultured with tumor cells (U373) in the presence of IL15 at an effector to target (E:T) ratio of 2:1. Every 7 days, CAR T cells were counted and re-stimulated with fresh tumor cells in the presence of IL15 at the same E:T ratio (2:1), as long as CAR T cells had killed tumor cells at the time of T-cell harvest. For effectors, T cells expressing HER2-CAR with a CD28.ζ endodomain (second generation CARs) or HER2-CAR with a ζ endodomain (first generation

CAR) were used. mRNA was extracted from these post-stimulation cell lines and was subjected to gene expression assay by microarray. The microarray data were analyzed using standard processes (see for example, Klaus and Reisenauer, *An end to end workflow for differential gene expression using Affymetrix microarrays*. bioconductor.org, 2018) to identify differentially expressed genes between the DNMT3A-knockout and the control (MC19 knockout) cells.

[00128] The design of the experiment is shown in Table 5. In the Table, “3a2” and “3a3” indicate guide RNAs guide 2 and guide 3, respectively, targeting DNMT3A (see Figure 12).

Table 5. Experimental design

ID	Knockout	Genotype	CAR Generation	Number of Stimulation
1	MC19	MC19-null	First (HER2.ζ)	Fourth
2	DNMT3A	3a2-null	First (HER2.ζ)	Fourth
3	DNMT3A	3a3-null	First (HER2.ζ)	Fourth
4	MC19	MC19-null	Second (HER2.CD28.ζ)	Fourth
5	DNMT3A	3a2-null	Second (HER2.CD28.ζ)	Fourth
6	DNMT3A	3a3-null	Second (HER2.CD28.ζ)	Fourth
7	MC19	MC19-null	First (HER2.ζ)	Fifth
8	DNMT3A	3a2-null	First (HER2.ζ)	Fifth
9	DNMT3A	3a3-null	First (HER2.ζ)	Fifth
10	DNMT3A	3a2-null	Second (HER2.CD28.ζ)	Fifth
11	MC19	3a3-null	Second (HER2.CD28.ζ)	Fifth

[00129] Principal component analysis (PCA) was performed to identify the key variables. Although there were a number of variables that could not be interrogated due to insufficient power, PCA analysis indicated that the majority of the variation in gene expression was explained by “Knockout” (DNMT3A vs MC19 control) and “Stimulation” (see Figure 9).

[00130] Because there was variation owed to stimulation, the data was analyzed twice, once comparing Fifth Stimulation DNMT3A-knockout to all MC19 samples, and once comparing Fourth Stimulation DNMT3A-knockout to all MC19 samples. The genes that were significantly upregulated in either Fourth Stimulation or Fifth Stimulation (or both) DNMT3A knockout CAR lines compared to control did not appear to predict patient response to CAR therapy (see Figure 10). However, after limiting the list of differentially expressed list to only include those genes that also exhibited a significant methylation difference between DNMT3A knockout and control, patient outcome could be predicted (see Figure 11).

[00131] These data demonstrate that only using gene expression of CAR T cells lacking DNMT3A is insufficient to determine the genes that are important for predicting CAR response; gene expression data must be integrated with or considered in the context of epigenetics (i.e., methylation targets of DNMT3A) in order to formulate accurate predictors of clinical outcome.

* * *

[00132] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[00133] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference in their entirety as if physically present in this specification.

WHAT IS CLAIMED IS:

1. A method for predicting a subject's responsiveness to an autologous T cell therapy, said method comprising:
 - a) determining gene expression level of one or more genes in a T cell sample isolated from the subject, wherein one or more of said genes are methylation targets of DNA (cytosine-5)-methyltransferase 3A (DNMT3A),
 - b) generating a Diagnostic Expression Score for the T cell sample isolated from the subject by calculating and summing absolute or weighted gene expression level(s) determined in step (a), or by calculating and summing relative gene expression level(s) relative to reference expression level(s) obtained using responders and non-responders in a reference dataset,
 - c) (i) determining that the subject is not likely to respond to an autologous T cell therapy if the Diagnostic Expression Score generated in step (b) is less than a threshold score; (ii) determining that the subject is likely to respond to an autologous T cell therapy if the Diagnostic Expression Score generated in step (b) is greater than the threshold score.
2. The method of claim 1, wherein the Diagnostic Expression Score is generated by Z-score summation and the threshold score is 0.
3. The method of claim 1, wherein the subject has a cancer, an infectious disease, an inflammatory disorder, or an autoimmune disease.
4. The method of any one of claims 1-3, further comprising improving the subject's T cell functioning in T cell therapies.
5. The method of claim 4, wherein improving the subject's T cell functioning in T cell therapies comprises inhibiting DNMT3A-mediated *de novo* DNA methylation and/or activating STAT5 signaling pathway in the subject's T cells.
6. The method of claim 5, wherein inhibiting DNMT3A-mediated *de novo* DNA methylation in the subject's T cells is achieved by inhibiting enzymatic activity of DNMT3A protein or making DNMT3A gene deleted or defective.

7. The method of claim 6, wherein the enzymatic activity of the DNMT3A protein is inhibited by exposing the cell to a DNMT3A active site inhibitor.
8. The method of claim 6, wherein the DNMT3A gene is mutated in DNMT3A catalytic domain so that the enzymatic activity of the DNMT3A protein is inhibited.
9. The method of any one of claims 5-8, wherein the level of functional DNMT3A protein in the cell is decreased by 50% or more.
10. The method of claim 5, wherein the STAT5 signaling pathway is activated by either stimulating the T cell with a signaling molecule or genetically modifying the T cell to express a signaling molecule.
11. The method of claim 10, wherein the signaling molecule is a common gamma chain cytokine.
12. The method of claim 11, wherein the cytokine is IL-15, IL-7, IL-2, IL-4, IL-9, or IL-21.
13. The method of claim 5, wherein the STAT5 signaling pathway is activated by modifying the T cell to express a constitutively active cytokine receptor or a switch receptor.
14. The method of claim 13, wherein the constitutively active cytokine receptor is a constitutively active IL7 receptor (C7R).
15. The method of claim 13, wherein the switch receptor is an IL-4/IL-7 receptor or an IL-4/IL-2 receptor.
16. The method of any one of claims 4-15, wherein said improving the subject's T cell functioning is conducted *ex vivo* or *in vitro*.
17. The method of any one of claims 4-16, further comprising repeating the method of claim 1 on the subject's T cells which were treated to improve the subject's T cell functioning.

18. The method of any one of claims 1-17, wherein the subject is determined in step (c) as not likely to respond to an autologous T cell therapy, further comprising administering to the subject an alternative therapy which is not a T cell therapy.

19. The method of claim 18, wherein the alternative therapy is selected from antiviral therapies, bone marrow transplant, chemotherapies, checkpoint blockade, and any combinations thereof.

20. The method of any one of claims 1-17, wherein the subject is determined in step (c) as likely to respond to an autologous T cell therapy, further comprising using the subject's T cells for an autologous T cell therapy.

21. A method for determining if T cells of a subject can be used for an allogeneic T cell therapy, said method comprising:

a) determining gene expression level of one or more genes in a T cell sample isolated from the subject, wherein one or more of said genes are methylation targets of DNA (cytosine-5)-methyltransferase 3A (DNMT3A),

b) generating a Diagnostic Expression Score for the T cell sample isolated from the subject by calculating and summing absolute or weighted gene expression level(s) determined in step (a), or by calculating and summing relative gene expression level(s) relative to reference expression level(s) obtained using responders and non-responders in a reference dataset,

c) (i) determining that the T cells of the subject cannot be used for an allogeneic T cell therapy if the Diagnostic Expression Score generated in step (b) is less than a threshold score; (ii) determining that the T cells of the subject can be used for an allogeneic T cell therapy if the Diagnostic Expression Score generated in step (b) is greater than the threshold score.

22. The method of claim 21, wherein the Diagnostic Expression Score is generated by Z-score summation and the threshold score is 0.

23. The method of claim 21, further comprising improving the subject's T cell functioning in T cell therapies.

24. The method of claim 23, wherein improving the subject's T cell functioning in T cell therapies comprises inhibiting DNMT3A-mediated *de novo* DNA methylation and/or activating STAT5 signaling pathway in the subject's T cells.

25. The method of claim 24, wherein inhibiting DNMT3A-mediated *de novo* DNA methylation in the subject's T cells is achieved by inhibiting enzymatic activity of DNMT3A protein or making DNMT3A gene deleted or defective.

26. The method of claim 25, wherein the enzymatic activity of the DNMT3A protein is inhibited by exposing the cell to a DNMT3A active site inhibitor.

27. The method of claim 25, wherein the DNMT3A gene is mutated in DNMT3A catalytic domain so that the enzymatic activity of the DNMT3A protein is inhibited.

28. The method of any one of claims 24-27, wherein the level of functional DNMT3A protein in the cell is decreased by 50% or more.

29. The method of claim 24, wherein the STAT5 signaling pathway is activated by either stimulating the T cell with a signaling molecule or genetically modifying the T cell to express a signaling molecule.

30. The method of claim 29, wherein the signaling molecule is a common gamma chain cytokine.

31. The method of claim 30, wherein the cytokine is IL-15, IL-7, IL-2, IL-4, IL-9, or IL-21.

32. The method of claim 24, wherein the STAT5 signaling pathway is activated by modifying the T cell to express a constitutively active cytokine receptor or a switch receptor.

33. The method of claim 32, wherein the constitutively active cytokine receptor is a constitutively active IL7 receptor (C7R).

34. The method of claim 32, wherein the switch receptor is an IL-4/IL-7 receptor or an IL-4/IL-2 receptor.

35. The method of any one of claims 21-34, wherein said improving the subject's T cell functioning is conducted *in vitro*.

36. The method of any one of claims 21-35, further comprising repeating the method of claim 21 on the subject's T cells which were treated to improve the subject's T cell functioning.

37. The method of any one of claims 20-36, wherein it is determined in step (c) that the T cells of the subject can be used for an allogeneic T cell therapy, further comprising using the subject's T cells for an allogeneic T cell therapy.

38. The method of any one of claims 1-37, comprising obtaining a sample of T cells from the subject prior to step (a).

39. The method of claim 38, wherein the sample of T cells is derived from blood, marrow, or tissue of the subject.

40. The method of claim 38, wherein the subject has cancer and the sample of T cells is derived from a tumor of the subject.

41. The method of any one of claims 1-37, comprising stimulating the T cells *in vitro* or *ex vivo* prior to step (a).

42. The method of claim 41, wherein the T cells are stimulated using anti-CD3 and anti-CD28 stimulation.

43. The method of any one of claims 1-42, wherein determining the gene expression level in step (a) comprises isolating mRNA from the T cells.

44. The method of any one of claims 1-43, wherein determining the gene expression level in step (a) is performed using mRNA sequencing, microarray gene expression profiling, or qPCR.

45. The method of any one of claims 1-44, further comprising banking the subject's T cells.

46. The method of any one of claims 1-45, wherein the DNMT3A target gene(s) is selected from the genes recited in Table 1.
47. The method of any one of claims 1-45, wherein the DNMT3A target gene(s) is selected from the genes recited in Table 2.
48. The method of any one of claims 1-45, wherein the DNMT3A target gene(s) is selected from the genes recited in Table 3.
49. The method of any one of claims 1-48, wherein the method comprises determining the expression level of 10 or more DNMT3A target genes in step (a).
50. The method of claim 49, wherein the method comprises determining the expression level of RORA, EOMES, STAT1, EGR2, ASCL1, BACH2, E2F5, ZBTB16, IRF4, HIC1, BCL3, CBFA2T3, TRPS1, NFKBIA, EGR3, KLF7, TCF7, NR4A3, SETBP1, EGR1, MYB, TFAP2A, BCL6, LEF1, and NRIP1 genes in step (a).
51. The method of any one of claims 1-50, wherein the T cell is selected from a CD8⁺ T cell, a CD4⁺ T cell, a cytotoxic T cell, an $\alpha\beta$ T cell receptor (TCR) T cell, a natural killer T (NKT) cell, a $\gamma\delta$ T cell, a memory T cell, a T-helper cell, and a regulatory T cell (Treg).
52. The method of any one of claims 1-51, wherein the subject is human.
53. The method of any one of claims 1-52, wherein the T cell therapy is a CAR T cell therapy.
54. The method of any one of claims 1-52, wherein the T cell therapy is an $\alpha\beta$ TCR therapy.
55. The method of any one of claims 1-52, wherein the T cell therapy is a $\gamma\delta$ TCR therapy.
56. The method of any one of claims 1-52, wherein the T cell therapy is an iNKT therapy.
57. The method of any one of claims 1-52, wherein the T cell therapy is a tumor-infiltrating lymphocyte (TIL) therapy.

58. The method of any one of claims 1-52, wherein the T cell therapy is an *in vitro* sensitized (IVS) T cell therapy.

59. The method of any one of claims 1-52, wherein the T cell therapy is an *in vivo* T cell therapy.

FIG. 1

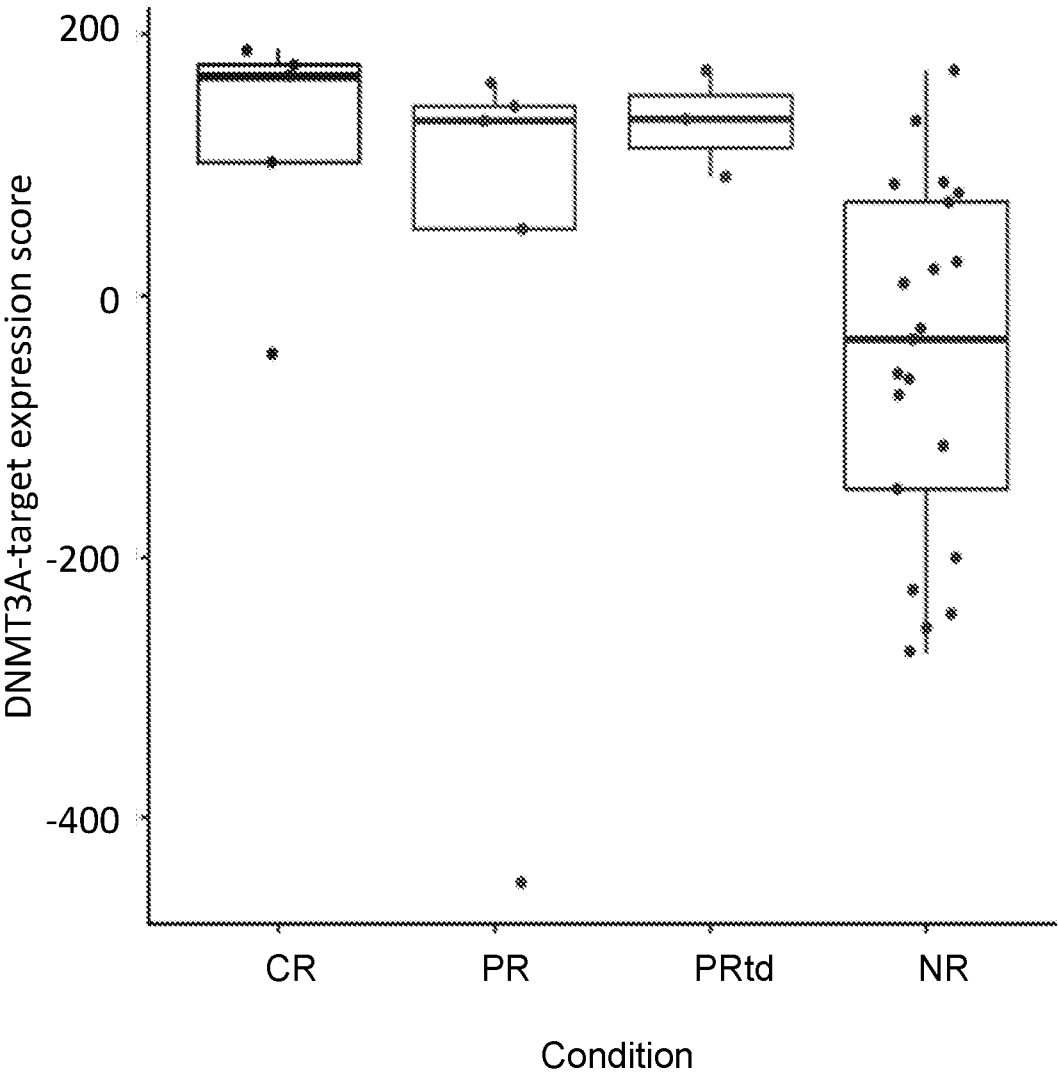


FIG. 2

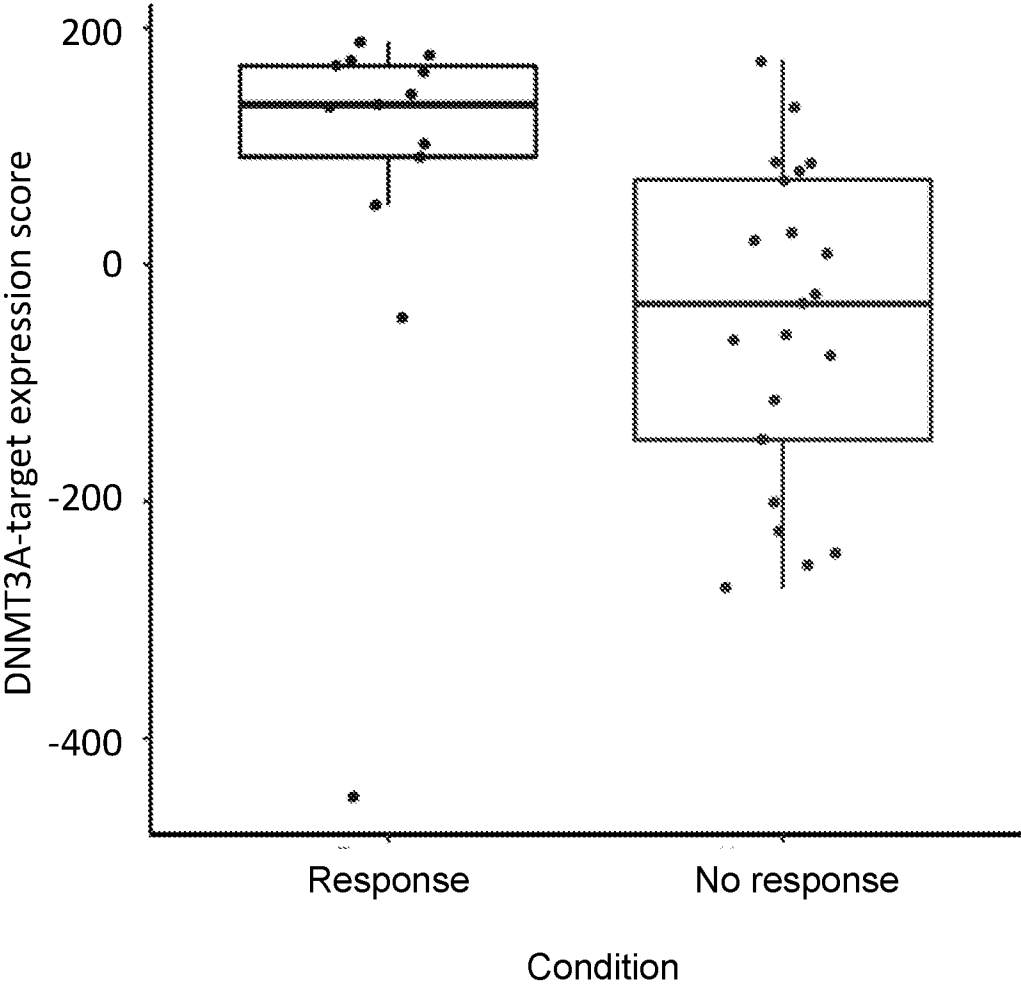


FIG. 3

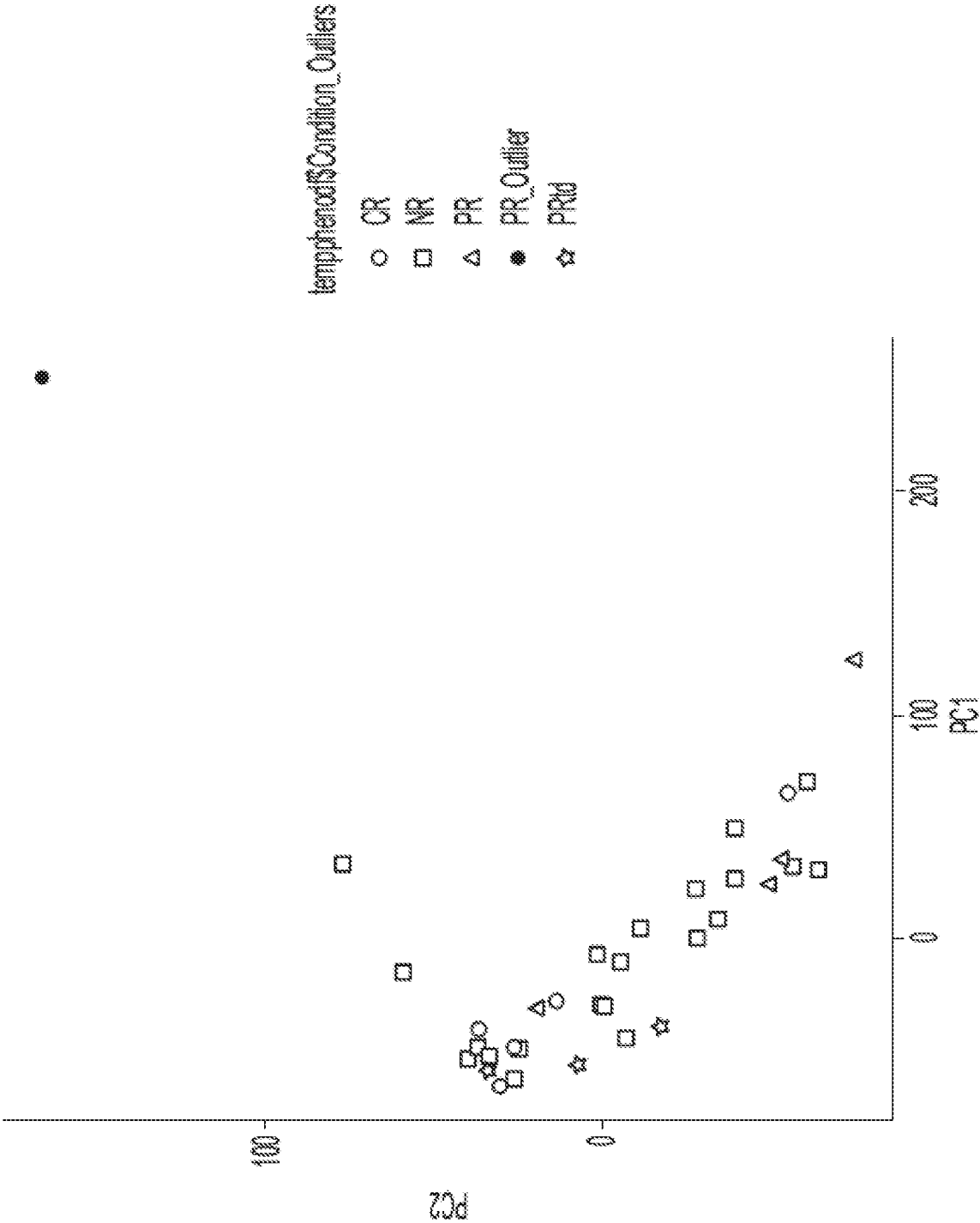


FIG. 4

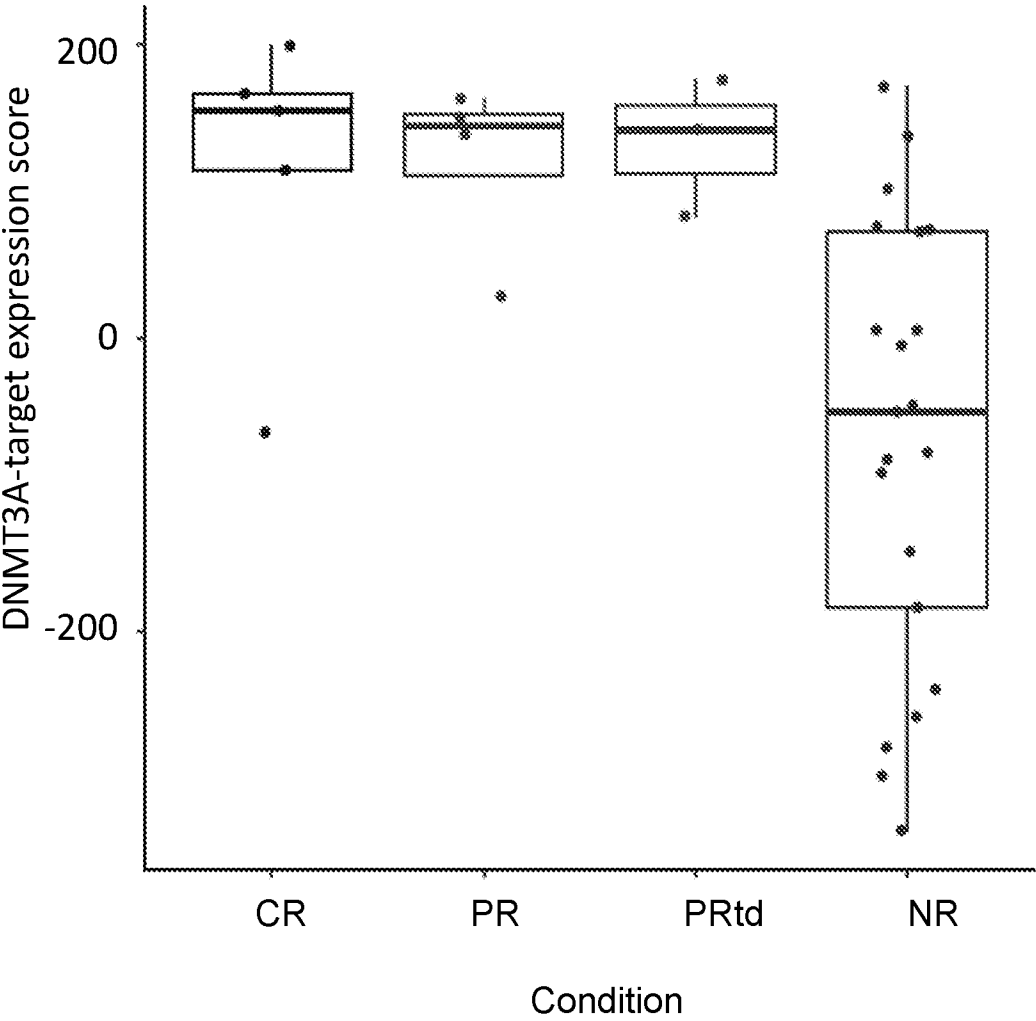


FIG. 5

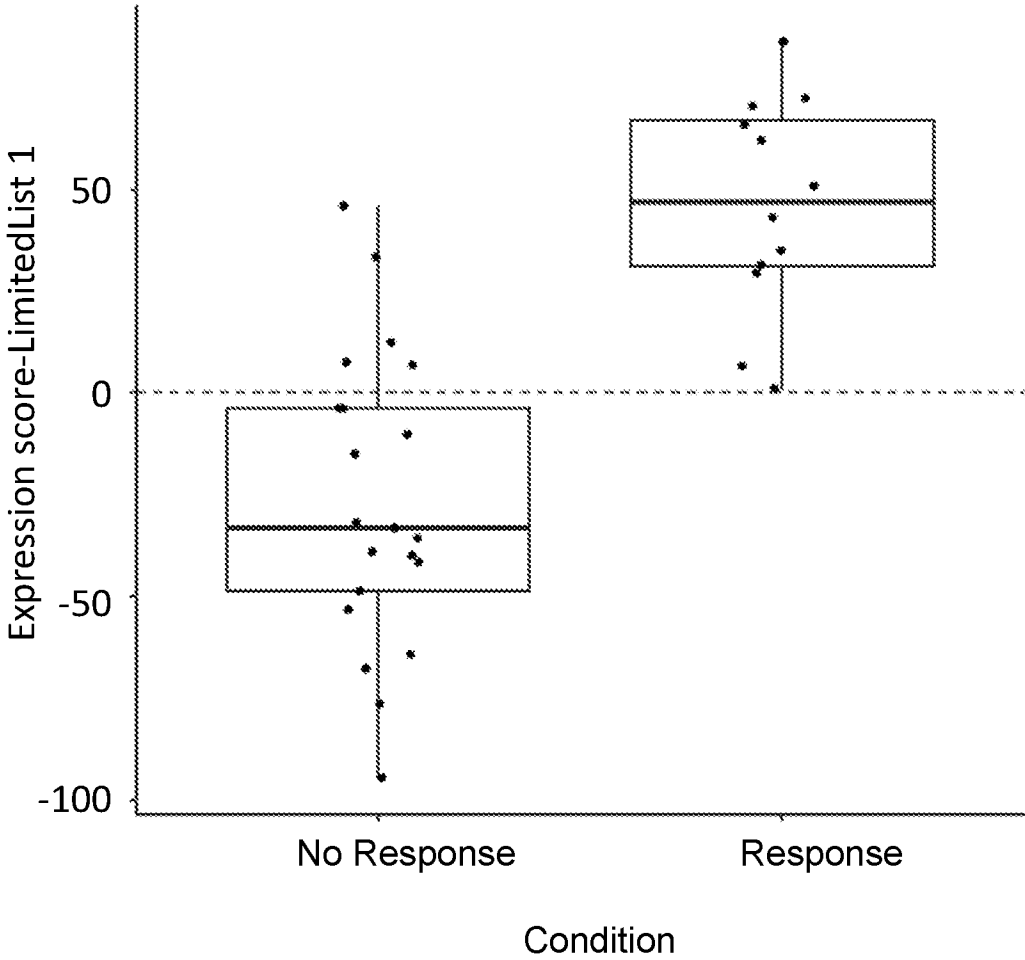


FIG. 6A

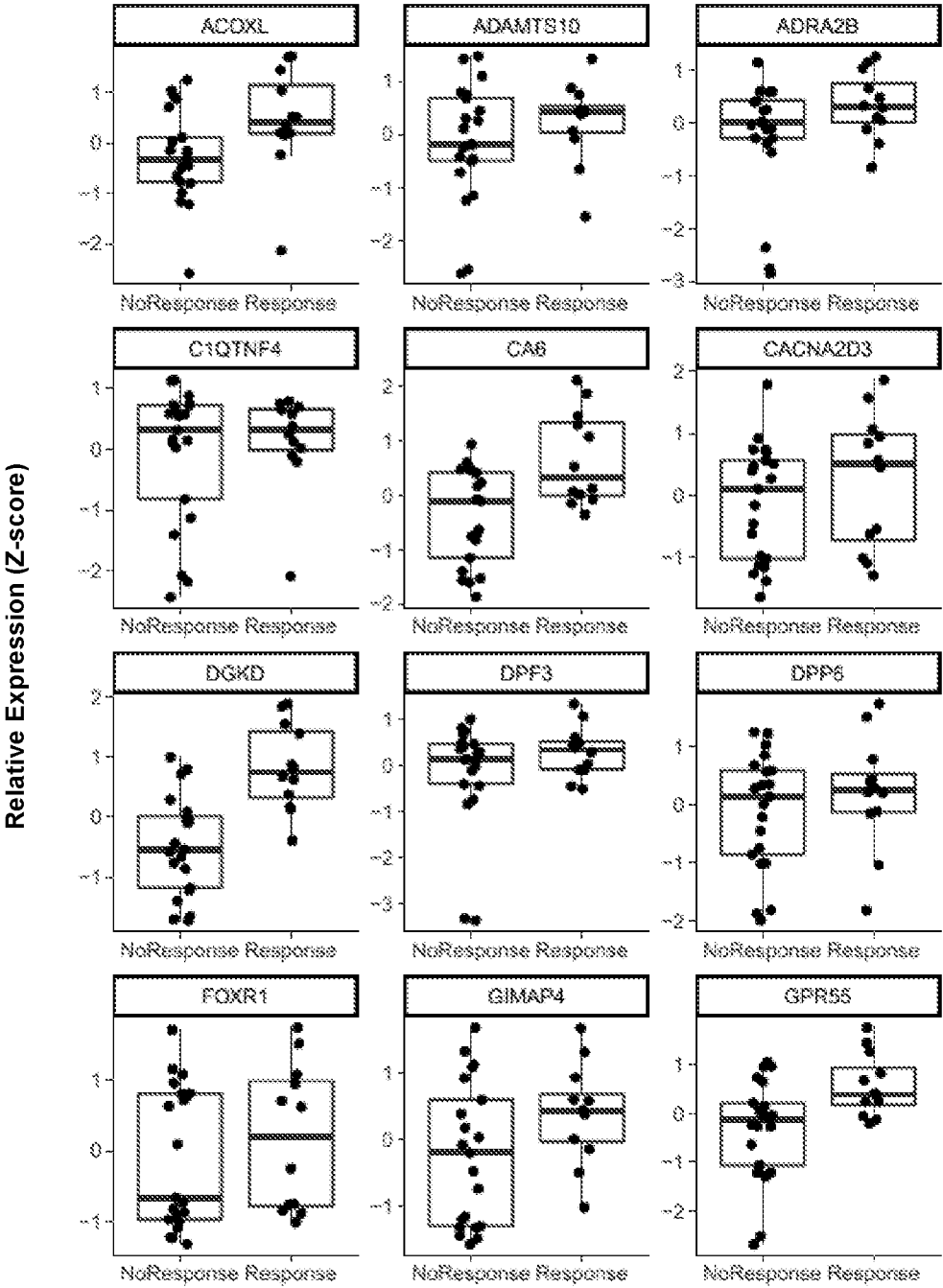


FIG. 6B

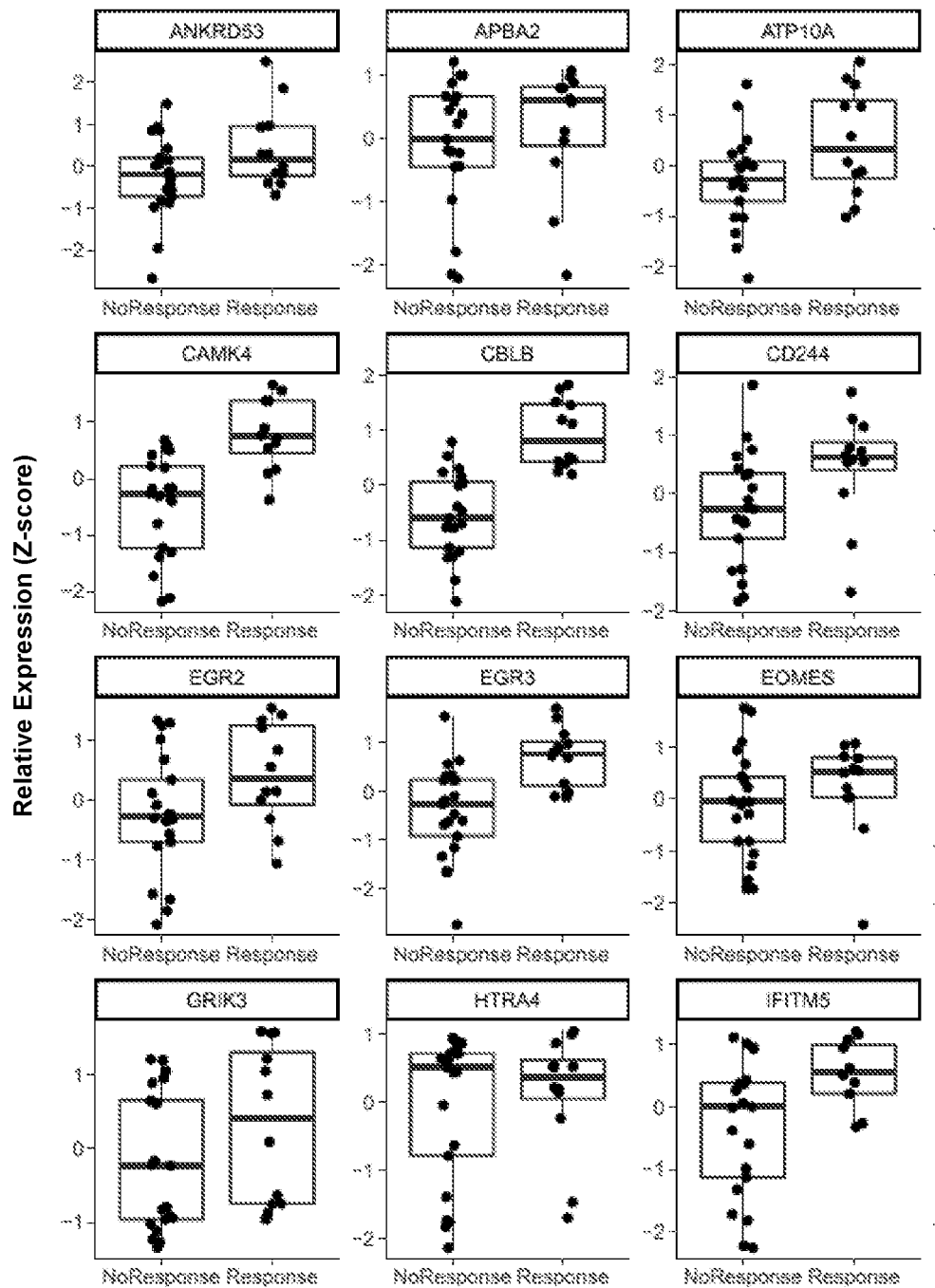


FIG. 6C

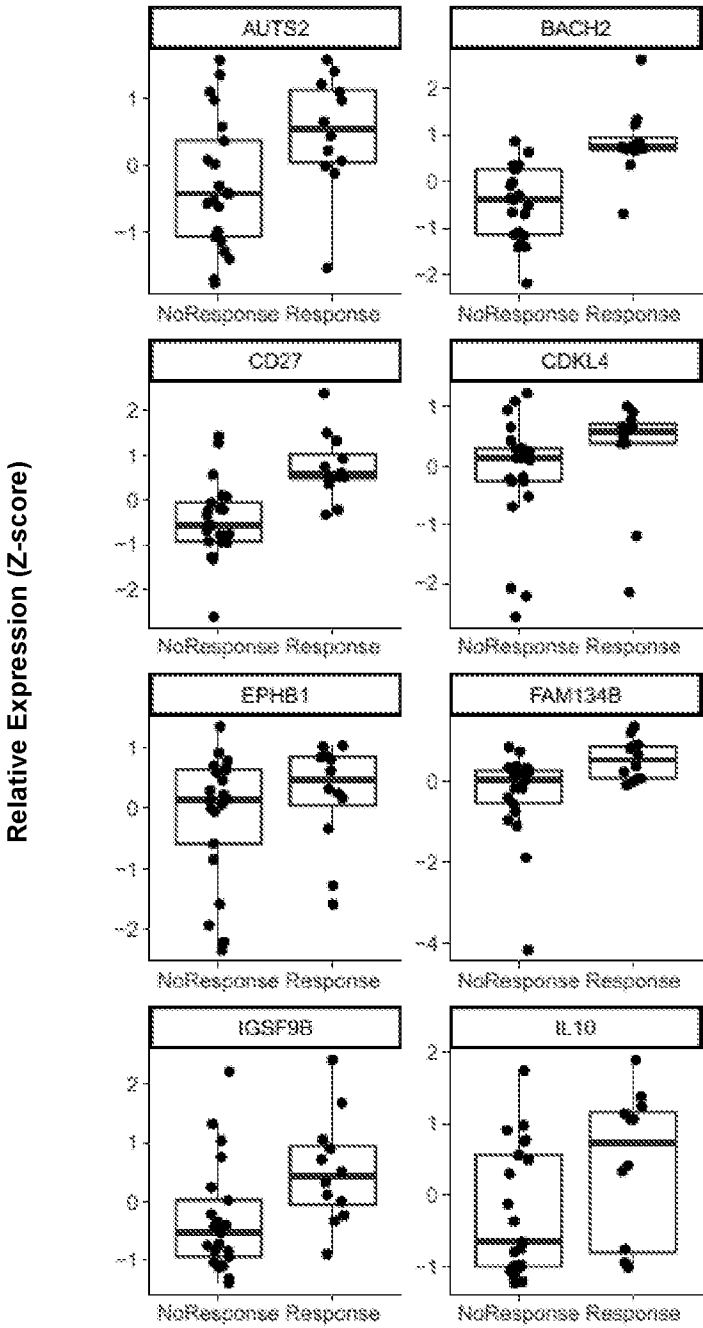


FIG. 6D

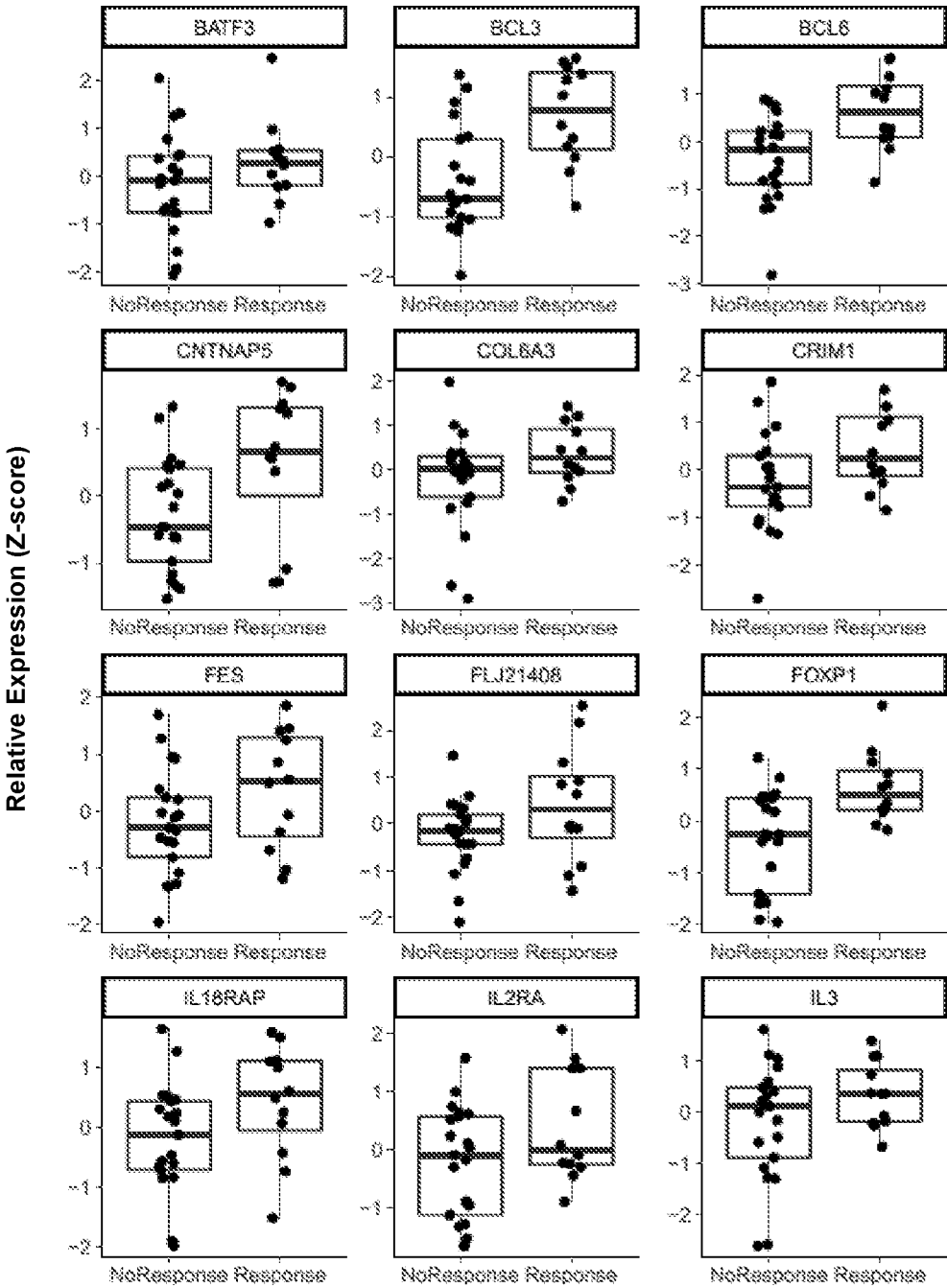


FIG. 6E

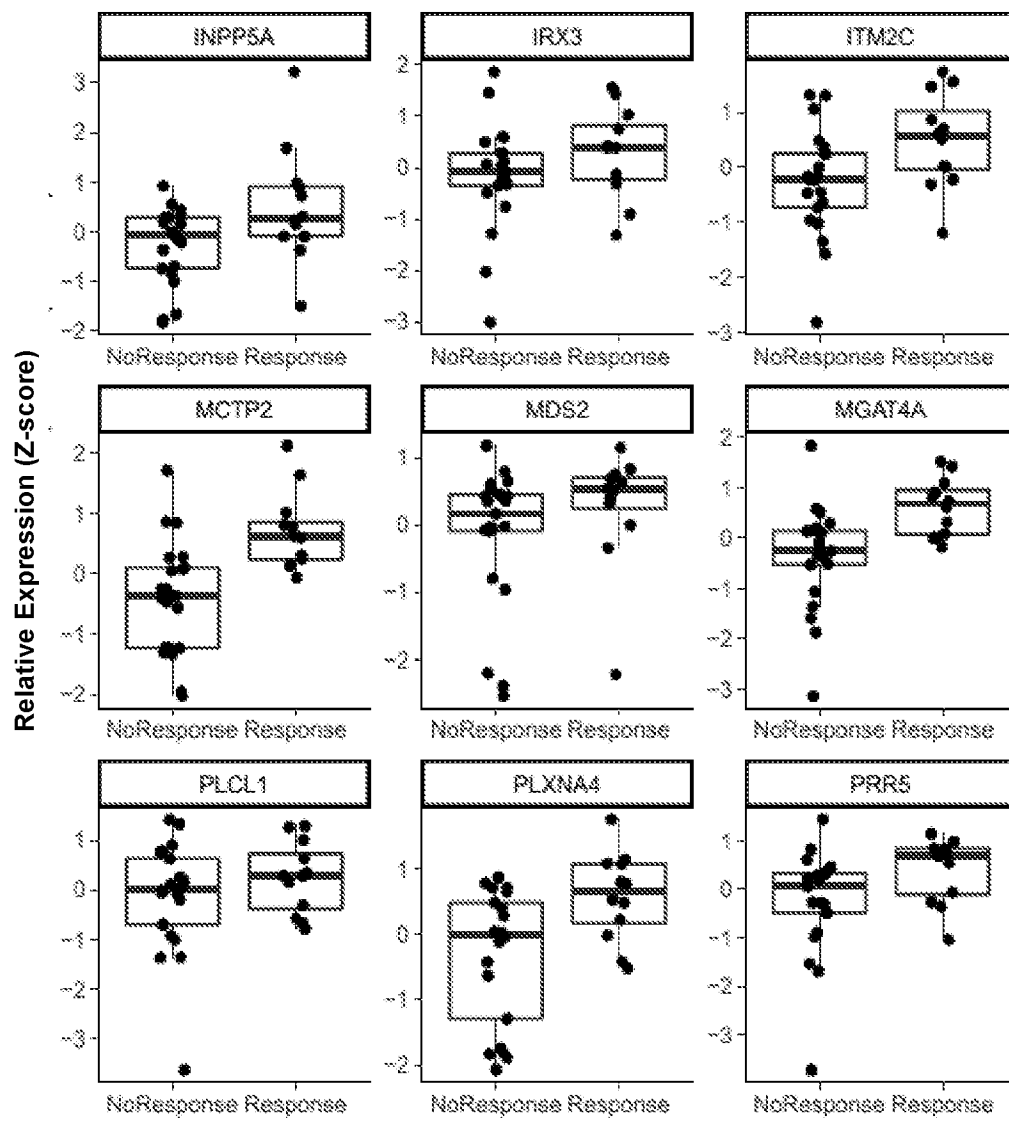


FIG. 6F

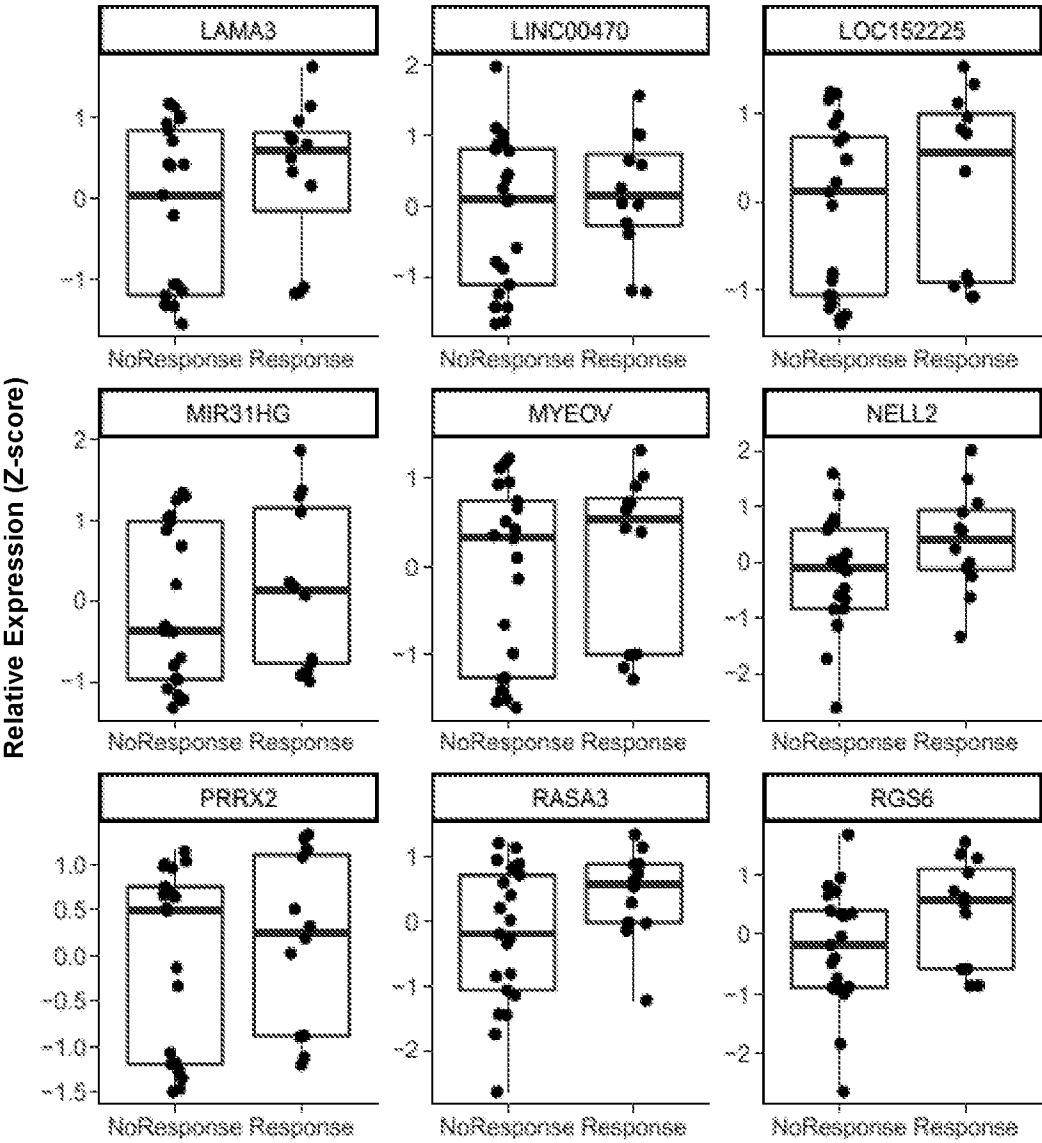


FIG. 6G

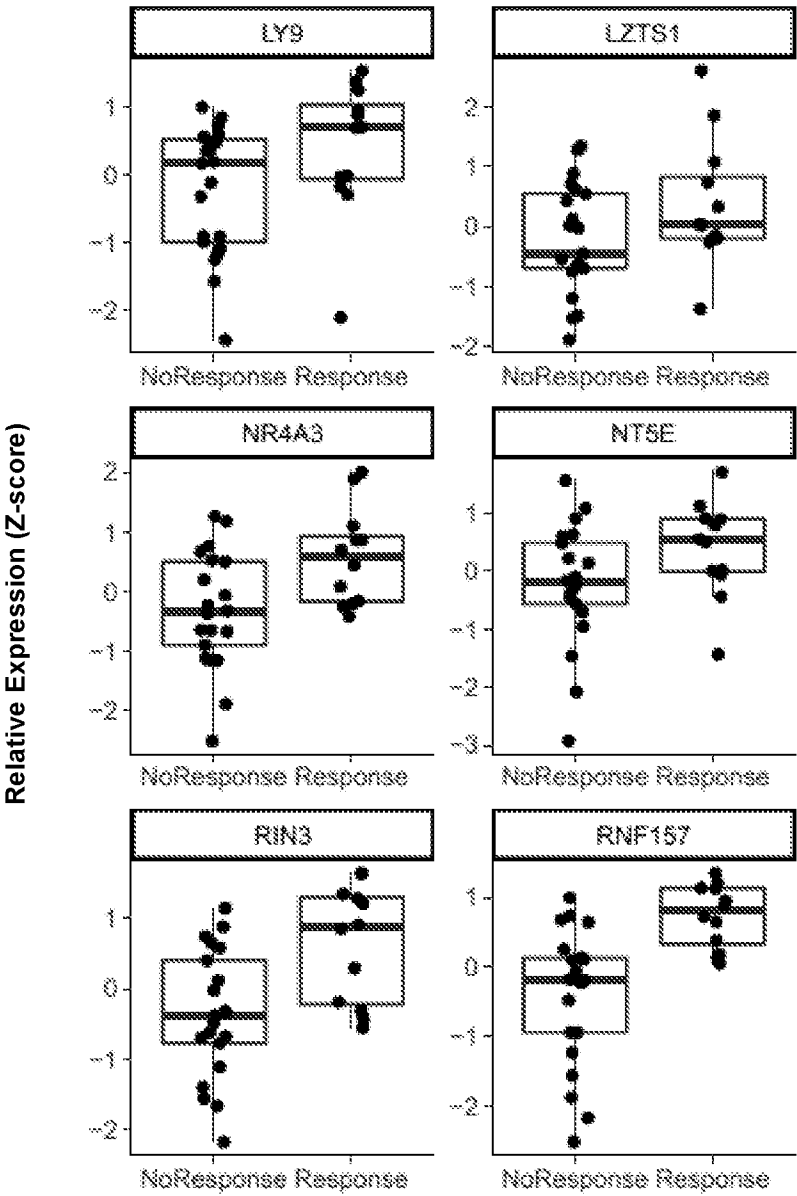


FIG. 6H

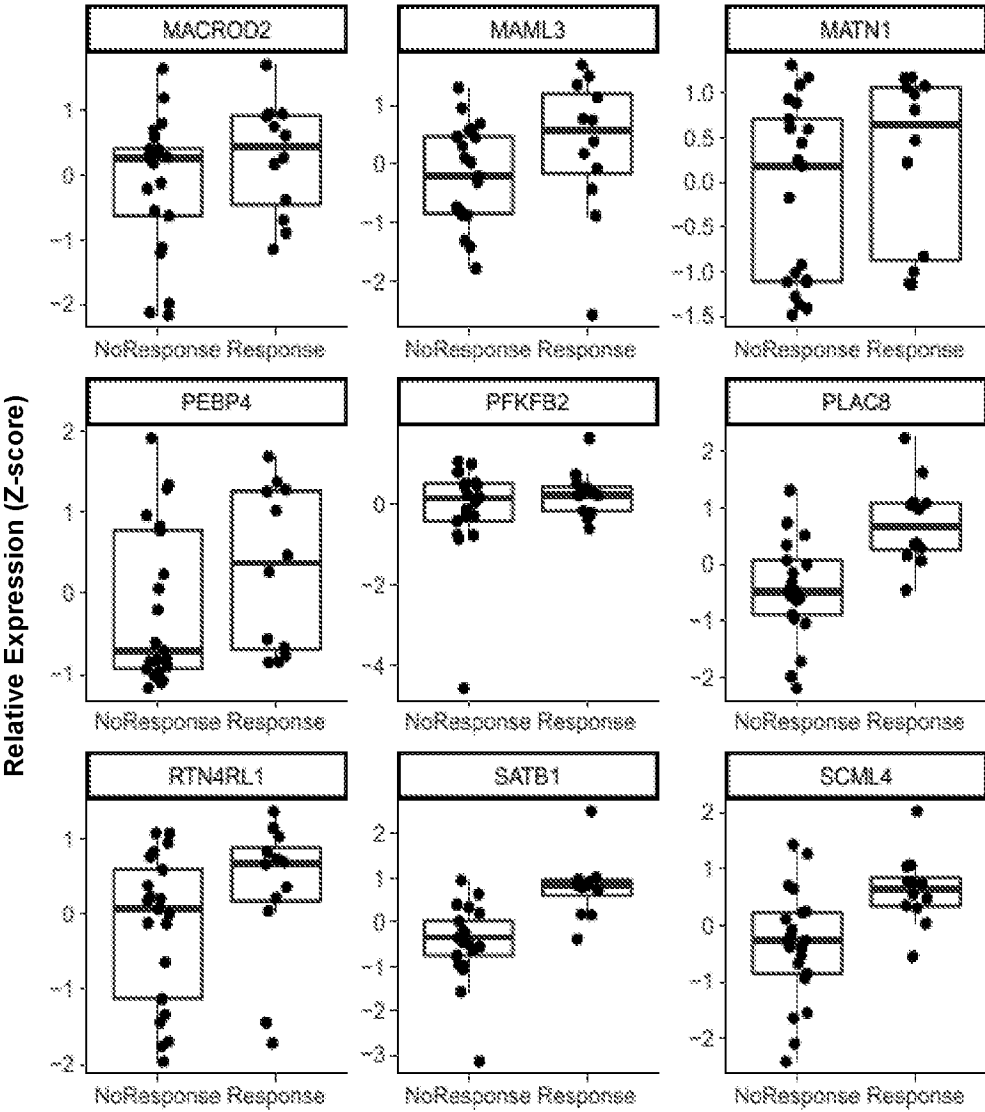


FIG. 6I

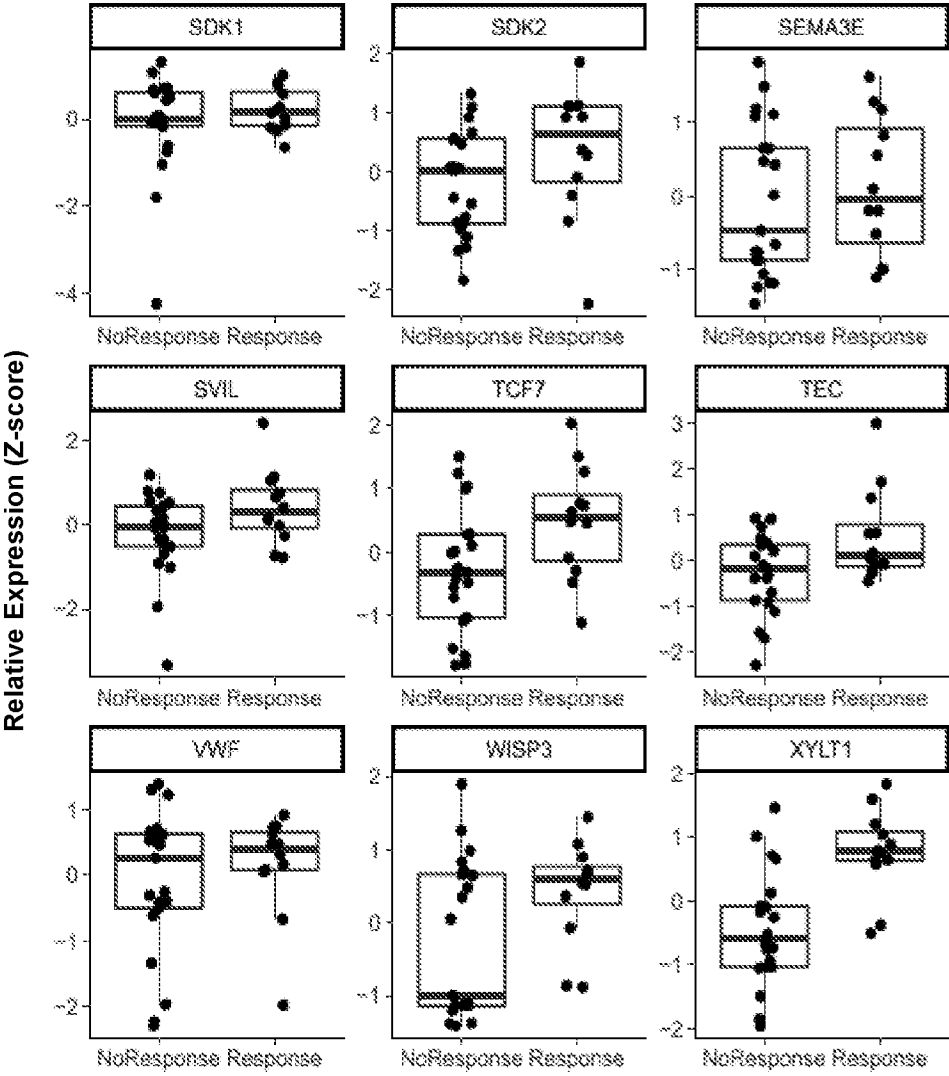


FIG. 6K

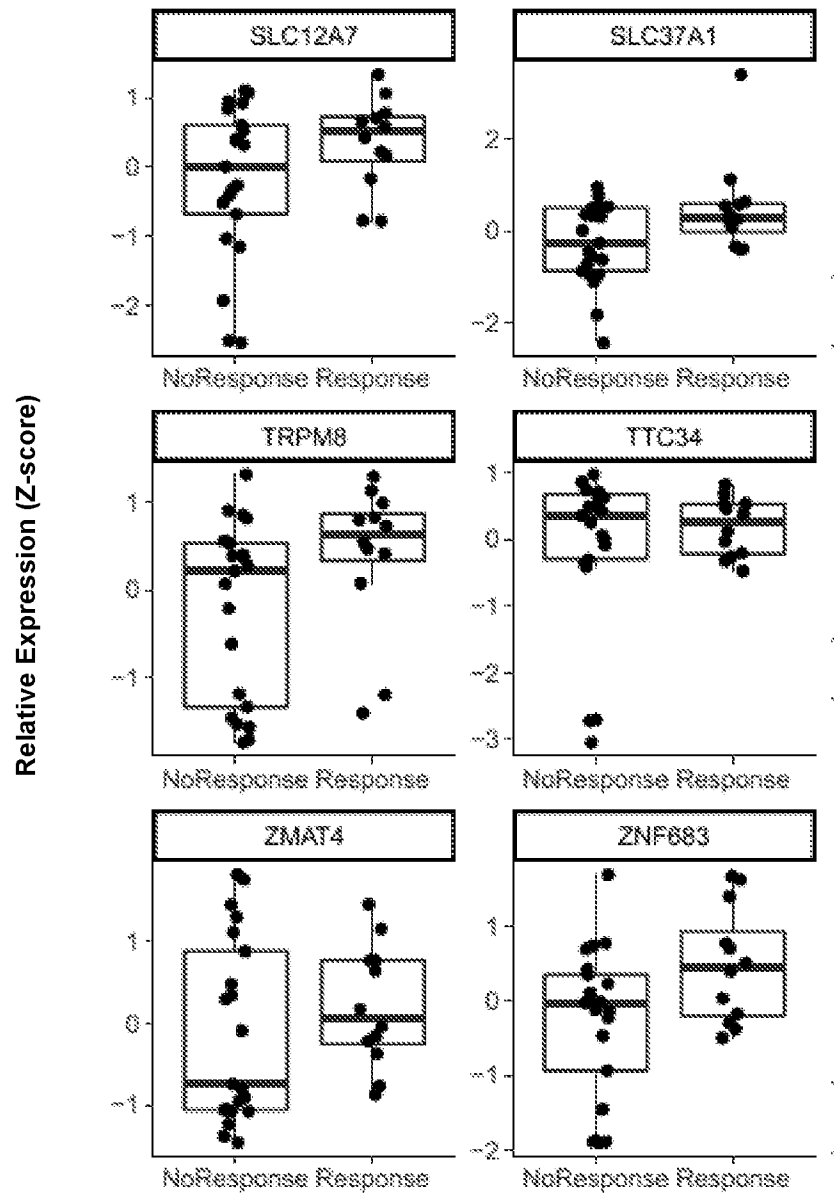


FIG. 6L

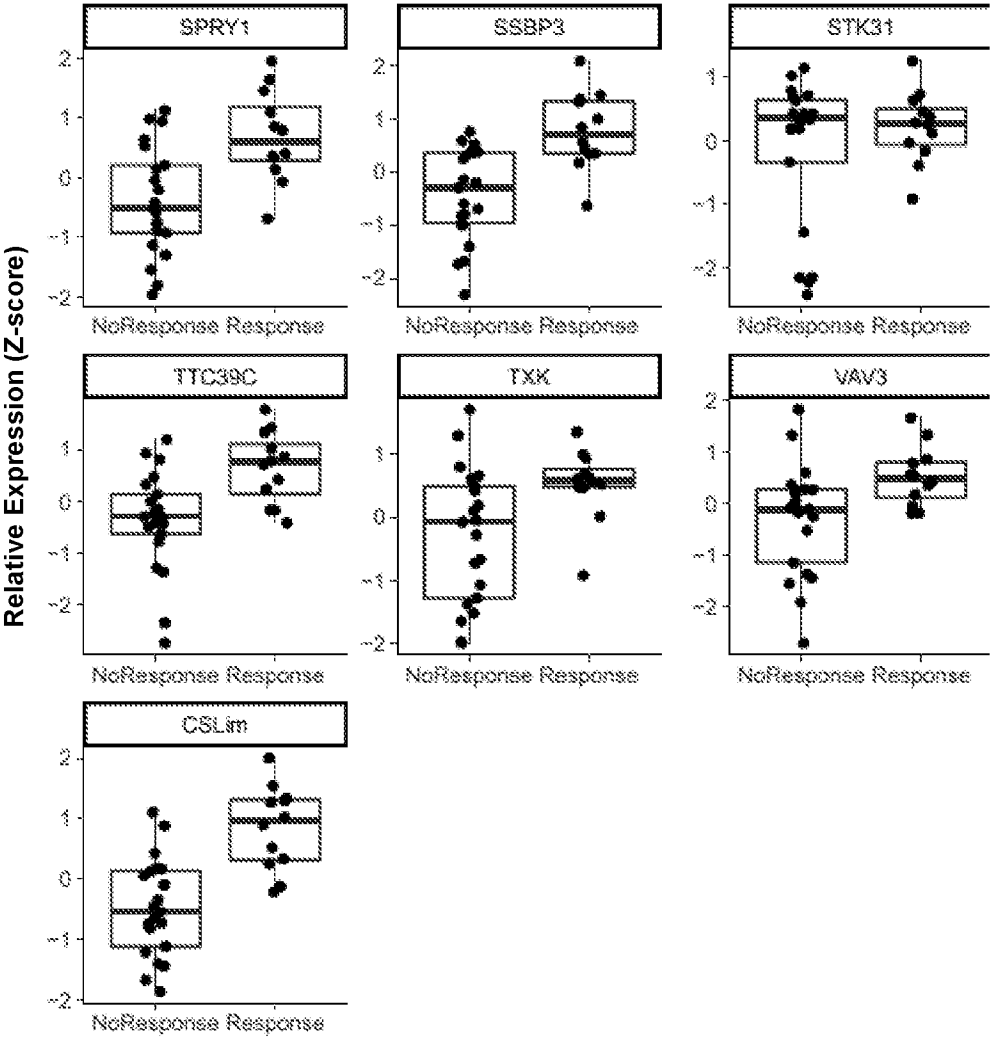


FIG. 7A

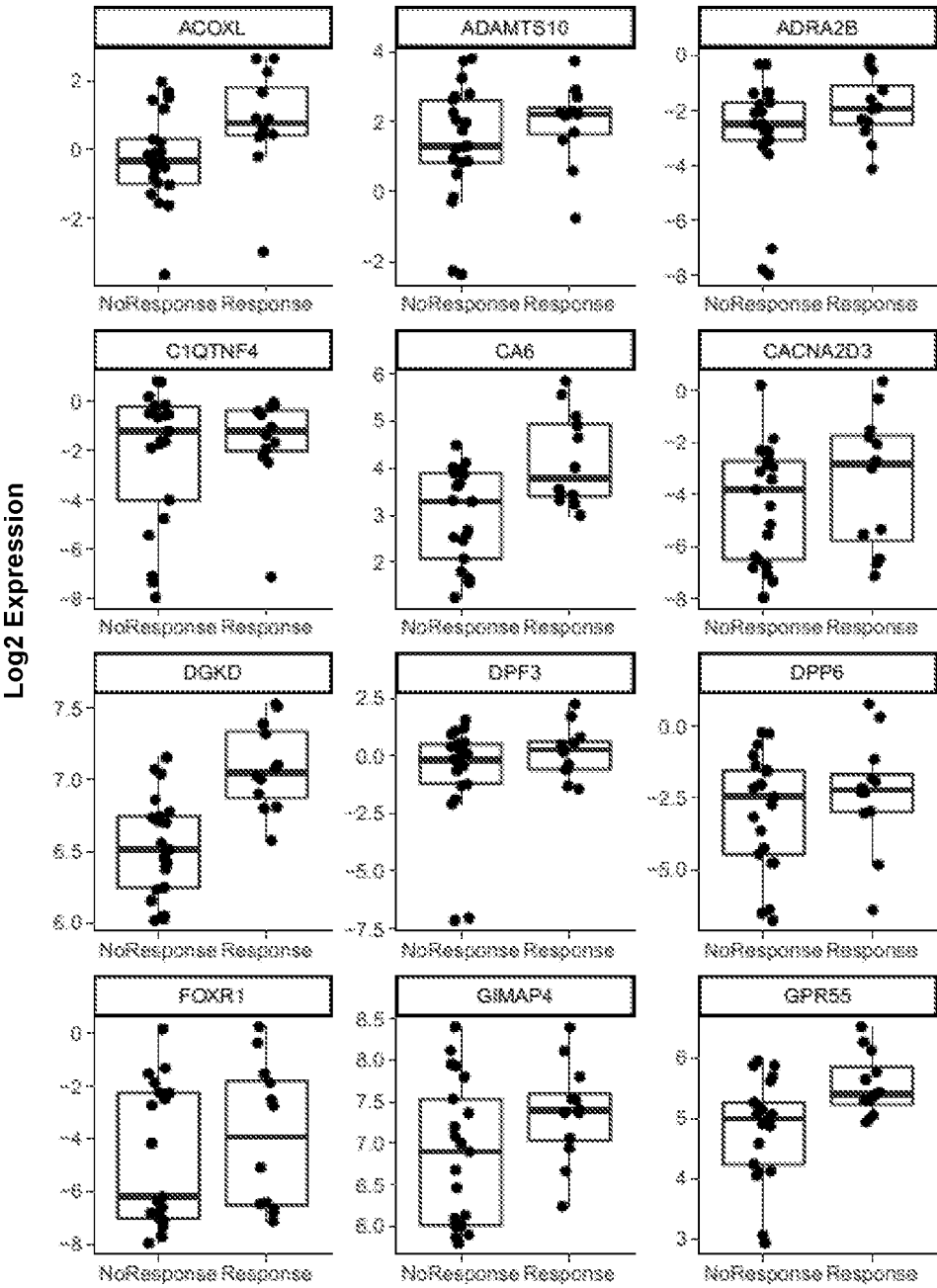


FIG. 7B

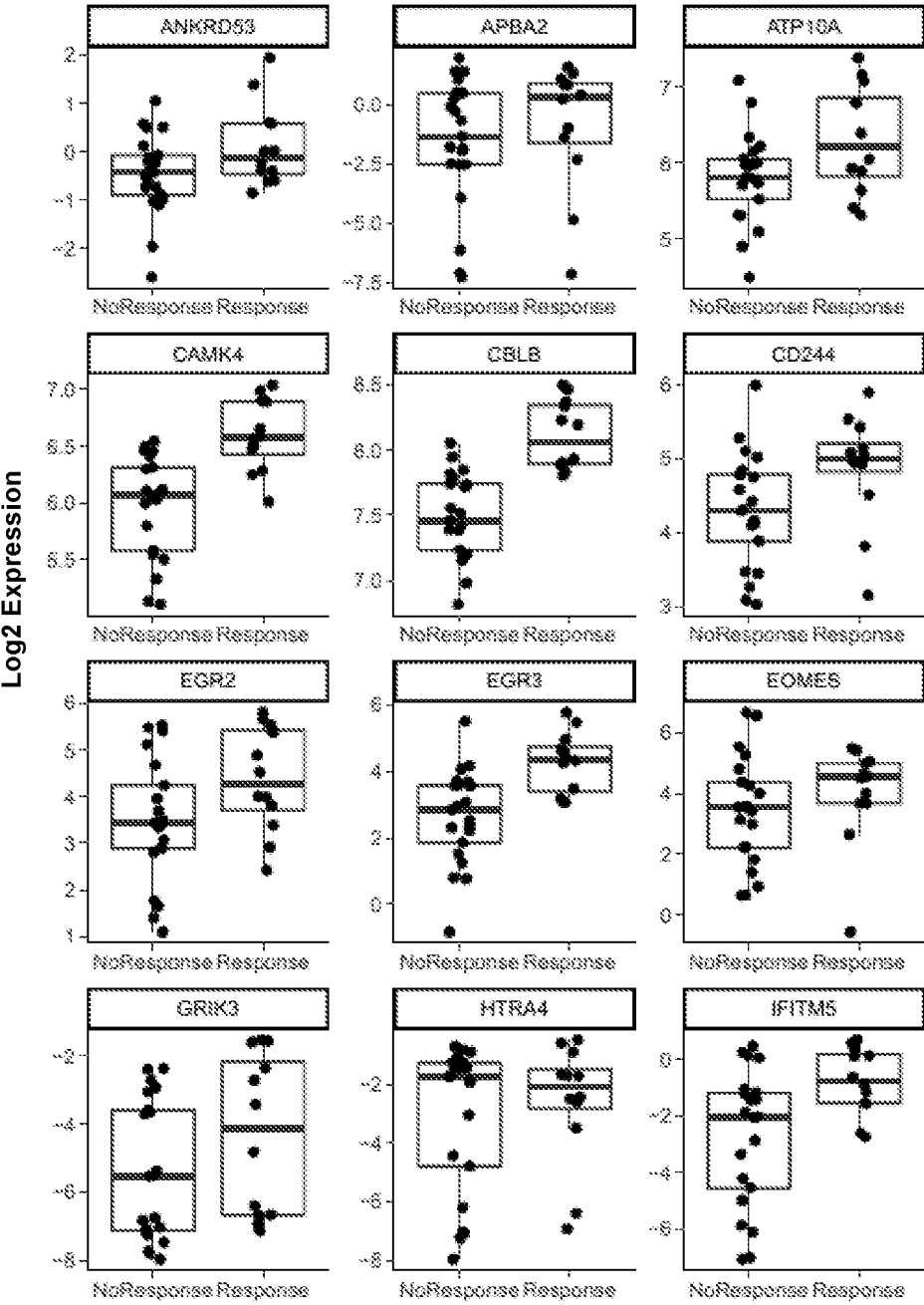


FIG. 7C

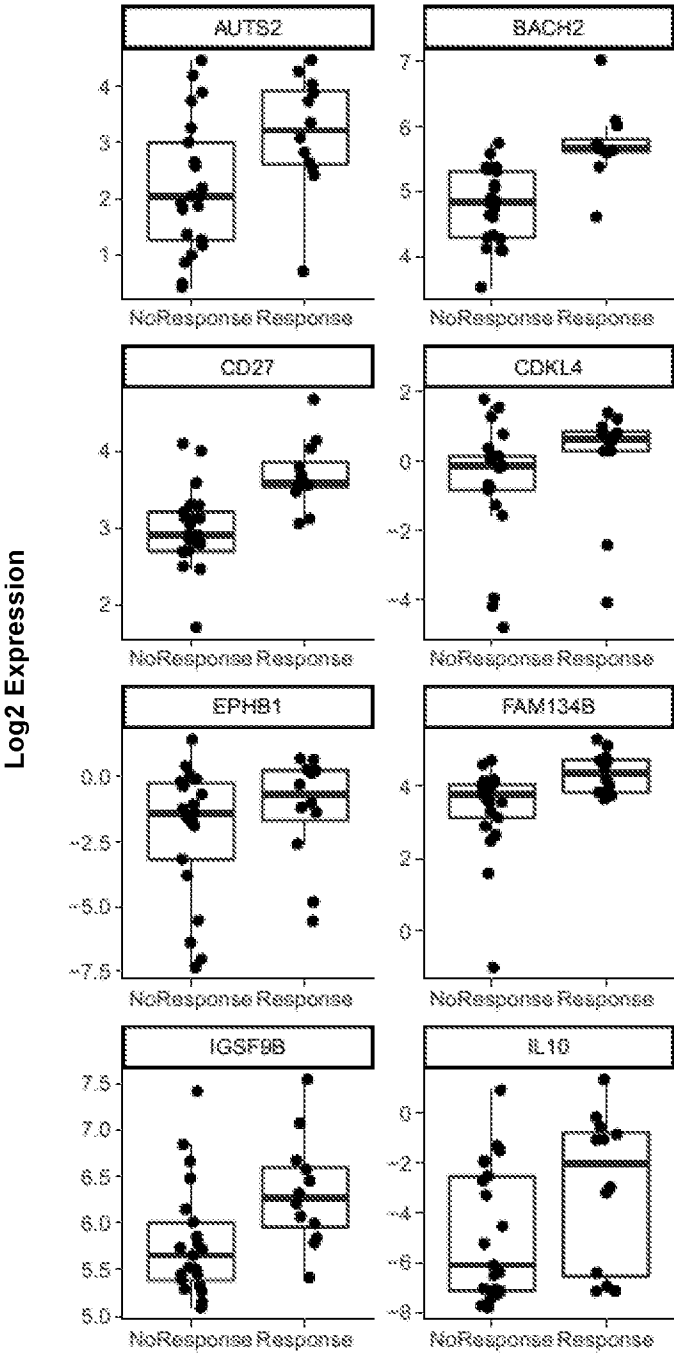


FIG. 7D

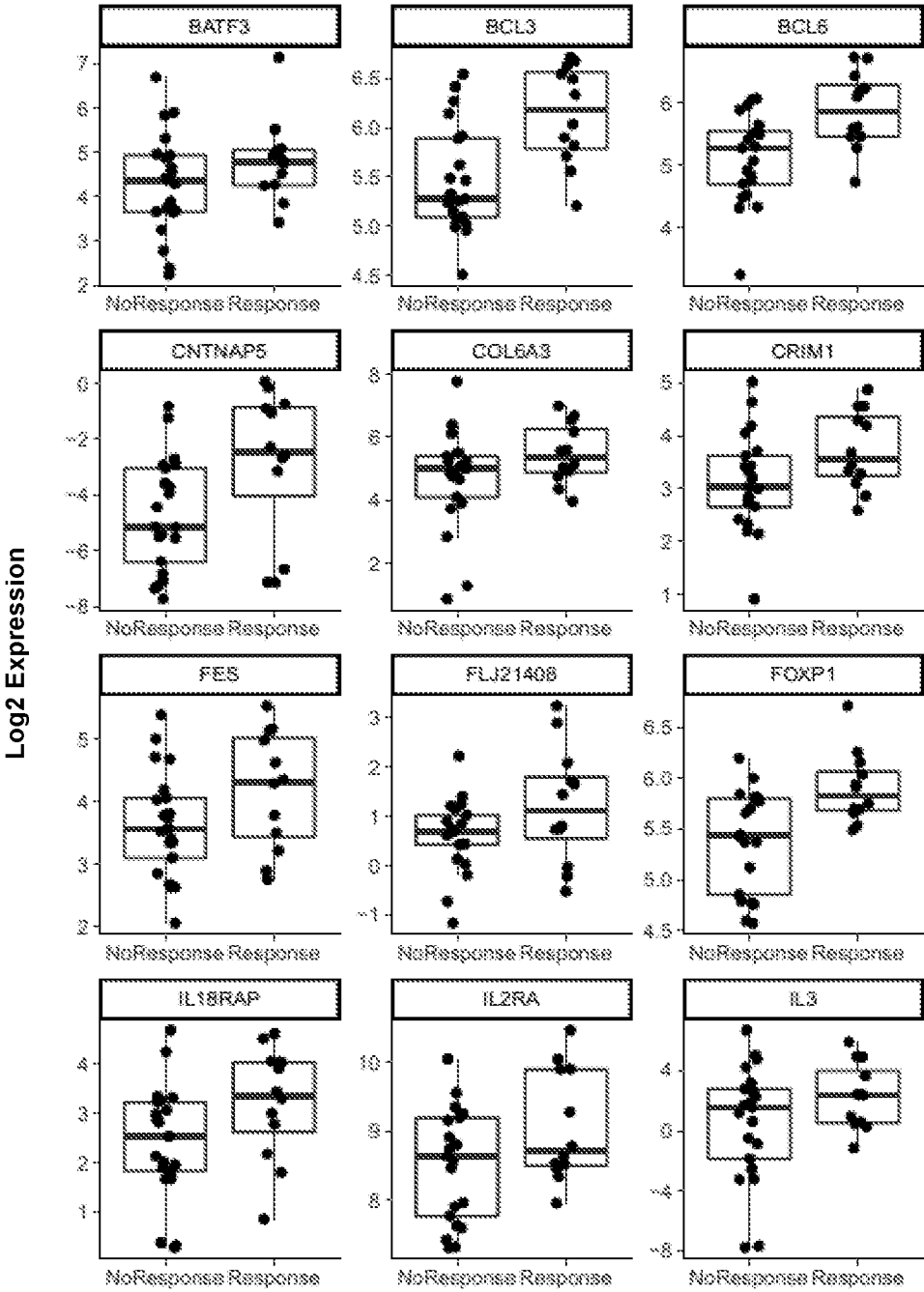


FIG. 7E

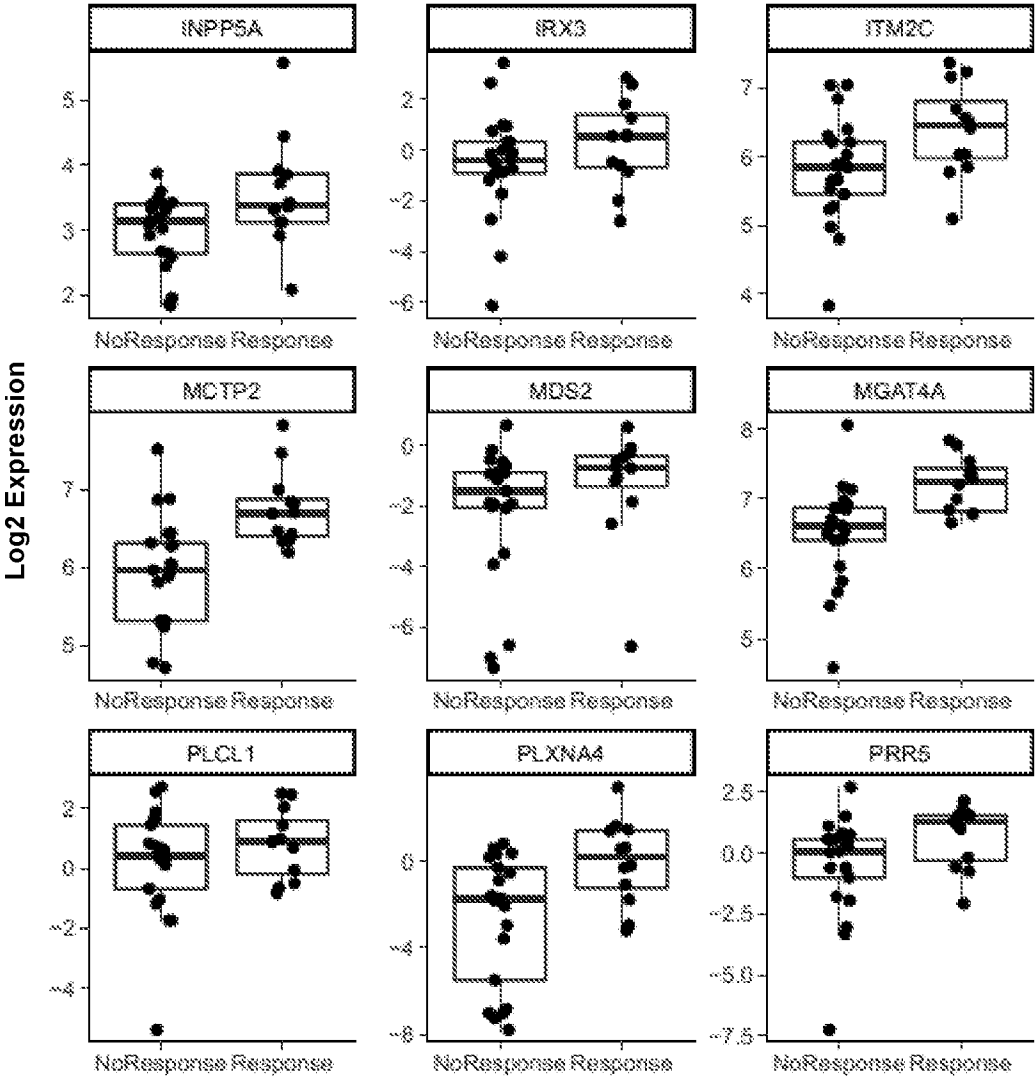


FIG. 7F

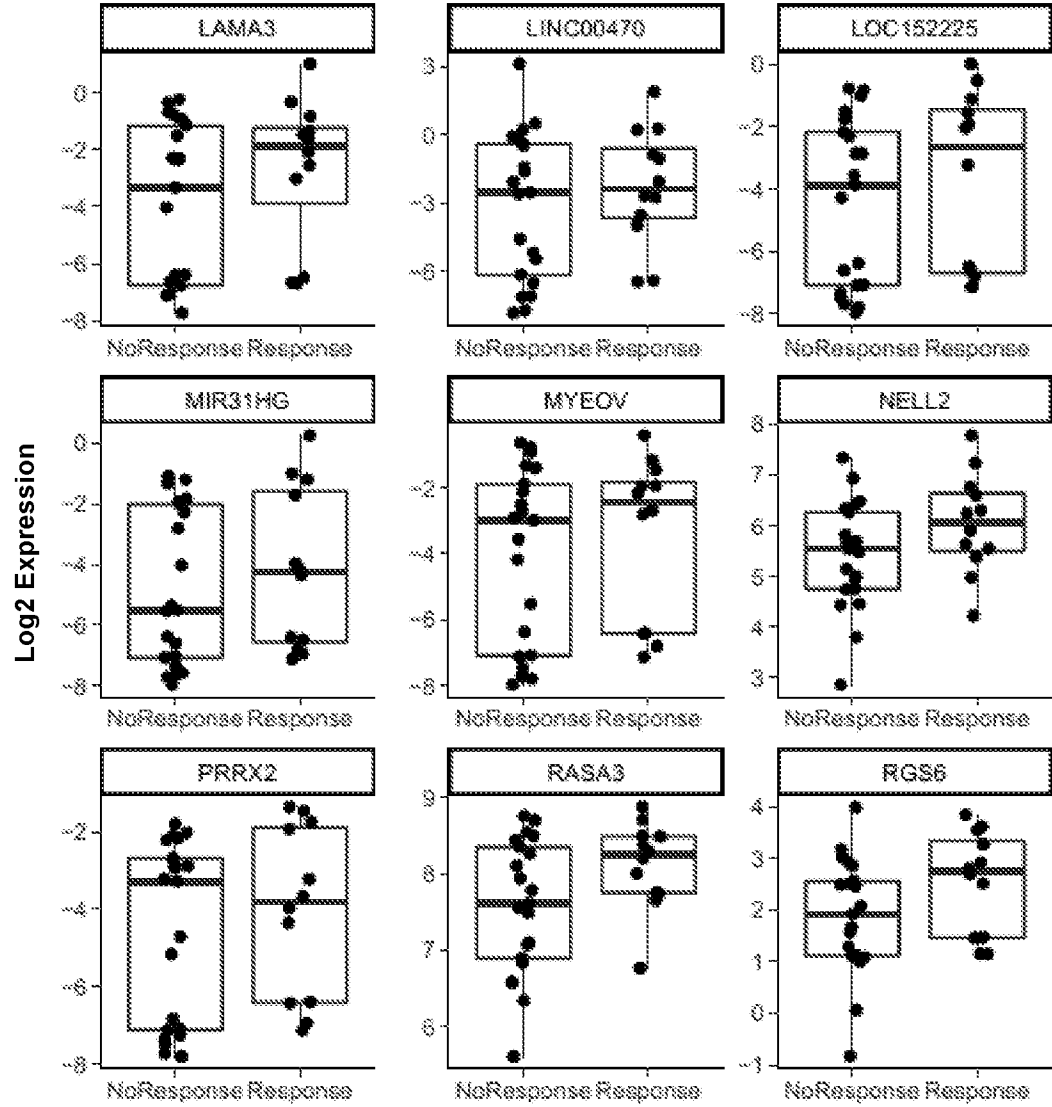


FIG. 7G

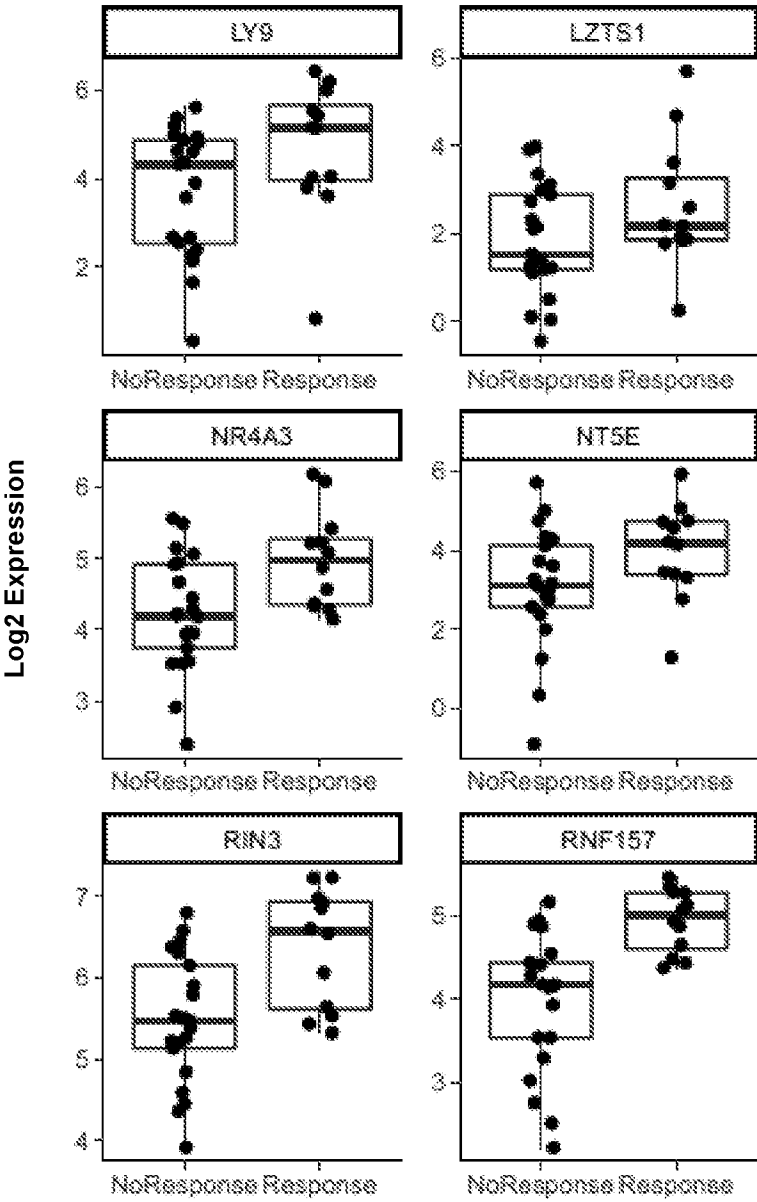


FIG. 7H

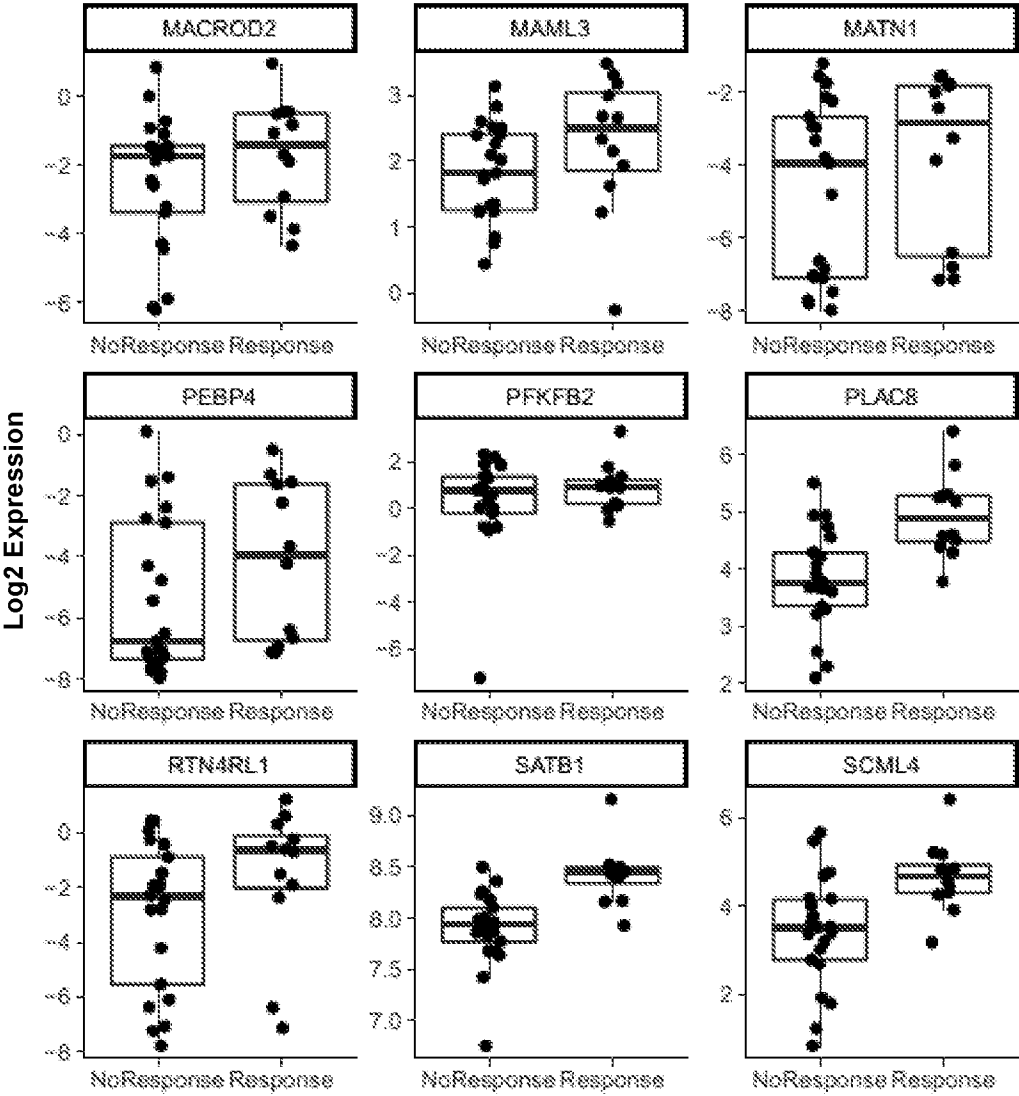


FIG. 7I

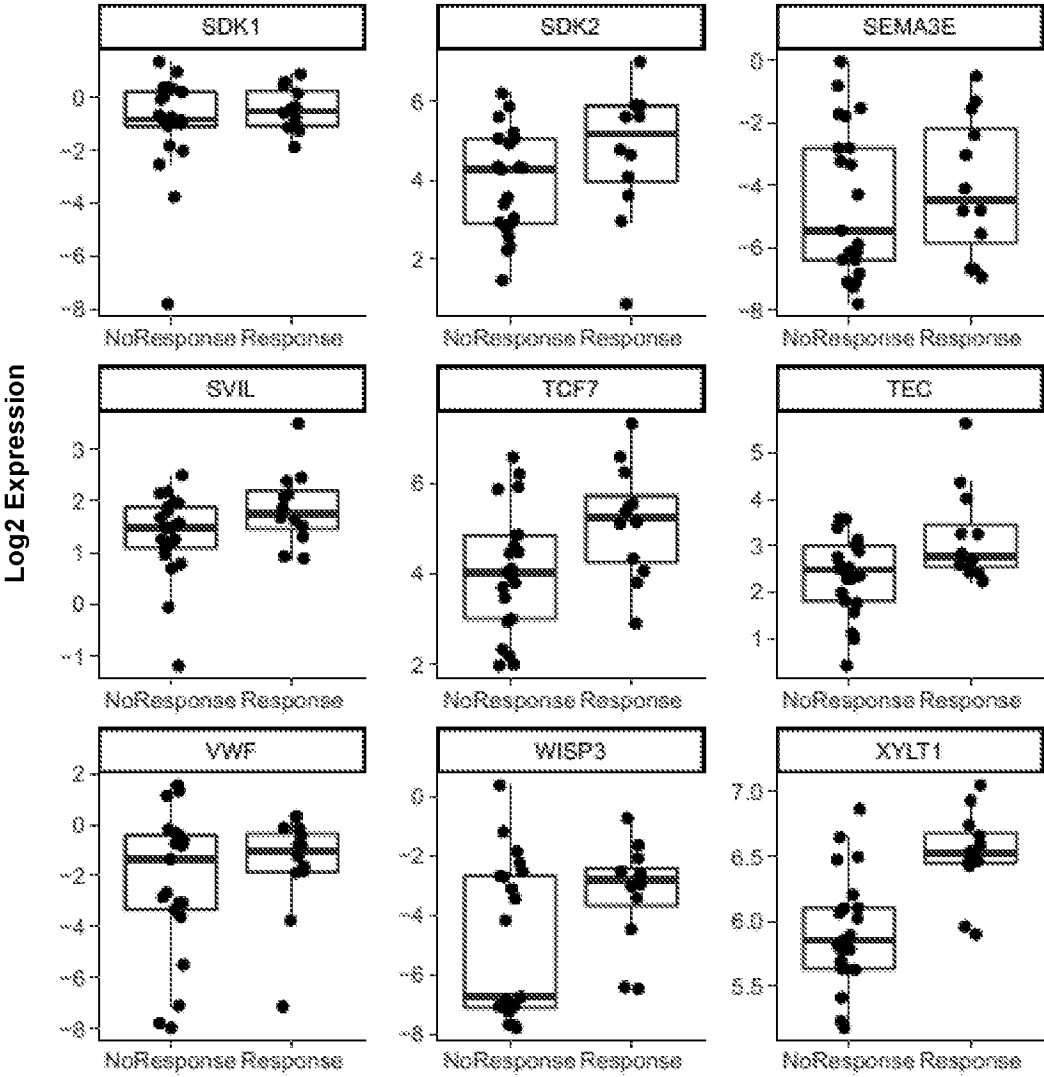


FIG. 7J

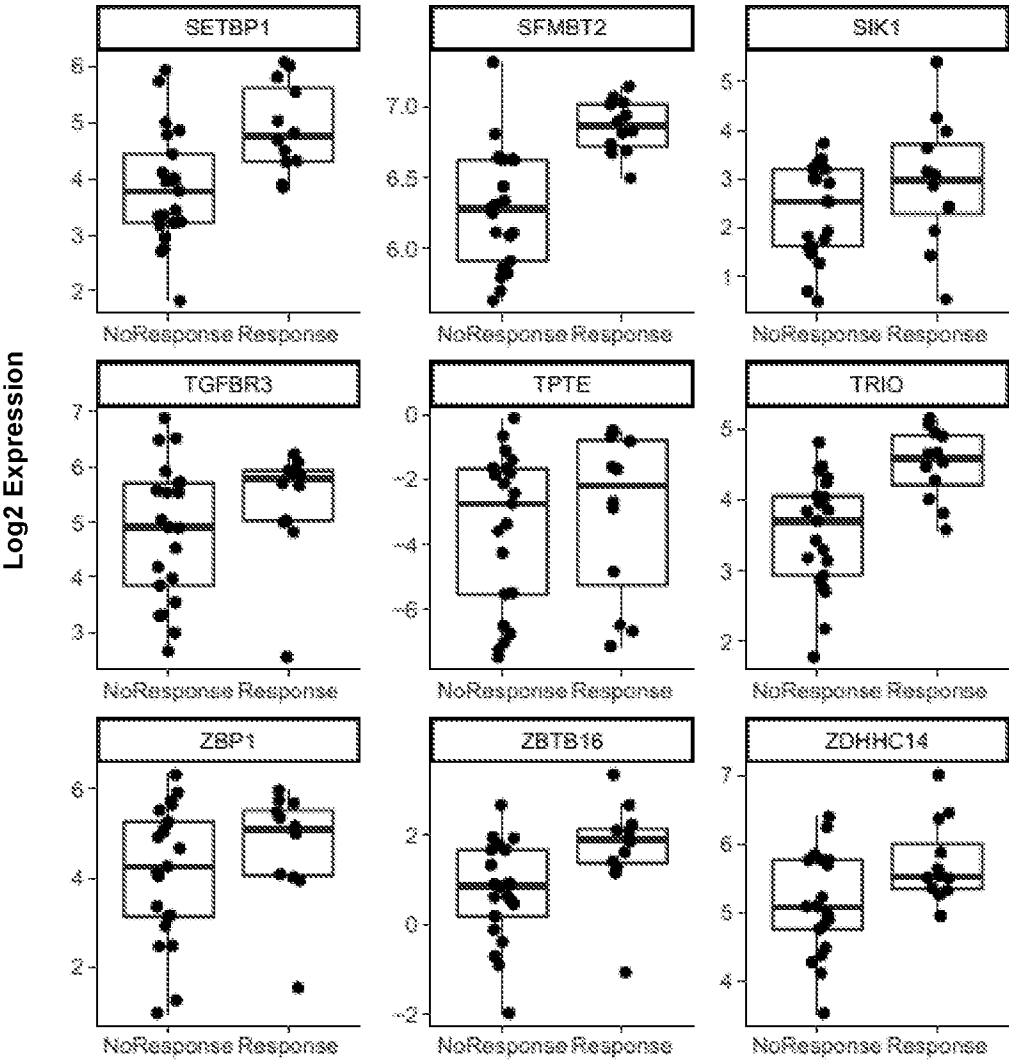


FIG. 7K

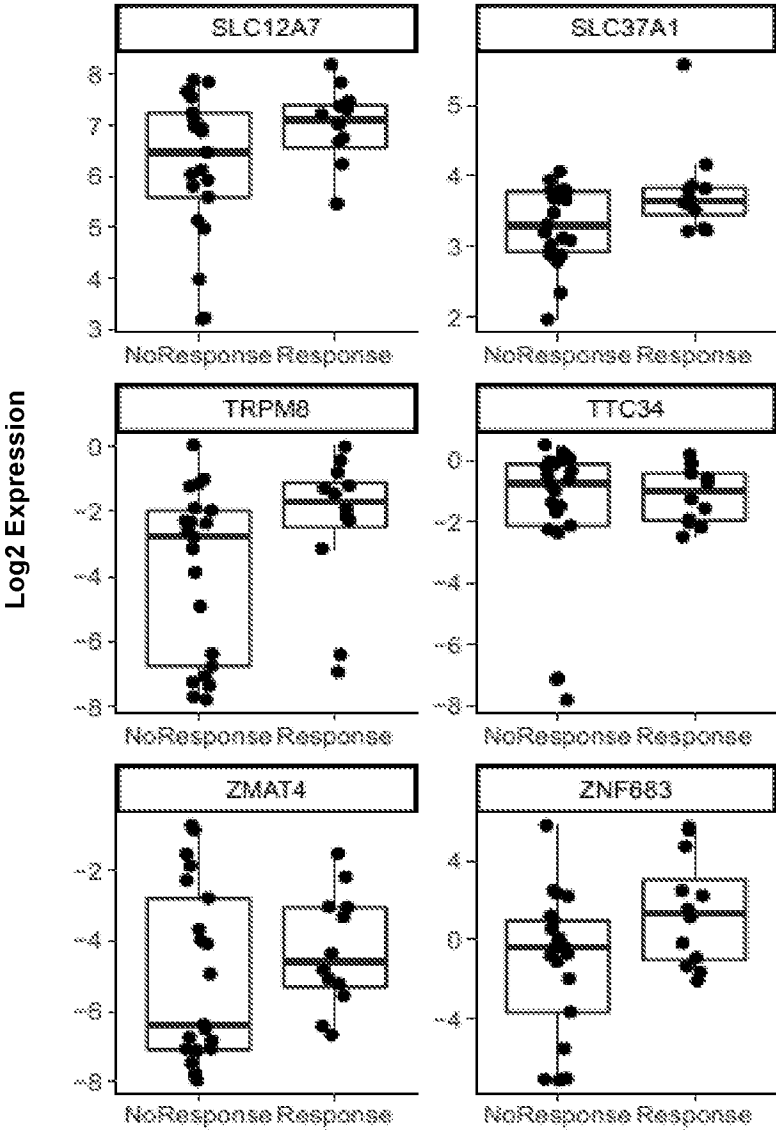


FIG. 7L

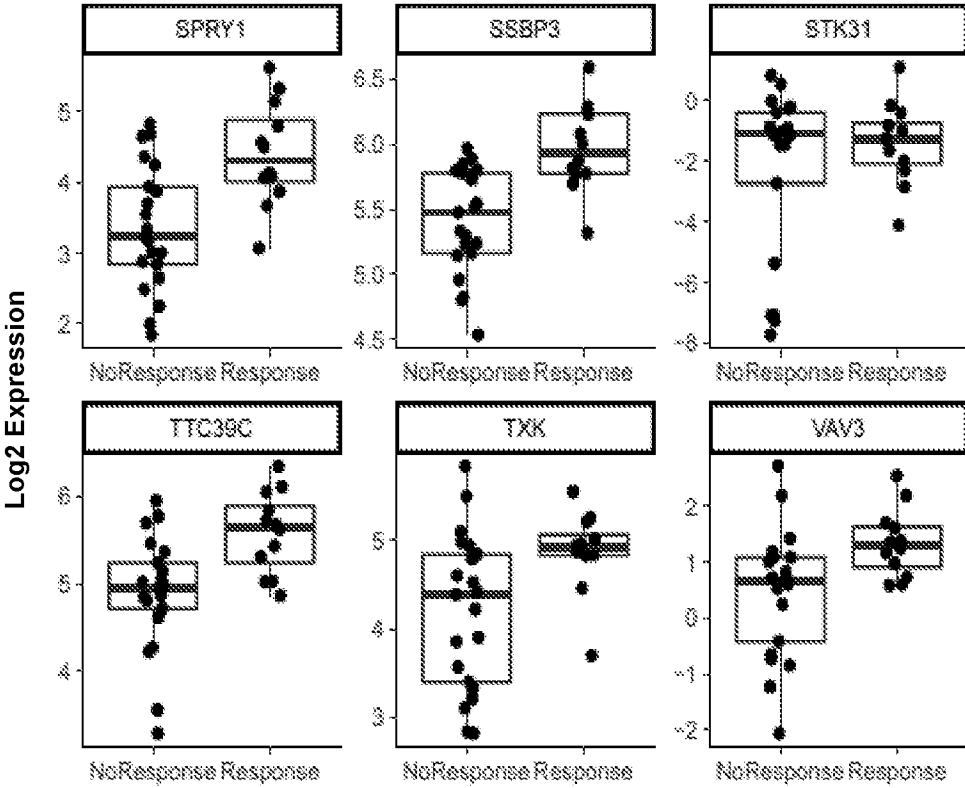


FIG. 8

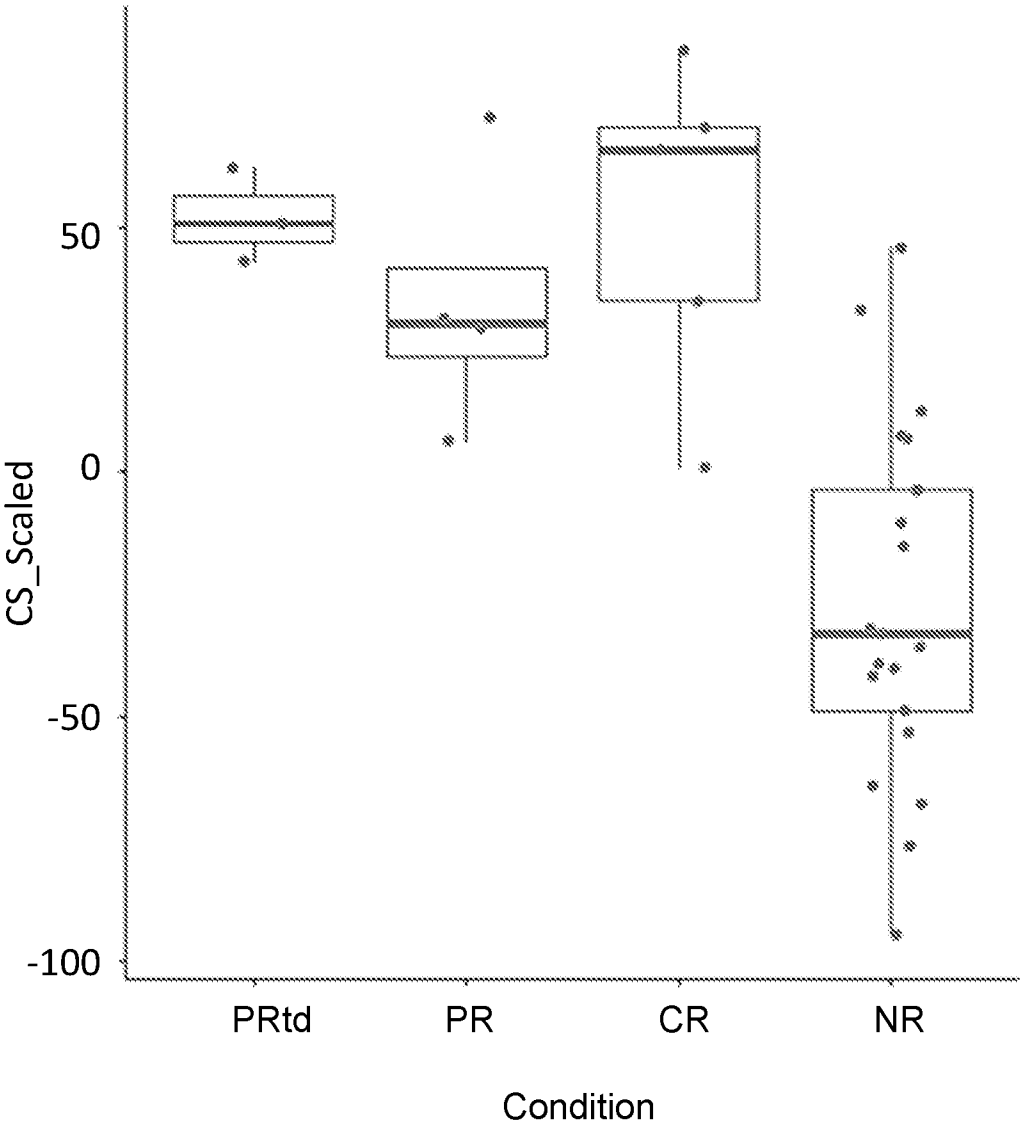


FIG. 9

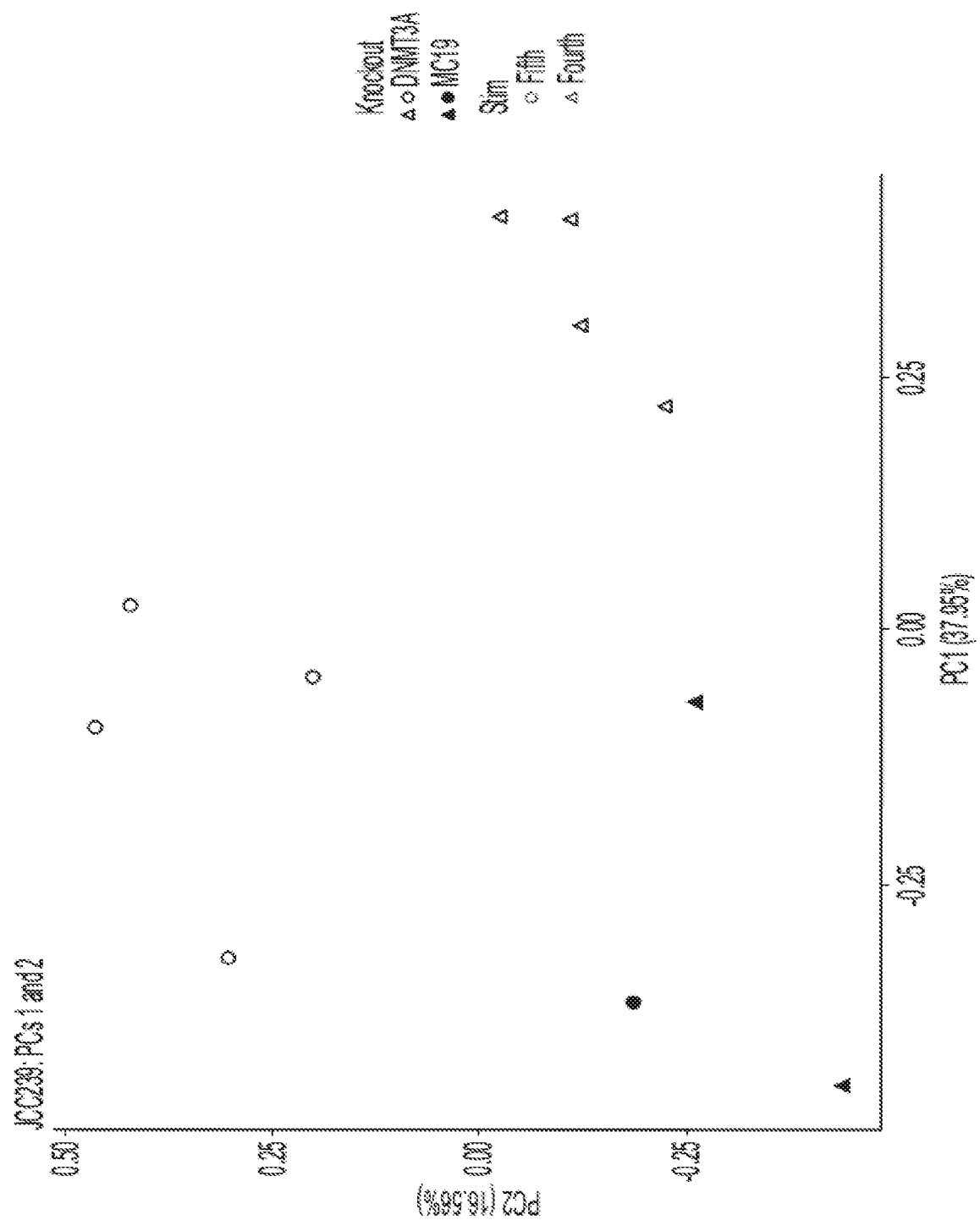


FIG. 10

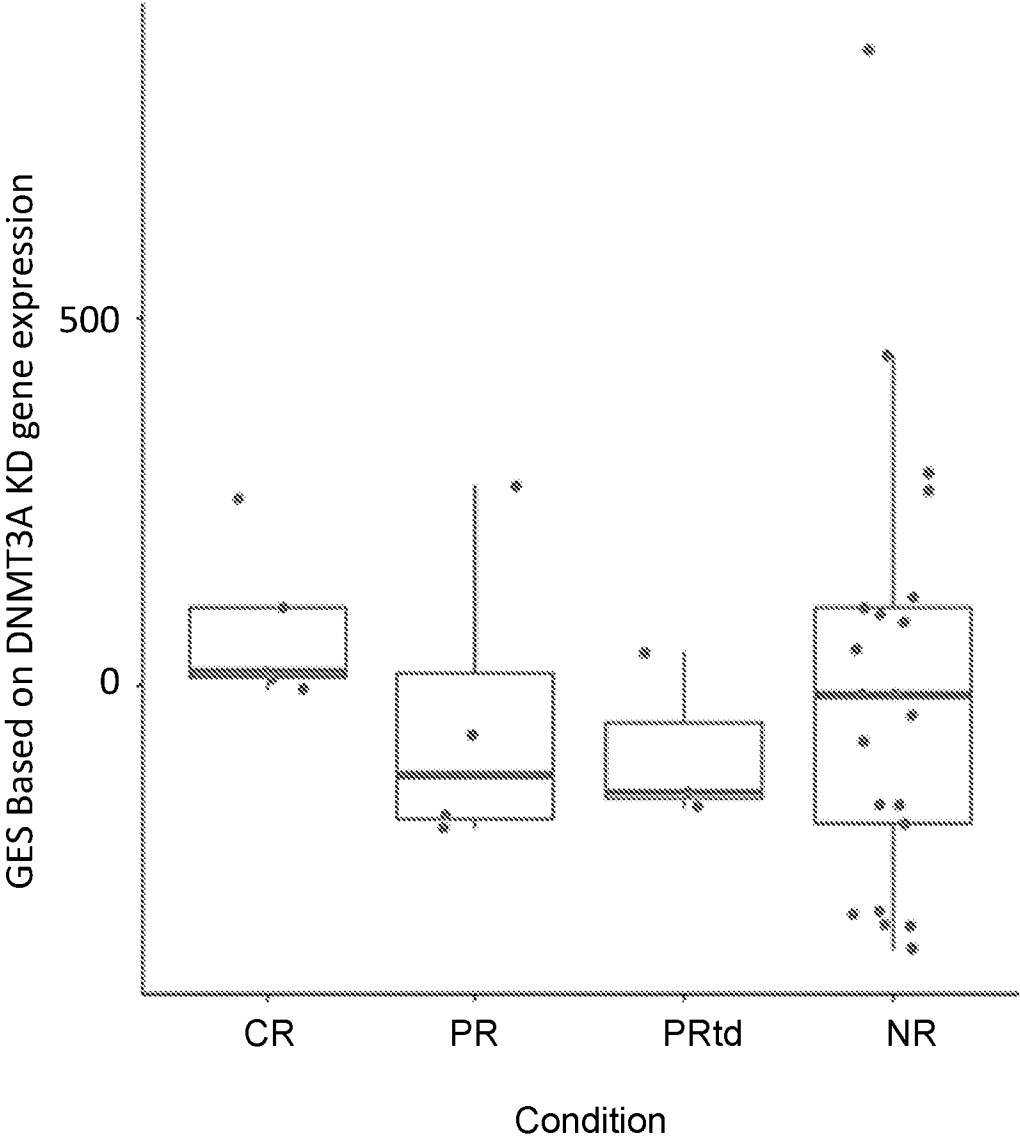


FIG. 11

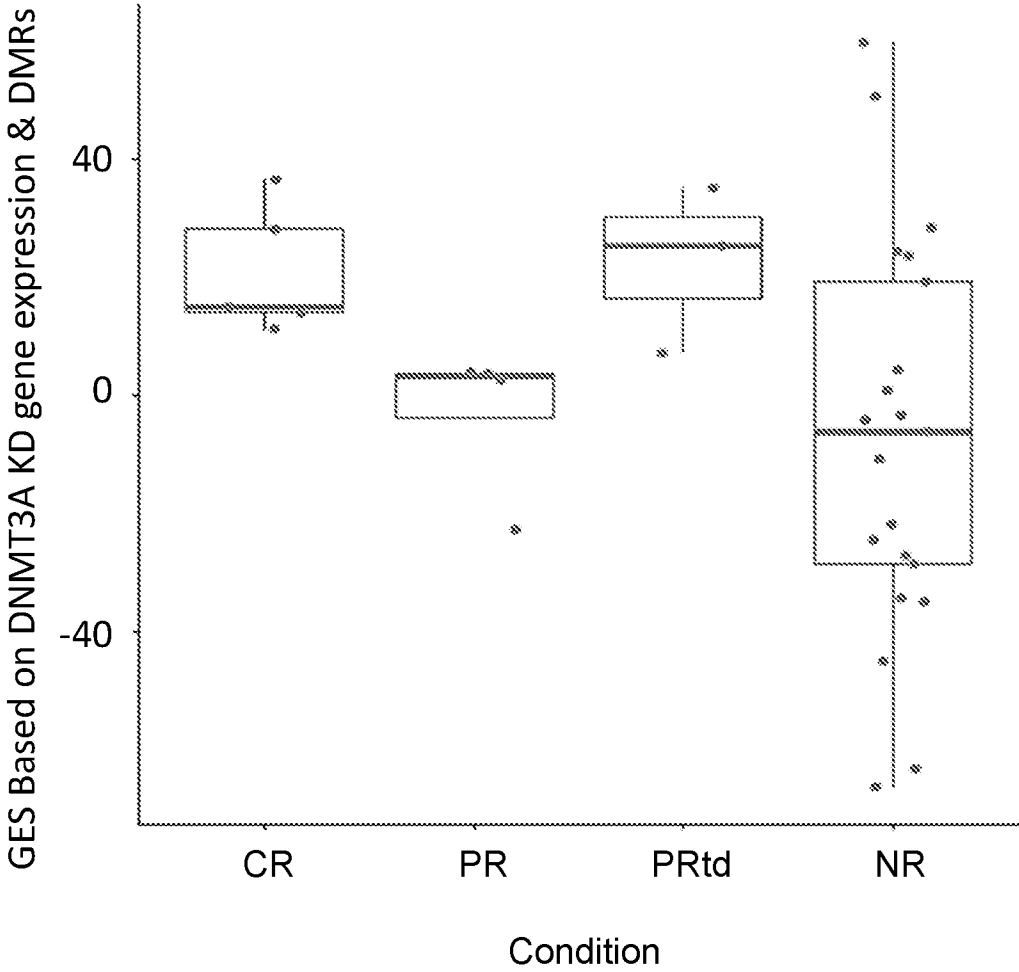
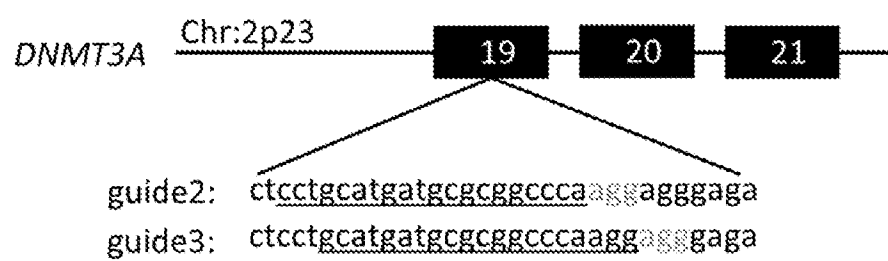


FIG. 12



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/27291

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/17, A61P 35/02 (2020.01)

CPC - C12N 5/0638, C12N 5/0692, C12N 5/0696, C12Q 2600/154, C12N 2501/2302, C12N 2506/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HITCHINGS. There's just something about them: predicting response to CAR T cell therapy. ACIR Weekly digest, 23 May 2018 [online]. [Retrieved on 30 June 2020]. Retrieved from the internet <URL: https://acir.org/weekly-digests/2018/may/theres-just-something-about-them-predicting-response-to-car-t-cell-therapy > pg 1-2	1-8, 10-15, 21-34
Y	GHONEIM et al. De Novo Epigenetic Programs Inhibit PD-1 Blockade-Mediated T Cell Rejuvenation. Cell, 29 June 2017, vol 170, no 1, pp142-157.E19, pg 144, col 1, para 1, pg 146, col 1, para 2, 152, col 1, para 3, col 2, para 2	1-8, 10-15, 21-34
Y	SZABLEWSKI et al. An epigenetic regulator-related score (EpiScore) predicts survival in patients with diffuse large B cell lymphoma and identifies patients who may benefit from epigenetic therapy. Oncotarget, 10 April 2018, vol 9, pp 19079-19099, abstract, pg 19093, col 1, para 2, col 2, para 1-3,	1-8, 10-15, 21-34
Y	US 2018/0318350 A1 (EMORY UNIVERSITY) 08 November 2018 (08.11.2018) abstract, para [0010], [0011], [0034], [0075], [0093]	5-8, 10-15, 24-34
Y	DERENZO et al. Genetic Modification Strategies to Enhance CAR T Cell Persistence for Patients With Solid Tumors. Front. Immunol., 15 February 2019, vol 10, article 218, pp 1-8, pg 4, col 1, para 4, pg 5, col 1, para 2, pg 5, col 1, para 4 - col 2, para 1, para 4, pg 6 col 1, para 2	5-8, 10-15, 24-34

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D" document cited by the applicant in the international application	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

30 June 2020

Date of mailing of the international search report

15 JUL 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Authorized officer

Lee Young

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/27291

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9, 16-20, 35-59
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/27291

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PIEKARZ et al. Epigenetic Modifiers: Basic Understanding and Clinical Development. Clin Cancer Res, 15 June 2009, vol 15, no 12, pp 3918-3926, abstract, pg 3919, col 2, para 2, Table 1	7, 26, 28/26