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(54) Title: METHOD FOR DETERMINING RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS

(57) Abstract: Provided are methods for determining responsiveness to immune checkpoint inhibition. The methods involve determining a mitochondrial haplogroup for an individual who has been diagnosed with cancer. Based on the determination of the mitochondrial haplogroup, an immune checkpoint inhibition therapy can be selected for the individual with improved likelihood of efficacy compared to selection of an immune checkpoint inhibition therapy selected without the mitochondrial haplogroup analysis. The mitochondrial haplogroup analysis can be performed prior to initiating an immune checkpoint therapy.



METHOD FOR DETERMINING RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is based on and claims the benefit of United States Provisional Patent Application No. 63/506,673, filed on June 7, 2023, and of United States Provisional Patent Application No. 63/508,734, filed on June 16, 2023, the entire disclosures of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under R01 CA227505 and F99 CA274650 awarded by the National Institutes of Health. The government has certain rights in the invention.

RELATED INFORMATION

While the prognosis for early-stage melanoma is favorable, with a 10-year survival rate of >99%, late-stage disease was long considered incurable. Immune checkpoint inhibitors (ICIs) have revolutionized melanoma treatment of advanced disease. Single-agent anti-PD1 or the combination of anti-CTLA4 and anti-PD1^{1,2} are now the primary frontline therapies for advanced melanoma, often producing curative outcomes. Nevertheless, ~40% of patients do not respond across treatment arms^{3,4} and ~80% of ICI-treated patients experience immune-related adverse events (irAEs), frequently resulting in treatment discontinuation that dramatically reduces therapy benefits.⁵⁻⁷ The availability of reliable biomarkers predictive of ICI efficacy and/or toxicity at the baseline (pre-treatment) would have significant clinical benefit for improving current ICI regimens and patient survival.

Existing biomarkers of ICI efficacy (response, survival), such as tumor mutation burden (TMB)⁸⁻¹⁰ or levels of infiltrating lymphocytes (TILs),¹¹ are primarily derived from the tumor and tumor microenvironment (TME) and have demonstrated clinical utility in predicting ICI efficacy.^{12,13} Nevertheless, these alone or in combination do not sufficiently explain the heterogeneity of the outcomes observed in ICI-treated patients. Furthermore, the current tumor-based predictors introduce several challenges for clinical practice. These include concerns regarding the feasibility of routine tumor-based assays, and the biological constraints of tumor heterogeneity, in particular for metastatic lesions eliciting diverse immune responses in distal metastatic sites or the shifting tumor/immunity dynamics during ICI treatments. A recent study also showed variation in TMB between different lesions

collected posthumously from the same patient,¹⁴ highlighting previous challenges inherent in reliable and reproducible tumor/immune repertoire assessment.

Over the last several years mitochondrial function has become increasingly implicated in T-cell proliferation and memory formation.^{15,16} Mitochondria are providers of energy in the cell via adenosine triphosphate (ATP) production and mitochondrial assembly requires proteins that arise from both the nuclear and mitochondrial genomes. Unlike the nuclear genome, the mitochondrial genome is inherited solely from the maternal parent and all mitochondrial diversity can be categorized into haplogroups which are defined by specific sets of single nucleotide polymorphisms (SNPs). Haplogroups are associated with different ethnic backgrounds representing major branch points in the mitochondrial phylogenetic tree as humans dispersed globally from Africa. Experiments using cybrids (cell lines with identical nuclear genomes but different mitochondrial genomes) have shown that different mitochondrial haplogroups are associated with changes in a cell's ability to resist chemotherapy drugs¹⁷ and reactive oxygen species (ROS).^{18,19}

Mitochondrial function has also been linked to ICI outcomes with modulation of T-cell efficacy being one proposed mechanism. Recent work has identified a subset of CD8+ cells present in both peripheral blood and tumor-infiltrating T-cells with high levels of oxidative phosphorylation (OXPHOS) as well as both cytotoxicity and exhaustion markers (CD8+ TOXPHOS cells). High levels of CD8+ TOXPHOS cells in the ICI-treated patient cohort were correlated with resistance to treatment.²⁰ Furthermore, they developed a predictive model based on the single-cell transcriptomic profile of CD8+ TOXPHOS cells which was able to predict patient outcomes with ~88% accuracy in their scRNAseq and validation cohorts.²⁰

In mouse models, reactive oxygen species (ROS) signaling has also been demonstrated to improve the tumor-killing effect seen when disrupting the PD-1/PD-L1 axis.²¹ Injection of a ROS precursor (Luperox) into mice with MC38-derived tumors did not increase antitumor activity alone. When administered in combination with anti-PD-L1 however, the antitumor effect was greater than what was observed with single-line anti-PD-L1 treatment, and this improved antitumor effect was lost when ROS was quenched.²¹ They further demonstrated that activation of AMPK and mTOR pathways, which are both downstream of ROS signaling, also synergized with anti-PD-L1 to increase antitumor activity with the activation of both simultaneously providing the best response.²¹ The AMPK and mTOR signaling pathways increase the expression of PGC-1 α which activates mitochondria through nuclear respiratory factors (NRFs) and peroxisome proliferator-activated receptors

(PPARs). Two drugs known to activate PGC-1 α /NRF2 (oltipraz) and PGC-1 α /PPARs (bezafibrate) were also tested for synergistic effects with anti-PD-L1 in mouse models and both showed improved tumor control when tested in combination with anti-PD-L1 but not when used as single-line therapies. Further studies demonstrated that activation of

5 mitochondria using bezafibrate can overcome the immunosuppressive factors secreted by some cancers that inhibit tumor control by anti-PD-L1,²² increasing both proliferation of naïve T-cells and the numbers of effector/memory T-cells.²³ While these studies provide evidence that mitochondria are an important regulator of ICI response, there remains an ongoing and unmet need for improved approaches to predicting ICI responses, and for
10 adjusting patient treatment based on such predictions. The present disclosure is pertinent to this need.

BRIEF SUMMARY

ICIs have revolutionized metastatic melanoma treatment; however, a significant proportion of patients do not respond to treatment. The present disclosure describes
15 minimally invasive pre-treatment biomarkers to stratify melanoma patients so that they can be treated with the correct treatment regimen. The present disclosure demonstrates that baseline host immunity and mitochondrial haplogroups represent novel biomarkers of ICI resistance. Using samples and clinical data from both clinical trials and a large standard-of-care (SOC) cohort, we assessed the association of peripheral CD8+ T cell expression and
20 underlying genomic and mitochondrial genetic repertoire with outcomes in multiple ICI treatment regimens. Combining RNA-seq, ATAC-seq, and High-coverage (30x) Whole Genome Sequencing (WGS) of baseline samples from 115 anti-PD-1 (nivolumab “NIVO”)-treated metastatic melanoma (MM) patients from the CheckMate 067 BMS Phase III clinical trial we determined transcriptomic and genetic underpinnings, comparing patients with NIVO
25 clinical benefit (CB) and no clinical benefit (NCB). We additionally assessed differences in pre-treatment single-cell transcription profiles of cytotoxic T-cells of these patients by performing single-cell (sc)RNA-seq on CD8+ T-cells from baseline PBMCs. We then validated these findings in additional NIVO-treated MM patients from the CheckMate 067 trial using 30x WGS, ultra-low-coverage (0.5x) (lc)WGS, and Whole Exome Sequencing
30 (WES). We further validated these findings in in NIVO-treated MM patients in the IO-GEM cohort, an international consortium of genomic data collected in the SOC setting. Mitochondrial haplogroups in IO-GEM were determined from both Global Screening Array (GSA) and 0.5x lcWGS. Additional validation of the findings was performed in anti-CTLA-4

ipilimumab (IPI)-treated CheckMate 067 and IO-GEM patients. We assessed the association of transcriptomes and haplogroups with combination therapies, first in IPI/NIVO-treated CheckMate-067 patients, and then IPI/NIVO IO-GEM patients. Based on the described analysis, the disclosure provides novel biomarkers detected by RNA-seq analyses of CD8+ T cells (HMAX) pointing to MTRNR2L8 as the most significantly associated with NIVO resistance, in addition to other informative markers as further described herein. Combined with genomic analyses the disclosure reveals that this association is driven by the genetic variation that determines specific mitochondrial haplogroup repertoire. The disclosure also provides an analysis showing that HMAX- patients are enriched by most common European haplogroup (H, ~45%), displaying a NIVO response distribution (~48% CB, 52% NCB). The disclosure demonstrates that HMAX association detected in CD8+ RNA-seq analysis (in HMAX+ patients) links with T haplogroup, as the most significant haplogroup that is ICI resistant. Response rates differ significantly when assessed across the other major European haplogroups. CheckMate-067 patients with the T haplogroup fare especially poorly when treated with NIVO, with a 79% NCB rate (NCB OR, T vs. Others = 3.99 (1.65-11.2), p-value=2.26E-03), a finding that has been validated in IO-GEM (NCB OR, T vs. Others = 3.00 (1.10-9.59), p-value=0.04. From scRNA-seq, the disclosure provides a novel CD8+ T cell-type-specific phenotype among T haplogroup patients, composed of predominantly naïve or undifferentiated T-cells. In contrast, T haplogroup patients treated with IPI tended to have better outcomes compared to the others. Outcomes for T haplogroup patients treated with combined IPI/NIVO are also significantly worse when compared to other haplogroups (69% NCB for HG-T compared to 37% NCB in other haplogroups).

Thus, the disclosure demonstrates for the first time that response to ICI differs significantly across mitochondrial haplogroups, and different ICI treatments produce different outcomes within mitochondrial haplogroups. The disclosure also reveals a unique pre-treatment cellular phenotype among cytotoxic T-cells in T haplogroup patients showing a greater proportion of naïve cells. Given the provided evidence that metabolic differences across haplogroups affect T-cell differentiation, the disclosure provides identification of a subset of patients for whom ICIs targeting cellular differentiation (e.g., anti-CTLA-4) will likely produce better treatment outcomes. However, the disclosure also provides identification of patients that would reach substantially better clinical benefit from ICI that reinvigorate exhausted cells (anti-PD-1) if treated by pembrolizumab. The disclosure therefore provides improved pre-treatment identification of patients across ICI therapies in melanoma and potentially other cancers, thereby providing for tailoring existing ICI

treatments to the appropriate patients and personalized combination ICI therapies. Identifying immune checkpoint inhibition responses as associated with other haplotypes are also provided, such as for K, J and U haplogroups. Markers for L haplogroups and others are also provided and can be analyzed for use in predicting immune checkpoint inhibition responses.

5 Accordingly, the disclosure identifies variants associated with a variety of mitochondrial haplogroups as an independent method of determining ICI outcomes at baseline. In examples, the disclosure accordingly provides a method comprising determining whether or not an individual is likely to respond to treatment with one or a combination of particular ICI's based on a determination of a mitochondrial haplogroup for the individual, and wherein the
10 haplogroup of the individual is determined prior to receiving immune checkpoint inhibition. In examples, the haplogroup is a T haplogroup, a K haplogroup, a U haplogroup, or a J haplogroup. In an example, the haplogroup is the T haplogroup or the K haplogroup or the U haplogroup, and wherein the T haplogroup or the K haplogroup or the U haplogroup indicates the individual is likely to be resistant to an anti-PD-1 ICI as a monotherapy. In this example,
15 a method of the disclosure may further comprise administering to the individual an ICI that is not anti-PD-1 therapy as a monotherapy. In an example, the haplogroup is the J haplogroup, thereby indicating the individual will likely be sensitive to an anti-PD1 ICI. In this example, the method may further comprise administering the anti-PD-1 ICI to the individual. Data presented in this disclosure demonstrate that individuals with haplogroup T are more
20 sensitive to pembrolizumab and resistant to nivolumab.

In examples, determining the haplogroup comprises determining one or more of the markers as defined by the haplogroup tables herein, including but not necessarily limited to Tables 1-4, and the Haplogroup Tables. In an example, the disclosure provides for quantitative next-generation sequencing analysis of RNA expressed from a locus that
25 includes MTRNR2L8 or ATAC-seq chromatin accessibility at this locus (e.g., a quantitative DNA-based method), to thereby indicate the individual has the T haplogroup. In this example, the method may further comprise administering to the individual an ICI that is not anti-PD-1 therapy as a monotherapy.

BRIEF DESCRIPTION OF THE FIGURES

30 **FIG. 1.** Population flow diagram illustrating the full patient population, sample collection, multi-omic material extraction, sequencing/genotyping, and haplogroup determination for the analytic cohorts.

FIG. 2A. Violin plot of the meta-analysis within the single-agent Discovery and Validation Cohorts showing the distribution of HMAX $\log_2$ (gene expression) in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by single-agent Nivolumab ICI by response status (n=116; Complete Response (CR), n=21; Partial Response (PR), n=27; Stable Disease (SD), n=9; Progressive Disease(PD), n=59). HMAX was identified by RNA-seq differential expression as the most significantly differentially expressed gene in this cohort (FDR adj. $p=2.79E-13$, $\log_2FC=4.03$).

FIG. 2B. Violin plot of the meta-analysis within the combination Discovery and Validation Cohorts showing the distribution of HMAX $\log_2$ (gene expression) in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by combination Ipilimumab/Nivolumab ICI by response status (n=115; Complete Response (CR), n=26; Partial Response (PR), n=42; Stable Disease (SD), n=15; Progressive Disease(PD), n=32). HMAX was identified by RNA-seq differential expression as the most significantly differentially expressed gene in this cohort (FDR adj. $p=3.12E-28$, $\log_2FC=4.95$).

FIG. 3A. Volcano plot of the meta-analysis within the single-agent Discovery and Validation Cohorts showing regions of differentially accessible chromatin identified by ATAC-seq analysis in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by single-agent Nivolumab ICI by clinical benefit (CB) status (n=116; CB (Complete Response (CR), n=21; Partial Response (PR), n=27) vs. No Clinical Benefit (NCB) (Stable Disease (SD), n=9; Progressive Disease (PD), n=59); Purple points are regions of open chromatin with a p-value for significant differential accessibility >0.005 ; gray points are regions with an $\text{abs}(\log_2FC) < 1$; pink points are regions with a p-value for significant differential accessibility ≤ 0.05 and an $\text{abs}(\log_2FC) \geq 1$; the green highlighted point is the region of significantly differentially accessible chromatin near the HMAX locus (chr11:10,507,914-10,509,996, build hg38) (FDR adj. $p=7.8686E-08$, $\log_2FC=-1.48$).

FIG. 3B. Violin plot of the meta-analysis within the single-agent Discovery and Validation Cohorts showing the distribution of $\log_2$ (chromatin accessibility) at the HMAX locus (chr11:10,508,542-10,509,994, build hg38) from the ATAC-seq analysis in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by single-agent Nivolumab ICI by response status (n=116; Complete Response (CR), n=21; Partial Response (PR), n=27; Stable Disease (SD), n=9; Progressive Disease (PD), n=59). The HMAX chromatin locus was identified by ATAC-seq differential chromatin accessibility as the most significantly differentially accessible region in this cohort (FDR adj. $p=7.86E-08$, $\log_2FC=-1.48$).

FIG. 4A. Volcano plot of the combination Meta-Analysis Cohort showing the regions of differentially accessible chromatin identified by ATAC-seq analysis in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by combination Ipilimumab/Nivolumab ICI by Clinical Benefit (CB) status (n=110; CB (n=65, Complete Response (CR), n=23; Partial Response (PR), n=42) vs. No Clinical Benefit (NCB) (n=45, Stable Disease (SD), n=16; Progressive Disease (PD), n=29). Purple points are regions of open chromatin with a p-value for significant differential accessibility >0.005 ; gray points are regions with an $\text{abs}(\log_2\text{FC}) < 1$ and a p-value >0.005 ; pink points are regions with a p-value for significant differential accessibility ≤ 0.005 and an $\text{abs}(\log_2\text{FC}) \geq 1$; the green highlighted point is the region of significantly differentially accessible chromatin near the HMAX locus (chr11: 10,508,160-10,509,996, build hg38) (FDR adj. $p=1.17\text{E-}03$, $\log_2\text{FC}=-1.28$).

FIG. 4B. Violin plot of the combination Meta-Analysis Cohort showing the distribution of $\log_2(\text{chromatin accessibility})$ at the HMAX locus (chr11: 10,508,160-10,509,996, build hg38) from the ATAC-seq analysis in baseline (pre-treatment) peripheral blood CD8+ T-cells of patients treated by combination Ipilimumab/Nivolumab ICI by response status (n=110; Complete Response (CR), n=23; Partial Response (PR), n=42; Stable Disease (SD), n=16; Progressive Disease (PD), n=29). The HMAX chromatin locus was identified by ATAC-seq differential accessibility among the top 10 most significantly differentially accessible regions in this cohort (FDR adj. $p=1.17\text{E-}03$, $\log_2\text{FC}=-1.28$).

FIG. 5. Phylogenetic tree depicting mtDNA lineages and CheckMate-067 NIVO treatment response. Subjects with NIVO Clinical Benefit (CB; Complete Response (CR) or Partial Response (PR)) are shown in navy. Subjects with NIVO No Clinical Benefit (NCB; Stable Disease (SD) or Progressive Disease (PD)) are shown in blue. Frequency counts of CB and NCB are plotted for the major European haplogroups (H, U, T, J, K, V, I, X, W). **Inset:** proportion of CB and NCB within each major haplogroup (H, U, T, J, K).

FIGS. 6A-D . CheckMate-067 and IO-GEM NIVO treatment efficacy frequencies by European haplogroup (H, U, T, J, K, and Other, including W, R, V, X, I). (A) CheckMate-067 NIVO discovery cohort, (B) CheckMate-067 NIVO validation cohort, (C) CheckMate-067 pooled analysis, and (D) IO-GEM validation cohort.

FIG. 7. Forest plots showing the odds ratios (ORs), log odds, and p-values for NIVO treatment outcomes among T, T and K, or J haplogroup and Other European Haplogroups. Black boxes represent the log odds point estimate and the lines extending from them represent the 95% confidence intervals. Point estimates to the left of the dotted line indicate increased odds of NIVO clinical benefit, while point estimates to the right indicate increased

odds of no NIVO clinical benefit. Confidence intervals that do not cross the dotted line indicate a statistically significant point estimate ($p < 0.05$).

FIG. 8. Forest plot showing the odds ratios (ORs), log odds, and p-values when comparing NIVO treatment outcomes by haplogroups, comparing T versus Other European Haplogroups. Black boxes represent the point estimate for the log odds and the lines extending from them represent the 95% confidence intervals. Point estimates to the left of the dotted line indicate increased odds of NIVO clinical benefit, while point estimates to the right indicate increased odds of no NIVO clinical benefit. Confidence intervals that do not cross the dotted line indicate a statistically significant point estimate ($p < 0.05$).

FIGS. 9A-D. Single-cell (sc)RNA-seq analysis for pre-treatment circulating CD8⁺ T cells from CheckMate-067 NIVO patients (n=21). FIG. 9A displays Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction plot. FIG. 9B displays a bubble plot showing per-cluster cell-type classification based on T cell marker gene expression. FIG. 9C displays a box plot showing single-cell CD8⁺ T-cell type proportions stratified by NIVO treatment response (Clinical Benefit, CB; No Clinical Benefit, NCB) and haplogroup (NIVO-resistant haplogroups, HGR; other haplogroups, HGO). FIG. 9D displays a scatterplot showing per-cluster mean expression of T cell exhaustion markers grouped by cell type.

FIG. 10A. Top 8 significantly differentially expressed genes per cluster from single-cell (sc)RNA-seq of peripheral blood CD8⁺ T cells isolated from n=21 NIVO-treated patients from CheckMate-067.

FIG. 10B. Per-cluster cell-type classification based on differential expression of marker genes from scRNA-seq of peripheral blood CD8⁺ T cells isolated from n=21 NIVO-treated patients from CheckMate-067.

FIG. 10C. Per-cluster gene expression of T cell exhaustion markers from scRNA-seq of peripheral blood CD8⁺ T cells isolated from n=21 NIVO-treated patients from CheckMate-067.

FIGS. 11A-F. Proportion of CB and NCB ICI treatment responses in NIVO, COMBO (IPI/NIVO), and IPI cohorts comparing HG-T and other (H, U, J, K, W, R, V, X, and I) MT-HGs in: (A) CM-067 NIVO cohort; (B) CM-067 COMBO cohort; (C) CM-067 IPI cohort; (D) IO-GEM NIVO cohort; (E) IO-GEM COMBO cohort (F) IO-GEM IPI cohort. Discovery (CheckMate-067, n=526) and Validation (IO-GEM, n=749). CB: clinical benefit (Complete Response CR; Partial Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progressive Disease PD).

FIGS. 12A-B. ICI treatment efficacy in NIVO, IPI, or IPI/NIVO cohorts comparing HG-T and other (H, U, J, K, W, R, V, X, and I) MT-HGs. CB: clinical benefit (Complete Response CR; Partial Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progression of Disease PoD). MT-HG: mitochondrial haplogroups. FIG. 12A displays a meta-analysis proportions of CB and NCB comparing HG-T and other MT-HGs for: CheckMate-067 and IO-GEM IPI cohort (CTLA-4); CheckMate-067 and IO-GEM NIVO cohort (PD-1); CheckMate-067 and IO-GEM COMBO cohort (Combo). FIG. 12B displays forest plots showing the odds ratios (ORs), log odds, and p-values for ICI treatment outcomes among T haplogroup and Other European Haplogroups. Black boxes represent the log odds point estimate and the lines extending from them represent the 95% confidence intervals. Point estimates to the left of the dotted line indicate increased odds of ICI clinical benefit compared to the reference (Other European Haplogroup patients treated with anti-CTLA-4, anti-PD-1, or Combo), while point estimates to the right indicate increased odds of no ICI clinical benefit compared to the reference. Confidence intervals that do not cross the dotted line indicate a statistically significant point estimate ($p < 0.05$).

FIG. 13A. ICI treatment efficacy in anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO combined), or combined IPI/NIVO (COMBO) cohorts. CB: clinical benefit (Complete Response CR; Partial Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progression of Disease PoD). MT-HG: mitochondrial haplogroups.

FIG. 13B. ICI treatment efficacy in anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO separated), or combined IPI/NIVO (COMBO) cohorts. CB: clinical benefit (Complete Response CR; Partial Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progression of Disease PoD). MT-HG: mitochondrial haplogroups.

FIG. 13C. ICI treatment efficacy in anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO combined), or combined IPI/NIVO (COMBO) cohorts comparing HG-T and other (H, U, J, K, W, R, V, X, and I) MT-HGs. CB: clinical benefit (Complete Response CR; Partial Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progression of Disease PoD). MT-HG: mitochondrial haplogroups.

FIG. 13D. ICI treatment efficacy in anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO separated), or combined IPI/NIVO (COMBO) cohorts comparing HG-T and other (H, U, J, K, W, R, V, X, and I) MT-HGs. CB: clinical benefit (Complete Response CR; Partial

Response PR; durable Stable Disease dSD). NCB: no clinical benefit (progressive Stable Disease pSD; Progression of Disease PoD). MT-HG: mitochondrial haplogroups.

FIG. 14. Volcano plot showing significant upregulation of ROS detoxification genes in baseline CD8⁺ T cells of HG-T patients from CheckMate-067 compared to HG-H (n=121)

5 DETAILED DESCRIPTION

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains.

Unless specified to the contrary, it is intended that every maximum numerical limitation given throughout this description includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

All nucleotide sequences described herein include the RNA and DNA equivalents of such sequences, i.e., an RNA sequence includes its cDNA. All nucleotide sequences include their complementary sequences. All protein sequences described herein include all isoforms of such proteins, e.g., proteins made from splice variants, and proteins that may vary from individual to individual in certain amino acids. All mRNAs described herein include all splice variants. The amino acid or polynucleotide sequence as the case may be associated any databased entry of this disclosure is incorporated herein by reference as presented in the database on the effective filing date of this application or patent.

In general, and as illustrated by the examples below and the figures, the present disclosure provides a method for predicting treatment response to immune checkpoint inhibitors (ICIs). The methods involve determining a mitochondrial haplogroup for an individual who has been diagnosed with cancer. Based on the determination of the mitochondrial haplogroup, an immune checkpoint inhibition therapy can be selected for the individual with improved likelihood of efficacy compared to selection of an immune checkpoint inhibition therapy selected without the mitochondrial haplogroup analysis. The mitochondrial haplogroup analysis can be performed prior to initiating an immune checkpoint therapy. Accordingly, the described methods are applicable to any individuals who are

diagnosed with cancer. In examples, the method comprises a) analyzing a biological sample obtained from the individual prior to a treatment with one or more ICIs for expression of one or more biomarkers described herein, including but not necessarily limited to any one or combination of single nucleotide polymorphisms (SNPs) or other nucleotide changes, such as insertions or deletions, to establish a baseline level of the one or more markers, and b) comparing the signature of the one or more biomarkers to a control value, and/or designating the individual to have be of a particular mitochondrial haplogroup. While the disclosure relates in some examples to haplogroups that are identified by polynucleotide sequencing, the disclosure also includes analysis of proteins that may have certain features that indicate the individual is a member of a particular haplogroup. Thus, both nucleic acid and protein detection are included in the method. In examples, RNA sequencing is used. In examples, any suitable RNA-Seq approach may be used. In examples, mitochondrial DNA or RNA may be sequenced to determine a haplogroup. In examples, whole genome or whole exome sequencing may be used. In examples, quantitative next-generation sequencing analysis of RNA expressed from a locus that includes MTRNR2L8, or ATAC-seq chromatin accessibility at this locus, indicates the individual has the T haplogroup.

In examples, a biological sample from an individual is analyzed. In examples, a liquid biological sample is used. In examples, peripheral blood lymphocytes are obtained from an individual and are tested to determine a haplogroup. In examples, in addition to determining a haplogroup, the disclosure also comprises analyzing CD8⁺ T cells, and includes but is not necessarily limited to determining biomarker expression and/or a haplogroup in the CD8⁺ T cells. In examples, the control to which the patient sample is compared comprises a sample from one or more individuals who respond to one or more checkpoint inhibitors as a treatment for cancer, and as such, a control may be a value for a known haplogroup. In examples, the control comprises a sample from one or more individuals who respond to a combination of checkpoint inhibitors as a treatment for cancer. In examples, the control may be a standardized curve(s), a cutoff or threshold value, and the like. Where described haplogroup is determined, the disclosure further comprises administering one or more ICIs to the individual. In examples, a second, third, fourth, sample, etc. can be obtained from an individual who is undergoing treatment and tested to monitor the effect of the treatment, and keep steady, change, adjust, discontinue, or not reinitiate treatment with a checkpoint inhibitor.

In one example, the sample is obtained from an individual who has any type of cancer and has not undergone any previous ICI treatment, and thus may be considered a baseline

sample. In one example, the individual has not received any ICI treatment within a period of at least six months. In one example, the cancer is melanoma, which may be metastatic melanoma, but the described methods can be used for other types of cancer, representative and non-limiting examples of which include lung cancer, breast cancer, ovarian cancer, pancreatic cancer, colorectal cancer, bladder cancer, brain cancer, cervical cancer, esophageal cancer, stomach cancer, head and neck cancer, kidney cancer, liver cancer, testicular cancer, prostate cancer, non-melanoma skin cancer, any sarcoma, uterine cancer, and liquid cancers of the blood or lymph, and any other type of cancer where use of any ICI treatment would be indicated.

The disclosure relates to predicting responses and recommending treatments, and providing treatments, for any of ICI that targets PD-1, non-limiting examples of which include Pembrolizumab, Nivolumab, and Cemiplimab, and any ICI that targets PD-L1, non-limiting examples of which include Atezolizumab, Avelumab, and Durvalumab, and any ICI that targets CTLA-4, non-limiting examples of which include Ipilimumab and Tremelimumab, and LAG-3 inhibitors, a non-limiting example of which includes Relatlimab, as well as combinations of ICI inhibitors, one non-limiting example of which is Relatlimab that is used in combination with nivolumab and is referred to as Opdualag, and any other ICI, such as anti-TIGIT and anti-TIM-3 agents.

The present disclosure is different from prior approaches in that the described biomarkers have not been previously shown to be predictive for responses to an immune checkpoint inhibitor or a combination of such inhibitors before treatment with an immune checkpoint inhibitor. For example, U.S. patent publication 20200347456 discloses biomarkers that include, for example, MTRNR2L8. However, this publication is focused on tumors and tumor infiltrating cells that are analyzed after treatment. Further, it is the post-treatment values that are used to select individuals for CDK4/6 treatment in combination with immune checkpoint inhibition. Accordingly, any resistance biomarker signature described in this publication is used for identification of cancer cells that have developed resistance to therapeutic agents as a consequence of treatment, e.g., oncogenic immune checkpoint resistances states. This publication also describes such resistance as an intrinsic property of tumor cells that arises only after immune checkpoint inhibition, and focuses on tumor microenvironment and its effect on tumor cells. This publication does not describe predicting responses by germline inherited genetics. This publication also does not describe predicting responses to combined immune checkpoint inhibition, nor does it describe any biomarkers, as

a part of an immune checkpoint resistance signature, or having any connection to T cell status.

In marked contrast to the 20200347456 publication, and as described above, the present disclosure relates at least in part to characterization of haplogroups and their connection to ICI responses. This allows for establishment of a pre-treatment baseline value. This approach can be used to predict immune checkpoint resistance before an individual has ever received a checkpoint inhibitor, or for individuals who have stopped taking an immune checkpoint inhibitor and are under consideration for additional rounds of immune checkpoint inhibition. In connection with this, the data provided and discussed below demonstrate that resistance to immune checkpoint inhibition is a property of the host immune system at the baseline, a germline inherited genetic factor. Thus, the present disclosure does not require determining any biomarker from cancer cells or a tumor. Instead, the present disclosure is agnostic towards tumor status, and accordingly provides compositions and methods for predicting *a priori* those patients who should not be treated with a particular immune checkpoint inhibitor, and by identifying those patients, determining which individuals are candidates for immune checkpoint inhibition going forward. Thus, in examples, a sample tested using the compositions of this disclosure may be free of any sample of a tumor or cancer cells.

In examples, the disclosure provides for determination of a haplogroup and determining that the individual is a candidate to receive a particular ICI or combination of ICIs, or the individual should not receive or should discontinue a particular ICI or combination of ICIs. In one example, the method comprises determining that the individual has a haplogroup that is any of a K haplogroup, a T haplogroup, or a U haplogroup, and based upon this determination, determining that the individual will not likely sufficiently respond to a specific anti-PD-1 or anti-PD-L1 antibody, wherein either case the ICI is used as a monotherapy. Data presented in this disclosure demonstrate that individuals with haplogroup T are more sensitive to pembrolizumab and resistant to nivolumab. In examples, determining that an individual has the T haplogroup therefore indicates the individual should receive an ICI that is not nivolumab. In an example, an individual who has the T haplogroup is treated with pembrolizumab, or any other anti-PD-1 agent that is not nivolumab, and wherein the other anti-PD-1 agent that is not nivolumab elicits an improved anti-cancer response, relative to an actual or predicted response to nivolumab. The same applies to individuals with the K haplogroup and U haplogroup who show a similar trend to the T haplogroup. As such,

individuals who are of the K, T or U haplogroup can be treated with a different ICI antibody or a combination thereof.

In examples, individuals who are of the K, T or U haplogroup may be treated with an ICI that is not anti-PD-1 or anti-PD-L1, or may include anti-PD-1 or anti-PD-L1 in

5 combination with a different ICI, such as a combination of anti-PD-1 or anti-PD-L1 with anti-CTLA4, or any other different ICI, examples of which are discussed herein.

In an example, the disclosure provides for determination of an individual as having a J haplogroup and determining that the individual is a candidate for a particular ICI therapy, such as an anti-PD-1 or anti-PD-L1 therapy. The disclosure also includes assessment of L and
10 other haplogroups that may be informative of ICI responses, and tailoring ICI treatments accordingly. The disclosure includes determining haplogroups using any one or combination of markers that are described herein by way of the haplogroup tables presented below.

In more detail, and without intending to be limited by any particular theory, this disclosure provides an analysis of the presence of novel surrogates of transcriptomic and
15 genetic diversity of peripheral immune cells, as putative more personalized biomarkers of ICI efficacy. In particular, we determined whether mitochondrial haplogroups represent novel biomarkers predictive of ICI outcome. Using peripheral blood samples from a large metastatic melanoma clinical trial (CheckMate-067) with extensive clinical information harmonized across study sites, we assessed if the transcriptomic and genetic diversity of
20 peripheral CD8⁺ effector cells associates with the response to ICI therapies (anti-PD1 single-line Nivolumab or “NIVO” and COMBO; treated by Ipilimumab or “IPI” and NIVO) and whether such changes link with patients’ mitochondrial genetic repertoire. Subsequently, we validated the results in the standard of care setting using patients collected by the Germline Immuno-Oncology Melanoma Consortium (IO-GEM).

25 The following description is intended to illustrate but not limit the disclosure.

Materials and Methods

Study Population

In this study, we used patient samples from the CheckMate-067 clinical trial (CM-
30 067, ClinicalTrials.gov identifier NCT01844505), a 3-arm Phase III study that enrolled therapy-naïve advanced melanoma patients treated between 2013-2015 with single-agent ipilimumab (IPI), nivolumab (NIVO), or the combination (COMBO, IPI/NIVO)(FIG. 1)^{1,2} In

the present CM-067 analysis, we sequenced pretreatment specimens of PBMCs or purified peripheral CD8+ T cells from n=155 NIVO and n= 126 COMBO patients and utilized previously generated data from whole blood-based whole exome sequencing (WES) of patients treated by NIVO, COMBO, and IPI (n=454). In total, n=526 CM-067 patient samples were used in this analysis. The best overall response (BOR) to treatment in CM-067 was defined as complete response (CR), partial response (PR), stable disease (SD), or progression of disease (PoD), based on Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1.^{1,24} Clinical benefit (CB) was defined as those with CR, PR, or durable (d)SD (stable disease without progression for at least 6 months).²⁵ Patients with no clinical benefit (NCB) were defined as PoD or SD who progressed within 6 months (pSD). Overall survival (OS) and progression-free survival (PFS) were defined as described previously.¹

For validation of the findings from CM-067, we employed the Immuno-Oncology Germline Genetics in Melanoma (IO-GEM) Consortium, a standard-of-care (SOC) cohort of ICI-treated melanoma patients treated from centers in the US, Europe, and Australia. Samples for this analysis were collected the following institutions: New York University Langone Health (NYULH), Memorial Sloan Kettering Cancer Center (MSK), University of California Los Angeles's Jonsson Comprehensive Cancer Center (UCLA-JCCC), Massachusetts General Hospital (MGH), Dana Farber Cancer Institute (DFCI), University of Chicago Comprehensive Cancer Center (UCCCC), University of Colorado Cancer Center (CUCC), Roswell Park Cancer Institute (RPCI), and the National Tumor Institute Fondazione G. Pascale in Naples, Italy (INT-IRCCS). All patients had metastatic melanoma (MM) treated with NIVO, COMBO, or IPI (FIG. 1). Each patient provided blood samples (whole blood or PBMC) for DNA extraction. The sample and data collection protocols were approved by each institution's IRB and patient informed consent was obtained prior to collection. We also obtained data from publicly available sequencing of ICI-treated MM patients.^{26,27} In total, the SOC validation cohort consisted of n=675 patients. Response status was determined by the centers and was similarly dichotomized into CB (CR, PR, and dSD) and NCB (pSD and PoD). Patients with SD for whom we could not determine the duration of their stable disease were removed from the analysis (n=5).

For prognostic analyses of survival stratified by haplogroups, we used a cohort of immunotherapy-naïve melanoma patients who were prospectively enrolled in the Interdisciplinary Melanoma Cooperative Group (IMCG) at NYULH from 2002-2018 (n=1,024 subjects).²⁸ Clinicopathological, follow-up, and biospecimen collection protocols for the IMCG have been previously described.²⁸⁻³⁰ For confirmatory analysis of the expected

MT-HG population distribution, we obtained MT-HGs from n=420 participants from the European populations in the 1000 Genomes Project (1kGP).³¹ Genomic data for this analysis were collected from 1kGP Phase 1 (n=244 subjects) and Phase 3 (n=176 subjects).

Sample isolation

5 Pre-treatment PBMCs were used to isolate CD8+ T-cell populations from 240 patients. The CD8+ cells were negatively selected from the overall PBMC population using an EasySep Human CD8+ Enrichment Kit (STEMCELL Technologies, Vancouver, BC, Canada) per manufacturer's instructions. The RNA/DNA were extracted using the AllPrep DNA/RNA kit for simultaneous purification of genomic DNA and total RNA from human
10 cells (Qiagen, Germantown, MD). For ATAC-seq, two ATAC-seq libraries per patient were generated from each patient to ensure data integrity. Each library preparation used ~50,000 viable CD8+ cells and was prepared as previously described.

RNA and ATAC Sequencing and Data Pre-Processing

15 The rRNA-seq depletion has been used for preparation of RNA libraries, sequenced on the Nova-seq using 150-base paired-end sequencing to a depth of ~30 million reads per sample.

 The SMARTer Stranded Total RNA-Seq Kit v2 Pico Input Mammalian (TaKaRa Bio USA, Mountain View, CA) was used to prepare rRNA-depleted RNA-seq libraries. Each
20 library was then quantified by qPCR and libraries were pooled for sequencing using 150-base paired-end sequencing to a depth of ~30 million reads per sample.

 Each library ATAC-seq library was quantified by qPCR and the libraries were pooled for sequencing on the Nova-seq using 150-base paired-end sequencing to a depth of ~30 million reads per sample.

25 30x WGS Sample Preparation and Data Processing

 We isolated genomic DNA from pre-treatment PBMCs from the CheckMate-067 clinical trial using the Qiagen Blood and Tissue Kit (Qiagen, Germantown, MD). Libraries for whole genome sequencing (WGS) were prepared per manufacturers' instructions. Samples were prepared using the Illumina TruSeq DNA PCR-Free kit (for samples with DNA
30 content >500ng) or the New England BioLabs NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (for samples with <500ng DNA). Samples were sequenced to a target of 30x genomic coverage on an Illumina HiSeq X or NovaSeq 6000 per manufacturer's instructions, using 2 x 150bp paired-end reads.

Sequencing FASTQ files were processed using the Genome Analysis Toolkit (GATK) workflow.³² Briefly, raw FASTQs were aligned to the Genome Reference Consortium Human Build 38 reference genome (GRCh38) using bwa mem (version 0.7.17).³³ Aligned Binary Alignment Maps (.bams) were merged using sambamba merge (version 0.6.8),³⁴ sorted with samtools sort (version 1.9),³⁵ and duplicates marked using sambamba markdup. We then performed two rounds of base quality score recalibration (BQSR) using GATK BaseRecalibrator (version 4.1.2.0). To identify the variants in the mitochondrial genome, Variant Call Format files (.vcfs) were generated by passing the aligned .bams to GATK's Mutect2 (version 4.2.1.0) using mitochondria-mode which is designed for sensitive variant detection at high sequencing depth. Finally, mitochondrial haplogroups were classified by passing the .vcfs containing mitochondrial variants to haplogrep3 (version 3.2.1).³⁶ To define the haplogroups, we used the mitochondrial phylogenetic tree from Phylotree (version 17.1)³⁷ aligned to the revised Cambridge Reference Sequence (rCRS) by specifying -tree phylotree-rcrs@17.1 in the haplogrep classify statement.

15 **Single-cell RNA-sequencing and data analysis**

CD8⁺ T-cells were negatively selected from pre-treatment PBMCs from 23 NIVO-treated patients using the EasySep Human CD8⁺ Enrichment Kit (STEMCELL Technologies, Vancouver, BC, Canada) per manufacturer's instructions. Single-cell suspensions for each patient were individually tagged with unique hashtag antibodies (TotalSeq-B Anti-Human antibodies, Biolegend) and each sample was washed 3 times with 1x PBS + 2% BSA + 0.01% Tween before pooling. Four patient samples were pooled as one sample in suspension (n~12,500 cells per hashtag, n~50,000 total cells per pool) for a total of six pooled samples. Single cells were partitioned into Gel Beads-in-Emulsion (GEMs) using the Chromium Next GEM Chip G and tagged with a common 10x barcode and a unique molecular identifier (UMI) (10x Genomics, Chromium Next GEM Single Cell 3' GEM Library & Gel Bead Kit). Reverse transcription, amplification, and library preparation of 3' gene expression libraries were performed per published protocols (10x Genomics).³⁸ Libraries were sequenced using the NovaSeq 6000 platform at a minimum of 20,000 read pairs per cell at the Perlmutter Cancer Center Genome Technology Center Shared Resource (GTC).

Single-cell (sc)RNA-seq QC and downstream analysis of CD8⁺ T cells were performed with the Seurat v3 R toolkit.³⁹⁻⁴² Demultiplexing of samples based on hash tag oligos (HTOs) and annotation of doublets removed from the analysis was performed using

“HTODemux” function with default parameters. Cells with gene counts >200 and <12,500 were kept for downstream analysis while genes expressed in <3 cells were filtered out. Cells expressing >25% mitochondrial genes and <0.05% ribosomal genes were discarded. 92,531 CD8⁺ T cells from 21 patients successfully passed QC and were used for the present analysis.

5 Batch correction was then performed in Seurat using the integration method to mitigate artefactual differences between sequencing pools. Normalization of the data was performed using “NormalizeData” with the “LogNormalize” method and the most variable genes were found using the “FindVariableGene” function with “nfeatures” set to 2000. PCA was run on 50 dimensions and after a visual inspection of the elbow plot, the first 15 PCs were chosen to
10 construct a Shared Nearest-neighbor (SNN) graph with the “FindNeighbors” function. “FindClusters” was applied to identify clusters with the resolution set to 0.5. The Uniform Manifold Approximation and Projection (UMAP) algorithm was used to visualize the clustering in 2D space.

Infinium Global Screening Array Sample Preparation and Data Processing

15 Mitochondrial genotypes were determined for a subset of the IO-GEM SOC cohort using the Infinium Global Screening Array Multi-Disease (GSA-MD) platform, version 2.0 and version 3.0, which includes a drop-in panel of an additional ~50,000 multi-disease-associated genomic markers. Genomic DNA from these patients was isolated from whole blood or PBMCs using the Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen, Germantown,
20 MD). Using Qubit and gel electrophoresis, we quantified DNA concentration and assessed DNA integrity. Only high-quality samples with no evidence of degradation and a minimum concentration of 10ng/μL were genotyped. Prior to genotyping, samples were identity-tracked using a panel of 25 previously curated variants⁴³ using the Sequenom MassArray System (Agena Bioscience Inc., CA), per manufacturer’s instructions. We observed high concordance
25 (>99%) between the identity panel and the GSA-MD, minimizing the likelihood of possible sample mismatches.

Using Illumina’s GenomeStudio (version 2.0), raw intensity data (.idat) files from the GSA-MD were converted to genotype calls with a GenCall score threshold of 0.15. We exported these genotype calls from GenomeStudio to PLINK^{44,45} format for downstream
30 analysis using the PLINK Input Report Plug-in (version 2.1.4). Genotype quality assurance (QC) was performed in PLINK (version 1.9/2.0) by first excluding SNPs not present in ≥20% of the samples (assumed to be poorly genotyped SNPs) using the --geno command in PLINK.

We then excluded any patients missing $\geq 20\%$ of the remaining SNPs using the PLINK --mind command (assumed to be patients with poor overall genotype quality).

As haplogroups are defined by the specific nucleotide at each position, and not only whether a subject has a reference or an alternate allele, haplogrep3 requires the SNPs in the input .vcf to be reported according to the rCRS reference sequence. GSA probes can target sequences on either the forward or the reverse DNA strand; SNPs called from probes on the forward strand are reported according to the rCRS, while those on the reverse strand are reported as the complementary base. We therefore identified all the mitochondrial SNPs with GSA probes on the reverse strand and translated them to the complementary base using the flip command in PLINK. Using the --exclude PLINK command, we additionally dropped several mitochondrial SNPs that were retained after the --geno filter but which are not well covered on the GSA. We then generated .bed files aligned to the GRCh37 reference genome using the --ref-from-fa PLINK command. While analysis can be run without aligning to a reference genome, PLINK's default behavior is to assign reference and alternate alleles according to the population frequency in the samples being analyzed, which may differ from the rCRS in many cases. Finally, we converted the .bed file to a .vcf using the --recode vcf PLINK command, and passed this to haplogrep3, including the --chip argument for sequencing array data.

Ultra-low-coverage (0.5x) WGS Sample Preparation and Data Processing

Previously, we have shown that ultra-low-coverage WGS (lcWGS; defined as approximately 0.5x average genomic coverage) is comparable to both 30x WGS and GSA.⁴⁶ An additional 121 CheckMate-067 samples and 307 IO-GEM samples were sequenced using lcWGS. Genomic DNA was isolated as described above (see 30x WGS Sample Preparation). Sequencing libraries were prepared using either the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina or the Illumina DNA Prep kit, including up to 8 cycles of PCR. Libraries were quantified using real-time PCR and sequenced on either an Illumina HiSeq X or NovaSeq6000 per manufacturer's instructions using 2 x 150bp paired-end reads.

lcWGS FASTQs were processed into .bams according to the GATK workflow as described above (see 30x WGS Data Processing). Using Mutect2's mitochondrial mode, we generated .vcfs containing mitochondrial variants, which were passed to haplogrep3 as described above. To ensure concordance between the three sequencing modalities employed in this analysis, we compared the haplogrep3 results from each platform for a subset of patients. We observed excellent concordance between 30x and 0.5x WGS. 30x WGS and

GSA also had very good concordance, however, one SNP defining a subgroup within Haplogroup V is not covered on the GSA, and therefore we are unable to detect this specific subpopulation. V Haplogroup patients are present in ~3% of the European population and have approximately 28 subpopulations, resulting in a potential misclassification rate of ~0.1% in the GSA data.

Whole Exome Sequencing

Accurate mitochondrial genotypes can also be determined using reads from Whole Exome Sequencing (WES), even using probes designed exclusively for nuclear DNA.^{47,48} This is due to the increased content of mitochondrial DNA per cell (each cell contains hundreds of mitochondria, with each mitochondrion containing up to 10 copies of mtDNA),⁴⁹ which produces a high sequencing read-depth even among off-target reads arising from nuclear probes targeting nuclear mitochondrial sequences (NUMTs).

For additional CM-067 patients not included in lpWGS or WGS sequencing (n=454), we obtained off-target mitochondrial sequences⁴⁸ from pre-treatment matched-normal whole blood WES as described previously.⁵⁰ To obtain the MT-HG information, we processed the raw WES FASTQ files using the same pipeline as the WGS samples described above (see 30x WGS Data Processing). Because not all haplogroup-defining SNPs are covered by the targeted exome probes, we performed QC following haplogrep3 to remove samples with low-confidence MT-HG calls.

To determine mitochondrial haplogroups from our control population (using the 1000 Genomes Project), we obtained existing haplogroup data from Phase 1 participants⁴⁷ derived from off-target WES reads. We further obtained WES data from Phase 3 participants and derived haplogroup data as outlined below:

We additionally obtained low-coverage (2-4x) WGS from a subset of these patients and obtained haplogroup information as outlined above for our own lcWGS analysis. We confirmed 100% concordance between the haplogroups obtained from WES and lcWGS for these patients.

Statistical analysis

Differences in treatment outcomes by haplogroup were assessed using non-parametric chi-square tests, likelihood ratio tests (LRTs), and logistic regression models. LRT p-values are reported as they are robust to variations in sample size. We compared patients with CB versus NCB and analyzed NIVO-, IPI-, and COMBO-treated cohorts separately. In the IO-GEM cohort, we also conducted a sensitivity analysis assessing the association between MT-

HG and patients with any NIVO treatment (NIVO as first- or second-line ICI therapy) and those with first-line NIVO ICI treatment only (ICI treatment naïve).

MT-HG phylogenetic trees were constructed using the R package “ape.”⁵¹ Forest plots for logistic regression odds ratios were plotted using the R package “gt.”⁵² Survival analysis was conducted using the R package “survival,”⁵³ with Kaplan-Meier curves calculated using the survfit function, and Cox Proportional Hazards Models generated using coxph. Pooled statistical models were adjusted for clinical parameters including age, sex, and disease stage when available. For a subset of patients for whom age was unknown (n=99), age was imputed based on the median age (61 years) for the overall cohort (n=1,254). Cox proportional hazard assumptions were confirmed using the Schoenfeld residuals test. A two-tailed p-value of <0.05 was considered statistically significant. All analyses were conducted in R version 4.2.2.

Results

RNA-seq Gene Expression Analysis and ATAC-seq Open Chromatin Analysis:

CheckMate-067 (NIVO) -treated patient cohort

RNA-seq was performed on the baseline peripheral CD8+ samples isolated from 70 single-agent NIVO patients constituting the discovery cohort. Differential expression analysis comparing CB (CR, n=14; PR, n=15) to NCB (SD, n=6; PD, n=35) identified 7 genes surpassing the FDR threshold for statistical significance (Table 1), with the most significant association observed for the transcript of HMAX, located in MTRNR2L8, a nuclear homolog of the mitochondrial peptide known as humanin (MTRNR2) (FDR adj. p-value 8.87E-09). The MTRNR2L8 sequenced can be accessed via Gene ID: 100463486. In the described data, HMAX was significantly associated with patients with NCB when compared to CB patients in NIVO-treated cohort (Table 1a). We optimized the quantitative resolution of the HMAX signal by refining the length of the RNA-seq reads. Without this optimization, the magnitude of the difference between CB and NCB at the HMAX locus is diminished.

The presence of HMAX signal was also most significantly associated with NCB response in the validation group of additional 46 patients treated with single-agent NIVO (Table 1b) and the most significant association was observed in the pooled analysis of ALL NIVO-treated patients (n=116)(FDR adj. p=2.79E-13, log2FC=4.03) (FIG. 2a, Table 1c).

We also performed independent RNA-seq analyses on baseline CD8+ samples from patients treated by COMBO (IPI/NIVO), and HMAX was the most significantly associated marker predictive of NCB in the discovery (FDR adj. p-value 2.00E-14, log2FC=4.97),

validation(FDR adj. p-value=1.40E-06, log2FC=3.69) and pooled metanalyses (FDR adj. p=3.12E-28, log2FC=4.95) (FIGS. 2b, Table 2).

HMAX association with NCB resistant patients in NIVO and COMBO has been confirmed by independent interrogation of open chromatin accessibility using ATAC-seq analysis. In Both NIVO (FIGS. 3a-b, Table 3) and COMBO arm (FIGS. 4a-b, Table 4), HMAX showed significant association.

After detailed analyses of both RNA-seq and ATAC-seq data, the disclosure reveals that the sequencing reads corresponding to HMAX signal in both NIVO and COMBO patients are derived from mitochondrial genome of tested patients. While overall, CD8+-specific RNA-seq and ATAC-seq analyses have identified associations with HMAX and NCB, these differences are qualitative, rather than quantitative: the presence/absence of HMAX marker defines specific genetic variation in mitochondrial haplogroups. This finding led us to analyze mitochondrial genetic variability that would explain HMAX associations seen in RNA-seq and ATAC-seq analyses. Without intending to be constrained by any particular interpretation, it is considered that such a link with mitochondrial diversity has never been assessed before in the context of ICI or the host anti-tumor immune response.

Genomic Analysis Discovery: CheckMate-067 NIVO

Given the HMAX association with MTRNR2L8, the disclosure provides a systematic analysis of the mitochondrial genetic diversity in the described studied population for their impact on ICI efficacy. As detailed in Material and Method section, we first used 30X WGS data from pre-treatment PBMCs from the CheckMate-067 clinical trial in NIVO cohort (Table 5). Applying the genetic analyses, as described, we observed that the distribution of haplogroups in the CheckMate-067 NIVO cohort is representative of the expected European population distribution (Table 6). Treatment response in H, the most common haplogroup in Europe (48%) and in CheckMate-067 NIVO (45%) shows 43% of patients experiencing clinical benefit (FIG. 5). However, when assessed individually, all other haplogroups have significantly different response rates than what is expected (FIG. 5). Some haplogroups are associated with poorer outcomes, such as T and K, while others, like J, appear to predict positive outcomes (FIG. 6a).

Of the major European haplogroups, we saw significant differences in NIVO efficacy for patients with the haplogroup T (HG-T) in the CM-067-D NIVO cohort (~10% of both the European and CM-067-D NIVO cohort)(FIG. 6a). Eighty-five percent of HG-T patients showed significantly worse NIVO efficacy compared to patients with all other haplogroups

(p-value = 0.04), with significantly higher odds of NCB (odds ratio (OR) for NCB, T vs. Other MT-HGs = 4.52, (95% confidence interval (CI): 1.14-30.2)). To validate these associations, we performed further analyses in an additional n=108 NIVO-treated patients from the CM-067 trial (CM-067-V)(Table 5). We again found a strong association between
5 NIVO NCB and HG-T (p-value = 0.02), with a 75% NCB rate in HG-T patients (OR for NCB, T vs. Other MT-HGs = 4.08 (95% CI: 1.31-15.5)) (FIG. 6b).

Combining all CM-067 NIVO patients (n=223) resulted in a highly significant association of NIVO resistance for HG-T patients (p-value=2.26E-03); compared to 48% CB in CM-067 NIVO, 79% of HG-T patients experienced NCB (OR for NCB, T vs. Other MT-
10 HGs = 3.99 (95% CI: 1.65-11.2))(FIG. 6c). Adjusting the logistic regression model for age and disease stage further strengthened the association of HG-T with NCB (adjusted OR for NCB, T vs Other MT-HGs = 4.19 (95% CI: 1.71-11.9)).

Because each haplogroup has a collection of SNPs that define it as described in tables of this disclosure, we sought to determine if there were any patients with private mutations in
15 these canonical SNPs to see if they fit a similar response pattern. One patient in this cohort has the canonical SNPs associated with the K haplogroup but also has the mitochondrial SNP G1888A. This SNP is among those which define the T haplogroup, and this patient also does not respond to treatment, however, they do not have the other T SNPs.

Other less common MT-HGs detected in both CM-067 and IO-GEM also showed
20 suggestive associations with NIVO efficacy (FIG. 7). For example, patients with HG-K, similarly to HG-T, showed association trends with NIVO NCB consistently across all analytical stages, with the most significant associations observed in a meta-analysis when combined with HG-T. Conversely, HG-J patients showed suggestive associations with NIVO CB, but again not reaching statistical significance in every analytical stage. Hence, a larger
25 more focused analysis will be needed to provide more definitive effect size estimates for these less common MT-HGs.

Validation: IO-GEM NIVO

To further validate these findings outside of a clinical trial, in the standard-of-care (SOC) setting, we used samples obtained from the IO-GEM, a collaborative international
30 consortium of immunotherapy-treated melanoma patients (n=129 NIVO samples). We also included data derived from previously published datasets (n=61).^{26,27} Clinical characteristics and MT-HG distribution of the final IO-GEM NIVO cohort (n=174) are consistent with those

seen in the CM-067 analytical cohort (Tables 5-6). Overall efficacy in the IO-GEM NIVO cohort is similar to the CM-067 analysis, with 47% of subjects experiencing CB (FIG. 7d).

The IO-GEM analyses confirmed the observed associations from CM-067, showing that HG-T is significantly associated with NIVO resistance (75% NCB rate, p-value=0.04;

OR for NCB, T vs Other MT-HGs = 3.00 (95% CI: 1.10-9.59)). This association is comparable with the effect size and the significance observed in the discovery analysis in CM-067 (FIG. 11a and 11d). A meta-analysis combining all NIVO-treated patients (CM-067 and IO-GEM, n=397) demonstrated a highly significant overall association with NIVO resistance (p-value = 2.22E-04; OR for NCB, T vs Other MT-HGs = 3.53 (95% CI: 1.81-7.47))(FIG. 8), and the association remained significant after adjusting for age and sex (adjusted OR for NCB, T vs Other MT-HGs = 3.50 (95% CI: 1.77-7.45)).

scRNA-seq

Given the growing evidence that mitochondrial metabolism impacts T cell differentiation,^{15,16,21,23,54} the disclosure provides an analysis of whether peripheral effector CD8+ T-cells, as the targets of anti-PD-1 therapy, show distinct phenotypic features in patients with NIVO-resistant MT-HGs (HG-T, HG-K) versus those of other haplogroups. We performed single-cell RNA-seq analysis (scRNA-seq) on CD8+ cells isolated from CM-067 NIVO-treated patients (n=21), which included 8 patients with CB (n=7 CR, n=1 PR) and 13 with NCB (n=13 PoD). To delineate the phenotypic differences associated with MT-HGs, we enriched the patient selection as follows: for NCB patients, 7 were selected with NIVO-resistant MT-HGs (NCB-HG^R) (n=6 HG-T, n=1 with HG-K), and 6 with other MT-HGs (NCB-HG^O); for CB patients, 1 with HG-T and 7 with other MT-HGs.

Using scRNA-seq pipelines,³⁹⁻⁴² we mapped the transcriptional profiles of the CD8+ cells using the Uniform Manifold Approximation and Projection (UMAP) algorithm and constructed a shared nearest neighbor (SNN) graph to identify 16 unique clusters (FIG. 9a). Based on differential gene expression profiles of T cell marker genes derived from established reference sets,^{42,55-57} we annotated these 16 clusters to specific cell types (FIGS. 10a-b). We defined major phenotypic states used for downstream analysis, ordering them by T cell differentiation state (FIG. 9b): naïve cells (cluster 0 and 5), effector cell cluster 6 (earliest stage of effector cell differentiation), cluster 1 (early exhaustion), and cluster 2 (late exhaustion), followed by dysfunctional T cells (cluster 10), defined as those with the highest mean expression levels of exhaustion markers (FIG. 10c).

When stratifying the CD8⁺ T cell phenotypes by NIVO response status and MT-HG, NCB-HG^R patients tended to have predominantly naïve cells and significantly fewer early exhausted cells (cluster 1, p-value = 0.02) and late exhausted cells (cluster 2, p-value = 0.04), compared to CB patients. Compared to NCB-HG^O, NCB-HG^R patients not only had fewer
 5 late exhaustion cells (cluster 2, p-value = 0.05) but also significantly fewer dysfunctional cells (cluster 10, p-value = 0.01) (FIG. 9c).

It has been shown that PD-1 inhibition can reinvigorate exhausted T cells and promote their effector function, however, only a subset of exhausted cells, characterized by high TBX21 (TBX21^{HI}) and low EOMES (EOMES^{LOW}), are capable of reinvigoration.^{58,59} Based
 10 on this model, we further refined exhausted phenotypes identified in our data and defined TBX21^{HI} exhausted cells, the primary target of reinvigoration by NIVO, and those with high EOMES (EOMES^{HI}) and/or high TOX (TOX^{HI}), terminally exhausted cells that cannot be reinvigorated.^{57,60-64} Cluster 1, with TBX21^{HI} and EOMES^{LOW} expression (FIG. 9d), likely represents the early exhaustion phenotype that can be reinvigorated and is predominantly
 15 present in CB patients. NCB-HG^O patients show elevated levels of both cluster 2 (EOMES^{HI}, TOX^{HI}, and TBX21^{LOW}) and cluster 10 (EOMES^{LOW}, TBX21^{LOW}, and TOX^{HI}) (FIG. 9d), both likely the terminally exhausted cells with no capacity for anti-PD-1 reinvigoration. In contrast, NCB-HG^R patients show a significantly lower proportion of all three states of exhausted cells, exhibiting a distinct baseline peripheral cell phenotype characterized by an
 20 overall lower fraction of differentiated CD8⁺ T cells in patients with these haplogroups.

Prediction of clinical efficacy by MT-HG status with other immune checkpoint regimens

To evaluate whether MT-HGs associate with outcomes of other frontline ICI therapies in MM, we tested MT-HG associations with the clinical efficacy of combination IPI and
 25 NIVO (anti-CTLA-4/anti-PD-1; COMBO), which is currently an approved standard of care first-line ICI regimen for patients with MM. In CM-067, COMBO-treated HG-T patients showed significantly worse efficacy compared to all other MT-HGs (p-value = 7.05E-04), with an 83% NCB rate (OR for NCB, T vs Other MT-HGs = 6.99 (95% CI: 2.21-30.9)) (FIG. 11b). The same trend was shown in the IO-GEM COMBO cohort, with HG-T patients
 30 showing the poorest outcomes after COMBO (54% NCB vs 33% NCB in other MT-HGs) (p-value = 0.12; OR for NCB, T vs Other MT-HGs = 2.39 (95% CI: 0.76-7.73)) (FIG. 11e). Combining COMBO-treated patients from both CM-067 and IO-GEM showed a highly significant association (p-value = 4.43E-04), with a 69% NCB rate in HG-T patients

compared to 37% NCB rate in the other MT-HGs (OR for NCB, T vs other MT-HGs = 3.73 (95% CI: 1.76-8.45)) (FIG. 12a). Adjusting the COMBO meta-analysis for age and sex demonstrated the same trend (adjusted OR = 3.67 (95% CI: 1.71-8.41)).

We also tested whether MT-HGs are associated with single-agent IPI (anti-CTLA-4).

5 The meta-analysis combining both CM-067 and IO-GEM (n=477) found no statistically significant association of IPI efficacy with any of the MT-HGs (p-value = 0.13; adjusted OR = 0.59 (95% CI: 0.31-1.18))(FIG. 12a). Overall, our data show that patients with HG-T have comparably poorer outcomes in both NIVO and COMBO, the ICI treatment regimens with the highest efficacy in the general MM population (FIG. 12b).

10 **Association between MT-HG and ICI progression-free (PFS) and overall survival (OS)**

As survival is the most definitive indicator of ICI therapy success, we tested whether MT-HGs are associated with PFS and OS after ICI, using well-harmonized data from CM-067. We found significantly decreased PFS among the HG-T NIVO-treated (age, sex, stage adjusted PFS HR = 1.91 (95% CI: 1.20-3.06), p-value=0.007) as well as COMBO-treated
15 patients (adjusted PFS HR = 1.98 (95% CI: 1.05-3.73), p-value = 0.04). 80% of all HG-T patients progressed within the first four months of treatment, and their overall survival (OS) was also significantly poorer than patients who experienced CB (NIVO adjusted p-value <0.001; COMBO adjusted p-value<0.001), and comparably poor to all other patients with NCB. These data show that HG-T patients are less likely to achieve a durable clinical
20 response from NIVO-based ICI (both single line and combination) compared to the other haplogroups.

Evaluation of MT-HGs as an ICI-predictive outcome biomarker independent of disease prognosis

Given the observed associations of HG-T with NIVO and COMBO resistance,
25 validated in multiple patient populations in this disclosure, we sought to evaluate whether MT-HG status is predictive of ICI outcomes or a reflection of overall disease prognosis. We assessed the association of MT-HGs and OS in a prognostic cohort of 1,024 ICI-naïve melanoma patients (not treated by ICI) enrolled at NYULMH,²⁸ with extensive follow-up. In this analysis, we found no difference in survival in early-stage (I-II) patients (n=915, p-value
30 for OS by haplogroup=0.41) nor later-stage (III) patients in both univariable and multivariable analyses (n=109, p-value for OS by haplogroup=0.78). These data indicate that

MT-HG status does not determine disease prognosis, but rather is an independent predictor of ICI treatment efficacy.

Evaluating response to different anti-PD-1 monoclonal antibodies by MT-HG

The present identification of a genomic biomarker of anti-PD-1 resistance has particular clinical significance given that single-agent anti-PD-1 treatments typically provide substantially improved clinical benefits and reduced toxicities compared to Ipilimumab (IPI), an ICI targeting the CTLA-4 receptor.⁶⁵ As anti-PD-1-based ICIs have now become front-line therapies in MM and other tumor types, identifying patients that are resistant to these treatments not only has important clinical implications for patient stratification to alternative regimens but may also aid in better biological understanding of anti-PD-1-based ICI resistance.

Two monoclonal antibodies (mAbs) targeting the PD-1 receptor are currently used in SOC: Nivolumab (NIVO) and Pembrolizumab (PEMBRO). Studies directly comparing the efficacy of the two drugs have found no statistical differences in melanoma^{66,67} or other cancers for which they are used, such as non-small cell lung cancer.⁶⁸ While this has led to a general consensus that there is no practical distinction between the two drugs, with both showing comparable efficacy in advanced malignancies, differences do exist in the structure of the NIVO and PEMBRO mAbs. These differences largely affect the mechanism by which they bind PD-1, with NIVO interacting primarily with the N-terminal loop^{69,70} and PEMRBO predominantly interacting with the C'D loop of PD-1.^{69,71} However, whether these structural differences impact response to treatment in individual patients, rather than in aggregated cohorts, has never been tested.

While we initially assessed the ICI-haplogroup association only in SOC patients treated with NIVO-based ICI, we also sought to identify if different MT-HGs showed comparable response to PEMBRO and/or whether the association with ICI efficacy and MT-HGs is anti-PD-1 mAb-specific. Using additional genomic data obtained from MM patients treated with SOC PEMBRO, we compared the association between mitochondrial haplogroups and efficacy for PEMBRO and NIVO-based ICI treatments.

We collected clinical and genomic data from 1,497 metastatic melanoma patients treated with first-line ICI to assess differences in anti-PD-1 ICI treatment efficacy by mitochondrial haplogroup (MT-HG). In particular, we sought to assess differences in outcome for haplogroup T (HG-T), which we have previously shown to be associated with significantly poorer outcomes to NIVO-based therapies, as discussed above. Overall, the

cohorts of patients treated by the PD-1 inhibitors Nivo and Pembro are demographically comparable, with similar distributions in age and sex (Table 7) and similar MT-HG distribution was observed in the reference population from the 1000 Genomes Project (Table 8).

5 In the aggregate, assessing each checkpoint inhibition treatment as one group, clinical benefit (CB) for single-agent anti-CTLA-4 (IPI) was 24% CB, anti-PD-1 (inclusive of both NIVO and PEMBRO) was 50% CB, and combined anti-CTLA-4/anti-PD-1 (COMBO, consisting of IPI/NIVO only) was 60% CB (FIG. 13a, Table 9). These differences in treatment outcomes were statistically significant in both univariable and multivariable
10 analyses (adjusted for age and sex; multivariable odds ratios (ORs) and p-values are reported). We observed significantly increased efficacy in anti-PD-1 compared to anti-CTLA-4 treatment (OR for NCB, IPI vs. anti-PD-1: 3.27 (95% CI: 2.51-4.28), p-value < 2.00E-16), as well as improved efficacy in COMBO compared to either anti-CTLA-4 (OR for NCB, IPI vs COMBO: 5.04 (95% CI: 3.77-6.79), p-value < 2.00E-16) or anti-PD-1 alone (OR for
15 NCB, anti-PD-1 vs COMBO: 1.54 (95% CI: 1.19-2.00), p-value = 9.80E-04).

When assessing NIVO and PEMBRO anti-PD-1 treatments separately without stratifying by MT-HGs, the results are similar, with 47% CB in NIVO and 53% CB in PEMBRO (OR for NCB, NIVO vs PEMBRO: 1.22 (0.88-1.67), p-value=0.23) (FIG. 13b, Table 10). IPI outcomes were significantly worse compared to either NIVO (p-value=1.14E-
20 11) or PEMBRO (p-value < 2.00E-16), and COMBO outcomes were significantly better (COMBO vs NIVO p-value=5.65E-04, COMBO vs PEMBRO p-value=0.04).

When separating patients by MT-HG, we find that HG-T patients have significantly poorer outcomes when treated with anti-PD-1 (both NIVO and PEMBRO) compared to the other MT-HGs (p-value = 0.02), with 37% CB for HG-T compared to 52% CB in other MT-
25 HGs (FIG. 13c, Table 11). This is consistent with our previous analysis showing that HG-T had poor outcomes to NIVO-based therapies compared to other MT-HGs, with the worst outcome observed in the single-agent NIVO cohort (23% HG-T NIVO CB). However, when combining NIVO and PEMBRO into one anti-PD-1 treated cohort, HG-T patients have slightly better outcomes to anti-PD-1 monotherapy (37% CB) compared to IPI monotherapy
30 (33% CB) or COMBO (29% CB). Although these distinctions are not statistically significant (p-value = 0.67 and 0.58 comparing HG-T anti-PD-1 to IPI and COMBO, respectively), this is in contrast the described findings showing HG-T patients had the worst outcomes with NIVO monotherapy.

These underlying differences are explained when assessing the association of HG-T with NIVO and PEMBRO efficacy separately by each treatment. While the difference between NIVO and PEMBRO outcomes in the other MT-HGs is negligible (OR for NCB, NIVO vs PEMBRO in other MT-HGs: 1.01 (0.72-1.41), p-value=0.98), HG-T patients have significantly poorer outcomes when treated with NIVO compared to PEMBRO (OR for NCB, NIVO vs PEMBRO in HG-T: 5.43 (1.87-16.9), p-value=2.40E-03) (FIG. 13d, Table 12). While there is no statistical difference in HG-T outcomes between NIVO and IPI or COMBO (p-value=0.30 and 0.45, respectively), HG-T patients treated with PEMBRO respond significantly better compared to both IPI (OR for NCB, IPI vs PEMBRO in HG-T: 3.31 (1.20-9.67), p-value=0.02) and COMBO (OR for NCB, COMBO vs PEMBRO: 3.62 (1.19-11.8), p-value=0.03).

While in other MT-HGs the response to COMBO treatment is significantly better than either NIVO (p-value=3.54E-03) or PEMBRO (p-value=4.41E-03), not only is there no difference in outcome between other MT-HGs treated with NIVO or PEMBRO compared to HG-T treated with PEMBRO (p-value=0.36 and 0.37, respectively), but there is also no difference between outcomes for HG-T treated with PEMBRO compared to other MT-HGs treated with COMBO (OR for NCB, other MT-HG COMBO vs HG-T PEMBRO: 0.93 (0.40-2.28), p-value=0.87) (FIG. 13d, Table 12).

In order to develop a potential biological hypothesis underpinning these significant differences, the disclosure provides an analysis of the association between anti-PD-1 efficacy, MT-HGs, and tumor PD-L1 status, as both NIVO and PEMBRO disrupt the PD-1/PD-L1 axis. Although PD-L1 status is not routinely captured in SOC ICI treatment of MM patients, it was recorded for all patients on the CheckMate-067 trial. We therefore performed a sub-analysis, stratified by MT-HG status, for the association between anti-PD-1-based treatment efficacy and PD-L1 status for NIVO- and COMBO-treated patients on CheckMate-067. While the majority of PD-L1+ patients with other haplogroups tended to experience NIVO CB (60 of 99 (61%) PD-L1+ NIVO CB), ALL HG-T NIVO CB patients were PD-L1+ (6 of 6 (100%) PD-L1+ NIVO CB) (Table 13). For patients treated with COMBO, the association with PD-L1 positivity was not as strong for the other MT-HGs (50 of 109 (46%) PD-L1+ COMBO CB), but remained high for HG-T (2 of 3 (67%) PD-L1+ COMBO CB) (Table 13). Combining the NIVO and COMBO cohorts (Table 2-6b), we observed a statistically significant enrichment of PD-L1 positivity among HG-T CB patients (8 of 9 (89%) PD-L1+ CB) compared to CB patients with other MT-HGs (110 of 208 (53%) PD-L1+ CB) (chi-square p-value=0.03).

These findings, from nearly 1,500 patients from a clinical trial and large international SOC consortium, collectively provide the first evidence of differences in the efficacy of two different anti-PD-1 mAbs based on mitochondrial genetic makeup. Specifically, HG-T patients are significantly more likely to experience CB when treated with PEMBRO as compared to IPI, NIVO, or COMBO. This is in contrast to patients with other MT-HGs, who are most likely to experience CB from COMBO. In CheckMate-067, we also observed significant enrichment of PD-L1 positivity among HG-T patients who experienced CB from NIVO-based ICI. Whereas approximately half of CB patients with other MT-HGs were PD-L1+, we observed almost 90% PD-L1 positivity in HG-T CB patients. These results strongly indicate a need for a parallel assessment of MT-HG status and PD-L1 expression in determination of a more personalized selection of anti-PD-1 therapy option with the improved expected treatment outcome.

Discussion of the foregoing examples

Without intending to be bound by any particular theory, it is considered that this disclosure provides for the first time, testing and validation of the association of inherited mitochondrial haplogroups (MT-HGs) with ICI efficacy in melanoma, providing a novel link between mitochondrial inheritance and ICI-mediated anti-tumor immune response.

Analyzing the mitochondrial genome of 1,254 ICI-treated metastatic melanoma (MM) patients, we found that ICI efficacy varies by MT-HGs. We discovered that mitochondrial haplogroup T (HG-T) is significantly associated with resistance to NIVO (pooled OR = 3.53, p-value = 2.22E-04), which we validated in both a clinical trial (CheckMate-067) as well as in an independent standard-of-care ICI cohort from an international consortium (IO-GEM). This is the first inherited genetic marker predicting patients before treatment who will not benefit from single-agent NIVO, a treatment option with otherwise high efficacy and low toxicity.⁶⁵ In alignment with these observations, NIVO-resistant HG-T patients show poor PFS as well as OS, when compared to other MT-HGs treated by single-agent NIVO.

This disclosure also shows that HG-T patients experience comparably poor outcomes with anti-PD-1/anti-CTLA-4 combination therapy (COMBO)(31% ORR, OR = 3.73, p = 4.43E-04), which is also a frontline ICI therapy in MM. With no statistical difference observed in anti-CTLA-4 efficacy between HG-T and other MT-HGs, these findings collectively indicate that HG-T is specifically associated with resistance to NIVO-based treatments (single-agent or in combination with anti-CTLA-4), and this also correlates with significantly poorer survival. The disclosure thus supports use of other ICI treatment

combinations that HG-T patients may benefit from, particularly in light of recent development of new combination ICIs that include blockade of other immune checkpoints (e.g. LAG-3, TIGIT, TIM-3, *etc.*⁵⁵) or alternative immunotherapies such as recently approved adoptive cell therapies in melanoma.⁷²

5 This disclosure reveals a potential link between mitochondrial inheritance, host immune cell repertoire, and ICI efficacy. It has been proposed that PD-1 inhibition returns the cytotoxic capacities of certain phenotypes of exhausted CD8⁺ T cells, possibly improving anti-tumor immunity. In contrast, dysfunctional cells (terminally exhausted) can no longer be reinvigorated by PD-1 inhibition.^{58,59,62} Our scRNA-seq data show that the peripheral
10 baseline distribution of these exhausted cell phenotypes varies by MT-HG and treatment efficacy in NIVO-treated patients. We have identified three distinct peripheral CD8⁺ effector T cell exhaustion phenotypes by NIVO response and MT-HG status. We observed that CB patients exhibit higher baseline levels of cell phenotypes capable of regaining cytotoxic activity by NIVO reinvigoration. In contrast, NCB patients of non-T MT-HGs show a lesser
15 fraction of these cells, but more dysfunctional phenotypes, no longer able to be reinvigorated by NIVO. NCB patients with HG-T show significantly lower overall baseline levels of all differentiated CD8⁺ T-cell states, neither dysfunctional nor those that can be PD-1 reinvigorated. This NIVO-resistance-associated CD8⁺ T cell phenotype possibly points to an altered differentiation cascade, which, in HG-T patients, retains CD8⁺ T cells at earlier
20 differentiation stages.

These data indicate that MT-HG status may be associated with the processes that drive CD8⁺ cells into effector states and this capacity affects response to ICI. While the biological mechanisms explaining these correlations are elusive, they are consistent with recent studies showing that T cell differentiation to effector states depends on differential tolerance to
25 reactive oxygen species (ROS),²³ which varies by MT-HGs. HG-T can better tolerate ROS damage compared to other MT-HGs.¹⁸ Upon PD-1 blockade, ROS also stimulate the AMPK/mTOR pathway, leading to an increase in TBX21 production and a decrease in EOMES.²¹ Based on this evidence, in individuals with greater tolerance to ROS damage, such as those with HG-T, more ROS may be needed to drive the cells into the activated state.
30 While not intending to be constrained by any particular interpretation, this may explain why we observed very few effector cells in the CD8⁺ repertoire of HG-T patients not responding to NIVO. The described results linking mitochondrial haplogroups and response to ICI support the involvement of mitochondria-associated ROS in T cell differentiation. The transcriptional profiles of CD8⁺ T cells from HG-T NIVO-resistant patients from

CheckMate-067 are enriched by gene signatures in pathways related to ROS detoxification, including GCLC, CPT1C, ANPEP, CYP1B1, and GPX3 (FIG. 14). This finding provides support for the hypothesis that the baseline CD8⁺ T cell phenotype in NIVO-resistant HG-T patients may be associated with their ability to resist ROS damage.

5 We additionally found that patients with mitochondrial haplogroup T (HG-T) are significantly more likely to respond to pembrolizumab (PEMBRO) compared to nivolumab (NIVO), with over 5 times the odds of no clinical benefit (NCB) in HG-T patients treated with NIVO compared to PEMBRO (HG-T NCB OR for NIVO vs PEMBRO: 5.43 (95% CI: 1.87-16.9), p-value=2.40E-03), both ICIs targeting the PD-1 receptor. The significant
10 difference in PEMBRO outcomes for HG-T was also present when assessing outcomes in the NIVO-based combination treatment, which again showed significantly poorer efficacy (HG-T NCB OR for COMBO vs PEMBRO: 3.62 (95%: 1.19-11.8), p-value=0.03), providing more evidence that the disclosure reveals a NIVO-based resistance marker. That other haplogroups follow the expected response distribution, with virtually identical outcomes when comparing
15 NIVO and PEMBRO (Other MT-HG NCB OR for NIVO vs. PEMBRO: 1.01 (95% CI: 0.72-1.41), p-value=0.98), possibly explains why there was little difference observed between the two therapies in the aggregate ICI analyses.^{67,73} It is also possible, therefore, that differences in the underlying haplogroup distributions may account for some of the observed variation in outcomes when the two treatments are compared in parallel. Taken together, these data
20 provide evidence that, for a subset of patients, these two drugs should be administered rationally. These findings also clearly point to possible differences in the mechanism of action for each of these mAbs when inhibiting the PD-1/PD-L1 axis. Although they target the same receptor, NIVO and PEMBRO are not structurally identical antibodies, and these structural variations may affect how they interact with the corresponding PD-L1 ligand.⁷⁴ The primary
25 difference is in the variable region of the two antibodies, which generates the antibody's paratope, the region of the antibody that binds to the antigen epitope.⁶⁹ Specifically, the PEMRBO epitope region covers a much greater extent of the PD-L1 binding site of the PD-1 receptor compared to the NIVO epitope region.⁶⁹ Despite their other similarities, there is almost no overlap in the PD-1 molecule binding sites between NIVO and PEMBRO,
30 including where they overlap with the PD-1/PD-L1 binding site.⁶⁹⁻⁷¹ Further, recent evidence shows that PD-1 is capable of dimerization in *cis* (on the same T cell surface), and this propensity for dimerization may be associated with decreased cytotoxic T cell function.⁷⁵

Given these structural differences between the two anti-PD1 mAbs in relation to the PD-L1 binding site, the present disclosure supports the interpretation that even HG-T patients

with lower tumor PD-L1 expression may benefit from PEMBRO treatment, and it can be expected that this improvement to be even more pronounced for patients with high PD-L1 expression. This is supported by the present data, showing that PEMBRO provides superior clinical benefit in HG-T patients, who are otherwise largely resistant to NIVO. All patients with HG-T that responded to NIVO are PD-L1 positive, further supporting a model that PD-L1 positivity may be an important factor in determining anti-PD-1 clinical benefit for HG-T patients. Of nine total HG-T patients experiencing CB from NIVO-based treatments on CheckMate-067, eight were PD-L1 positive. The link between PD-L1 status and HG-T NIVO-resistance also remained when we assessed melanoma patients treated with adjuvant NIVO or COMBO therapy on the CheckMate-915 clinical trial (data not shown). When evaluating only the patients with disease recurrence (the clearest measure of treatment efficacy in the adjuvant setting wherein some patients are cured by surgery alone), 12 of 13 HG-T patients who recurred were PD-L1 negative, including all single-agent NIVO-treated HG-T patients.

This association between efficacy and PD-L1 status is consistent with our bulk and single-cell (sc)RNA-seq data that lead us to hypothesize that the overall lower proportion of differentiated effector T cells in NIVO-resistant HG-T patients may be related to their ability to resist reactive oxygen species (ROS). ROS have been shown to induce tumor PD-L1 expression⁷⁶⁻⁷⁸ while ROS scavenging tends to repress PD-L1.⁷⁶ The one HG-T patient with scRNA-seq data who responded to NIVO showed a much more differentiated baseline immune portfolio and was PD-L1 positive, while five of the six that were NIVO-resistant were PD-L1 negative. It is therefore possible that, for HG-T patients, there is a synergistic relationship between their ability to resist ROS, the extent of differentiation in their immune repertoire, and their level of tumor PD-L1 positivity.

The present disclosure includes the large sample size (n=1,254 ICI-treated patients), with a discovery and validation dataset generated from well-controlled clinical trial samples (CheckMate-067) and a standard-of-care treatment cohort from a multinational study (IO-GEM), ensuring the rigor and reproducibility of the findings. Combining these cohorts generated the largest genomic analysis to date of ICI-treated MM patients, and the first to explore the role of inherited mitochondrial genetics in ICI efficacy. Further, unlike most previous studies that combine patients into one “ICI-treated” population, in the present disclosure, stratifying by treatment allowed analysis of outcomes separately by ICI therapy as well as by haplogroup. The addition of scRNA-seq data is an aspect of the disclosure, extending the observational findings to identify a relevant peripheral host cell phenotype

connecting MT-HGs with a putative biological model of HG-T impacting ROS resistance and ICI efficacy.

As discussed above, it is believed this disclosure provides the first evidence that the MT-HG status is associated with ICI outcomes. The disclosure supports the use of MT-HG genetic assessment for use as a clinically viable, minimally invasive baseline biomarker that can identify patients who are less likely to respond to current NIVO-based treatment regimens. In an aspect, it is considered the disclosure reveals for the first time the connection between mitochondrial genetics and the peripheral blood immune cell repertoire in the context of ICI resistance.

The results demonstrate that the T haplogroup correlates with poor response to current NIVO-based therapy and indicate that other haplogroups also respond differently relative to the expected overall response. Given the present description of better response in T haplogroup patients receiving PEMBRO, the disclosure includes analysis of outcomes of T haplogroup patients treated with novel anti-PD-1-based treatments such as those combining NIVO with anti-LAG-3 therapy. As all currently FDA-approved predictive biomarkers (PD-L1⁷⁹ and MSI status⁸⁰ TMB⁸¹) require tumor samples, mitochondrial haplogrouping represents an approach for a novel, conveniently obtainable baseline predictor of subsequent treatment response. Thus, the disclosure supports the use of mitochondrial haplogrouping of patients as a clinically viable, minimally invasive baseline biomarker that can identify patients who are less likely to respond to current NIVO-based treatment regimens and that these patients would respond better to PEMBRO treatment. Given the ease of germline genetic testing along with the clear evidence of this disclosure demonstrating heterogeneous responses to mainstream ICI therapies across haplogroups, the disclosure includes analyzing historic ICI combinations that have been discarded due to their worse/unaltered response rates to test if they are actually benefiting patients with specific haplogroups. Accordingly, these blood tests may maximize the utility of existing immunotherapies while improving response to treatment by creating new personalized treatment regimens.

This reference listing is not an indication that any reference is material to patentability.

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Table 1: Results of CD8+ T-cell differential expression analysis of:

- A. Discovery Cohort of patients treated by single-agent Nivolumab ICI (n=70) (Complete Response (CR), n=14; Partial Response (PR), n=15; Stable Disease (SD), n=6 Progressive Disease (PD), n=35).
- B. Validation Cohort of patients treated by single-agent Nivolumab ICI (n=46) (CR, n=7; PR, n=12; SD, n=3; PD, n=24).
- C. Meta-Analysis (Discovery and Validation Cohorts) of patients treated by single-agent Nivolumab ICI (n=116) (CR, n=21; PR, n=27; SD, n=9; PD, n=59).

The expression is derived from RNA-seq analysis of baseline (pre-treatment) peripheral blood CD8+ T-cells. The table lists the top 10 genes that are most significantly differentially expressed ($p < 0.01$, $|\log_2FC| > 1$) in each response comparison.

A. NIVO RNA-seq Discovery Cohort, n=70

CB (CR, n=14; PR, n=15) vs NCB (SD, n=6; PD, n=35)

Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	4.124786034	4.37E-13	8.87E-09	protein coding	HMAX
2	HLX	2.247898968	8.60E-07	0.008730808	protein coding	H2.0 like homeobox
3	FFAR2	3.006203835	1.88E-06	0.012717011	protein coding	free fatty acid receptor 2
4	LYZ	1.702383589	4.54E-06	0.015365051	protein coding	lysozyme
5	SERPINA1	1.467126048	4.20E-06	0.015365051	protein coding	serpin family A member 1
6	C5AR1	1.917016154	3.47E-06	0.015365051	protein coding	complement C5a receptor 1
7	HOXA7	-2.034423079	6.09E-06	0.017651538	protein coding	homeobox A7
8	SLC11A1	1.598853412	2.82E-05	0.063883114	protein coding	solute carrier family 11 member 1
9	FCN1	1.729496242	3.64E-05	0.063883114	protein coding	ficolin 1
10	WTIP	-1.431375059	3.94E-05	0.063883114	protein coding	WT1 interacting protein

CB (CR, n=14; PR, n=15) vs NCB (PD, n=35)

1	HMAX	4.313132797	2.03E-13	4.17E-09	protein coding	HMAX
2	LYZ	1.778638509	1.44E-06	0.011205081	protein coding	lysozyme

3	C5AR1	2.018301272	1.64E-06	0.011205081	protein coding	complement C5a receptor 1 serpin family A member 1 homeobox A7 ficolin 1 free fatty acid receptor 2 WT1 interacting protein chloride voltage-gated channel 5 solute carrier family 11 member 1
4	SERPINA1	1.401995364	5.23E-06	0.026819112	protein coding	
5	HOXA7	-2.058985011	1.22E-05	0.049895199	protein coding	
6	FCN1	1.811497847	2.03E-05	0.054323819	protein coding	
7	FFAR2	2.675487121	1.63E-05	0.054323819	protein coding	
8	WTIP	-1.506273733	2.76E-05	0.054323819	protein coding	
9	CLCN5	1.527630214	2.91E-05	0.054323819	protein coding	
10	SLC11A1	1.634678133	4.06E-05	0.067247951	protein coding	

B. NIVO RNA-seq Validation, n=46

CB (CR, n=7; PR, n=12) vs. NCB (SD, n=3; PD, n=24)

Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	5.131617118	7.70E-11	1.63E-06	protein coding	MT-RNR2 like 8
2	WDFY4	3.255290925	2.74E-09	2.90E-05	protein coding	WDFY family member 4
3	GUCY1A1	2.604583926	7.97E-07	0.005618073	protein coding	Guanylate cyclase soluble subunit alpha-1
4	CD79A	1.653473616	1.65E-05	0.070383653	protein coding	Cluster of differentiation CD79A
5	NIBAN3	2.33059601	1.66E-05	0.070383653	protein coding	niban apoptosis regulator 3
6	ADAM28	1.889793852	3.15E-05	0.072088372	protein coding	ADAM metalloproteinase domain 28
7	COL19A1	2.464579374	3.31E-05	0.072088372	protein coding	collagen type XIX alpha 1 chain
8	PTPRF	3.355842884	2.75E-05	0.072088372	protein coding	protein tyrosine phosphatase receptor type F
9	TSPAN33	1.775605012	3.41E-05	0.072088372	protein coding	tetraspanin 33
10	PROS1	3.366300942	3.16E-05	0.072088372	protein coding	protein S

CB (CR, n=7; PR, n=12) vs. NCB (PD, n=24)

1	WDFY4	2.560772356	3.14E-08	0.000656156	protein coding	WDFY family member 4
2	CD22	2.811976558	6.62E-07	0.004621567	protein coding	CD22 molecule
3	HMAX	4.12399036	5.59E-07	0.004621567	protein coding	HMAX

4	GUCY1A1	2.623291772	1.48E-06	0.007735769	protein coding	guanylate cyclase 1 soluble subunit alpha 1
5	PTPRF	3.538340111	7.99E-06	0.033463773	protein coding	protein tyrosine phosphatase receptor type F
6	NIBAN3	2.403339229	1.12E-05	0.03918731	protein coding	niban apoptosis regulator 3
7	CD79A	1.547500575	2.01E-05	0.060148593	protein coding	Cluster of differentiation CD79A
8	COL19A1	2.497925557	4.78E-05	0.118368878	protein coding	collagen type XIX alpha 1 chain
9	HOMER2	2.163677712	5.09E-05	0.118368878	protein coding	homer scaffold protein 2
10	MAP1B	1.983631641	0.000123201	0.161161872	protein coding	microtubule associated protein 1B

C. NIVO RNA-seq Meta-Analysis, n=116

CB (CR, n=21; PR, n=27) vs. NCB (SD, n=9; PD, n=59)

Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	4.033424762	1.32E-17	2.79E-13	protein coding	HMAX
2	TSPAN33	1.441624756	2.59E-08	0.000273373	protein coding	tetraspanin 33
3	LTF	3.403239928	1.41E-06	0.008603064	protein coding	lactotransferrin
4	G0S2	1.50300118	1.89E-06	0.008603064	protein coding	G0/G1 switch 2
5	RTN1	2.058299101	2.11E-06	0.008603064	protein coding	reticulon 1
6	SHROOM4	2.156006671	2.44E-06	0.008603064	protein coding	shroom family member 4
7	FCN1	1.569170022	3.56E-06	0.009534198	protein coding	ficolin 1
8	PADI2	2.195336963	4.51E-06	0.009534198	protein coding	peptidyl arginine deiminase 2
9	MPP1	1.025128729	4.29E-06	0.009534198	protein coding	membrane palmitoylated protein 1
10	PROS1	1.943637848	3.72E-06	0.009534198	protein coding	Protein S

CB (CR, n=21; PR, n=27) vs. NCB (PD, n=59)

1	HMAX	4.237953364	1.31E-18	3.03E-14	protein coding	MT-RNR2 like 8
2	SHROOM4	2.267730422	1.08E-06	0.012498032	protein coding	shroom family member 4
3	RTN1	2.106095618	1.77E-06	0.013711018	protein coding	reticulon 1

4	G0S2	1.486499348	2.70E-06	0.014977281	protein coding	G0/G1 switch 2
5	CLCN5	1.401975596	3.23E-06	0.014977281	protein coding	chloride voltage-gated channel 5
6	HOMER2	1.298055409	5.14E-06	0.019879051	protein coding	homer scaffold protein 2
7	TSPAN9	1.706161799	7.92E-06	0.022134117	protein coding	tetraspanin 9
8	TSPAN33	1.003964191	7.08E-06	0.022134117	protein coding	tetraspanin 33
9	EHD3	1.088502844	1.33E-05	0.030927829	protein coding	EH domain containing 3
10	PTGIR	1.866182294	1.54E-05	0.032439464	protein coding	prostaglandin I2 receptor
CB (CR, n=21) vs. NCB (PD, n=59)						
1	HMAX	4.15500316	7.57E-10	1.75E-05	protein coding	HMAX
2	CDC42BPA	1.595030251	6.64E-06	0.076675514	protein coding	CDC42 binding protein kinase alpha
3	MEF2C	1.292225447	6.14E-05	0.159891137	protein coding	myocyte enhancer factor 2C
4	GRK3	1.044256851	2.38E-05	0.159891137	protein coding	G protein-coupled receptor kinase 3
5	MYOM1	1.137032235	0.000124778	0.159891137	protein coding	myomesin 1
6	ARHGEF17	1.349159733	0.000140873	0.159891137	protein coding	Rho guanine nucleotide exchange factor 17
7	FRK	1.911875303	8.02E-05	0.159891137	protein coding	fyn related Src family tyrosine kinase
8	FFAR2	3.363590635	4.84E-05	0.159891137	protein coding	free fatty acid receptor 2
9	FCGR2A	1.909027655	3.56E-05	0.159891137	protein coding	Fc fragment of IgG receptor IIa
10	FARP1	1.750862825	9.97E-05	0.159891137	protein coding	FERM, ARH/RhoGEF and pleckstrin domain protein 1

Table 2: Results of CD8+ T-cell differential expression analysis of:

- A. Discovery Cohort of patients treated by combination Ipilimumab/Nivolumab ICI (n=69) (Complete Response (CR), n=16; Partial Response (PR), n=25; Stable Disease (SD), n=9 Progressive Disease (PD), n=19).
- B. Validation Cohort of patients treated by combination Ipilimumab/Nivolumab ICI (n=46) (CR, n=10; PR, n=17; SD, n=6; PD, n=13).
- C. Meta-Analysis (Discovery and Validation Cohorts) of patients treated by combination Ipilimumab/Nivolumab ICI (n=115) (CR, n=26; PR, n=42; SD, n=15; PD, n=32).

The expression is derived from RNA-seq analysis of baseline (pre-treatment) peripheral blood CD8+ T-cells. The table lists the top 10 genes that are most significantly differentially expressed ($p < 0.01$, $|\log_2FC| > 1$) in each response comparison.

A. IPI/NIVO RNA-seq Discovery Cohort, n=69

CB (CR, n=16; PR, n=25) vs NCB (SD, n=9; PD, n=19)

Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	4.966551831	9.16E-19	2.00E-14	protein coding	HMAX
2	S100A8	2.456008677	1.07E-06	0.0092445	protein coding	S100 calcium binding protein A8
3	S100A9	2.218862956	1.27E-06	0.0092445	protein coding	S100 calcium binding protein A9
4	FCGR1A	3.837487592	6.77E-06	0.0368785	protein coding	Fc fragment of IgG, high affinity 1a, receptor (CD64)
5	OPRM1	3.286355219	1.16E-05	0.0504726	protein coding	opioid receptor mu 1
6	TMPRSS4	3.939884333	2.77E-05	0.0670619	protein coding	Transmembrane protease, serine 4
7	FHIT	1.5199198	2.65E-05	0.0670619	protein coding	fragile histidine triad diadenosine triphosphatase
8	GRM4	2.661142111	4.40E-05	0.0799335	protein coding	glutamate receptor, metabotropic 4

9	MT-CO2	1.091799821	4.40E-05	0.0799335	protein coding	mitochondrially encoded cytochrome c oxidase II solute carrier family 1 member 2
10	SLC1A2	2.183350547	7.34E-05	0.1142566	protein coding	
<u>CB (CR, n=16; PR, n=25) vs NCB (PD, n=19)</u>						
1	HMAX	4.297722354	6.15E-14	9.32E-10	protein coding	HMAX
2	S100A8	2.850908847	2.44E-08	0.0001234	protein coding	S100 calcium binding protein A8
3	S100A9	2.590726446	2.15E-08	0.0001234	protein coding	S100 calcium binding protein A9
4	KCNHI	5.916300996	1.36E-06	0.0041346	protein coding	potassium voltage-gated channel subfamily H member 1
5	FHIT	1.843480915	2.31E-06	0.0048356	protein coding	fragile histidine triad diadenosine triphosphatase
6	PLBD1	1.913572208	2.80E-05	0.0256695	protein coding	phospholipase B domain containing 1
7	C5AR1	1.931220639	2.88E-05	0.0256695	protein coding	complement C5a receptor 1
8	HK2	1.096253177	3.64E-05	0.0264168	protein coding	hexokinase 2
9	MNDA	2.061980341	4.56E-05	0.0276352	protein coding	myeloid cell nuclear differentiation antigen
10	SLC11A1	1.73512988	4.81E-05	0.0280181	protein coding	solute carrier family 11 member 1
B. IPI/NIVO RNA-seq Validation Cohort, n=46						
<u>CB (CR, n=10; PR, n=17) vs NCB (SD, n=6; PD, n=13)</u>						
Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	3.693479185	6.45E-11	1.40E-06	protein coding	HMAX
2	RASGRF2- AS1	-3.704948821	1.34E-06	0.0145572	lncRNA	RASGRF2 antisense RNA 1
3	HLA-DRB5	2.385559837	9.22E-06	0.0667842	protein coding	major histocompatibility complex, class II, DR beta 5

4	FLRT2	-4.370790275	2.37E-05	0.1070885	protein coding	fibronectin leucine rich transmembrane protein 2 Paired box protein Pax-6 branched chain amino acid transaminase 1 ephrin A3 spectrin beta, non-erythrocytic 4 DENN domain containing 5B gap junction protein, gamma 1
5	PAX6	-3.605357357	7.77E-05	0.126297	protein coding	
6	BCAT1	1.588074398	7.30E-05	0.126297	protein coding	
7	EFNA3	1.873762135	5.46E-05	0.126297	protein coding	
8	SPTBN4	-1.853123322	5.89E-05	0.126297	protein coding	
9	DENND5B	2.626494392	3.77E-05	0.126297	protein coding	
10	GJC1	-3.213283995	8.14E-05	0.126297	protein coding	

CB (CR, n=10; PR, n=17) vs NCB (PD, n=13)

1	HMAX	4.196858127	4.08E-12	8.13E-08	protein coding	HMAX
2	TF	-3.808153615	2.52E-07	0.002507	protein coding	transferrin
3	RP11-632K20.2	-1.973951765	5.68E-06	0.0376955	unprocessed pseudogene	M-phase phosphoprotein 10 (MPHOSP10) pseudogene
4	BCAT1	1.890956587	1.97E-05	0.0580939	protein coding	branched chain amino acid transaminase 1
5	SRGAP1	-2.747506699	2.04E-05	0.0580939	protein coding	SLIT-ROBO Rho GTPase activating protein 1
6	AC099661.1	4.585930226	1.51E-05	0.0580939	lncRNA	novel transcript
7	AL096711.2	-1.606257776	2.45E-05	0.0608837	protein coding	novel protein
8	RAPGEF3	-1.956527908	5.40E-05	0.0849395	protein coding	Rap guanine nucleotide exchange factor 3
9	PLCB4	-2.602793463	5.04E-05	0.0849395	protein coding	1-phosphatidylinositol 4,5-bisphosphate
10	OTOF	-2.353715485	4.77E-05	0.0849395	protein coding	phosphodiesterase beta-4 Otoferlin

C. IPI/NIVO RNA-seq Meta-Analysis, n=115

CB (CR, n=26; PR, n=42) vs NCB (SD, n=15; PD, n=32)

Rank	Gene	log ₂ FoldChange	p-value	FDR adj. p	gene biotype	description
1	HMAX	4.953431843	1.36E-32	3.12E-28	protein coding	HMAX
2	S100A9	2.574907212	8.26E-12	9.49E-08	protein coding	S100 calcium binding protein A9
3	S100A8	1.977407428	3.38E-07	0.0025925	protein coding	S100 calcium binding protein A8
4	VCAN	1.728222061	3.12E-06	0.0175438	protein coding	versican
5	FLRT2	-3.20174567	3.82E-06	0.0175438	protein coding	fibronectin leucine rich transmembrane protein 2
6	DNAH11	-2.29648695	7.82E-06	0.0275913	protein coding	dynein axonemal heavy chain 11
7	PADI2	2.142975507	1.06E-05	0.0275913	protein coding	peptidyl arginine deiminase 2
8	BRDT	-1.857179141	9.44E-06	0.0275913	protein coding	bromodomain, testis-specific
9	S100A12	2.291381363	1.32E-05	0.0275913	protein coding	S100 calcium binding protein A12
10	MNDA	1.565055378	1.24E-05	0.0275913	protein coding	myeloid cell nuclear differentiation antigen

CB (CR, n=26; PR, n=42) vs NCB (PD, n=32)

1	HMAX	4.819378762	3.83E-29	6.50E-25	protein coding	HMAX
2	S100A9	2.996357403	2.51E-14	2.13E-10	protein coding	S100 calcium binding protein A9
3	FCN1	2.519162482	3.68E-12	2.08E-08	protein coding	ficolin 1 [Source:HGNC Symbol;Acc:HGNC:3623]
4	S100A8	2.314132341	1.87E-08	7.93E-05	protein coding	S100 calcium binding protein A8
5	VCAN	2.144782057	6.58E-08	0.0002232	protein coding	versican
6	SERPINA1	1.75020927	1.98E-07	0.0005605	protein coding	serpin family A member 1
7	NGFRAP1	1.165972407	2.58E-07	0.0006255	protein coding	nerve growth factor receptor (TNFRSF16) associated protein 1

8	LYZ	1.773591843	4.96E-07	0.0007918	protein coding	lysozyme
9	S100A12	2.838544579	5.14E-07	0.0007918	protein coding	S100 calcium binding protein A12
10	C5AR1	1.740576074	4.94E-07	0.0007918	protein coding	complement C5a receptor 1
<u>CB (CR, n=26) vs NCB (PD, n=32)</u>						
1	HMAX	4.904365273	6.90E-15	6.48E-11	protein coding	HMAX
2	HK2	1.248898089	2.55E-06	0.0065649	protein coding	hexokinase 2
3	ADD2	1.200353304	8.27E-05	0.0707151	protein coding	adducin 2
4	ITGAX	-1.144619135	7.87E-05	0.0707151	lncRNA	integrin subunit alpha X
5	FHIT	1.693915777	0.000129	0.075879	protein coding	fragile histidine triad
6	VCAN	1.922832732	0.000259	0.0776774	protein coding	diadenosine triphosphatase versican
7	NGFRAP1	1.126397773	0.000227	0.0776774	protein coding	nerve growth factor receptor (TNFRSF16) associated protein 1
8	GPX1	1.08970375	0.000283	0.0776774	lncRNA	glutathione peroxidase 1
9	SERPINA1	1.47313709	0.000835	0.0969447	protein coding	serpin family A member 1
10	AC007278.1	-1.154800346	0.001267	0.1042573	lncRNA	novel transcript

Table 3: Results of CD8+ T-cell differential chromatin accessibility analysis from:

- A.** Discovery Cohort of patients treated by single-agent Nivolumab ICI (n=70) (Complete Response (CR), n=14; Partial Response (PR), n=15; Stable Disease (SD), n=6 Progressive Disease (PD), n=35).
- B.** Validation Cohort of patients treated by single-agent Nivolumab ICI (n=46) (CR, n=7; PR, n=12) vs. NCB (SD, n=3; PD, n=24).
- C.** Meta-Analysis (Discovery and Validation Cohorts) of patients treated by single-agent Nivolumab ICI (n=116) (CR, n=21; PR, n=28) vs. NCB (SD, n=11; PD, n=60).

The chromatin accessibility is derived from ATAC-seq analysis of baseline (pre-treatment) peripheral blood CD8+ T-cells. The table lists the top 10 chromatin regions that are most significantly differentially accessible ($p<0.05$) in each response comparison. All chromosomal positions are based on human genome build Human GRCh38 (hg38).

A. NIVO ATAC-seq Discovery Cohort, n=70
CB (CR, n=14; PR, n=15) vs. NCB (SD, n=6; PD, n=35)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chrX	126472363	126473271	909	5.58	3.91	6.15	-2.24	1.18E-15	1.03E-10
2	chr10	9996	10430	435	0.95	-0.95	1.55	-2.5	4.65E-12	2.03E-07
3	chr10	10654837	10655483	647	0.83	-0.81	1.39	-2.2	1.14E-10	3.30E-06
4	chr3	96617245	96618433	1189	6.66	7.39	5.75	1.64	1.29E-09	2.80E-05
5	chr14	99419499	99420981	1483	6.8	6.08	7.15	-1.07	7.17E-09	0.000125
6	chr8	123864127	123864571	445	0.44	-0.93	0.96	-1.89	2.60E-08	0.000378
7	chr5	134923786	134924739	954	7.61	6.28	8.13	-1.85	5.72E-08	0.000712
8	chr3	157879844	157880120	277	2.42	1.52	2.82	-1.31	3.22E-07	0.0034
9	chr18	80262779	80263269	491	1.52	0.3	2.01	-1.72	3.84E-07	0.0034
10	chr11	10508542	10509994	1453	6.27	5.28	6.71	-1.43	4.18E-07	0.0034

CB (CR, n=14; PR, n=15) vs. NCB (PD, n=35)

1	chr3	96617245	96618433	1189	6.81	7.63	5.39	2.23	1.27E-18	1.08E-13
2	chrX	126472363	126473270	908	5.9	4.15	6.56	-2.42	5.68E-17	2.40E-12
3	chr5	134923786	134924694	909	7.96	6.5	8.57	-2.06	3.73E-09	0.000105
4	chr11	10508542	10509993	1452	6.6	5.51	7.12	-1.61	3.74E-08	0.00079
5	chr10	9996	10430	435	0.59	-0.72	1.17	-1.89	6.46E-08	0.00109
6	chr17	36213002	36213300	299	3.69	4.06	3.29	0.77	2.15E-07	0.00303
7	chr10	10654837	10655480	644	0.56	-0.57	1.09	-1.66	4.30E-07	0.00519
8	chr21	5355319	5364232	8914	3.73	3.03	4.14	-1.11	6.71E-07	0.00708
9	chr21	35457210	35457535	326	2.43	2.82	1.99	0.83	4.32E-06	0.0405
10	chrX	87546486	87547013	528	2.68	2.28	2.93	-0.65	9.89E-06	0.0835

B. NIVO ATAC-seq Validation Cohort, n=46
CB (CR, n=7; PR, n=12) vs. NCB (SD, n=3; PD, n=24)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr1	630286	631368	1083	9.8	7.44	10.45	-3.01	1.23E-11	1.48E-06
2	chr11	10508201	10509996	1796	6.35	4.98	6.87	-1.89	8.43E-10	5.07E-05
3	chr21	44138161	44138672	512	1.03	1.81	0.03	1.78	8.34E-09	0.000334
4	chr21	44142993	44144801	1809	2.48	3.34	1.26	2.07	6.93E-08	0.00208
5	chr1	101370924	101371524	601	4.25	4.64	3.9	0.73	1.17E-07	0.00282
6	chr2	225308519	225309297	779	4.3	4.58	4.06	0.52	1.87E-07	0.00327
7	chr2	65027065	65027405	341	2.99	2.33	3.32	-1.0	1.90E-07	0.00327
8	chrY	9506636	9506948	313	1.17	1.85	0.37	1.48	2.86E-07	0.0043
9	chr3	3573220	3574148	929	4.8	5.1	4.55	0.56	4.36E-07	0.00583
10	chr1	26136768	26138340	1573	3.64	3.18	3.9	-0.72	9.72E-07	0.0117

CB (CR, n=7; PR, n=12) vs. NCB (PD, n=24)

1	chrY	9506636	9506948	313	1.15	1.87	0.08	1.8	3.00E-10	3.66E-05
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2	chr20	1588225	1589116	892	3.71	4.55	2.27	2.27	7.74E-08	0.00472
3	chr20	19284131	19284746	616	4.3	4.59	4.01	0.59	1.76E-07	0.00716
4	chr4	49297201	49299369	2169	0.98	1.52	0.32	1.21	3.71E-07	0.0113
5	chr8	58309472	58310363	892	4.58	4.87	4.3	0.57	5.08E-07	0.0124
6	chrX	89202927	89207888	4962	4.02	3.62	4.27	-0.65	7.40E-07	0.0126
7	chr5	178585453	178585741	289	6.03	5.57	6.32	-0.75	8.02E-07	0.0126
8	chr20	22828543	22829220	678	4.48	4.73	4.24	0.5	8.29E-07	0.0126
9	chr5	116452916	116453425	510	4.24	4.49	4.02	0.47	1.28E-06	0.0155
10	chr5	8879519	8880669	1151	5.14	5.33	4.96	0.37	1.50E-06	0.0155
\\										
193	chr11	10508198	10509996	1799	5.42	4.95	5.71	-0.75	8.54E-05	0.0537

C. NIVO ATAC-seq Meta-Analysis Cohort, n=116

CB (CR, n=21; PR, n=27) vs. NCB (SD, n=9; PD, n=59)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr11	10507914	10509996	2083	6.09	5.05	6.54	-1.48	6.75E-13	7.86E-08
2	chr10	9996	10430	435	0.43	-1	0.96	-1.96	1.02E-12	7.86E-08
3	chrY	9506636	9506948	313	0.24	0.79	-0.34	1.13	2.78E-10	1.43E-05
4	chr10	10654837	10655483	647	0.44	-0.65	0.91	-1.56	1.87E-09	7.20E-05
5	chr14	99419499	99420981	1483	6.38	5.82	6.67	-0.85	4.21E-09	0.000126
6	chr5	134923786	134924739	954	7.66	6.51	8.13	-1.62	4.92E-09	0.000126
7	chr20	27460597	27472688	12092	2.76	1.82	3.18	-1.36	1.37E-08	0.000302
8	chrX	144668451	144669276	826	2.86	2.46	3.08	-0.63	4.38E-08	0.000845
9	chr17	36212925	36213413	489	3.72	4.08	3.39	0.69	1.44E-07	0.00246
10	chrY	11781410	11781758	349	0.88	1.36	0.41	0.95	2.01E-07	0.00311

CB (CR, n=21; PR, n=27) vs. NCB (PD, n=59)

1	chr11	10507916	10509996	2081	6.38	5.29	6.9	-1.61	5.72E-14	8.43E-09
2	chr5	134923786	134924694	909	7.98	6.74	8.53	-1.79	3.69E-10	2.72E-05
3	chr17	36212925	36213413	489	3.94	4.32	3.54	0.78	8.02E-09	0.000394
4	chr20	27460597	27463030	2434	2.71	1.66	3.21	-1.55	2.40E-08	0.000885
5	chr7	63829616	63831474	1859	3.87	4.04	3.72	0.32	1.56E-07	0.00409
6	chr16	73334011	73334565	555	4.05	4.24	3.87	0.37	1.90E-07	0.00409
7	chr9	118230252	118230910	659	3.72	3.91	3.54	0.37	1.94E-07	0.00409
8	chrY	11781410	11781758	349	1.14	1.59	0.63	0.97	2.98E-07	0.00549
9	chr13	80063194	80063651	458	3	3.21	2.8	0.41	6.52E-07	0.00962
10	chr3	3683879	3685072	1194	4.88	5.03	4.75	0.28	6.52E-07	0.00962

CB (CR, n=21) vs. NCB (PD, n=59)

1	chrX	146619661	146620596	936	2.78	2.07	2.98	-0.91	1.08E-07	0.00734
2	chr8	58309472	58310634	1163	4.25	4.59	4.11	0.48	1.16E-07	0.00734
3	chr7	75179900	75180804	905	-0.21	0.51	-0.6	1.11	2.17E-07	0.00915
4	chr17	36212925	36213413	489	3.97	4.56	3.68	0.88	4.29E-07	0.0136
5	chr8	58360770	58361608	839	4.2	4.6	4.02	0.57	9.10E-07	0.0209
6	chr12	76985090	76985542	453	2.69	2.25	2.83	-0.58	1.09E-06	0.0209
7	chr15	22253285	22254838	1554	6.05	6.42	5.88	0.54	1.15E-06	0.0209
8	chr5	107519735	107520744	1010	5.01	5.37	4.86	0.51	1.80E-06	0.0285
9	chr15	20353157	20354538	1382	6.5	6.87	6.34	0.53	2.23E-06	0.0302
10	chrX	18404751	18405223	473	1.67	0.94	1.87	-0.93	2.67E-06	0.0302
\\										
580	chr11	10507916	10509996	2081	6.44	5.69	6.64	-0.95	0.0011	0.239

Table 4: Results of CD8+ T-cell differential chromatin accessibility analysis from:

- A. Discovery Cohort of patients treated by combination Ipilimumab/Nivolumab ICI (n=62) (Complete Response (CR), n=13; Partial Response (PR), n=25; Stable Disease (SD), n=9; Progressive Disease (PD), n=15).
- B. Validation Cohort of patients treated by combination Ipilimumab/Nivolumab ICI (n=48) (CR, n=10; PR, n=17) vs. NCB (SD, n=7; PD, n=14).
- C. Meta-Analysis (Discovery and Validation Cohorts) of patients treated by combination Ipilimumab/Nivolumab ICI (n=110) (CR, n=23; PR, n=42) vs. NCB (SD, n=16; PD, n=29).

The chromatin accessibility is derived from ATAC-seq analysis of baseline (pre-treatment) peripheral blood CD8+ T-cells. The table lists the top 20 chromatin regions that are most significantly differentially accessible ($p<0.05$) in each response comparison. All chromosomal positions are based on human genome build Human GRCh38 (hg38).

A. IPI/NIVO ATAC-seq Discovery Cohort, n=62
CB (CR, n=13; PR, n=25) vs. NCB (SD, n=9; PD, n=15)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr2	46369712	46370382	671	4.69	4.97	4.07	0.91	6.81E-17	3.47E-12
2	chr1	93758517	93759524	1008	5.21	5.47	4.67	0.79	2.28E-13	5.80E-09
3	chr5	80650506	80652039	1534	6.71	5.48	7.64	-2.16	2.36E-12	4.00E-08
4	chrX	126472163	126473271	1109	5.29	4.21	6.17	-1.97	7.76E-12	9.87E-08
5	chr1	6293961	6295281	1321	5.42	5.68	4.87	0.8	1.27E-11	1.30E-07
6	chr14	51596167	51597163	997	4.61	4.85	4.13	0.72	3.84E-11	3.26E-07
7	chr3	4748650	4749475	826	3.97	4.21	3.49	0.72	6.32E-11	3.97E-07
8	chr2	28508255	28508974	720	5.12	5.38	4.57	0.8	6.32E-11	3.97E-07
9	chr19	54905695	54907066	1372	4.93	5.15	4.49	0.65	7.02E-11	3.97E-07
10	chr10	110459869	110461229	1361	5.3	5.5	4.91	0.59	3.67E-10	1.87E-06

20

chr11

10508160

10509996

1837

5.65

4.91

6.35

-1.45

8.34E-10

2.12E-06

CB (CR, n=13; PR, n=25) vs. NCB (PD, n=15)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr2	46369712	46370382	671	4.76	4.97	3.99	0.98	2.24E-13	1.19E-08
2	chrX	126472163	126473271	1109	5.26	4.21	6.47	-2.27	4.43E-13	1.19E-08
3	chr5	80650506	80652039	1534	6.66	5.48	7.93	-2.45	1.43E-12	2.56E-08
4	chr7	66124802	66126711	1910	5.49	5.75	4.52	1.23	1.03E-10	1.38E-06
5	chr1	93758517	93759524	1008	5.27	5.47	4.63	0.83	4.48E-10	4.23E-06
6	chr10	110459869	110461229	1361	5.34	5.5	4.8	0.7	4.73E-10	4.23E-06
7	chr17	3693852	3694293	442	3.95	4.14	3.35	0.79	5.90E-10	4.53E-06
8	chr1	6293961	6295281	1321	5.48	5.68	4.8	0.88	9.16E-10	6.14E-06
9	chr7	139886098	139886705	608	3.93	4.11	3.32	0.79	2.26E-09	1.35E-05
10	chr3	4748650	4749475	826	4.03	4.21	3.43	0.79	3.36E-09	0.000018
\\										
29	chr11	10508160	10509996	1837	5.49	4.91	6.38	-1.47	3.59E-08	6.57E-05

B. COMBO ATAC-seq Validation Cohort, n=48

CB (CR, n=10; PR, n=17) vs. NCB (SD, n=7; PD, n=14)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr1	234314884	234315819	936	4.74	5.15	3.95	1.2	5.75E-07	2.40E-02
2	chrX	126472360	126473271	912	6.16	4.96	6.95	-1.99	6.30E-07	2.40E-02
3	chr5	80650505	80651993	1489	7.17	6.11	7.91	-1.81	2.95E-06	7.49E-02
4	chr13	18995117	18995749	633	3.89	3.64	4.16	-0.51	8.62E-06	1.45E-01
5	chr3	14216276	14216968	693	4.13	4.44	3.6	0.84	9.50E-06	1.45E-01

6	chr6	87915022	87915455	434	3.36	2.95	3.75	-0.8	1.58E-05	1.75E-01
7	chr8	42926351	42926943	593	3.91	3.66	4.18	-0.52	1.61E-05	1.75E-01
8	chr7	44051097	44052080	984	3.73	3.52	3.96	-0.44	1.96E-05	1.82E-01
9	chr18	26195406	26196192	787	2.35	2.61	1.92	0.69	2.15E-05	1.82E-01
10	chr5	257282	262414	5133	2.84	2.56	3.13	-0.57	2.66E-05	2.03E-01
\\										
439	chr11	10508199	10509996	1798	7.17	6.58	7.68	-1.09	6.90E-03	9.04E-01
CB (CR, n=10; PR, n=17) vs. NCB (PD, n=14)										
Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr1	234314884	234315819	936	5.12	5.47	4.04	1.43	9.37E-08	4.68E-03
2	K1270733.1	121318	125255	3938	6.08	6.39	5.19	1.2	1.84E-07	4.68E-03
3	chrX	126472361	126473270	910	6.53	5.28	7.61	-2.33	2.03E-07	4.68E-03
4	chr17	22524064	22524530	467	9.75	10.23	7.71	2.53	1.58E-06	2.73E-02
5	chr5	80650506	80651991	1486	7.48	6.43	8.49	-2.06	2.86E-06	3.26E-02
6	chr2	62443332	62444092	761	3.84	4.02	3.43	0.59	3.20E-06	3.26E-02
7	chr17	23693638	23699882	6245	1.07	1.51	-0.59	2.1	3.30E-06	3.26E-02
8	chr18	19470330	19481576	11247	2.3	1.88	2.87	-0.99	1.53E-05	1.33E-01
9	chr3	14216276	14216968	693	4.51	4.76	3.83	0.93	1.98E-05	1.51E-01
10	chr7	44051097	44052080	984	4.03	3.84	4.34	-0.49	2.18E-05	1.51E-01
\\										
104	chr11	10508199	10509993	1795	7.62	6.9	8.44	-1.53	9.90E-04	6.46E-01

C. COMBO ATAC-seq Meta-Analysis Cohort, n=110
CB (CR, n=23; PR, n=42) vs. NCB (SD, n=16; PD, n=29)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chrX	126472163	1.26E+08	1109	5.66	4.51	6.5	-1.99	4.09E-17	3.03E-12
2	chr5	80650505	80652039	1535	6.85	5.69	7.69	-2.0	1.33E-16	4.90E-12
3	chr2	46369671	46370382	712	4.68	4.87	4.36	0.5	1.83E-08	4.51E-04
4	chr11	10508160	10509996	1837	6.39	5.73	7.01	-1.28	6.32E-08	1.17E-03
5	chr22	21689511	21690533	1023	4.17	4.35	3.85	0.51	1.71E-07	2.54E-03
6	chr5	1151973	1152966	994	4.54	4.66	4.33	0.33	6.48E-07	7.99E-03
7	chr12	101743121	1.02E+08	1620	5.34	5.48	5.12	0.36	1.11E-06	1.11E-02
8	chr20	20181196	20181916	721	4.04	4.2	3.77	0.44	1.20E-06	1.11E-02
9	chr18	58887276	58887912	637	3.62	3.73	3.43	0.3	1.56E-06	1.28E-02
10	chr4	139528523	1.40E+08	655	4.03	4.19	3.76	0.43	2.16E-06	1.49E-02

CB (CR, n=23; PR, n=42) vs. NCB (PD, n=29)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chrX	126472163	1.26E+08	1109	5.67	4.51	6.83	-2.32	1.22E-18	8.69E-14
2	chr5	80650506	80652039	1534	6.82	5.69	7.96	-2.27	1.08E-16	3.83E-12
3	chr11	10508160	10509996	1837	6.44	5.73	7.34	-1.61	4.96E-09	1.17E-04
4	chr2	46369671	46370382	712	4.71	4.87	4.28	0.59	7.21E-09	1.28E-04
5	chr10	394203	395234	1032	3.99	4.12	3.66	0.46	2.40E-08	3.21E-04
6	chr6	144197814	1.44E+08	1405	4.54	4.68	4.17	0.51	2.71E-08	3.21E-04
7	K1270733.1	121311	125255	3945	5.68	5.9	5.01	0.89	4.97E-08	4.42E-04
8	chr6	167121825	1.67E+08	1178	4.51	4.62	4.23	0.4	4.97E-08	4.42E-04
9	chr5	1151973	1152966	994	4.55	4.66	4.27	0.4	7.02E-08	5.54E-04
10	chr22	39205110	39206487	1378	4.77	4.94	4.32	0.62	1.62E-07	1.15E-03

CB (CR, n=23) vs. NCB (PD, n=29)

Rank	Chromosome	Start	End	Width	Mean accessibility	Mean Accessibility CB	Mean Accessibility NCB	Fold	p-value	FDR adj. p
1	chr5	80650506	80652039	1534	7.81	6.32	8.41	-2.09	1.54E-07	5.45E-03
2	chr22	45341482	45341844	363	3.98	3.29	4.36	-1.07	1.88E-07	5.45E-03
3	chr3	58355586	58356374	789	3.86	4.21	3.52	0.69	2.98E-07	5.45E-03
4	chr11	102474165	1.02E+08	1013	3.95	4.31	3.57	0.74	3.28E-07	5.45E-03
5	chr6	130766562	1.31E+08	728	3.73	4.01	3.46	0.54	7.30E-07	9.69E-03
6	chr5	175507299	1.76E+08	666	4.37	3.94	4.64	-0.7	9.66E-07	1.07E-02
7	chrX	126472163	1.26E+08	1109	6.72	5.4	7.28	-1.88	1.31E-06	1.24E-02
8	chr5	95773291	95773694	404	3.57	3.91	3.21	0.7	2.07E-06	1.35E-02
9	chr10	5291433	5294429	2997	6.85	6.54	7.06	-0.52	2.14E-06	1.35E-02
10	chr1	63892419	63893606	1188	5.32	5.57	5.09	0.49	2.45E-06	1.35E-02
	==									
20	chr11	10508160	10509996	1837	7.26	6.09	7.79	-1.7	4.19E-06	1.35E-02

Table 5. Demographic overview for patients in the final analytic cohort

	CheckMate-067 Clinical Trial				COMBO Discovery (N=205)
	Total (N=1,254)	NIVO Discovery (N=115)	NIVO Validation 1 (N=108)	IPI Discovery (N=98)	
Sex					
Male	797 (63.6%)	65 (56.5%)	66 (61.1%)	65 (66.3%)	137 (66.8%)
Female	457 (36.4%)	50 (43.5%)	42 (38.9%)	33 (33.7%)	68 (33.2%)
Age at treatment start ^b (years)					
Mean (SD)	60.1 (14.0)	57.9 (14.7)	58.6 (13.6)	61.8 (14.9)	59.2 (14.1)
Median [Min, Max]	61.0 [19.0, 90.6]	59.0 [25.0, 88.0]	60.0 [28.0, 89.0]	63.0 [21.0, 89.0]	62.0 [23.0, 87.0]
Haplogroup					
H	580 (46.3%)	52 (45.2%)	50 (46.3%)	49 (50.0%)	98 (47.8%)
U	160 (12.8%)	17 (14.8%)	14 (13.0%)	16 (16.3%)	24 (11.7%)
T	125 (10.0%)	13 (11.3%)	16 (14.8%)	7 (7.1%)	18 (8.8%)
J	127 (10.1%)	9 (7.8%)	11 (10.2%)	11 (11.2%)	21 (10.2%)
K	133 (10.6%)	7 (6.1%)	12 (11.1%)	10 (10.2%)	12 (5.9%)
Other ^c	129 (10.3%)	17 (14.8%)	5 (4.6%)	5 (5.1%)	32 (15.6%)
Treatment Response					
Clinical Benefit					
CR	168 (13.4%)	14 (12.2%)	21 (19.4%)	5 (5.1%)	36 (17.6%)
PR	270 (21.5%)	29 (25.2%)	30 (27.8%)	11 (11.2%)	59 (28.8%)
dSD	89 (7.1%)	5 (4.3%)	6 (5.6%)	12 (12.2%)	17 (8.3%)
No Clinical Benefit					
pSD	117 (9.3%)	9 (7.8%)	6 (5.6%)	22 (22.4%)	30 (14.6%)
PoD	610 (48.6%)	58 (50.4%)	45 (41.7%)	48 (49.0%)	63 (30.7%)

^a Unique patients (does not include n=21 2nd-line patients treated with 1st-line IPI (n=14) or COMBO (n=7))

^b Value for participants missing age (n=99) imputed using cohort median (61 years)

^c Other European haplogroups (I, X, W, R, V)

IO-GEM Standard-of-Care Cohort			
NIVO Validation 2	NIVO <i>Ist-line only</i>	IPI Validation	COMBO Validation
(N=174)	(N=153) ^a	(N=379)	(N=196)
117 (67.2%)	103 (67.3%)	240 (63.3%)	121 (61.7%)
57 (32.8%)	50 (32.7%)	139 (36.7%)	75 (38.3%)
62.2 (12.0)	62.6 (12.1)	60.6 (14.9)	59.7 (12.3)
61.0 [20.0, 90.6]	61.0 [20.0, 90.6]	61.0 [19.9, 90.5]	61.0 [19.0, 83.7]
77 (44.3%)	68 (44.4%)	180 (47.5%)	83 (42.3%)
22 (12.6%)	19 (12.4%)	45 (11.9%)	25 (12.8%)
20 (11.5%)	19 (12.4%)	39 (10.3%)	13 (6.6%)
16 (9.2%)	13 (8.5%)	43 (11.3%)	19 (9.7%)
25 (14.4%)	23 (15.0%)	40 (10.6%)	29 (14.8%)
14 (8.0%)	11 (7.2%)	32 (8.4%)	27 (13.8%)
19 (10.9%)	18 (11.8%)	26 (6.9%)	48 (24.5%)
47 (27.0%)	40 (26.1%)	35 (9.2%)	66 (33.7%)
16 (9.2%)	10 (6.5%)	24 (6.3%)	15 (7.7%)
12 (6.9%)	12 (7.8%)	25 (6.6%)	13 (6.6%)
80 (46.0%)	73 (47.7%)	269 (71.0%)	54 (27.6%)

Table 6. Frequency of European Haplogroups in each analytic cohort compared to the population frequency in the 1000 Genomes Project

Reference Population (1000 Genomes Project)			
Haplogroup	1k Genomes (Reference)	% of Population	
H	201	49.8%	
U	79	19.6%	
T	36	8.9%	
J	33	8.2%	
K	24	5.9%	
Other	31	7.7%	
Total	404		

Total meta-analysis (BMS-067 and IO-GEM)			
Haplogroup	Overall Meta	%	p-value
H	589	46.2%	0.23
U	163	12.8%	0.001
T	126	9.9%	0.63
J	130	10.2%	0.27
K	135	10.6%	0.01
Other	132	10.4%	0.14
Total	1275		

p-value for two-sided t-test for difference in proportions

Haplogroup	BMS-067 Nivo			BMS-067 Ipi			BMS-067 Combo		
		%	p-value		%	p-value		%	p-value
H	102	45.7%	0.38	49	50.0%	0.99	98	47.8%	0.71
U	31	13.9%	0.09	16	16.3%	0.56	24	11.7%	0.02
T	29	13.0%	0.14	7	7.1%	0.72	18	8.8%	0.99
J	20	9.0%	0.85	11	11.2%	0.45	21	10.2%	0.48
K	19	8.5%	0.29	10	10.2%	0.20	12	5.9%	0.99
Other	22	9.9%	0.43	5	5.1%	0.50	32	15.6%	0.004
Total	223			98			205		

IO-GEM (Validation)									
Haplogroup	IO-GEM Nivo			IO-GEM Ipi			IO-GEM Combo		
		%	p-value		%	p-value		%	p-value
H	77	44.3%	0.26	180	47.5%	0.58	83	42.3%	0.11
U	22	12.6%	0.06	45	11.9%	0.004	25	12.8%	0.05
T	20	11.5%	0.42	39	10.3%	0.59	13	6.6%	0.43
J	16	9.2%	0.81	43	11.3%	0.17	19	9.7%	0.64
K	25	14.4%	0.002	40	10.6%	0.03	29	14.8%	6.00E-04
Other	14	8.0%	0.99	32	8.4%	0.79	27	13.8%	0.03
Total	174			379			196		

Meta-analysis (BMS-067 and IO-GEM)									
Haplogroup	Nivo Meta			Ipi Meta			Combo Meta		
		%	p-value		%	p-value		%	p-value
H	179	45.1%	0.21	229	48.0%	0.65	181	45.1%	0.21
U	53	13.4%	0.02	61	12.8%	0.008	49	12.2%	0.006
T	49	12.3%	0.14	46	9.6%	0.80	31	7.7%	0.63
J	36	9.1%	0.74	54	11.3%	0.15	40	10.0%	0.44
K	44	11.1%	0.01	50	10.5%	0.02	41	10.2%	4.00E-02
Other	36	9.1%	0.56	37	7.8%	0.99	59	14.7%	0.002
Total	397			477			401		

Table 7. Patient demographics of the analytic cohort comparing NIVO and PEMBRO

	Total (N=1,497)	Anti-CTLA-4		Anti-PD-1		Anti-CTLA-4/Anti-PD-1 Ipilimumab/Nivolumab (N=401)
		Ipilimumab (N=477)	Nivolumab (N=323)	Pembrolizumab (N=296)		
Sex						
Male	951 (63.5%)	305 (63.9%)	195 (60.4%)	193 (65.2%)		258 (64.3%)
Female	546 (36.5%)	172 (36.1%)	128 (39.6%)	103 (34.8%)		143 (35.7%)
Age at treatment start ^a (years)						
Mean (SD)	60.9 (13.8)	60.8 (14.9)	60.0 (13.9)	63.7 (12.5)		59.6 (13.2)
Median	62	61.7	62	62		62
[Min, Max]	[19.0, 95.4]	[19.9, 90.5]	[20.0, 90.6]	[27.0, 95.4]		[19.0, 87.0]
European Haplogroup						
H	697 (46.6%)	229 (48.0%)	145 (44.9%)	142 (48.0%)		181 (45.1%)
U	192 (12.8%)	61 (12.8%)	44 (13.6%)	38 (12.8%)		49 (12.2%)
T	145 (9.7%)	46 (9.6%)	44 (13.6%)	24 (8.1%)		31 (7.7%)
J	159 (10.6%)	54 (11.3%)	29 (9.0%)	36 (12.2%)		40 (10.0%)
K	149 (10.0%)	50 (10.5%)	32 (9.9%)	26 (8.8%)		41 (10.2%)
Other ^b	155 (10.4%)	37 (7.8%)	29 (9.0%)	30 (10.1%)		59 (14.7%)
Treatment Response						
Clinical Benefit						
CR	221 (14.8%)	31 (6.5%)	52 (16.1%)	54 (18.2%)		84 (20.9%)
PR	331 (22.1%)	46 (9.6%)	84 (26.0%)	76 (25.7%)		125 (31.2%)
dSD	112 (7.5%)	36 (7.5%)	16 (5.0%)	28 (9.5%)		32 (8.0%)
No Clinical Benefit						
pSD	117 (7.8%)	47 (9.9%)	21 (6.5%)	6 (2.0%)		43 (10.7%)
PD	716 (47.8%)	317 (66.5%)	150 (46.4%)	132 (44.6%)		117 (29.2%)

^a Value for participants missing age (n=194) imputed using cohort median

^b Includes haplogroups I, R, V, W, and X

Table 8. Frequency of European Haplogroups in each analytic cohort compared to the population frequency in the 1000 Genomes Project, p-value for two-sided t-test for difference in proportions

Reference Population (1000 Genomes Project)

Haplogroup	1k Genomes (Reference)	% of Population
H	201	49.8%
U	79	19.6%
T	36	8.9%
J	33	8.2%
K	24	5.9%
Other	31	7.7%
<i>Total</i>	404	

Total analytic population (CheckMate-067 and IO-GEM)

Haplogroup	Overall Meta	%	<i>p-value</i>
H	697	46.6%	0.25
U	192	12.8%	0.0006
T	145	9.7%	0.64
J	159	10.6%	0.15
K	149	10.0%	0.01
Other	155	10.4%	0.11
<i>Total</i>	1497		

CheckMate-067 and IO-GEM by ICI

Haplogroup	IPI	%	<i>p-value</i>
H	229	48.0%	0.6
U	61	12.8%	0.006
T	46	9.6%	0.71
J	54	11.3%	0.12
K	50	10.5%	0.02
Other	37	7.8%	0.96
<i>Total</i>	477		

Haplogroup	NIVO	%	<i>p-value</i>
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H	145	44.9%	0.19
U	44	13.6%	0.03
T	44	13.6%	0.04
J	29	9.0%	0.7
K	32	9.9%	0.05
Other	29	9.0%	0.05
<i>Total</i>	323		

Haplogroup	PEMBRO	%	<i>p-value</i>
H	142	48.0%	0.44
U	38	12.8%	0.07
T	24	8.1%	0.99
J	36	12.2%	0.03
K	26	8.8%	0.07
Other	30	10.1%	0.12
<i>Total</i>	296		

Haplogroup	COMBO	%	<i>p-value</i>
H	181	45.1%	0.19
U	49	12.2%	0.004
T	31	7.7%	0.54
J	40	10.0%	0.37
K	41	10.2%	0.03
Other	59	14.7%	0.002
<i>Total</i>	401		

Table 9. Logistic regression results for treatment outcome (clinical benefit vs no clinical benefit) for anti-CTLA-4 (IPI), anti-PD-1 (NIVO/PEMBRO), and combined anti-CTLA-4/anti-PD-1 (COMBO) immune checkpoint inhibitors (ICI) in the CheckMate-067 and IO-GEM cohorts (n=1,497)

Treatment Comparison	OR	95% CI	p-value
<i>Univariable</i>			
IPI vs anti-PD-1 ^a	3.23	(2.49-4.22)	< 2.00E-16
IPI vs COMBO	4.85	(3.64-6.51)	< 2.00E-16
Anti-PD-1 ^a vs COMBO	1.50	(1.16-1.94)	1.75E-03
<i>Multivariable^b</i>			
IPI vs anti-PD-1 ^a	3.27	(2.51-4.28)	< 2.00E-16
IPI vs COMBO	5.04	(3.77-6.79)	< 2.00E-16
Anti-PD-1 ^a vs COMBO	1.54	(1.19-2.00)	9.84E-04

^a Anti-PD-1 group includes both NIVO and PEMBRO-treated patients.

^b Multivariable regression model adjusted for age and sex.

Table 10. Logistic regression results for treatment outcome (clinical benefit vs no clinical benefit) for anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO separately), and combined anti-CTLA-4/anti-PD-1 (COMBO) immune checkpoint inhibitors (ICI) in the CheckMate-067 and IO-GEM cohorts (n=1,497)

Treatment Comparison	OR	95% CI	p-value
NIVO			
<i>Univariable</i>			
IPI vs Nivo	2.86	(2.12-3.89)	1.14E-11
Pembro vs Nivo	0.78	(0.57-1.06)	0.12
COMBO vs Nivo	0.59	(0.44-0.79)	4.82E-04
<i>Multivariable^a</i>			
IPI vs Nivo	2.98	(2.19-4.05)	3.17E-12
Pembro vs Nivo	0.82	(0.60-1.13)	0.23
COMBO vs Nivo	0.59	(0.44-0.80)	5.65E-04
Treatment Comparison			
PEMBRO			
<i>Univariable</i>			
IPI vs Pembro	3.69	(2.71-5.04)	< 2.00E-16
Nivo vs Pembro	1.29	(0.94-1.77)	0.12
COMBO vs Pembro	0.76	(0.56-1.03)	0.08
<i>Multivariable^a</i>			
IPI vs Pembro	3.62	(2.65-4.96)	8.47E-16
Nivo vs Pembro	1.22	(0.88-1.67)	0.23
COMBO vs Pembro	0.72	(0.53-0.98)	0.04

^a Multivariable regression model adjusted for age and sex.

Table 11. Logistic regression results, stratified by mitochondrial haplogroup, for treatment outcome (clinical benefit vs no clinical benefit) for anti-CTLA-4 (IPI), anti-PD-1 (NIVO/PEMBRO), and combined anti-CTLA-4/anti-PD-1 (COMBO) immune checkpoint inhibitors (ICI) in the CheckMate-067 and IO-GEM cohorts (n=1,497)

Treatment Comparison	OR	95% CI	p-value
Other MT-HGs			
<i>Univariable</i>			
IPI vs anti-PD-1 ^a	3.64	(2.76-4.83)	1.71E-19
IPI vs COMBO	5.71	(4.21-7.80)	1.68E-28
Anti-PD-1 ^a vs COMBO	1.57	(1.20-2.06)	1.03E-03
<i>Multivariable^b</i>			
IPI vs anti-PD-1 ^a	3.69	(2.79-4.91)	1.37E-19
IPI vs COMBO	5.91	(4.34-8.10)	5.56E-29
Anti-PD-1 ^a vs COMBO	1.60	(1.22-2.10)	6.81E-04
Treatment Comparison	OR	95% CI	p-value
HG-T			
<i>Univariable</i>			
IPI vs anti-PD-1 ^a	1.20	(0.55-2.68)	0.65
IPI vs COMBO	0.85	(0.31-2.26)	0.74
Anti-PD-1 ^a vs COMBO	0.70	(0.27-1.73)	0.45
<i>Multivariable^b</i>			
IPI vs anti-PD-1 ^a	1.19	(0.54-2.66)	0.67
IPI vs COMBO	0.92	(0.33-2.47)	0.86
Anti-PD-1 ^a vs COMBO	0.77	(0.30-1.91)	0.58

^a Anti-PD-1 group includes both NIVO and PEMBRO-treated patients.

^b Multivariable regression model adjusted for age and sex.

Table 12. Logistic regression results, stratified by haplogroup, for treatment outcome (clinical benefit vs no clinical benefit) for anti-CTLA-4 (IPI), anti-PD-1 (NIVO and PEMBRO separately), and combined anti-CTLA-4/anti-PD-1 (COMBO) immune checkpoint inhibitors (ICI) in the CheckMate-067 and IO-GEM cohorts (n=1,497)

2-5a. Other MT-HGs

Treatment Comparison	OR	95% CI	p-value
Other MT-HGs, NIVO			
<i>Univariable</i>			
IPI vs Nivo	3.52	(2.55-4.89)	3.31E-14
Pembro vs Nivo	0.94	(0.67-1.31)	0.69
COMBO vs Nivo	0.62	(0.45-0.84)	2.65E-03
<i>Multivariable^a</i>			
IPI vs Nivo	3.68	(2.66-5.13)	7.86E-15
Pembro vs Nivo	0.99	(0.71-1.40)	0.98
COMBO vs Nivo	0.62	(0.45-0.86)	3.54E-03
Other MT-HGs, PEMBRO			
<i>Univariable</i>			
IPI vs Pembro	3.77	(2.72-5.24)	2.15E-15
Nivo vs Pembro	1.07	(0.77-1.49)	0.69
COMBO vs Pembro	0.66	(0.48-0.91)	0.01
<i>Multivariable^a</i>			
IPI vs Pembro	3.70	(2.67-5.16)	8.03E-15
Nivo vs Pembro	1.01	(0.72-1.41)	0.98
COMBO vs Pembro	0.63	(0.45-0.86)	4.41E-03

2-5b. HG-T

Treatment Comparison	OR	95% CI	p-value
HG-T, NIVO			
<i>Univariable</i>			

IPI vs Nivo	0.61	(0.23-1.54)	0.30
Pembro vs Nivo	0.18	(0.06-0.51)	1.75E-03
COMBO vs Nivo	0.72	(0.25-2.08)	0.54

Multivariable^a

IPI vs Nivo	0.61	(0.23-1.55)	0.30
Pembro vs Nivo	0.18	(0.06-0.53)	2.40E-03
COMBO vs Nivo	0.67	(0.23-1.94)	0.45

Treatment Comparison**HG-T, PEMBRO****OR****95% CI****p-value***Univariable*

IPI vs Pembro	3.44	(1.25-9.99)	0.02
Nivo vs Pembro	5.67	(1.97-17.5)	1.75E-03
COMBO vs Pembro	4.07	(1.35-13.2)	0.02

Multivariable^a

IPI vs Pembro	3.31	(1.20-9.67)	0.02
Nivo vs Pembro	5.43	(1.87-16.9)	2.40E-03
COMBO vs Pembro	3.62	(1.19-11.8)	0.03

2-5c. Other MT-HGs vs HG-T NIVO or PEMBRO**Treatment Comparison****Other MT-HGs vs HG-T NIVO****OR****95% CI****p-value***Univariable*

Other IPI vs HG-T Nivo	1.00	(0.45-2.03)	0.99
Other Nivo vs HG-T Nivo	0.28	(0.13-0.58)	8.93E-04
Other Pembro vs HG-T Nivo	0.27	(0.12-0.54)	4.75E-04
Other COMBO vs HG-T Nivo	0.17	(0.08-0.35)	3.43E-06

Multivariable^a

Other IPI vs HG-T Nivo	1.01	(0.46-2.06)	0.97
Other Nivo vs HG-T Nivo	0.28	(0.12-0.56)	7.15E-04
Other Pembro vs HG-T Nivo	0.27	(0.12-0.56)	6.87E-04
Other COMBO vs HG-T Nivo	0.17	(0.08-0.35)	3.03E-06

Treatment Comparison**Other MT-HGs vs HG-T
PEMBRO****OR****95% CI*****p*-value***Univariable*

Other IPI vs HG-T Pembro	5.67	(2.44-13.9)	7.25E-05
Other Nivo vs HG-T Pembro	1.61	(0.69-3.94)	0.28
Other Pembro vs HG-T Pembro	1.50	(0.65-3.69)	0.35
Other COMBO vs HG-T Pembro	0.99	(0.43-2.42)	0.98

Multivariable^a

Other IPI vs HG-T Pembro	5.50	(2.36-13.5)	1.06E-04
Other Nivo vs HG-T Pembro	1.49	(0.64-3.69)	0.36
Other Pembro vs HG-T Pembro	1.49	(0.64-3.67)	0.37
Other COMBO vs HG-T Pembro	0.93	(0.40-2.28)	0.87

^a Multivariable regression model adjusted for age and sex.

Table 13. Clinical benefit for NIVO-based ICI treatment (NIVO or COMBO) for patients on CheckMate-067, stratified by MT-HG status (HG-T or other MT-HGs)

13a. Single-agent NIVO cohort

	CheckMate-067 NIVO			
	HG-T		Other MT-HGs	
	CB	NCB	CB	NCB
PD-L1⁺	6	8	60	27
PD-L1⁻	0	15	39	68

13b. Combined IPI/NIVO (COMBO) cohort

	CheckMate-067 COMBO			
	HG-T		Other MT-HGs	
	CB	NCB	CB	NCB
PD-L1⁺	2	9	50	29
PD-L1⁻	1	6	59	49

13c. NIVO and COMBO cohorts

	CheckMate-067 NIVO and COMBO			
	HG-T		Other MT-HGs	
	CB	NCB	CB	NCB
PD-L1⁺	8	17	110	56
PD-L1⁻	1	21	98	117

In the tables that follow, the Haplogroup Markers indicate the position in the mitochondrial genome and the corresponding base pair for top-level mitochondrial haplogroup definitions (those denoted by a single capital letter, e.g., U). These positions are based on mtDNA Tree build 17 (18 Feb 2016 [phylotree.org/tree/index.htm](https://www.phylotree.org/tree/index.htm) from which the entire description of haplogroups is incorporated herein by reference as it exists as of the effective filing date of this application). Unless in parentheses, each marker is present in at least 80% of the listed haplogroup. Parentheses indicate markers present in at least 50% but less than 80% of top level haplogroups. Lowercase nucleotide letters (a, c, t, g) indicate transversions (e.g., 186a). Lowercase "d" indicates a deletion following the (range of) position number(s) (e.g., 522d or 8281-8289d). Insertions are indicated by a dot after the mitochondrial reference sequence and the inserted base(s) (e.g., 2156.1A). Back mutations (markers that are reversions to the ancestral base) are indicated with an exclamation mark (!; !! for double back mutation) (e.g., A15301G!). Markers with the same value as the rCRS mitochondrial reference genome are indicated with an equals sign (e.g., 150C=). These markers are used to define the top level haplogroup, but will not be called as variants/SNPs by bioinformatics tools or genetic test results as they are identical to the reference sequence. The lack of any of the following high frequency variants, common to most haplogroup lineages, may also be informative in determining haplogroup identity: 73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G, & 16519C.

Haplogroup Markers Tables

Lineage	Top Level Haplogroup	Ancestral Marker Motif (the "RSRS50")	Haplogroup Markers	Other Selected Markers
L	L0	247A, 750G, 769A, 825A, 1018A, 2758A, 2885C, 3594T, 4104G, 4312T, 4769G, 7028T, 7146G, 7256T, 7521A, 8468T, 8655T, 8701G, 8860G, 9540C, 10398G, 10664T, 10688A, 10810C, 10873C, 10915C, 11719A, 11914A, 12705T, 13105G, 13276G, 13506T, 13650T, 14766T, 15326G, 16187T, 16189C, 16223T, 16230G, 16311C	1048T, 3516a, 5442C, 6185C, 9042T, 9347G, 9755A, 10589A, 12007A, 12720G	(263A=)
L	L1	(73G, 146C, 152C, 195C, 1438G, 2706G, 16129A, 16519C) 73G, 152C, 247A, 263G, 750G, 769A, 825A, 1018A, 2706G, 2758A, 2885C, 3594T, 4104G, 4769G, 7028T, 7146G, 7256T, 7521A, 8468T, 8655T, 8701G, 8860G, 9540C, 10398G, 10688A, 10810C, 10873C, 11719A, 12705T, 13105G, 13506T, 13650T, 14766T, 15326G, 16187T, 16189C, 16223T, 16278T, 16311C, 16519C	(522d, 4586C, 9818T, 16172C) 182T, 3666A, 7055G, 7389C, 13789C, 14178C, 14560A	(151T, 186a, 189c, 316A, 522d, 2395d, 5951G, 6071C, 8027A, 9072G, 10586A, 12810G, 13485G, 14000A, 14911T, 16294T, 16360T)

L	L2	73G, 146C, 152C, 195C, 263G, 750G, 769A, 1018A, 1438G, 2706G, 3594T, 4104G, 4769G, 7028T, 7256T, 7521A, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 13650T, 14766T, 15326G, 16223T, 16278T	2416C, 8206A, 9221G, 10115C, 11944C, 13590A, 15301A, 16390A	150C=, 16311T=
		(11914A)	(2789T, 7175C, 7274T, 7771G, 12693G, 13803G, 14566G, 15784C, 16294T, 16309G)	
L	L3	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16223T	15301A	3594C=, 769G=, 1018G=, 16311T=
		(16519C)	(150T)	
L	L4	73G, 263G, 750G, 769A, 1018A, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16223T, 16311C	3918A, 15301A, 16362C	3594C=, L4b2: 146C
		(146C, 16519C)		
L	L5	73G, 247A, 263G, 750G, 769A, 825A, 1018A, 1438G, 2706G, 3594T, 4104G, 4769G, 7028T, 7256T, 7521A, 8655T, 8701G, 8860G, 9540C, 10398G, 10688A, 10810C, 10873C, 11719A, 12705T, 13506T, 13650T, 14766T, 15326G, 16129A, 16223T, 16278T, 16311C	(244G, 315CC, 1413C, 8104C, 9855G, 12609C, 13470G, 16293T, 16355T, 16399G) 182T, 3423C, 5147A, 7972G, 12432T, 12950G, 14581C, 16148T, 16166G	Length variants in the 455-459 region [459CCsor 455TTTC or 455TTTTC].

(152C, 195C, 13105G, 16189C)		(522d, 709A, 851G, 1822C, 5111T, 5656G, 6182A, 6297C, 7424G, 8155A, 8188G, 8582T, 9305A, 9329A, 11025C, 11881T, 12236A, 13722G, 14212C, 14239T, 14905A, 14971C, 15217A, 15884A, 16355T, 16362C)
L	L6	73G, 146C, 152C, 263G, 750G, 769A, 1018A, 1438G, 2706G, 3594T, 4769G, 7028T, 7256T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 13650T, 14766T, 15326G, 16223T, 16278T, 16311C, 16519C
L3 M CZ	C	(265C, 309CCT, 310C) 249d, 489C, 3552A, 4715G, 7196a, 8584A, 9545G, 10400T, 13263G, 14318C, 14783C, 15043A, 15301A, 15487T, 16298C, 16327T
L3 M	D	(16519C) 73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16223T
L3 M	E	489C, 3010A, 4883T, 5178a, 8414T, 10400T, 14668T, 14783C, 15043A, 15301A, 16362C 489C, 3027C, 3705A, 4248C, 4491A, 7598A, 10400T, 10834T, 13254C, 13626T, 14577C, 14783C, 15043A, 15301A, 16362C, 16390A

L3 M	G	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16223T	(6620C, 16291T) 489C, 709A, 4833G, 5108C, 10400T, 14569A, 14783C, 15043A, 15301A, 16362C
L3	M	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16223T	(5601T, 13563G) 489C, 10400T, 14783C, 15043A, 15301A
L3 M	Q	(16519C) 73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 15326G, 16129A, 16311C	89C, 489C, 4117C, 5460A, 5843G, 8790A, 8964T, 10400T, 12940A, 13500C, 14025C, 14783C, 15043A, 15301A, 16144C, 16148T, 16241G, 16265c
L3 M CZ	Z	(146C, 16223T) 73G, 152C, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8701G, 8860G, 9540C, 10398G, 10873C, 11719A, 12705T, 14766T, 16223T	(92A, 16343G) 249d, 489C, 4715G, 6752G, 7196a, 8584A, 9090C, 10400T, 14783C, 15043A, 15301A, 15487T, 15784C, 16185T, 16260T, 16298C
L3 N	A	(15326G) 73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G, 16223T	(310C) 235G, 522d, 663G, 1736G, 4248C, 4824G, 8794T, 16290T, 16319A, 16362C

L3 N R	B	(146C)	(153G, 310C, 8027A, 12007A, 16111T)	Branches B4 and B9: 10398A=s8271 9bp deletions [8271_8279del or 8281_8289del]; Branch B4: 514_515delCA Branch B5: 146T=, 16217T=
		73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G, 16189C, 16519C	16183c, 16217C	
		(146C)	3970T, 6392C, 10310A, 13928C, 16304C	
L3 N R	F	(146C)	(249d, 522d, 6962A, 9053A, 10609C, 12406A, 12882T, 13759A)	2706A=, 7028C=
		73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G		
		(16129A, 16519C)		
L3 N R HV	H	263G, 750G, 1438G, 4769G, 8860G, 15326G		14766C=
		(16519C)	(310C)	
		263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 15326G	199C, 204C, 250C, 1719A, 4529T, 8251A, 10034C, 10238C, 12501A, 13780G, 15043A, 15924G, 16391A	
L3 N R	HV	(16519C)		295T, 489C, 4216C, 11251G, 12612G, 13708A, 15452a, 16069T, 16126C
		73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 10398G, 11719A, 12705T, 14766T, 15326G, 16129A, 16223T, 16519C		
L3 N R JT	J	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 10398G, 11719A, 14766T, 15326G	(462T, 3010A, 14798C)	

L3 N R U	K	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 10398G, 11719A, 14766T, 15326G, 16311C, 16519C	1189C, 1811G, 3480G, 9055A, 9698C, 10550G, 11299C, 11467G, 12308G, 12372A, 14167T, 14798C, 16224C
			(315CC, 497T)
L3	N	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G, 16223T	8701A=, 9540T=, 10398A=, 10400C=, 10873T=, 15301G=
		(16519C)	
L3 N	O	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G, 16223T	6755A, 9140T, 16213A
		(152C, 16189C, 16519C)	(794C, 5563A, 16183c)
L3 N R	P	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G	15607G
L3 N	R	263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 14766T, 15326G	12705C=, 16223C=
		(73G, 11719A, 16519C)	
L3 N	S	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G	8404C
		(152C, 16223T)	
L3 N R JT	T	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G, 16519C	709A, 1888A, 4216C, 4917G, 8697A, 10463C, 11251G, 13368A, 14905A, 15452a, 15607G, 15928A, 16126C, 16294T
			10398A=

L3 N R	U	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 14766T, 15326G	(11812G, 14233G) 11467G, 12308G, 12372A
L3 N R HV	V	263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 15326G	72C, 4580A, 15904T, 16298C
L3 N	W	73G, 195C, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G, 16223T, 16519C	(310C) 189G, 204C, 207A, 709A, 1243C, 3505G, 5046A, 5460A, 8251A, 8994A, 11674T, 11947G, 12414C, 15884C, 16292T
L3 N	X	73G, 195C, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 11719A, 12705T, 14766T, 15326G, 16189C, 16223T, 16278T, 16519C	(194T) 153G, 1719A, 6221C, 6371T, 13966G, 14470C
L3 N	Y	73G, 263G, 750G, 1438G, 2706G, 4769G, 7028T, 8860G, 10398G, 11719A, 12705T, 14766T, 15326G (146C, 16519C)	(225A) 5417A, 8392A, 14178C, 14693G, 16126C, 16231C (310C, 3834A, 7933G, 16266T)

Unique SNPs associated with sub-clades of top-level haplogroups J, T, U, or K (e.g., T2). These SNPs may be present in multiple sub-clades.

9966A	7258C	4025T	16243C	14325C	11227T	11497T	14034C	16129c	3348G	5567C	8104C
9932A	7245G	4013T	16242T	14323A	11204C	11539T	14037G	16134T	334C	5583T	8119C
9899C	7211A	4011T	16242a	14296G	11177T	11549T	14053G	16144C	337G	5592G	8137T
9872G	7184G	3992T	16241G	14281T	11176A	11566G	14063C	16154C	338a	55a	8164T
9861C	7150C	3915A	16240c	14274G	11087C	11582G	14070G	16166d	3397G	560T	8167C
9843G	709A	3882A	16239T	14233G	11084G	11590G	14094C	16167T	340T	5686t	8188G
9836C	7076G	3867T	16235G	14182C	11062T	11620G	14110C	16168T	3434G	5746A	8251A
97A	7022C	3866C	16231C	14180C	11016A	11653G	14139G	16169a	3480G	5752d	8260C
9755A	7001G	385G	16224C	14133G	11002G	11674T	14142g	16173T	3498T	575T	8276.1C
9725C	6998T	384G	16222T	14118G	10C	11725G	14167T	16174T	3504C	5788C	8277C
9667G	6962t	3847C	16218T	1406C	10966C	11732C	14178C	16176T	3507a	5790a	827G
9635c	6915A	3834A	16214T	14061T	10942G	11734G	14179A!	16179T	3507T	5821A	8282T
9632G	6911C	3826C	16213A	14040A	10822T	11788T	14179G	16181G	3513T	5894c	8296G
9617G	6893T	3820T	16195C	14029G	10750G	11840T	14198A	16184T	3535a	5899.1C	8308G
9612A	6887T	3721G	16193T	14016A	10685A	11869a	14212C	16189a	3546a	5913A	8395T
9548A	6830a	3705A	16192T	13980A	10601a	11878C	14215C	16189C	3552C	5918C	8407a
9545G	6716G	3531A	16189C!	13973t	10598G	1187C	14245T	16189T!!	3564G	5984G	8410T
9494G	6671C	3456C	16188T	13966G	10559G	11881T	14258A	16192C!	3591A	5999C	8413G
9438A	6635C	3447G	16187T!	13965C	10499G	11893G	14259A	16206c	3654T	6023A	8428a
9422c	6620C	3398C	16186T	13956G	10497T	1189C	14278C	16207G	3666A	6041T	8433C
9324G	6554T	3394C	16172C	13953C	10496G	11923G	14305A	16209C	3672G	6045T	8440G
930A	6524C	3350C	16169T	13934T	10490C	11929C	14308C	16214a	3688c	6047A!	8473C
9300A	6491T	3338C	16163G	13933G	10463C	11935C	14319C	16219A!	368G	6047G	8490C
9254G	6489a	3308g	16153A	13928c	10454C	11938T	14384A	16219G	3696T	6053T	8496C
9234G	6464a	3290C	16148T	13917G	10446G	1193C	14388G	16223T!	3702G	6060G	8522T
9233C	6445T	321C	16147T	13899C	10410C	11941G	14420T	16230G!	3714G	6071C	8531G
9224C	6425C	3213G	16146G	13879C	10410a	11947G	1442A	16234T	3720G	6146G	8557c
9219g	6392C	3203G	16145A	13834G	10398G!	11965T	14518G	16245T	3738T	6164T	8567C
91T	6383A	319C	16140C	13830C	10398A!!	11971T	14530C	16246t	373G	6182A	8574T

9192A	634C	310.1T	16136C	13813A	10389C	11984C	14533T	16259T	3741T	6221C	8598C
9181G	6345C	3010A	16129A!	13791T	10376G	12017G	14562T	16270C!	3750T	6257A	8610C
9180G	6293C	2969G	16126T!	13785T	10345C	12019T	14574T	16278C!!	3768G	6260G!	8642G
9145A	6261A	295T	16126C	13768C	10334T	12022T	14577C	16293G	3777C	6284G	8676T
9139A	6260A	2850C	16114T	13759A	10321C	12094T	14577g	16295T	3816G	629C	8684T
9120G	6249A	279C	16104T	13753C	10289G	12097T	14605t	16311C	3849A	6341T	8701G!
9117C	6216C	271T	16092C	13743C	10274C	12103a	14620T	16311T!!	3861G	6359G	8702T
9111C	6152C	2707c	16086C	13722G	10237C	12106T	14684T	16318t	394T	6367C	8705C
9103C	6045a	2706A	16069T	13708A	10227C	1211A	146T!!	16320T	3969T	6386T	8706G
9090C	6026A	266C	16066G	13692T	10192T	12134C	1472A	16327T	4021G	63C	8766T
9083C	59C	247A!	16063C	13681G	10172A	12135a	14767C	16336A	4053G	6407C	8778T
9055A	5964C	242T	16051G	13590A	10166C	12136C	14831A	16342C	4059T	6413C	8790A
9052G	593C	2404C	159C	13581C	10143A	12161C	14866T	16343G	4062C	6452T	8812G
9041G	5915T	2387C	15965G	13557G	10101C	12188C	14869c	16352C	408a	6455T	8818T
9016G	5836G	2380T	15947G	13506T!	10084C	12246T	14883T	16353T	4093G	6461G	8842c
8994A	5817T	235G	15941C	13500C	1007A	12297C	14893G	16354T	4113A	64T	8856c
8974T	5811G	2308G	15936t	13470G	1005C	12308A!	14921A	16356C	4129G	6518T	8870C
8958T	57C	228A	15935G	13434G	10029G	12331G	14926G	16356T!	4132A	6527G	8901G
8940T	5774C	222T	15928A	13419G	10031C	12338C	14927G	16362T!	4137T	6528T	8933T
8934T	5773A	215G	15924G	13398G	10127G	12346T	14935C	16398A	4158G	6546T	8938G
8923G	5747G	2158C	15916C	13392C	10142T	12372A	14956C	16399G	4172a	6584T	8950A
8868C	573.XC	214G	15913T	13368A	10154G	12376g	14971C	16400T	4188G	6599G	8952C
8865A	5656G	2141C	15894A	13308G	10202T	12491T	15031T	16428A	4209C	6629G	896G
8864C	5633T	2140A	15884A	13281C	10203A	12501A	15049T	16463G	4216C	663G	8982G
8854A	5585A	207A	15853T	13260C	10253C	12530G	15061G	16465T	4224T	6665T	9006G
8853G	5582G	204C	15836G	13188T	10262G	12535T	15077A	16497G	4295G	6674C	9031T
8847T	5580C	203c	15812A	13145A	10283G	12582G	15097C	16524G	431a	6680C	9070g
8843C	5558G	200G	15787C	13135A	10301G	12603T	15106A	16525G	4336C	6719C	9093G
8839A	5527G	199C	15784C	13132T	10326a	12609C	15115C	1692t	437T	6836T	9094T
8838A	5501G	198T	15758G	13032G	10336C	12615G	15172A	1694C	4502C	6845T	9100G

8775T	55.1T	1977C	15725T	13020C	10364A	12616C	15191C	16t	455.1T	6881G	9101C
8734a	5498G	195C!	15679G	13015C	10397G	12618A	1520C	1700C	4553C	6890G	9128C
8730G	5493C	194T	15672C	12957C	103A	12630A	15217A	1719A	455d	6899A	9148C
8727T	5483C	189G	15670C	12891T	1040C	12654G	15218G	1721T	4561C	6905G	9196A
8715C	5471A	188G	15662G	12879C	10478T	12678C	15244G	1809C	4562G	6920a	9266A
8711G	5463T	1888A	15617A	12771A	1047G	12681C	15289C	1811A!	4592C	6929G	9287A
8697A	5460A	1871G	15607G	12753G	10506G	12696C	15301A!!	1819C	4616T	6938T	9293T
8659G	5442C	185A	15607A!	12741T	10544T	12720G	15314A	1822C	4640a	6956C	9299G
8633G	5426C	1850C	15601C	12672G	10550G	12727C	15325G	1834C	4640T	6975C	9335a
8596G	5414G	1836G	15596A	12634G	10589A	12738g	15326A	183G	4646C	7028C	9338G
8572A	5378G	1829G	15530C	12633a	10619T	12768G	15327T	1842G	4654T	7031T	9365T
8558T	5322c	1811G	15514C	12612G	10654T	12793C	15355A	1926t	4655A	7055G	9371T
8557A	5297T	1766C	15511C	12570G	10676T	12795A	15373G	195T!!	466C	7082T	9377G
8530G	5277C	1733Ta	15509G	12561A	10692T	12842C	15380G	1978G	4674G	7085C	9389G
8504C	5276G	1709A	15499T	12557T	10697T	12843C	15381T	2083C	4703C	7109T	93G
8460G	5262A	16527T	15490T	12477C	10698T	12870T	15383C	217C	471C	7118G	9428C
8455T	5237A	16526A	15466A	12453C	10700G	12937G	15431A	2217T	4732G	7151T	942G
8419C	5231A	16438A	15454C	12441C	10709c	12950c	15497A	2218T	4736C	7202G	9448G
8412C	5201C	16390A	15453C	12408C	10733T	12954C	15519C	2225a	4745t	7226A	9469T
8404C	5198G	16370A	15452a	12406A	10754G	13002a	15533G	2246G	4748T	723c	9477A
8386T	5187T	16368C	15445C	12397G	10768G	13017G	15544a	2294G	4775G	723G	9480C
8290A	5162C	16366T	15442G	12396C	10784G	1303A	15544T	234G	4796T	7253G	94A
8281-8289d	5147A	16362C	153G	12379T	10819G	13104G	15553A	2352C	4811G	7265G	9571T
8271G	513A	16357C	15367T	12373G	10858C	13105G!	15613G	2405.1C	4820A	7277G	9575A
8270T	512G	16355T	1530G	12363T	10861C	13111C	15625a	2483C	4843T	7278C	960.1C
8269A	5108C	16335G	152T!!	12358G	10876G	13117G	15626T	249d	4856C	7340A	960.XC
8256C	507C	16325C	152C!!	12311C	10885C	13158G	15631G	249G	4924c	7382A	960d
8230T	5054A	16324C	152C!	12308G	108G	13161C	15632T	2526T	4936T	7385G	9614G
8222C	5024T	16319A	15262C	12223G	10907C	13194A	15634G	252C	4965G	7388G	961C
8155A	499A	16311C!	15257A	12172G	10927C	13269G	15650A	262T	4970G	7403G	9647C

8152A	4991A	16309G	15213C	12171G	10972G	13285G	15652G	263A	497T	742C	965.XC
8149G	4959A	16304T!	151T	12127A	10978G	13293T	15661T	2672G	498d	745G	9656C
8078A	4931T	16304C	15199T	12123T	11009C	13326C	15693C	2707G	5081C	7471T	9664G
8041G	4924A	16301T	15172t	12083g	11013T	13351T	15721C	270G	508G	750A	9670G
8020A	4917G	16300G	15148A	12028C	11017C	13359A	15734A	2755G	5093C	7559G	9682C
7984A	4917A!	16298C	15119A	12020T	11025C	13395G	15777A	2757G	5120G	7569G	9698C
7963G	489C	16297C	15113G	12007A	11038G	13401C	15781T	2766a	512c	7581C	9716C
789C	4853A	16296T	15110A	11944C	11050C	13404C	15789T	2772T	5130C	7624C	9738A
7897A	482C	16294T	150T	11914A!	11071T	13413G	15790T	2792G	5153G	7642A	9767T
7891T	4829G	16294C!	15090C	11857T	11107T	1341T	15791G	280g	5165T	7657C	9770C
7873T	4823C	16292T	15067C	11827C	11116C	13420T	15799G	282C	516T	7700T	9777A
7870C	4808T	16291T	15043A	11818G	11143T	13422G	15803A	2831A	5177A	7705C	9779T
7853A	4802C	16290T	1503A	11812G	11151T	13431T	15850g	2836T	517t	7729G	9800C
7789A	4790G	16288C	15028a	11812A!	11167G	13440T	15862C	285T	5186t	7735G	9801A
7783C	4772C	16287T	15014C	11800G	11172G	13528G	15880G	291.1A	5240G	7768G	980C
7747T	4739T	16286T	14950T	11797G	11191T	13565T	15883A	295a	5250C	7775A	9852G
7711C	469T	16284G	14905A	11778A	11197T	13569C	15891T	296T	5261A	7792T	9852t
769A!	4696C	16278T!	14861A	11647T	11233C	13602C	15892C	3027C	5263T	7805A	988A
7679C	4695C	16274A	14839G	11623T	11268T	13617C	15900C	309d	5264T	7843G	9891C
7521A!	4688C	16271C	14836G	11533T	11272G	13630G	15903G	3105G	5265G	7859A	9896G
751t	462T	16270T	14798C	11518A	11296T	13635C	15905C	310C	5298G	7894G	9903C
7501C	458T	16269c	14793G	11503T	11299C	13637G	15907G	3116T	5319G	7912A	9938C
7476T	456T	16266T	14769G	114T	11326T	13656C	15927A	3158.1T	5319t	7918T	9948A
73A	4491A	16265G	146C!	11467G	11329G	13674C	15930A	3170a	533G	7927g	9962A
7391C	4464A	16265c	14687G	11440A	11332T	13710G	15944d	3192T	5360T	7930G	9977C
7364G	4454C	16263C	14605G	11416T	11339C	13734C	15946T	3197C	5390G	793T	9989C
7337A	430C	16261T	14569A	11386C	11348T	13740C	15954c	3198G	5437T	794a	
7310C	4232C	16261a	14544A	11383C	11350G	13789C	16048A	3204T	5452T	7960c	
7302C	4225G	16260T	14500G	11377A	11353C	13802T	16067T	3212T	5465C	7978T	
7299G	41T	16257T	14470C	11287C	11374G	13827G	16074G	3229.1A	5486T	8005C	

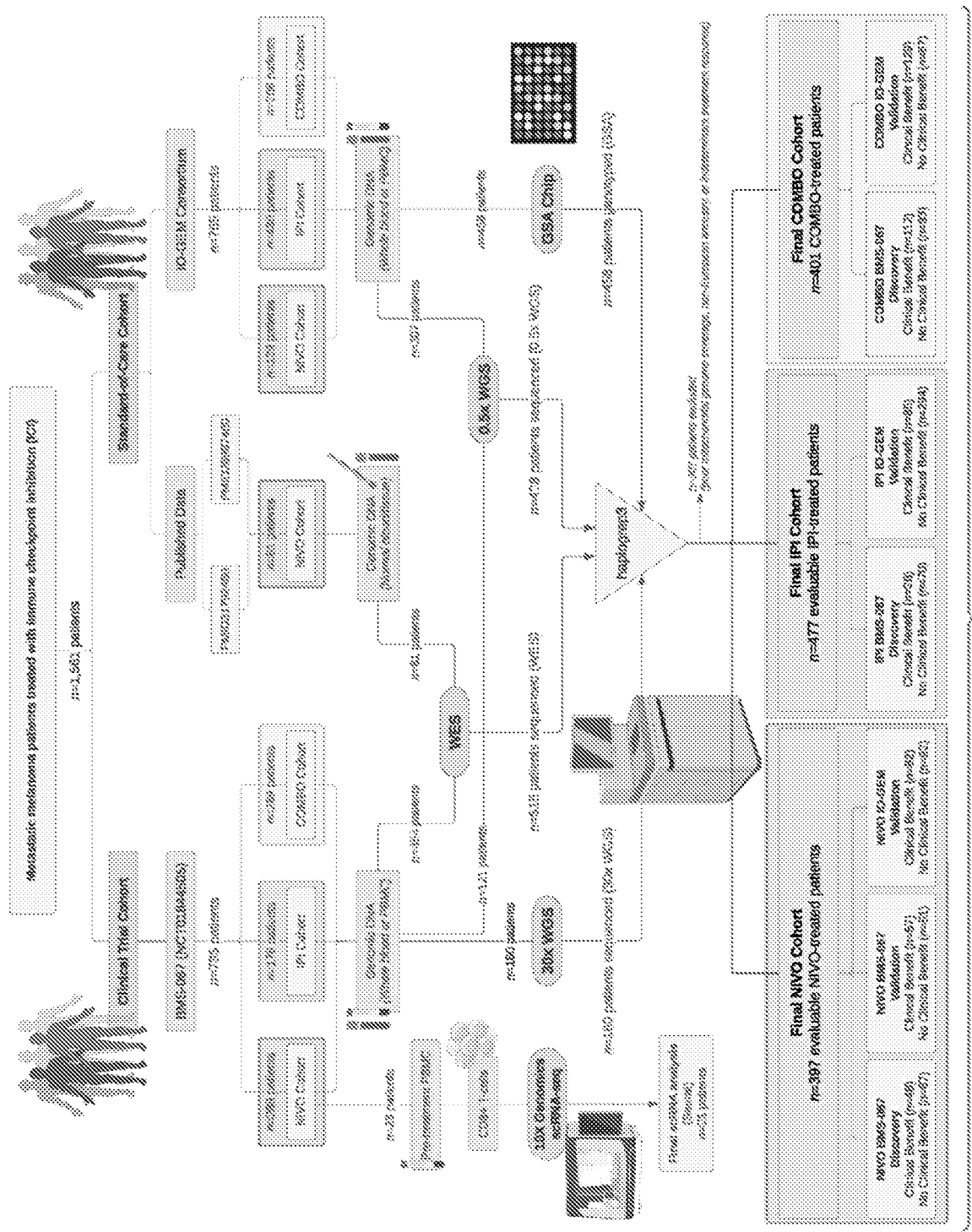
7295G	41C!	16257d	14455T	11266T	11465C	13928A	16079T	324T	5495C	8023C
7283C	4155T	16256T	143A	11252G	11470G	13967T	16093C	325T	5508C	8027A
7269A	4107T	16249C	1438A	11251G	11485C	14002G	16111T	3316A	5554a	8065A
7268C	4084A	16248T	14364A	11242g	11491G	14003T	16114a	3337A	5557C	8098G

What is claimed is:

1. A method comprising determining whether or not an individual is likely to respond to treatment with one or more immune checkpoint inhibitors (ICI's) based on a determination of a mitochondrial haplogroup for the individual, and wherein the haplogroup of the individual
5 is determined prior to receiving immune checkpoint inhibition.
2. The method of claim 1, wherein the haplogroup that is determined is a T haplogroup, a K haplogroup, a U haplogroup, or a J haplogroup.
3. The method of claim 2, wherein the wherein the haplogroup that is determined is the T haplogroup or the K haplogroup or the U haplogroup, and wherein the T haplogroup or the
10 K haplogroup or the U haplogroup indicates the individual is likely to be resistant to a particular anti-PD-1 ICI antibody as a monotherapy.
4. The method of claim 3, further comprising administering to the individual an ICI that is not the particular ICI anti-PD-1 antibody as a monotherapy.
5. The method of claim 2, wherein the haplogroup is the J haplogroup, and wherein the J
15 haplogroup indicates the individual will likely be sensitive to an anti-PD1 ICI.
6. The method of claim 5, further comprising administering the anti-PD-1 ICI to the individual.
7. The method of claim 2, wherein the wherein the haplogroup that is determined is the T haplogroup or the K haplogroup or the U haplogroup, and wherein the T haplogroup or the
20 K haplogroup or the U haplogroup indicates the individual is likely to be sensitive to an anti-PD-1 ICI antibody that is not nivolumab, and wherein optionally the anti-PD-1 antibody that is not the nivolumab is used as a monotherapy.
8. The method of claim 7, further comprising administering the anti-PD-1 ICI that is not the nivolumab to the individual.
- 25 9. The method of any one of claims 1-8, wherein determining the haplogroup comprises determining one or more of the markers as defined by the haplogroup tables herein.
10. The method of any one of claims 1-4, or 7, comprising quantitative next-generation sequencing analysis of RNA expressed from a locus that includes MTRNR2L8, or ATAC-seq

chromatin accessibility of the locus that includes the MTRNR2L8, to thereby indicate the individual has the T haplogroup.

11. The method of claim 10, further comprising administering to the individual an anti-PD-1 ICI that the individual is likely to be sensitive to based on a determined haplogroup,
5 and wherein optionally the anti-PD-1 ICI that the individual is likely to be sensitive is not nivolumab used as a monotherapy.



n=1,254 evaluable ICI-treated metastatic melanoma patients

FIG. 1

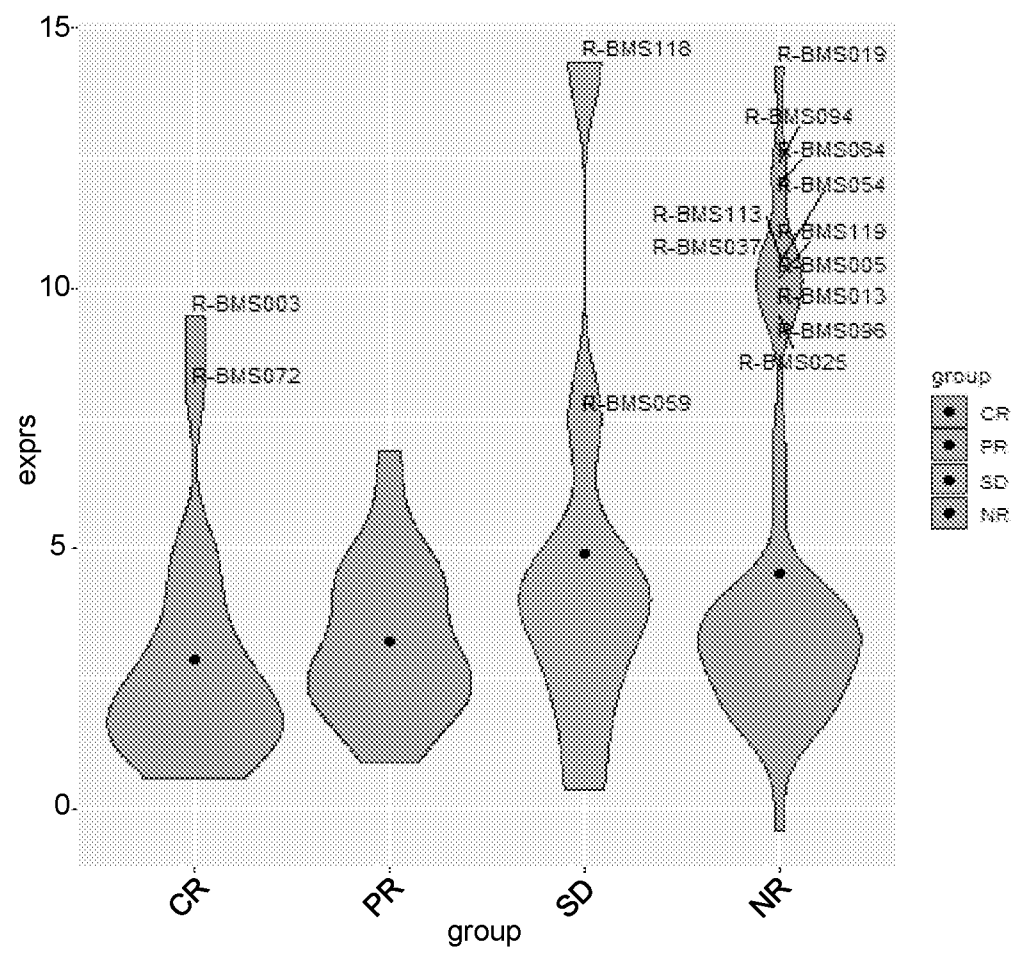


FIG. 2A

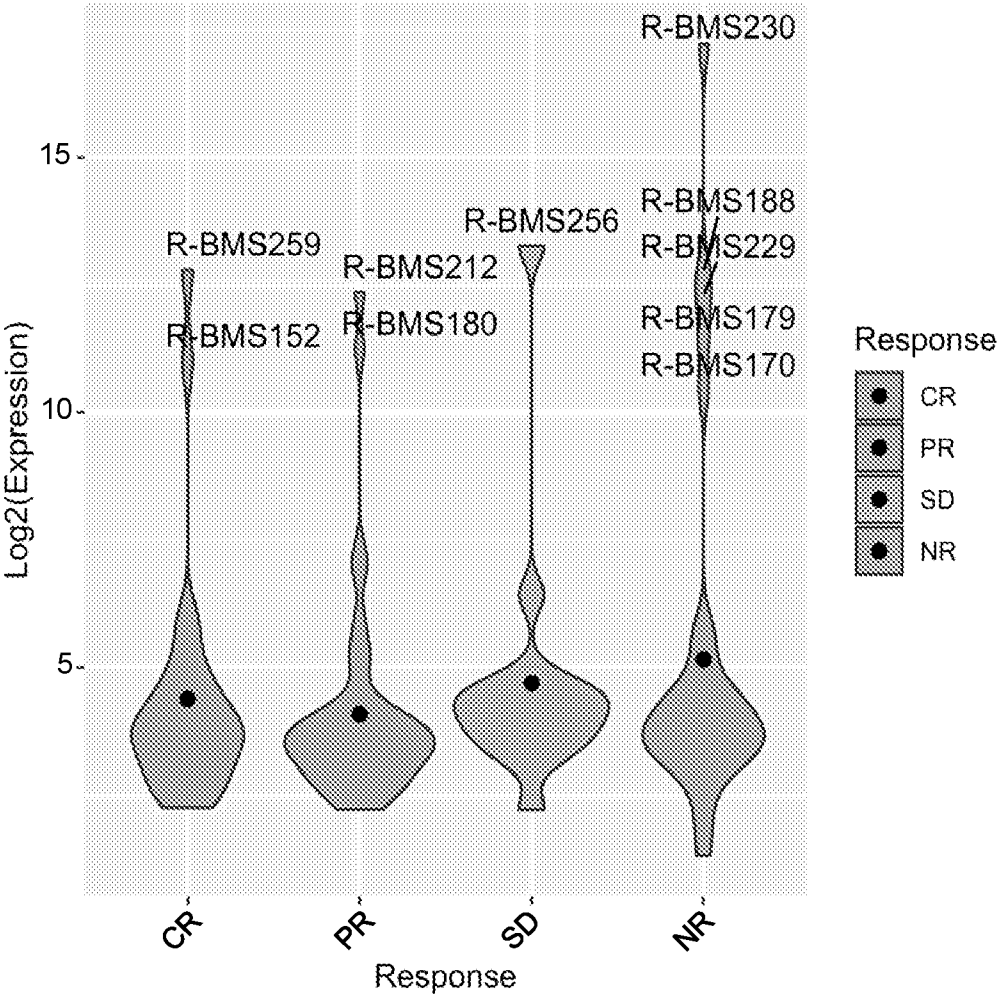


FIG. 2B

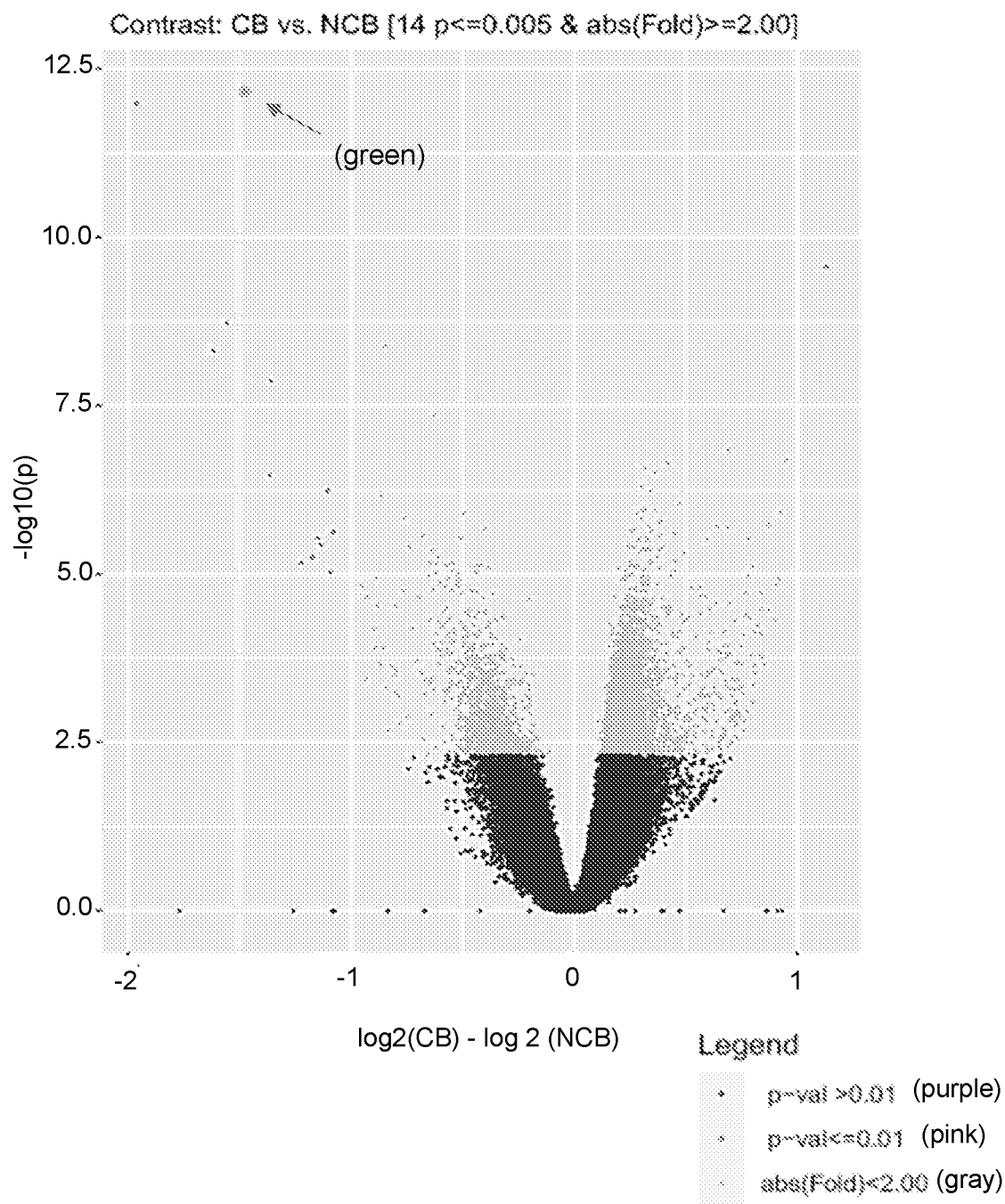


FIG. 3A

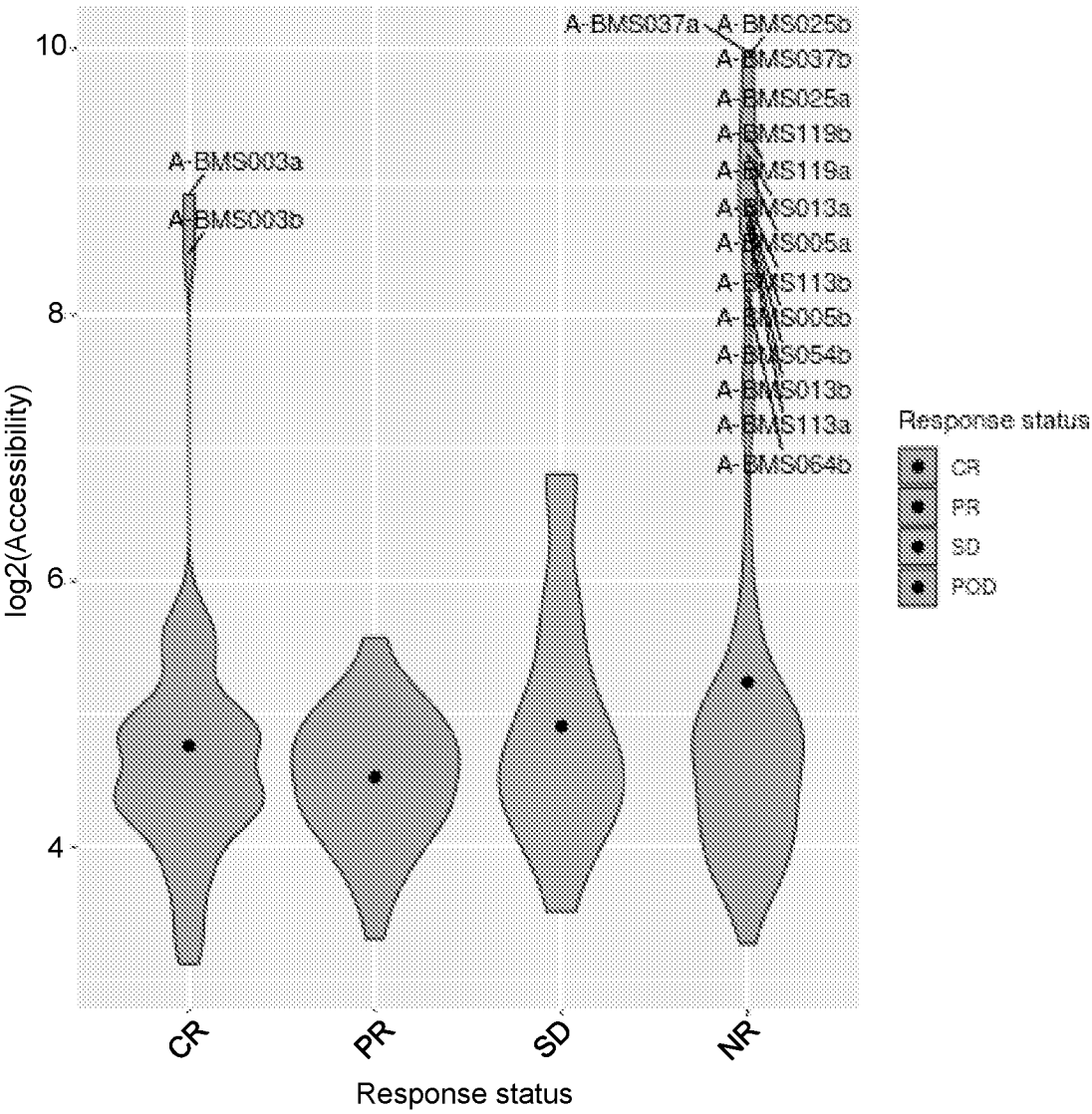


FIG. 3B

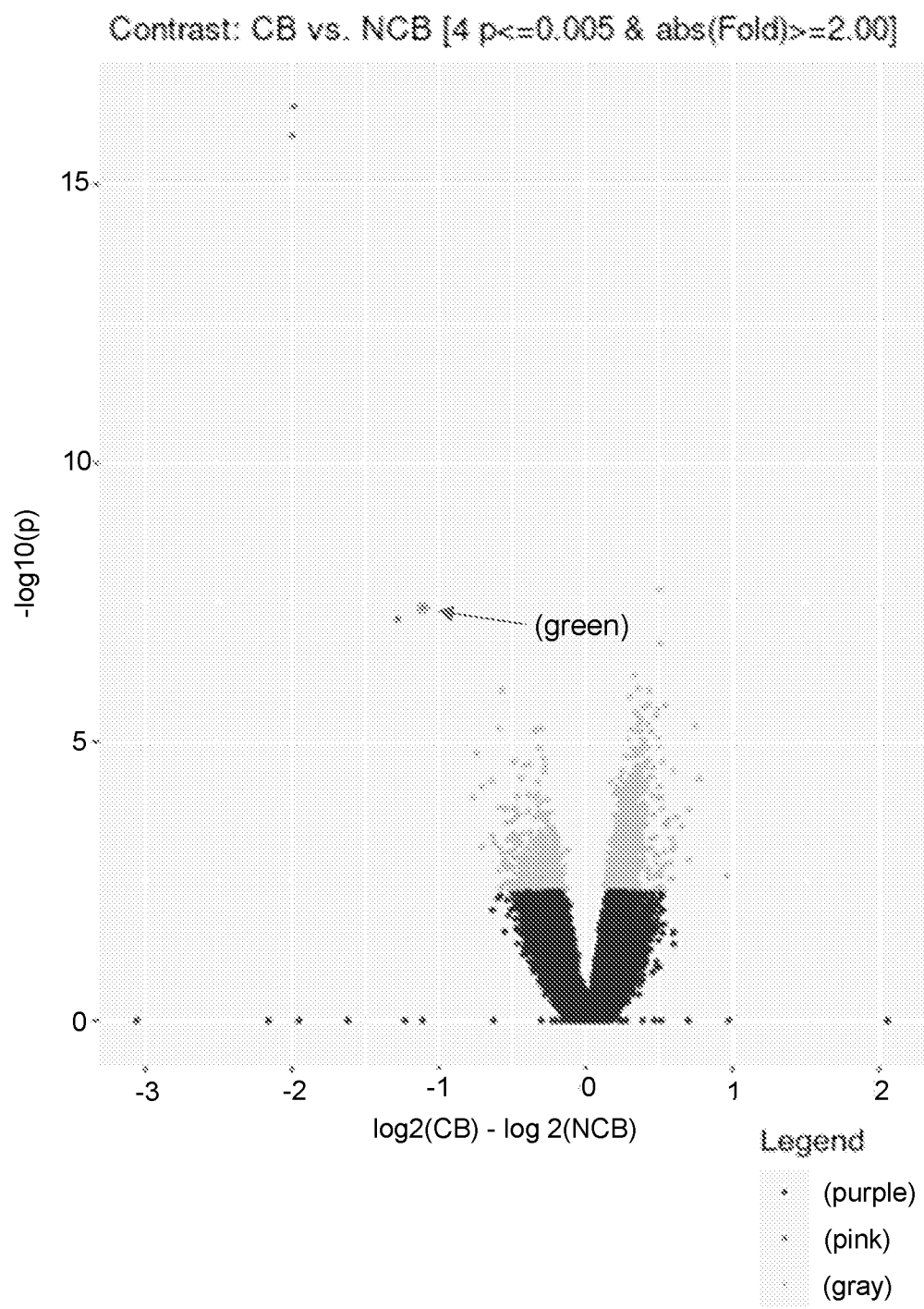


FIG. 4A

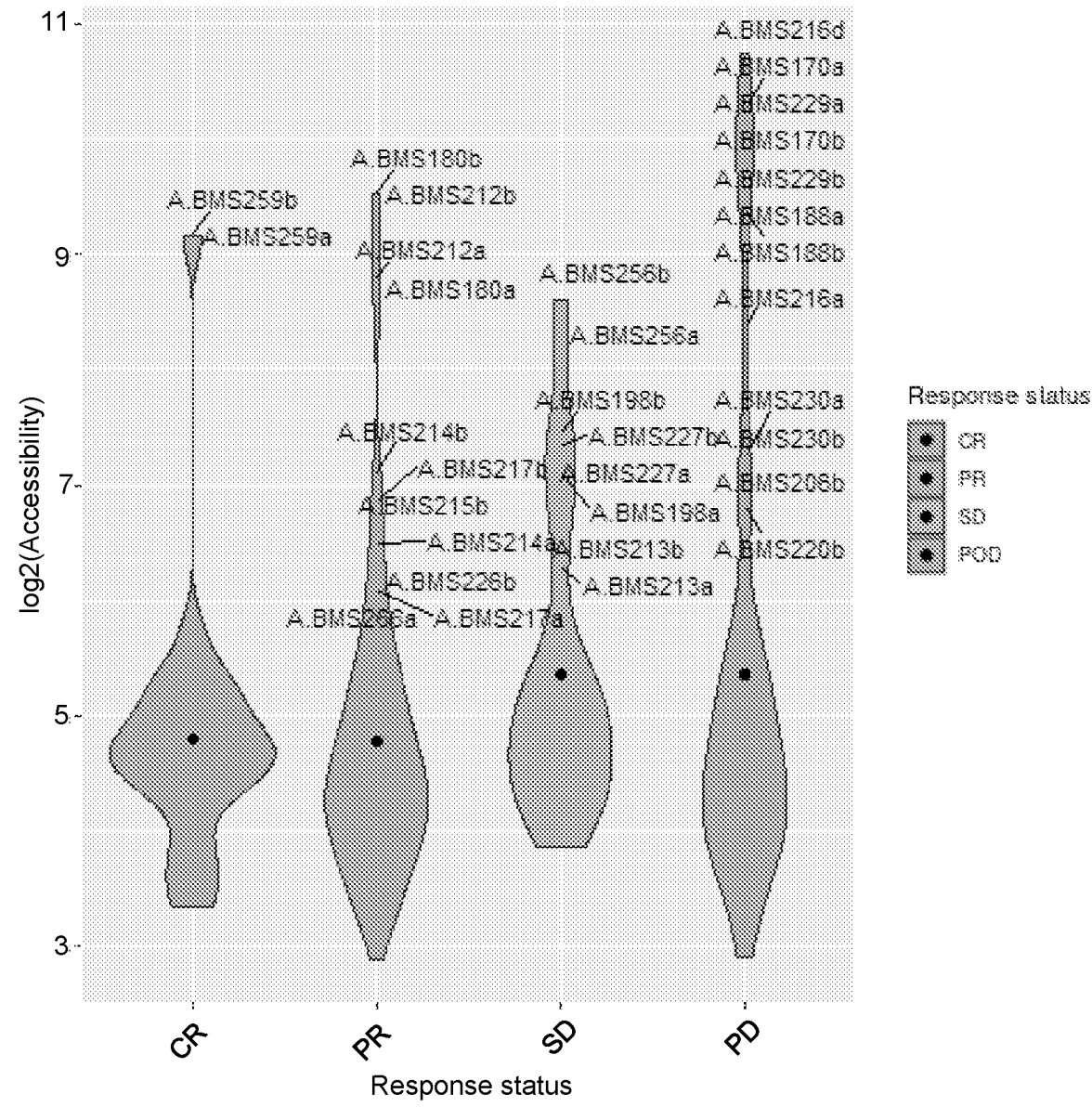


FIG. 4B

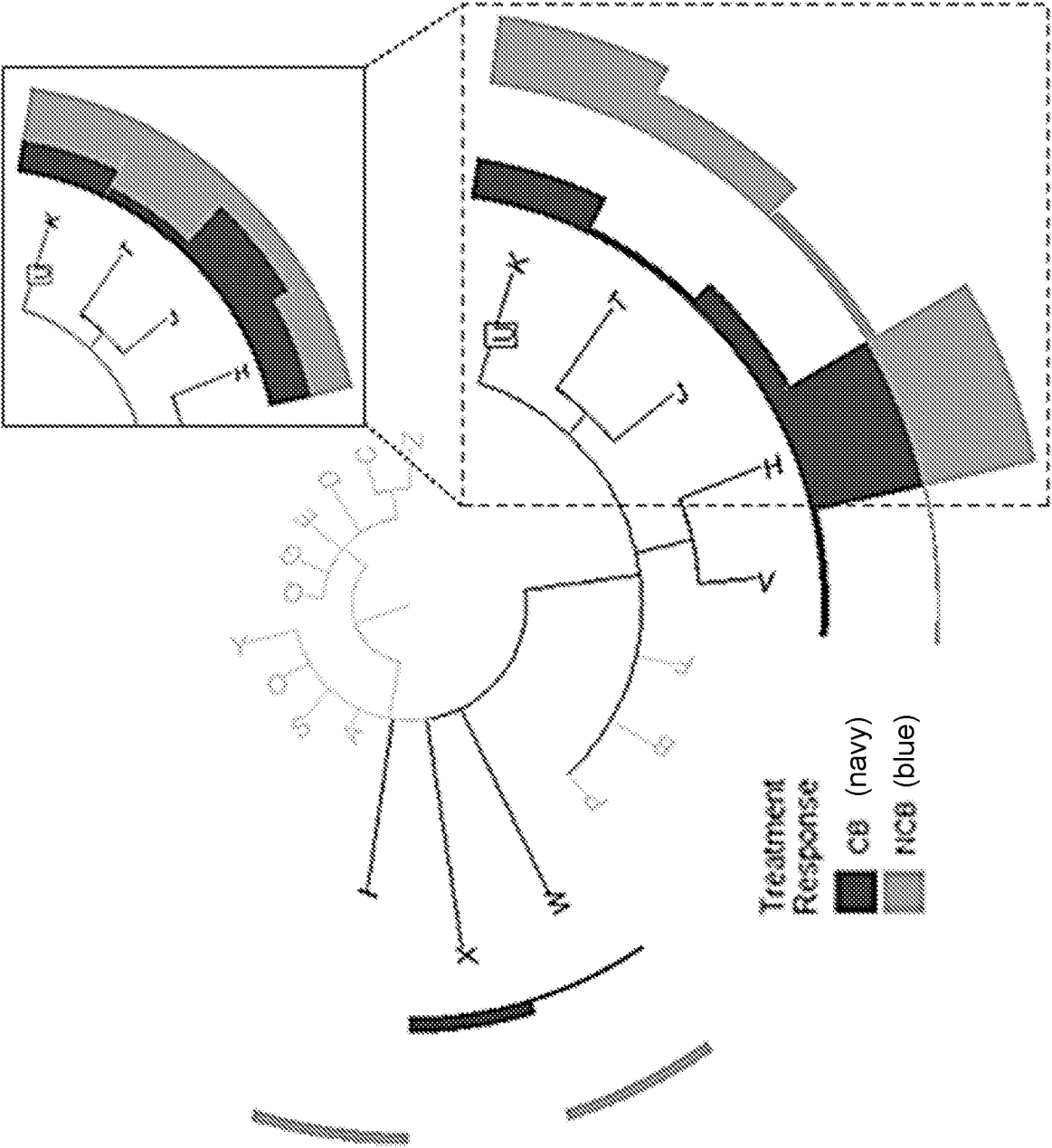


FIG. 5

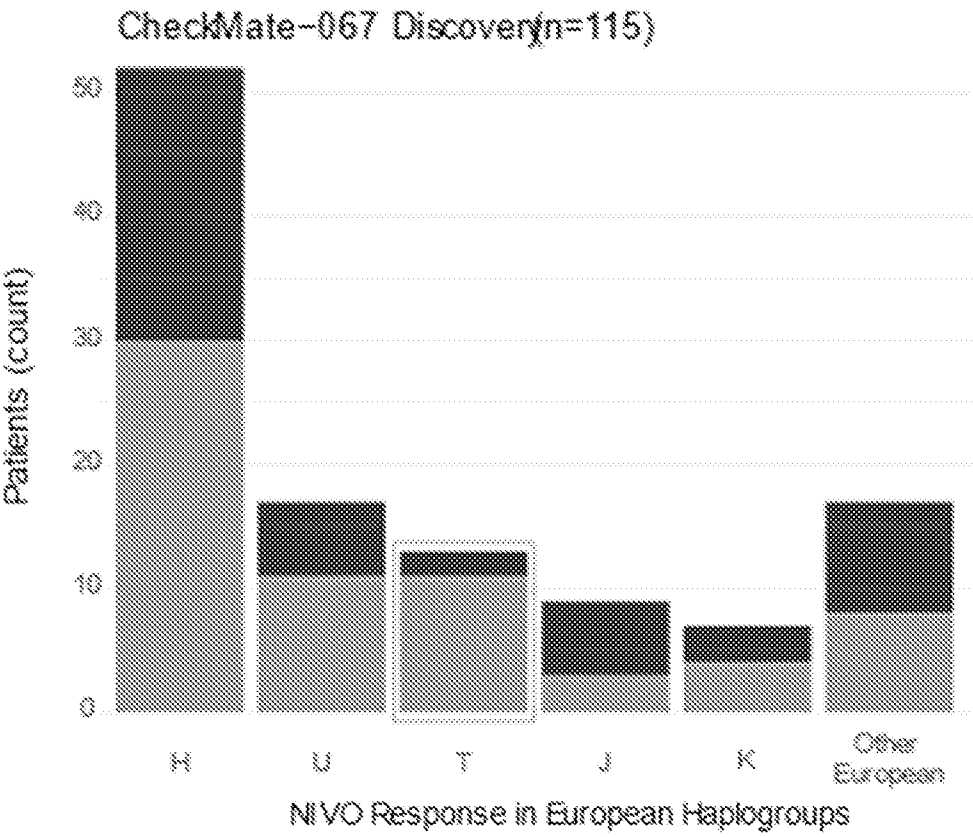


FIG. 6A

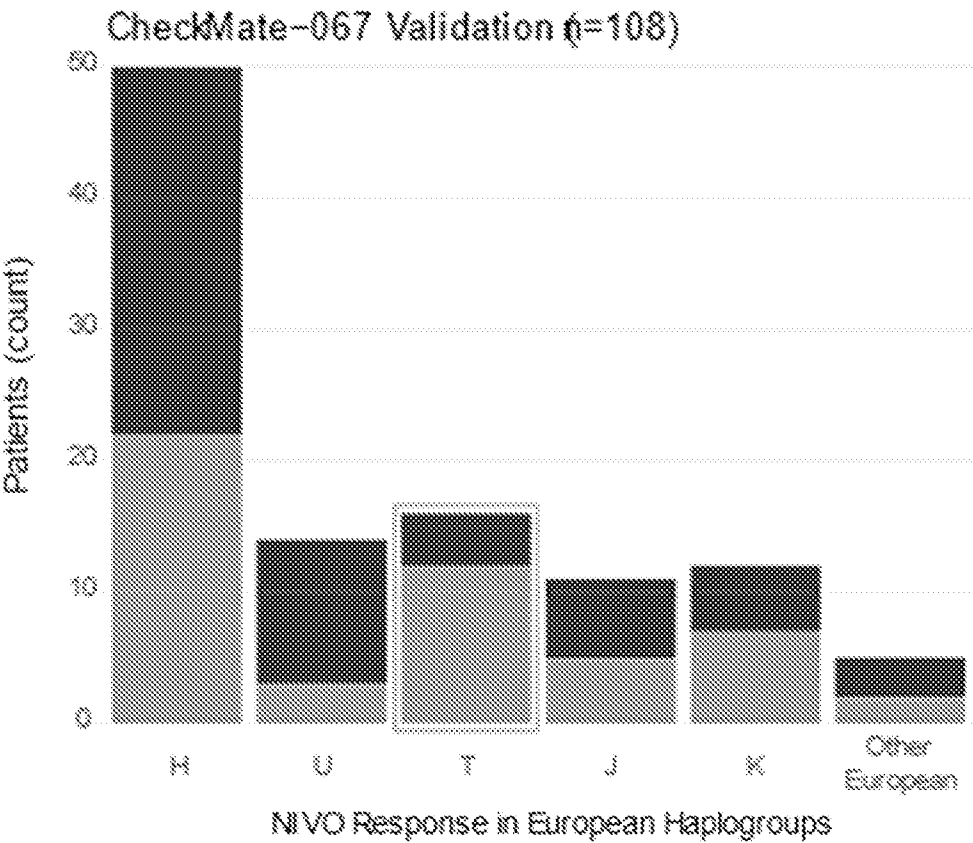


FIG. 6B

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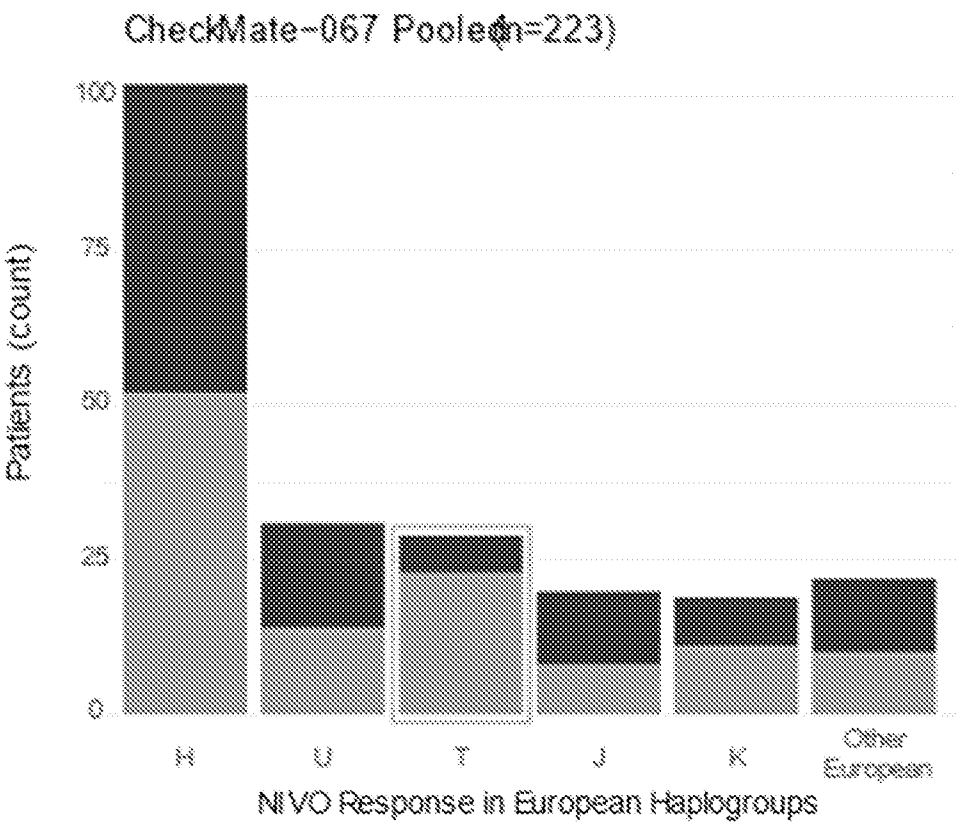


FIG. 6C

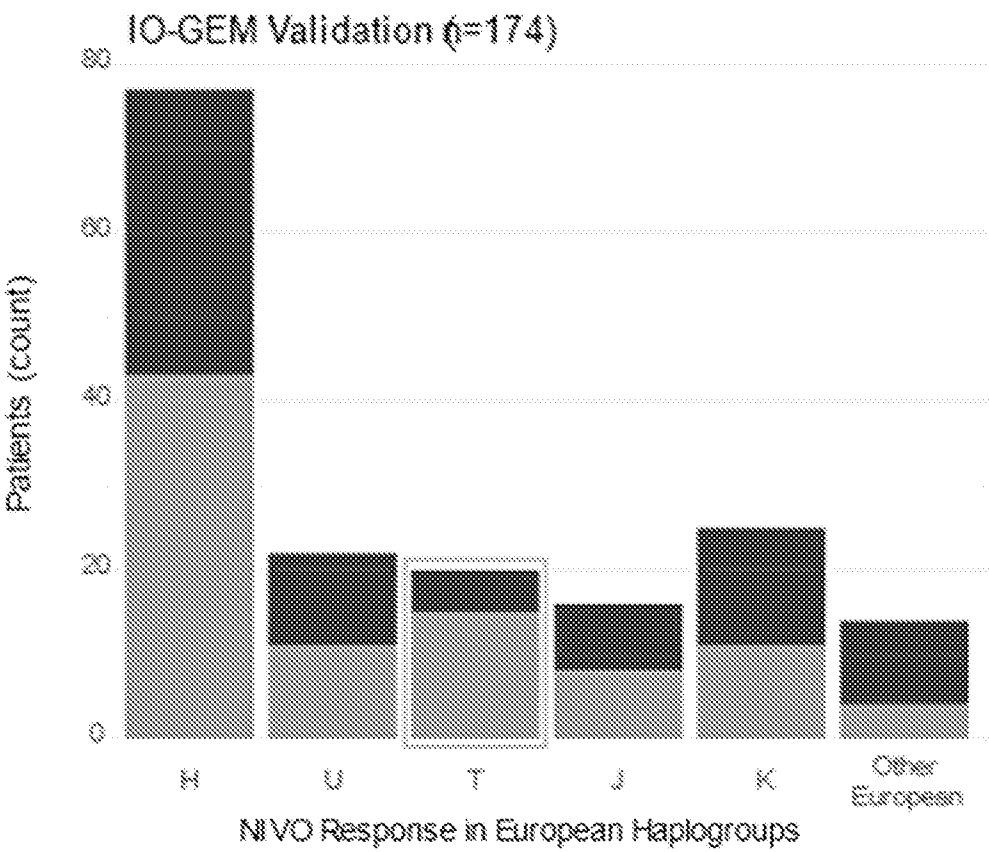


FIG. 6D

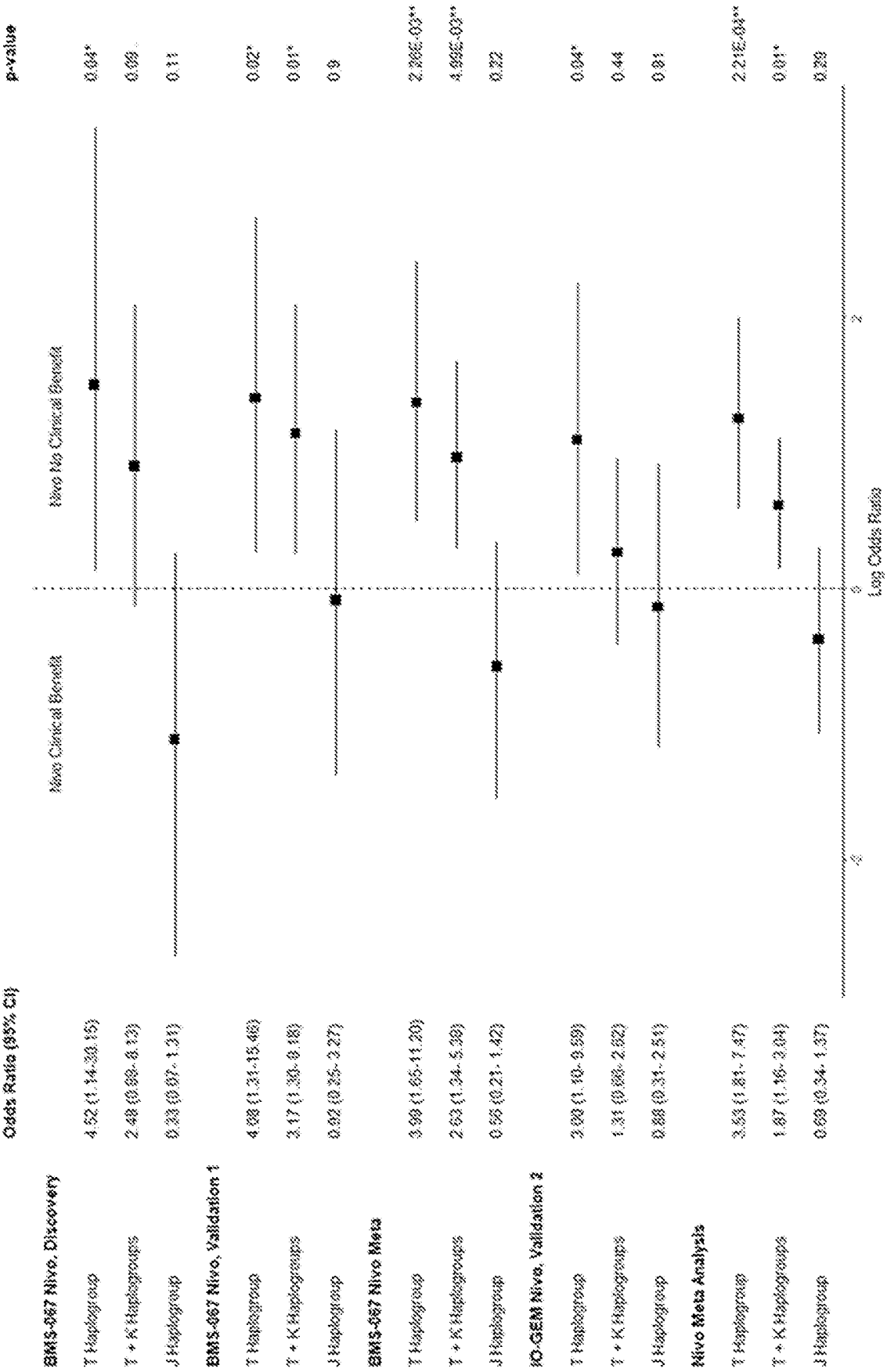


FIG. 7

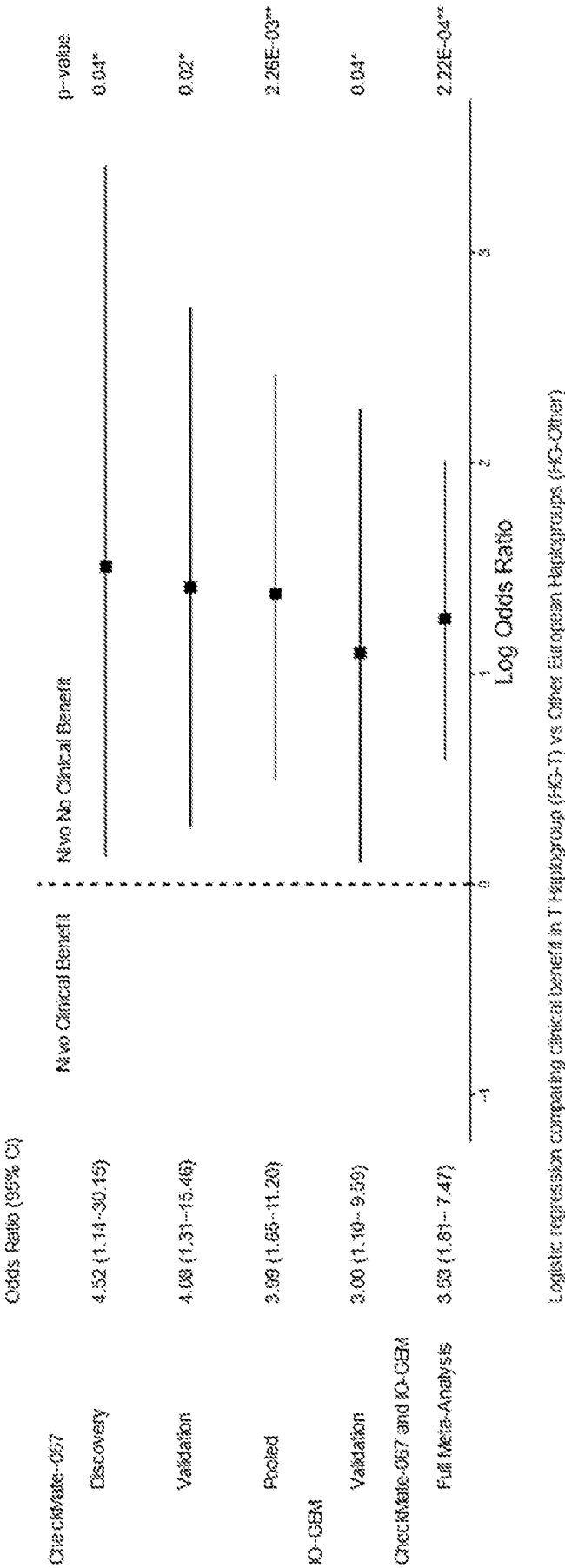


FIG. 8

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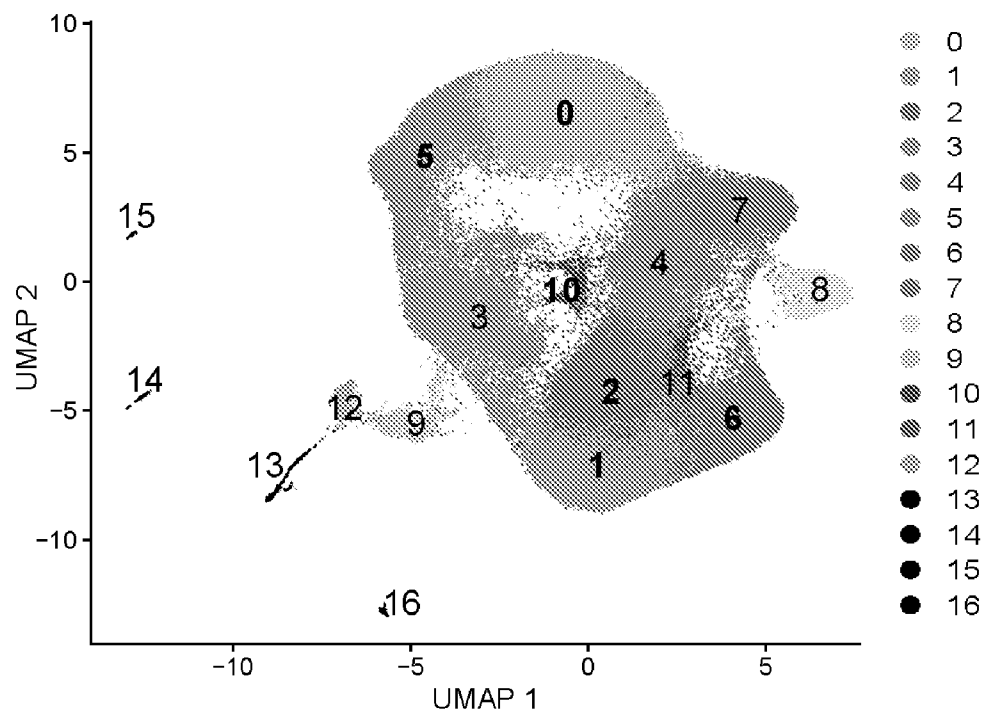


FIG. 9A

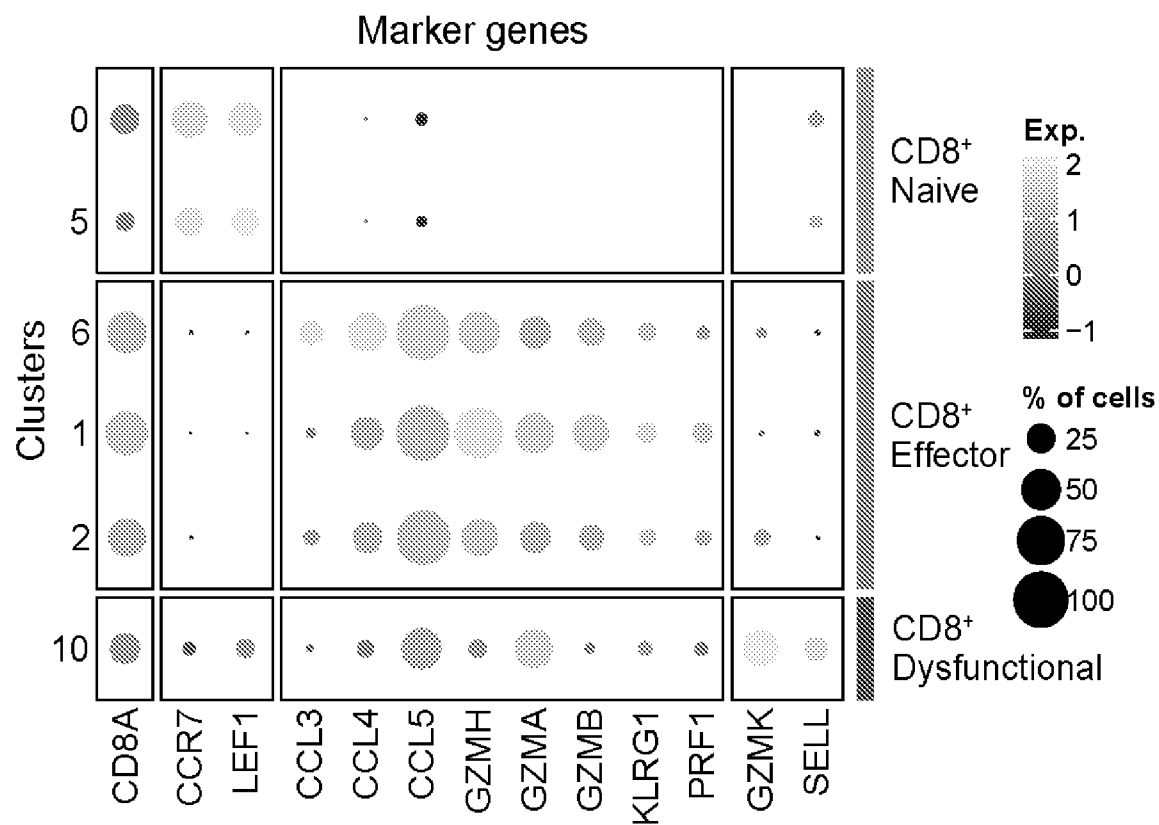


FIG. 9B

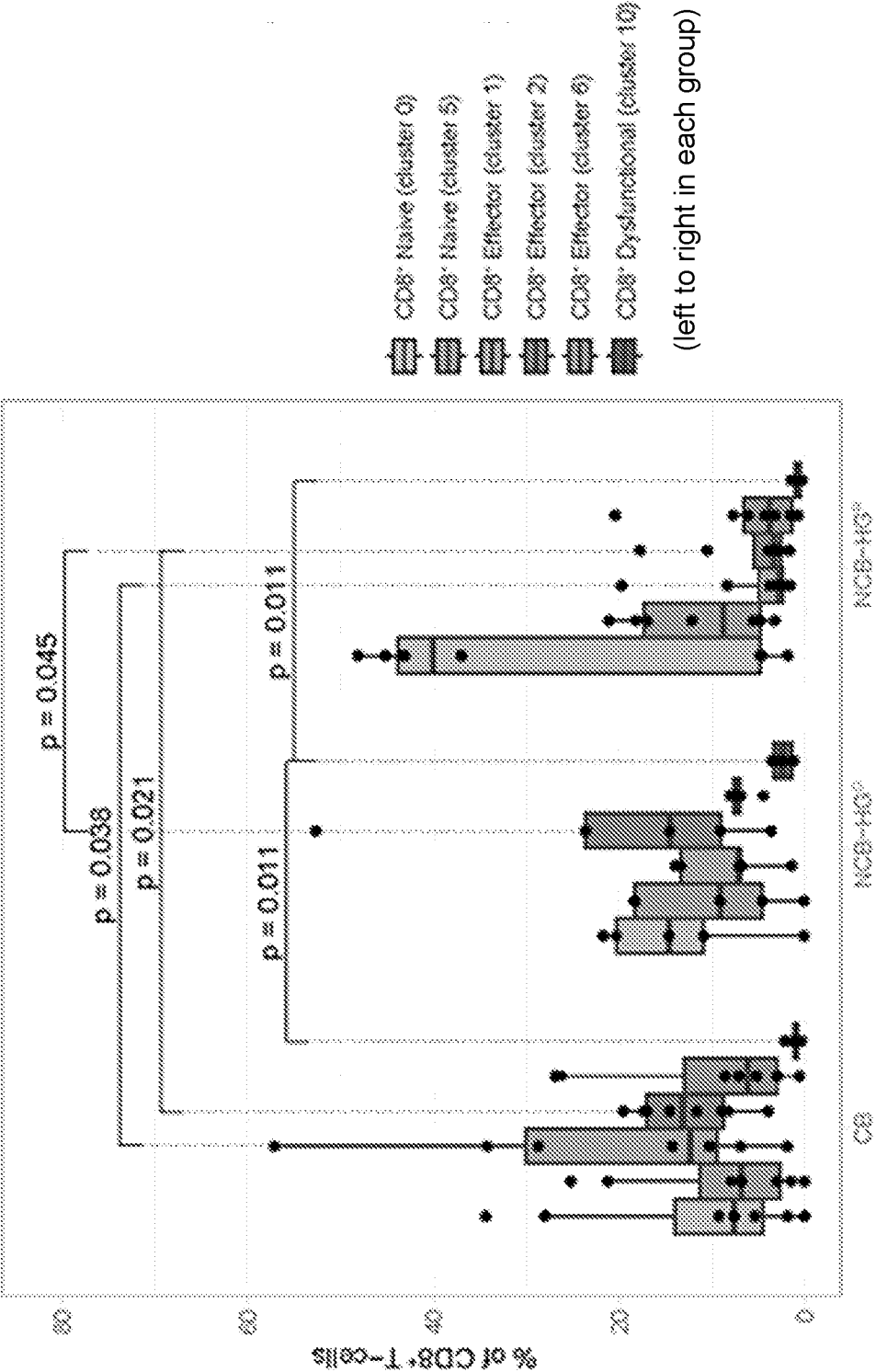


FIG. 9C

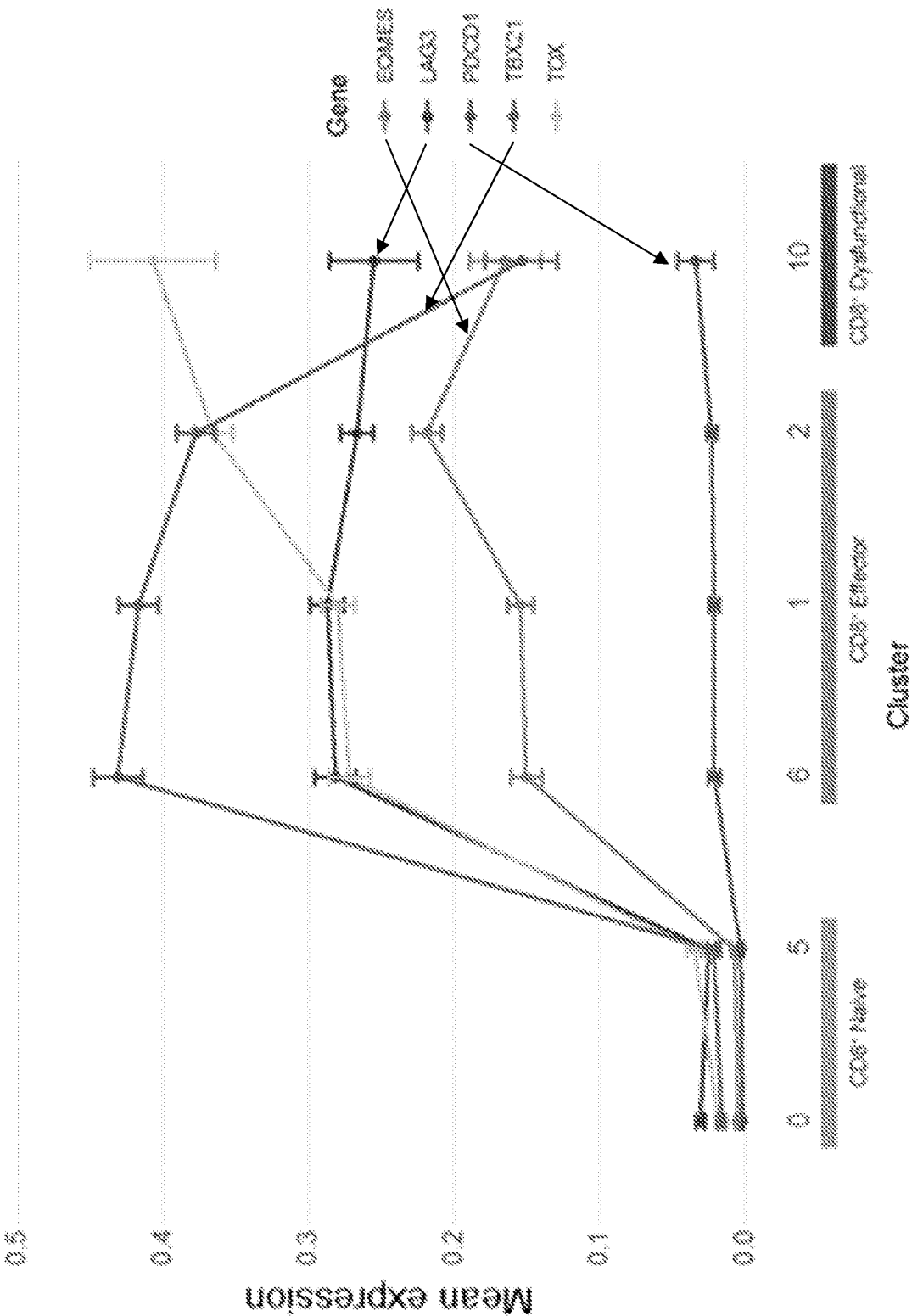


FIG. 9D

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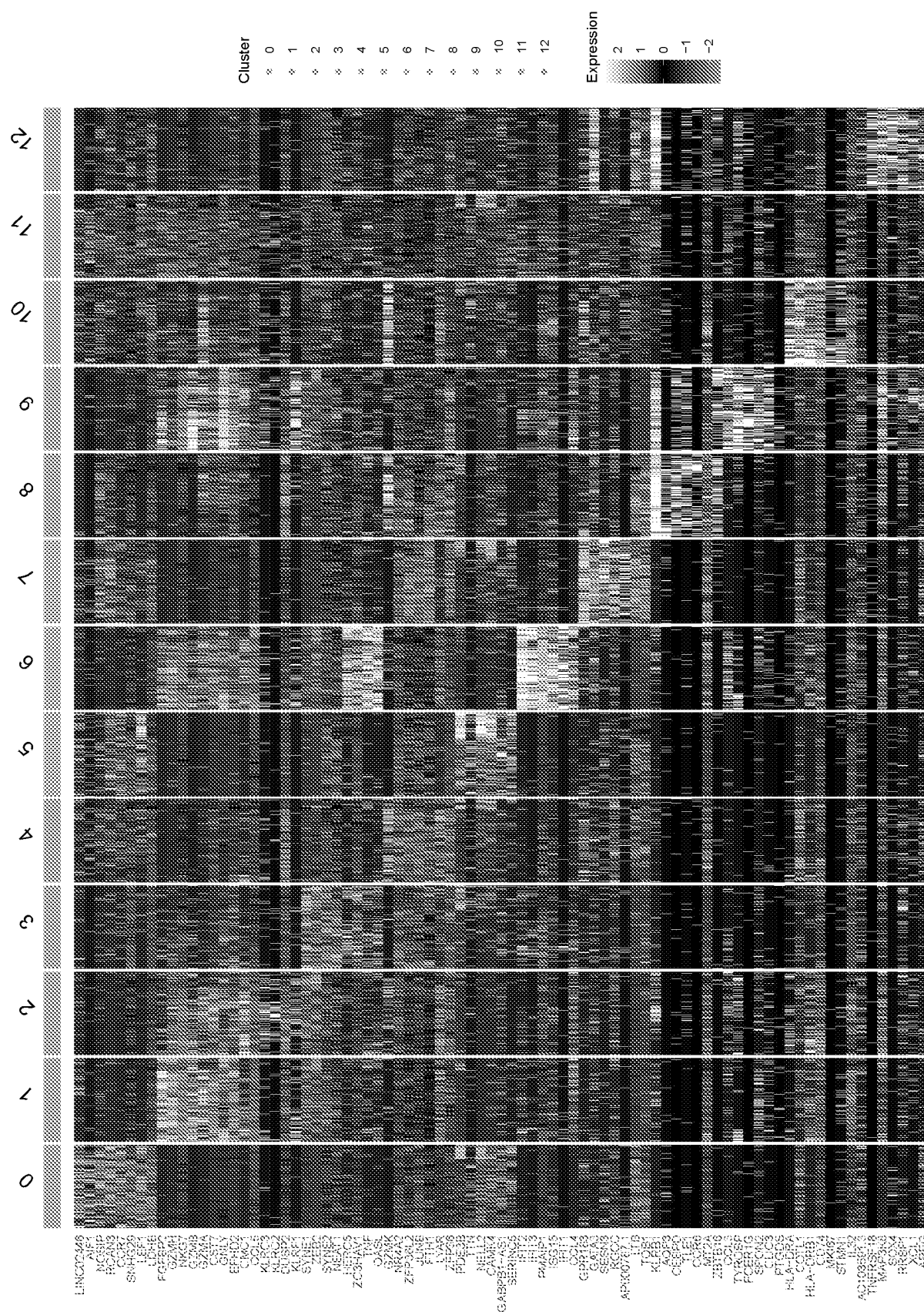


FIG. 10A

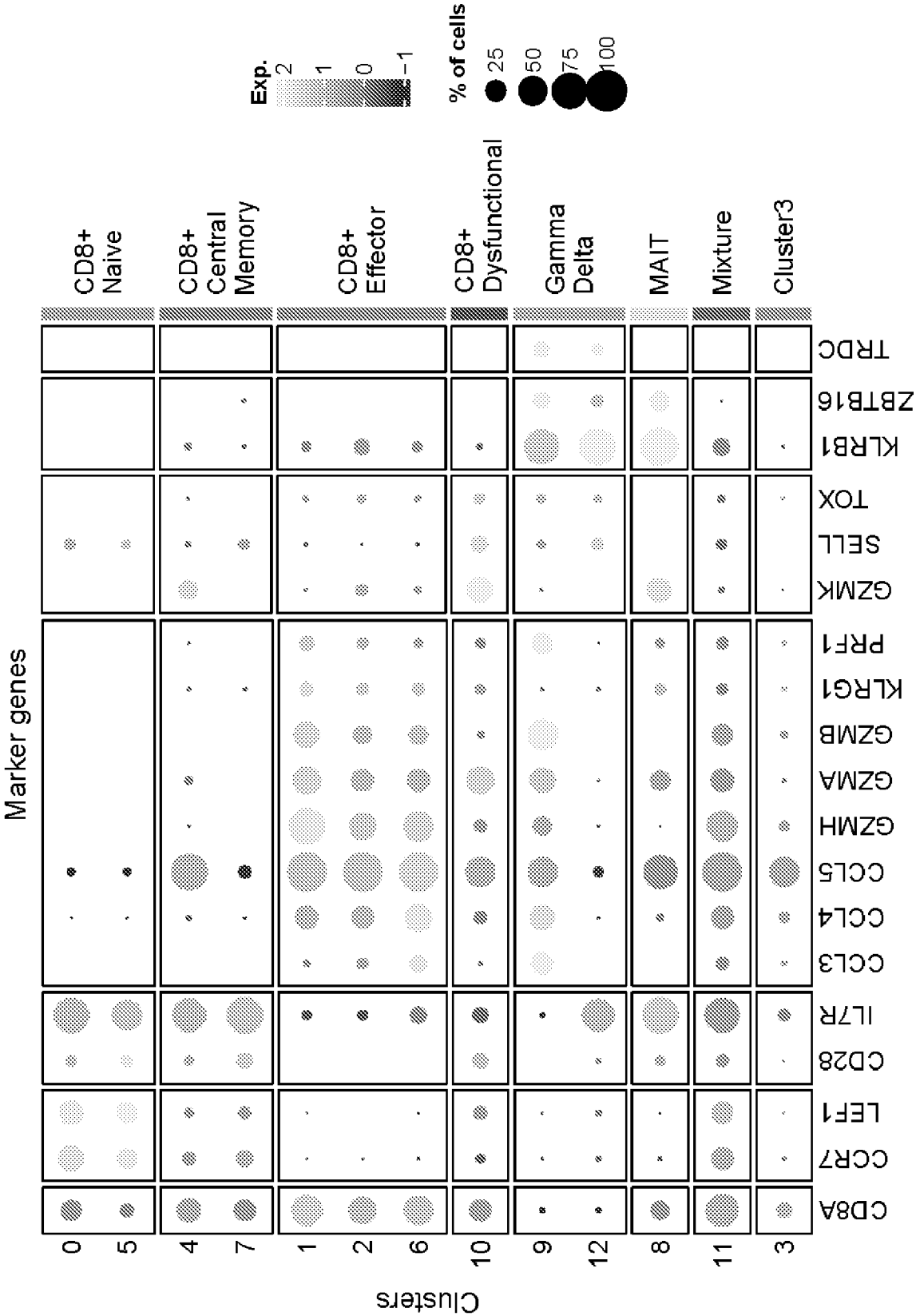


FIG. 10B

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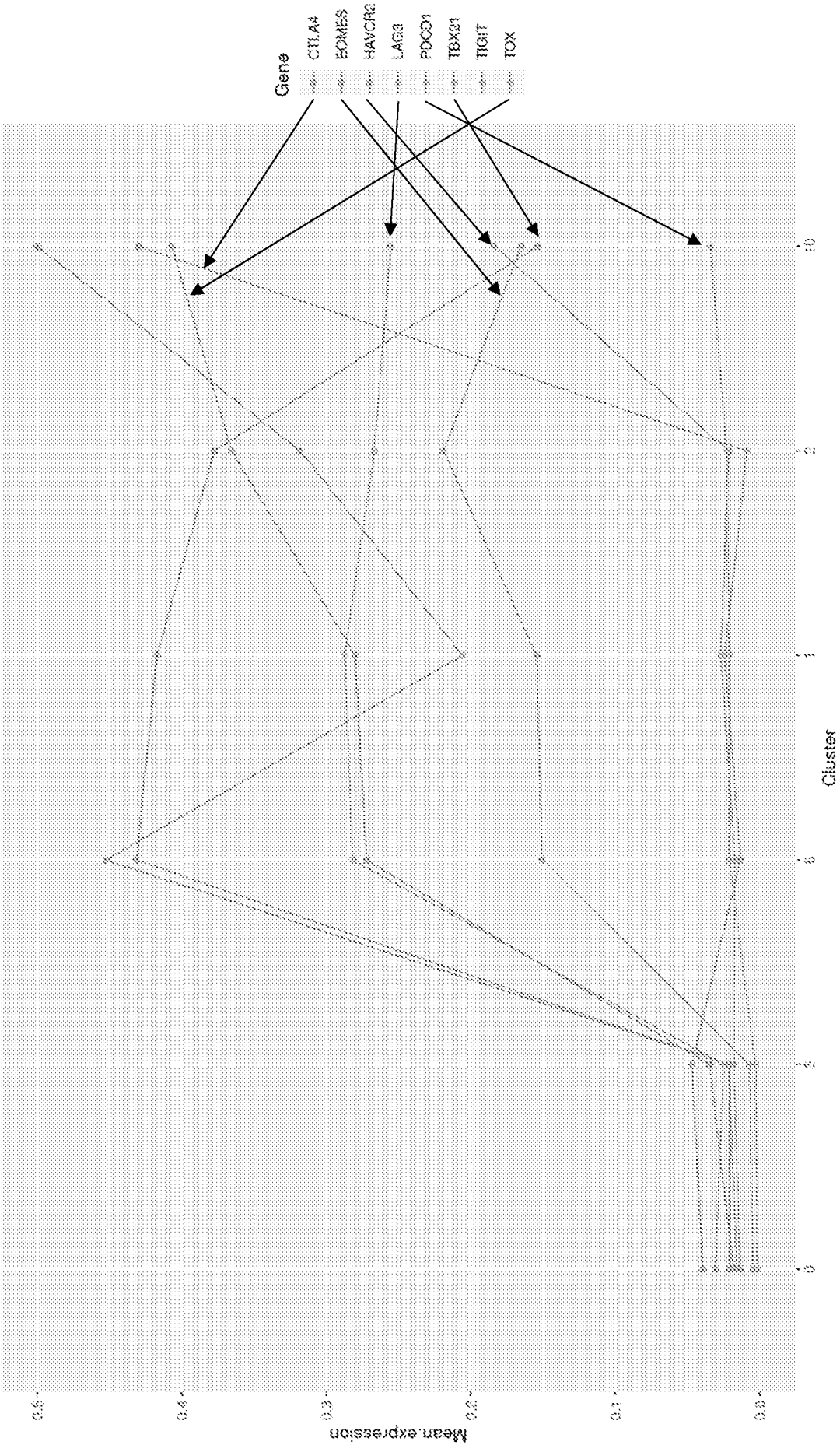


FIG. 10C

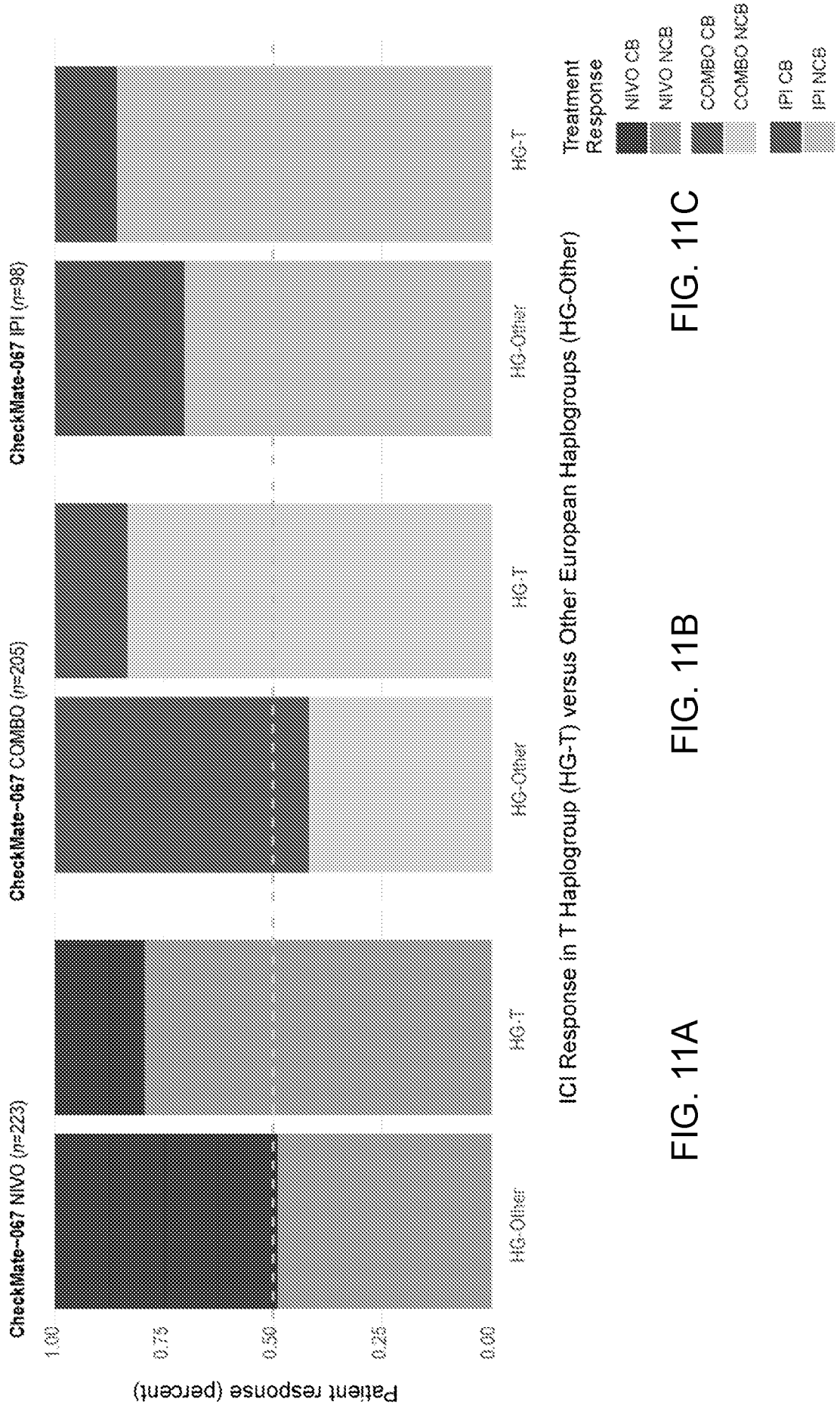
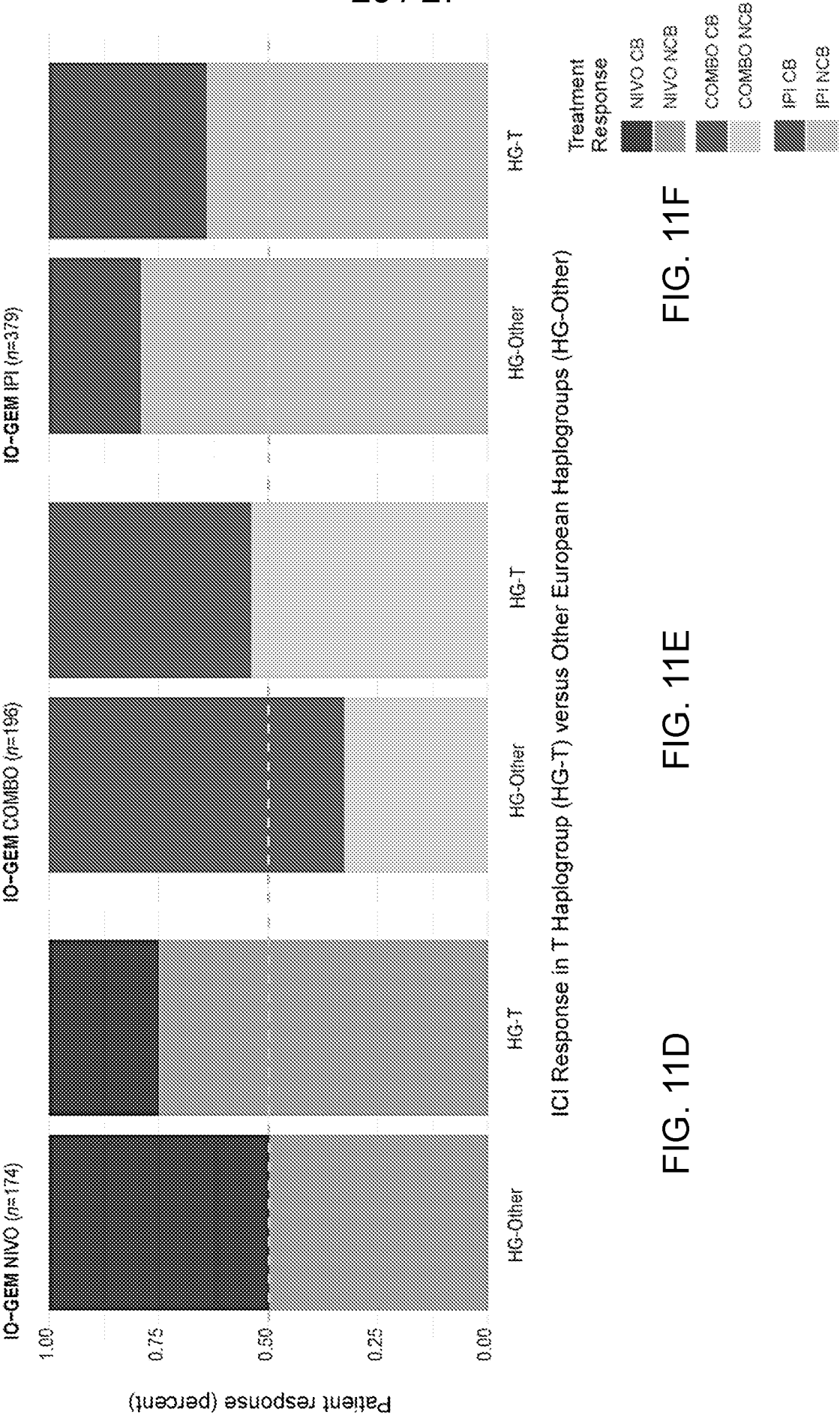


FIG. 11A

FIG. 11B

FIG. 11C



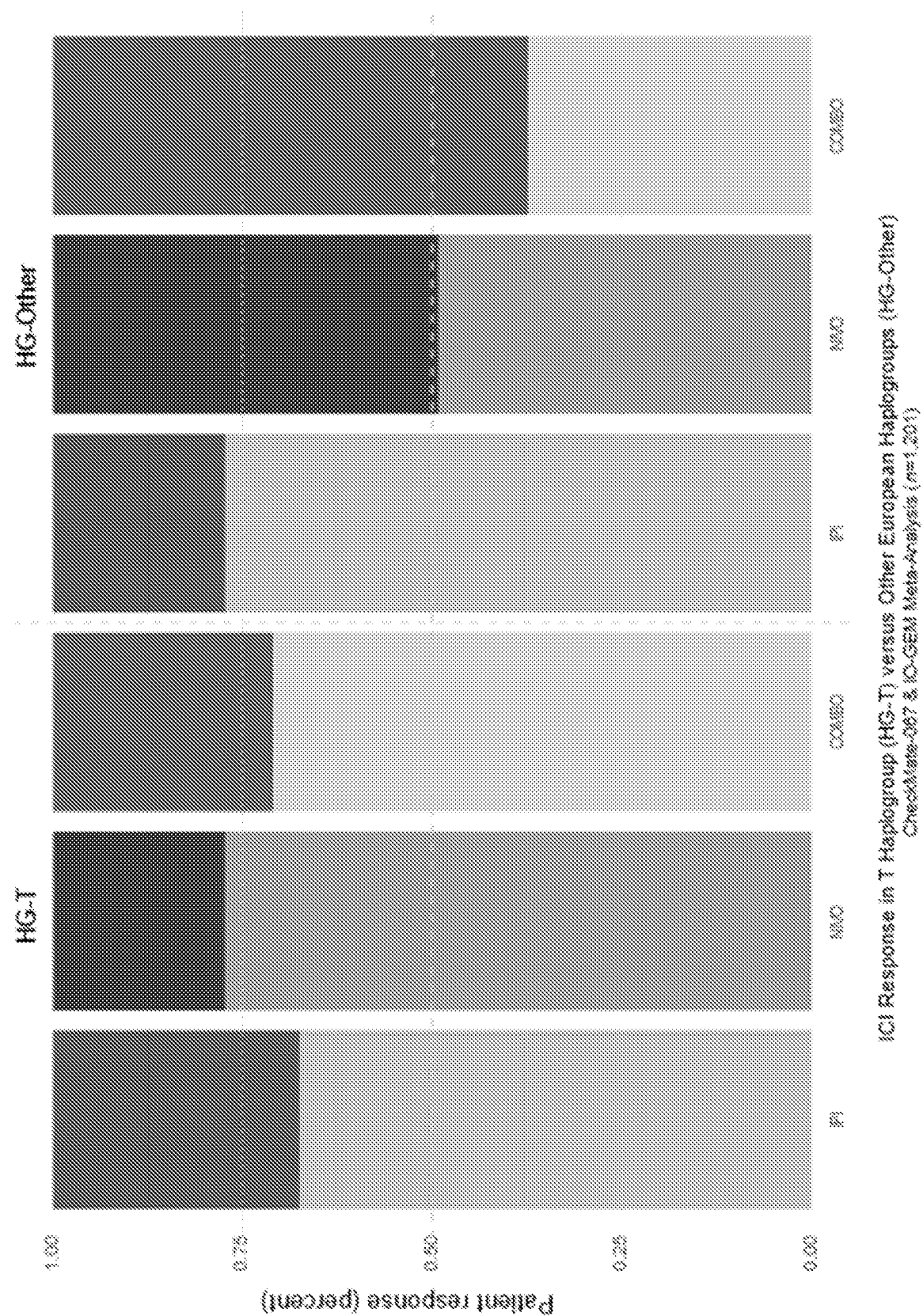


FIG. 12A

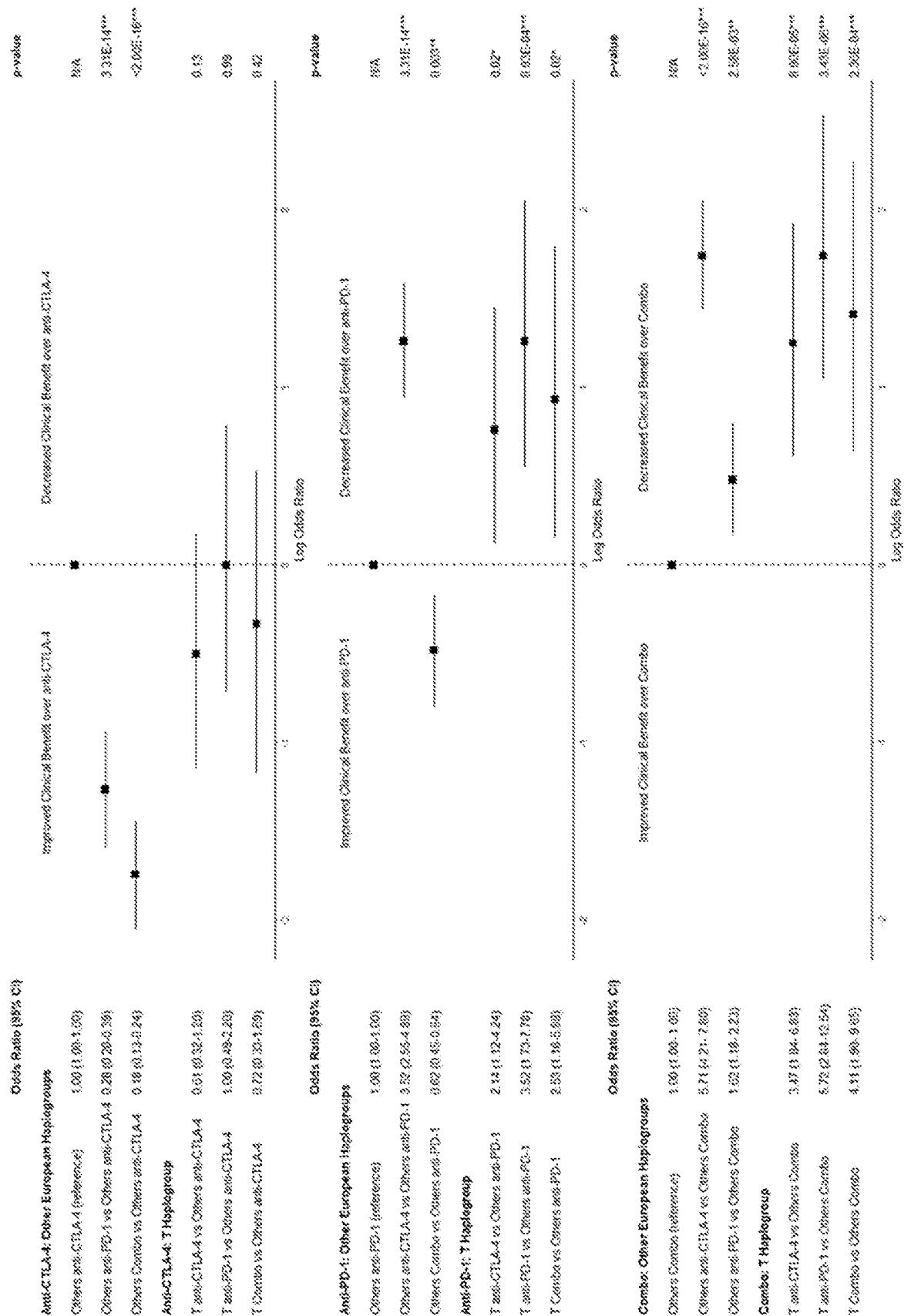


FIG. 12B

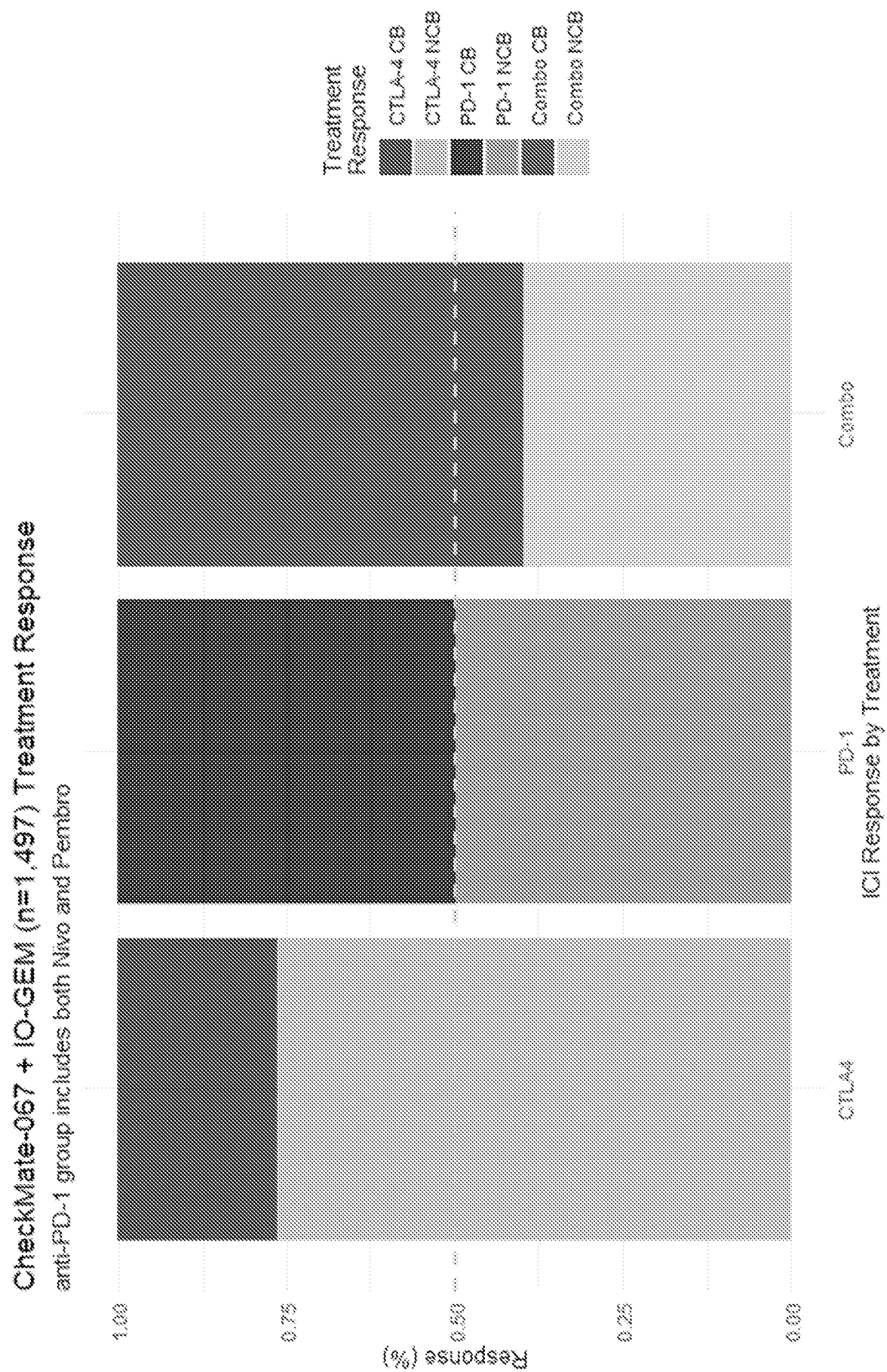


FIG. 13A

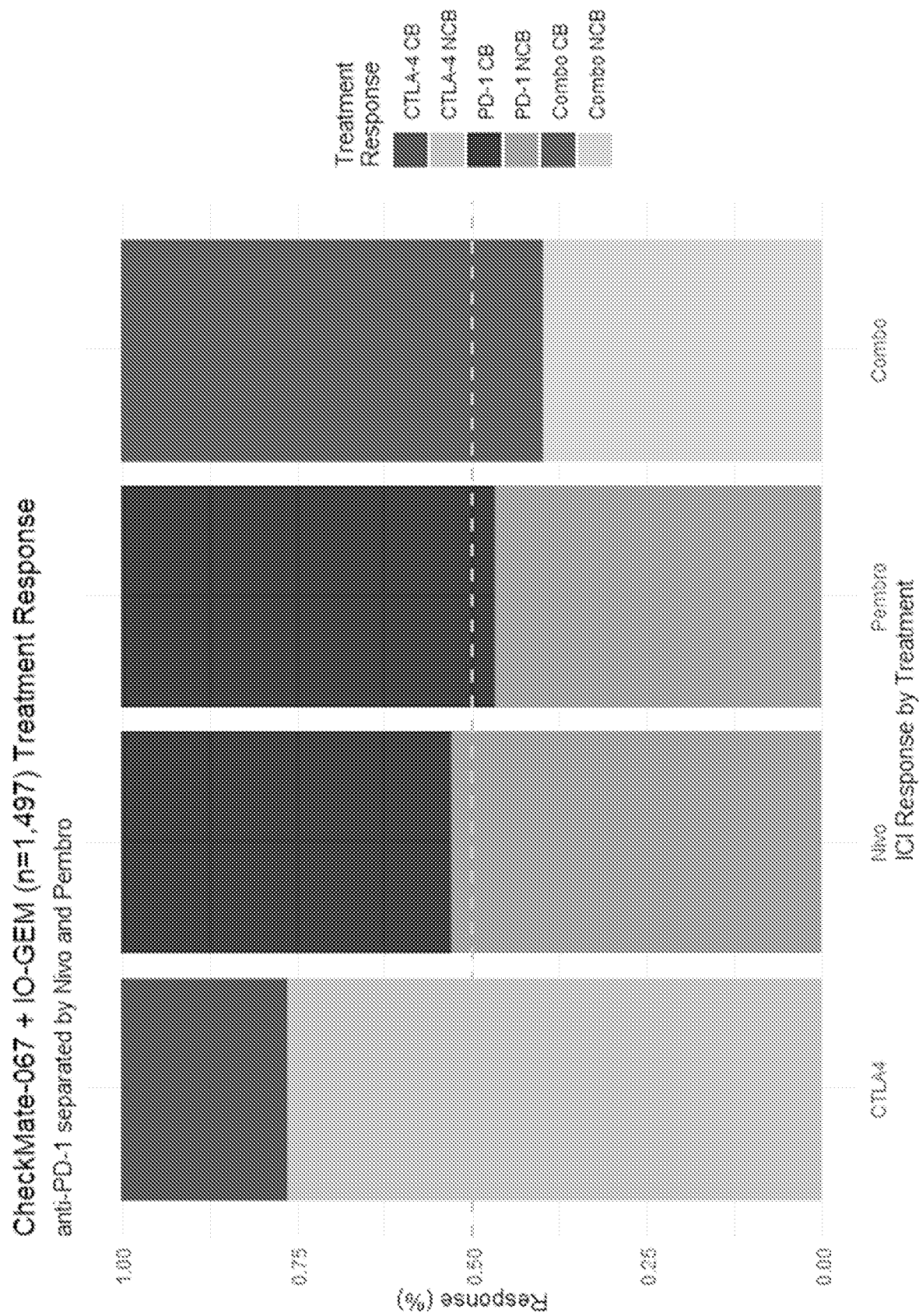


FIG. 13B

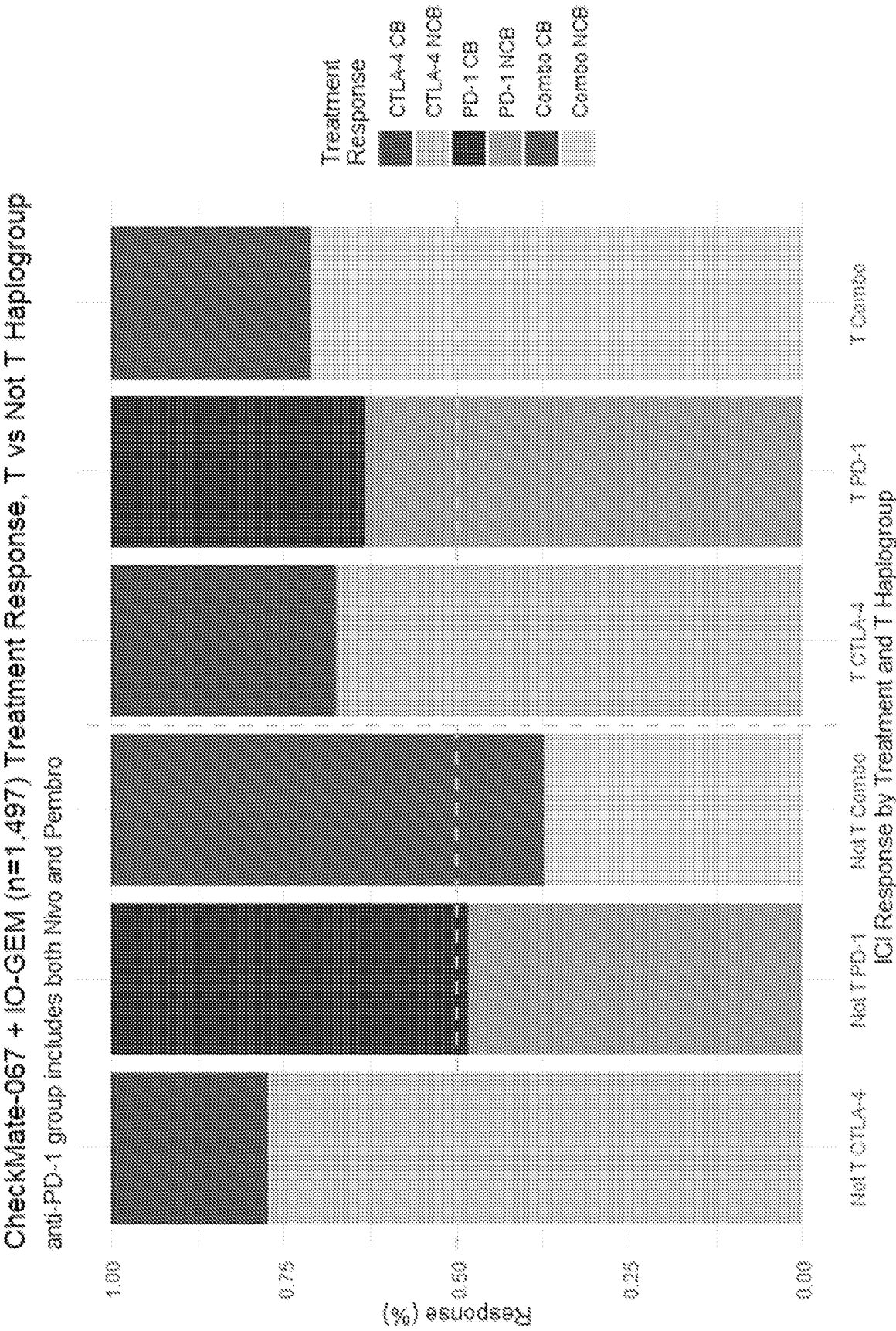


FIG. 13C

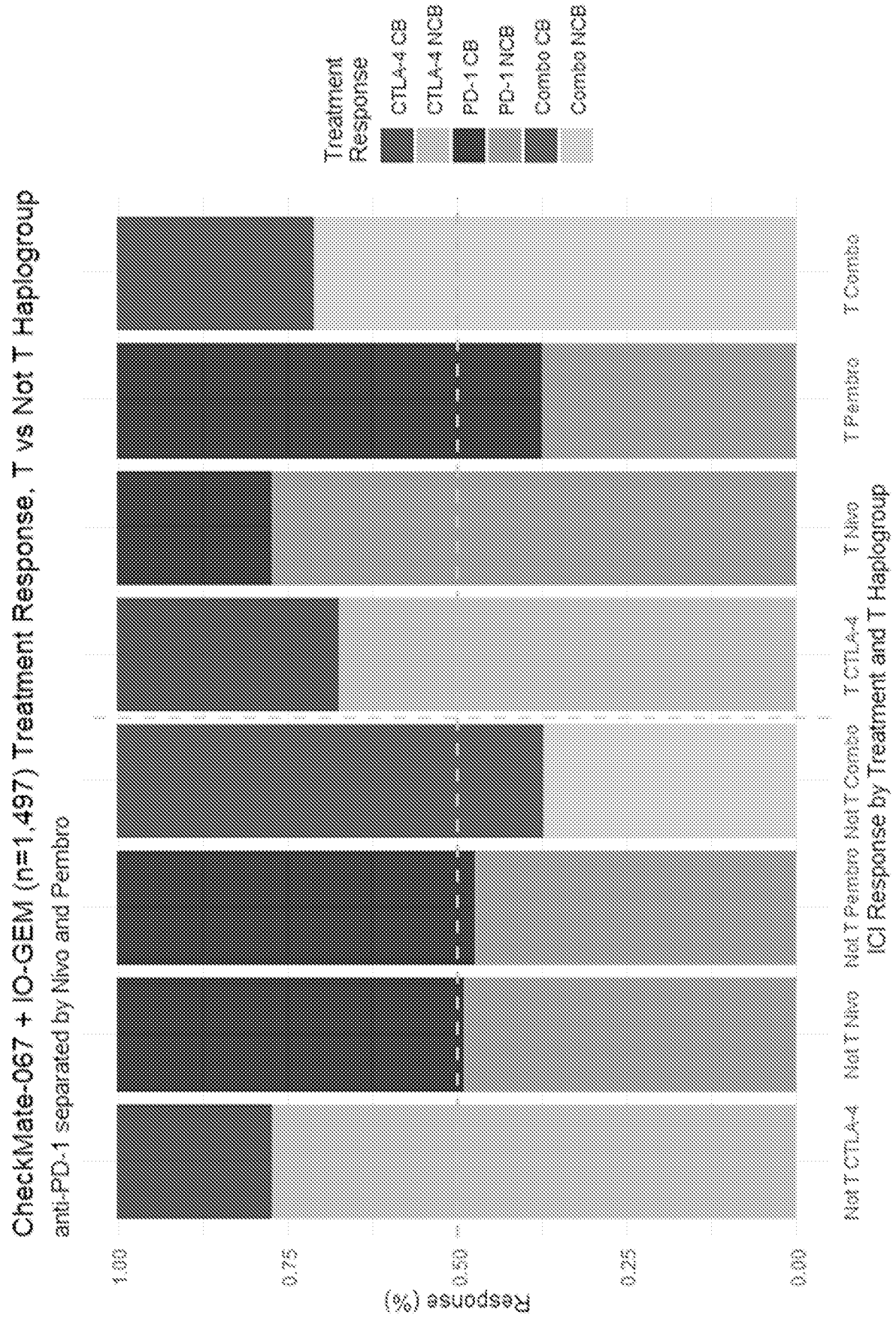


FIG. 13D

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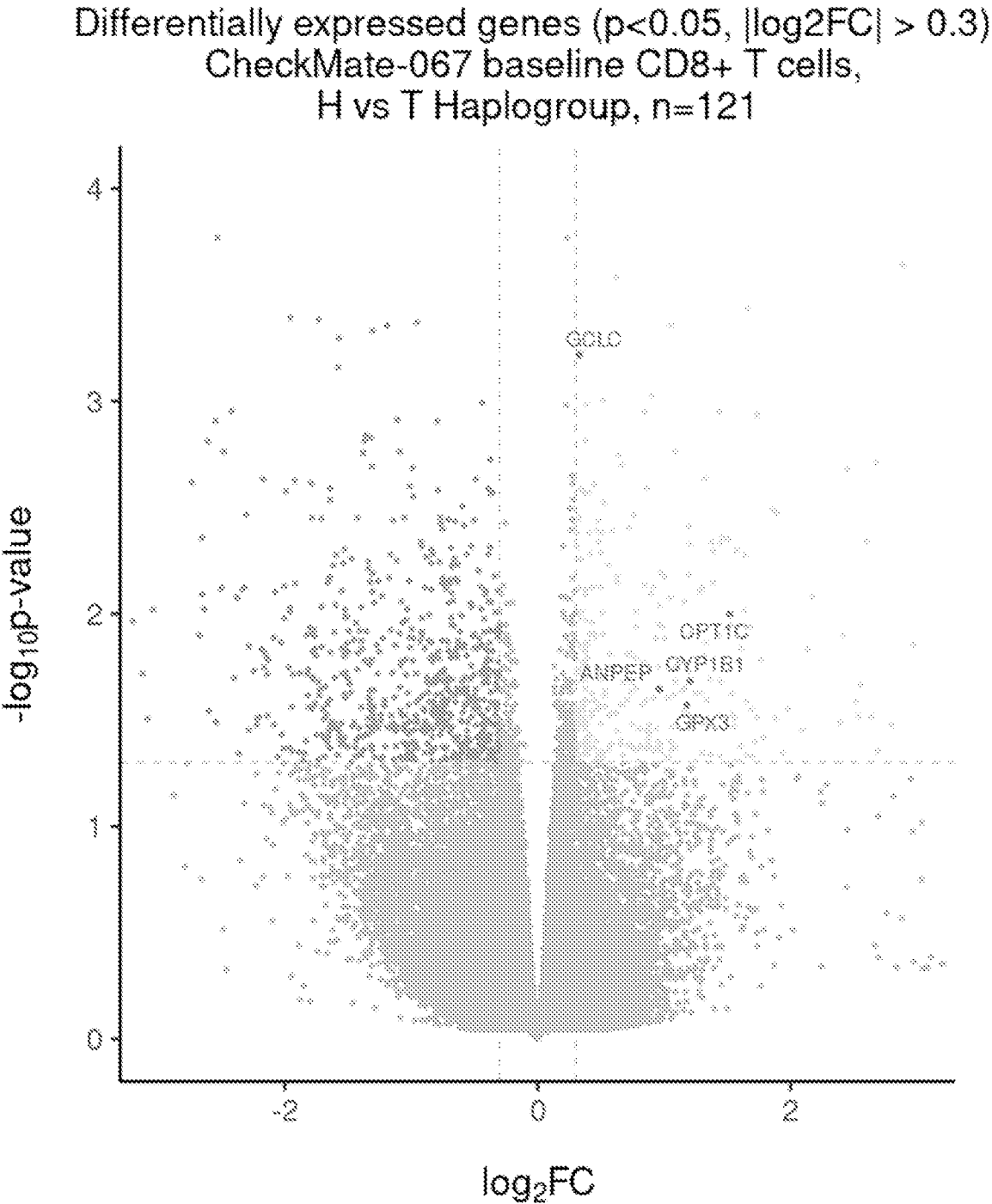


FIG. 14