Supplementary Figure Legends

Supplementary Figure 1. Immune profiling of pre-treatment, on-treatment and progression CTLA-4 blockade samples by immunohistochemistry. Immune profiling was performed via a 12-marker immunohistochemistry panel. CD45RO **(a)**, CD3 **(b),** CD20 **(c)**, CD57 **(d)**, CD68 **(e)**, FoxP3 **(f)**, Granzyme B (**g**), PD-1 **(h)**, and LAG-3 **(i)** were assessed for density by quantitative IHC. Error bars represent standard error mean. n.s.= not significant.

Supplementary Figure 2. Myeloid cell profiling of pre-treatment, on-treatment and progression CTLA-4 blockade samples by immunohistochemistry. Immune profiling was performed via a 4-marker immunohistochemistry panel. CD14 **(a)**, CD33 **(b),** CD163 **(c)**, and CD206 **(d)** were assessed for density by quantitative IHC. Shown in **e-h** are representative IHC images in responders and non-responders at the pre-treatment timepoint. Error bars represent standard error mean. n.s.= not significant. Statistical analysis was not possible between responders and non-responders at on-treatment time point as only one sample was available per group.

Supplementary Figure 3. Increased contact between CD8 T cells and CD68 myeloid cells in non-responding patients to anti-CTLA-4 and anti-PD-1 therapy at pre-treatment CTLA-4 blockade, pre-treatment PD-1 blockade, and on-treatment PD-1 blockade time points. a) Immunofluorescence staining showing nuclei by DAPI (blue), CD8 (red) and CD68 (yellow) cells in a responder and non-responder. **b)** Semi-quantitative pathological assessment of percentage of CD8 and CD68 cells in contact in responders and non-responders pre-treatment and on treatment with anti-CTLA-4 and anti-PD-1 therapy.

Supplementary Figure 4. Immune profiling of pre anti-PD-1, on-treatment anti-PD-1 and progression anti-PD-1 samples by immunohistochemistry. Immune profiling was performed via a 12-marker immunohistochemistry panel. CD45RO **(a),** CD20 **(b)**, CD57 **(c)**, CD68 **(d)**, FoxP3 **(e)**, and Granzyme B **(f)** were assessed for density by quantitative IHC. Error bars represent standard error mean. *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$, n.s.= not significant.

Supplementary Figure 5. Longitudinal increase in CD8, PD-1, and PD-L1 expression in responders to anti-PD-1 therapy. Five paired responder (**a, c, e**) and 14 paired non-responder (**b, d, f**) samples were evaluated for changes in CD8 (**a-b**) and PD-1 (**c-d**) counts/mm^2 and PD-L1 (**e-f**) H-Score at pre/on-treatment and on/post-treatment time points by immunohistochemistry. Lines link paired samples.

Supplementary Figure 6. Relative increase in CD8 T cell infiltrate at tumor center in responders to anti-PD-1 on treatment. Pie charts depicting the CD8 counts/mm^2 at pre-treatment anti-CTLA-4 (**a-b**), pre-treatment anti-PD-1 (**c, d**), and on treatment anti-PD-1 (**e, f**) time points in responders and non-responders at tumor margin (**g**) and center (**h**). Numbers represent average counts per treatment time point. Blue = Tumor margin, Red = Tumor center. Pre-treatment anti-CTLA-4: Responders (n=3), Non-responders (n=15); Pre-treatment anti-PD-1: Responders (n=2), Non-responders (n=8); On treatment anti-PD-1: Responders (n=2), Non-responders (n=2).

Supplementary Figure 7. Significant increase in immune infiltrate between responders and non-responders to PD-1 blockade in absence of prior anti-CTLA-4 therapy. a) Timeline illustrating

breakdown of anti-CTLA-4-naïve patient samples by response and treatment time point and planned analyses. CD4 **(b)**, CD8 **(c),** FoxP3 **(d)**, GzmB **(e)**, PD-1 **(f)**, PD-L1 **(g)**, and LAG-3 (**h**) were assessed for density by quantitative IHC. Error bars represent standard error mean. n.s.= not significant. Black dots depict anti-CTLA-4-naïve patients. *= p≤0.05, **= p≤0.01, ***= p≤0.001, n.s.= not significant.

Supplementary Figure 8. Immune profiling of myeloid cells atpre-treatment and on-treatment PD-1 blockade time pointsby immunohistochemistry. CD14 **(a)**, CD33 **(b),** CD163 **(c)**, and CD206 **(d)** were assessed for density by quantitative IHC. Shown in **e-h** and **i-l** are representative IHC images in responders and non-responders at the pre-treatment and on treatment timepoints, respectively. Error bars represent standard error mean. n.s.= not significant.

Supplementary Figure 9. Heatmap of 54 NanoString samples. Values are log2-transformed normalized mRNA count. Samples are ordered by treatment time point and by responsiveness to anti-CTLA-4 or anti-PD-1 therapy. Color pattern is relative with respect to the row within each time point, with red indicating gene up-regulation and green indicating gene down-regulation.

Supplementary Figure 10. Gene-specific NanoString concordance with immune profiling by IHC in pre-treatment, on-treatment and progression CTLA-4 blockade samples. Gene expression profiling was performed via NanoString on 54 tumor biopsies. Of the custom-designed 795 probe code set, 10 probes were represented in our immune profiling analysis by IHC, namely CD3, CD4, CD8, CD45RO, CD68, FoxP3, Granzyme B, LAG-3, PD-1 and PD-L1. All values represented by box and whisker plots. *= p≤0.05, **= p≤0.01, ***= p≤0.001, n.s.= not significant.

Supplementary Figure 11. Gene-specific NanoString concordance with immune profiling by IHC in pre-treatment, on-treatment and progression PD-1 blockade samples. Gene expression profiling was performed via NanoString on 54 tumor biopsies. Of the custom-designed 795 probe code set, 10 probes were represented in our immune profiling analysis by IHC, namely CD3, CD4, CD8, CD45RO, CD68, FoxP3, Granzyme B, LAG-3, PD-1 and PD-L1. All values represented by box and whisker plots. *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$, n.s.= not significant.

Supplementary Figure 12. Prior CTLA-4 blockade is not required for PD-1 early on-treatment profile. Heatmap of 28 anti-PD-1 samples, which included 7 pre-treatment samples (4 responders, 3 non-responders) and 8 on-treatment samples (3 responders, 5 non-responders) with prior CTLA-4 exposure, as well as 8 pre-treatment samples (6 responders, 2 non-responders) and 5 on-treatment samples (2 responders and 3 non-responders) that were CTLA-4 blockade-naïve. Values are median-centered and $\log_2$-transformed. Hierarchical clustering was performed on gene expression (higher expression in dark blue, lower expression in light blue).

Supplementary Figure 13. Hierarchical clustering of gene expression across 54 samples confirms lack of batch effect. Hierarchical clustering of gene expression across 54 samples with samples ordered by response to therapy, treatment time points, and batch to test for batch effects. Hierarchical clustering was performed using average linkage method with a correlation metric distance by heatmap. R function and gene expression values were scaled by rows (genes). After hierarchical clustering was performed, batch was not correlated with either response to therapy or time point. Responders (blue), non-responders (red), time points (pre-treatment anti-CTLA-4, on-treatment anti-CTLA-4, pre-treatment anti-PD-1, and on-treatment anti-PD-1), and batch (1, 2, 3, 4, 5).