Fig S1. Consort diagram.

Fig 1. Change in tumor burden, time to treatment failure, and overall survival.
A) Percentage change from baseline in the sum of the diameters of the target lesions in patients receiving ipilimumab (gold) or ipilimumab plus nivolumab (teal). Triangles denote the presence of a new lesion. Two patients in the ipilimumab plus nivolumab arm did not have post-baseline target lesion measurements and are not displayed on the plot (clinical progression; presence of a new lesion (counted as radiographic progressive disease) but no measurement of the target lesions).
B) Maximum percentage change from baseline in the sum of the diameters of the target lesions at Week 18. A star denotes that the response (complete or partial) was confirmed by a second scan by Week 18. Two patients, one in each arm, had a maximum change in the sum of target lesion diameters of less than 20% but had a best overall response of progressive disease due to the presence of a new lesion.
C) Kaplan-Meier curves for time to treatment failure.
D) Kaplan-Meier curves for overall survival.

Fig 2. Trends in peripheral immune characteristics and clinical benefit over the course of therapy.
A) Trends in CD4+ and CD8+ T cells by clinical benefit (pink= no clinical benefit [n=6], green= clinical benefit, [n=10]). Thick lines represent mean fold change +/- 95% confidence interval (CI) of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients. C1= baseline, C2=pre-cycle 2 of therapy, C3= pre-cycle 3, of therapy, C4= pre-cycle 4 of therapy, FU1= post 4 cycles of therapy.
B) Trends in CD4+ T cells by clinical benefit. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
C) Comparison of fold change from baseline in CD4+ and CD8+ T cell populations by clinical benefit prior to cycle 2 of therapy. *=$p<0.05$, **= $p<0.01$ by 2-sided Wilcoxon rank-sum test.
D) Trends in CD4+ T cell cytokine Polyfunctional Strength Index (PSI) and Effector PSI by clinical benefit (pink= no clinical benefit, [n= 7], green= clinical benefit, [n=11]). Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
E) Trends in Interferon-gamma secretion frequency and signal intensity from CD4+ T cells by clinical. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
F) Differences in Regulatory PSI and Interluikin-10 secretion frequency and from CD4+ T cells at baseline by clinical benefit (pink= no clinical benefit, [n=6], green= clinical benefit, [n=11]).

Fig S2. CyTOF profiling comparing trends in CD4+ and CD8+ T cell populations between and within treatment arms.
A) Trends in CD4+ and CD8+ T cells by treatment arm (gold= ipilimumab arm, [n=8], teal= ipilimumab + nivolumab arm, [n=8]). Thick lines represent mean fold change +/- 95% confidence interval (CI) of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients. C1= baseline, C2=pre-cycle 2 of therapy, C3= pre-cycle 3, of therapy, C4= pre-cycle 4 of therapy, FU1= post 4 cycles of therapy.
B) Trends in CD4+ T cell populations by clinical benefit (pink= no clinical benefit, green= clinical benefit) in the ipilimumab arm. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.

C) Trends in CD4+ T cell populations by clinical benefit (pink= no clinical benefit, green= clinical benefit) in the ipilimumab plus nivolumab arm. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
D) Trends in CD4+ T regulatory populations by clinical benefit (pink= no clinical benefit, green= clinical benefit). Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
E) Trends in CD8+ T cell populations by clinical benefit (pink= no clinical benefit, green= clinical benefit) in the ipilimumab + nivolumab arm. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.
F) Trends in CD8+ T cell populations by clinical benefit (pink= no clinical benefit, green= clinical benefit) in the ipilimumab monotherapy arm. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.

Fig S3. Comparison of iCOS on CD4+ and CD8+ T cells by clinical benefit within treatment arms
Trends in iCOS by CyTOF on CD4+ and CD8+ T cells within the A) Ipilimumab monotherapy and B) Ipi + Nivo treatment arms by clinical benefit (pink=no clinical benefit, green- clinical benefit). Thick lines represent mean fold change +/- 95% confidence interval (CI) of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients. C1= baseline, C2=pre-cycle 2 of therapy, C3= pre-cycle 3, of therapy, C4= pre-cycle 4 of therapy, FU1= post 4 cycles of therapy.

Fig S4. Comparison of CD4+ T cell cytokine profiles within treatment arms by IsoPlexis IsoCode.

A) Trends in CD4+ T cell cytokine Polyfunctional Strength Index (PSI) and Effector PSI in the ipilimumab arm by clinical benefit (pink= no clinical benefit, n=2, green= clinical benefit, n=6). Thick lines represent mean fold change +/- 95% confidence interval (CI) of group cell population over time, light colored lines represent measurements of individual patients.
B) Trends in CD4+ T cell cytokine Polyfunctional Strength Index (PSI) and Effector PSI in the ipilimumab plus nivolumab arm by clinical benefit (pink= no clinical benefit, [n=4], green= clinical benefit, [n=5]). Thick lines represent mean fold change +/- 95% CI of group cell population over time, light colored lines represent measurements of individual patients.
C) Trends in CD4+ T cell Interferon gamma secretion frequency and signal intensity in the ipilimumab monotherapy arm by clinical benefit. Thick lines represent mean fold change +/- 95% CI of group cell population over time, light colored lines represent measurements of individual patients.
D) Trends in CD4+ T cell Interferon gamma secretion frequency and signal intensity in the ipilimumab plus nivolumab arm by clinical benefit. Thick lines represent mean fold change +/- 95% CI of group cell population over time, light colored lines represent measurements of individual patients.
E) Boxplot of Regulatory PSI at baseline in the ipilimumab arm by clinical benefit (pink= no clinical benefit, [n=2], green= clinical benefit, [n=6]).
F) Boxplot of Regulatory PSI at baseline in the ipilimumab plus nivolumab arm by clinical benefit (pink= no clinical benefit, n=4, green= clinical benefit, n=5).
G) Boxplot of IL-10 secretion frequency at baseline in the ipilimumab monotherapy arm by clinical benefit.
H) Boxplot of IL-10 secretion frequency at baseline in the ipilimumab plus nivolumab arm by clinical benefit.

Fig 3. Features of the tumor microenvironment in melanoma patients treated with ipilimumab or ipilimumab plus nivolumab.
A) Mutational landscape indicating melanoma and immunotherapy related genes with alterations in patients (n=10) at all timepoints. BOR = best overall response; PD = progressive disease; PR = partial response; SD = stable disease.
B) Heatmap of expression profiling of patient tumors (n=10) by RNA sequencing at the on-treatment timepoint. The list of genes comprising each signature (heatmap columns) are detailed in Supplementary Table S2.
C) Heatmap showing immune population scores calculated by EPIC deconvolution of the gene expression data for all patients with on-treatment samples sent for RNA sequencing (n=10).

Fig. S5. Lack of association between TMB and clinical benefit at baseline and on-treatment timepoints.
A) Bar plot showing total mutation burden (per mb) of pre-treatment samples sent for whole exome sequencing. Height of bars represents TMB value at baseline, color represents clinical benefit classification (pink= no clinical benefit, [n=4], green= clinical benefit, [n=9]).
B) Bar plot showing total mutation burden (per mb) of post-treatment samples sent for whole exome sequencing. Height of bars represents TMB value post-treatment, color represents clinical benefit classification (pink= no clinical benefit, [n=3], green= clinical benefit, [n=7]).
C) Line plot showing change in total mutation burden (per mb) from baseline to on-treatment in the subset of patients with whole exome sequencing at both timepoints (pink= no clinical benefit, [n=3], green= clinical benefit, [n=7)].
D) Box plot comparing total mutation burden (per mb) of baseline TMB by clinical benefit classification. Color of dots indicates treatment arm (gold= ipilimumab, teal= ipilimumab plus nivolumab) test by 2-sided Wilxocon Rank-Sum. ns = not significant.
E) Box plot comparing total mutation burden (per mb) of post-treatment TMB by clinical benefit classification. Color of dots indicates treatment arm (gold= ipilimumab, teal= ipilimumab plus nivolumab), test by 2-sided Wilxocon Rank-Sum. ns = not significant.

Fig S6. Heatmaps of tumor signatures and immune populations by RNA sequencing at additional timepoints and by sample processing type.
A) Heatmap showing tumor signature scores for all patients with pre-treatment samples sent for RNA sequencing. BOR = best overall response; PD = progressive disease; PR = partial response; SD = stable disease.
B) Heatmap showing relative change in tumor signature scores from baseline to post-treatment for all patients with pre- and post-treatment samples sent for RNA sequencing.
C) Heatmap showing immune population enrichment scores by EPIC for all patients with pre-treatment samples sent for RNA sequencing.
D) Heatmap showing relative change in immune population enrichment scores by EPIC from baseline to post-treatment for all patients with pre- and post-treatment samples sent for RNA sequencing.

Fig S7. Peripheral NK cell trends over time and clinical benefit by CyTOF.
A) Line plot showing trends in peripheral NK cell populations by clinical benefit (pink= no clinical benefit, [n=6], green= clinical benefit, [n= 10]) over time by CyTOF. Thick lines represent mean fold change +/- 95% CI of group cell population over time as percent of total leukocytes, light colored lines represent measurements of individual patients.

Fig S8. HLA gene expression from patient tumor samples by clinical benefit. Boxplots showing expression in fragments per kilobase of transcript per million mapped reads (fpkm) of HLA-A, HLA-B, HLA-C at A) Baseline and B) On-treatment from patient tissue samples, n=10 respectively. Pink represents patients with lack of clinical benefit, green are patients who experienced clinical benefit, pairwise p-values by Mann-Whitney.

Fig S9. Peripheral CyTOF profiling in 2 patients with discordant tumor transcriptional profiles.
A) Trends in CD4+ and CD8+ T cells over time. Lines represent mean fold change over baseline, color represents patient and respective clinical benefit status. C1= baseline, C2=pre-cycle 2 of therapy, C3= pre-cycle 3, of therapy, C4= pre-cycle 4 of therapy, FU1= post 4 cycles of therapy.
B) Trends in CD4+ Central Memory and Naïve T cells over time. Lines represent mean fold change over baseline, color represents patient and respective clinical benefit status.
C) Comparison of fold change from baseline in CD4+ and CD8+ T cell populations of interest at C2 between patients.