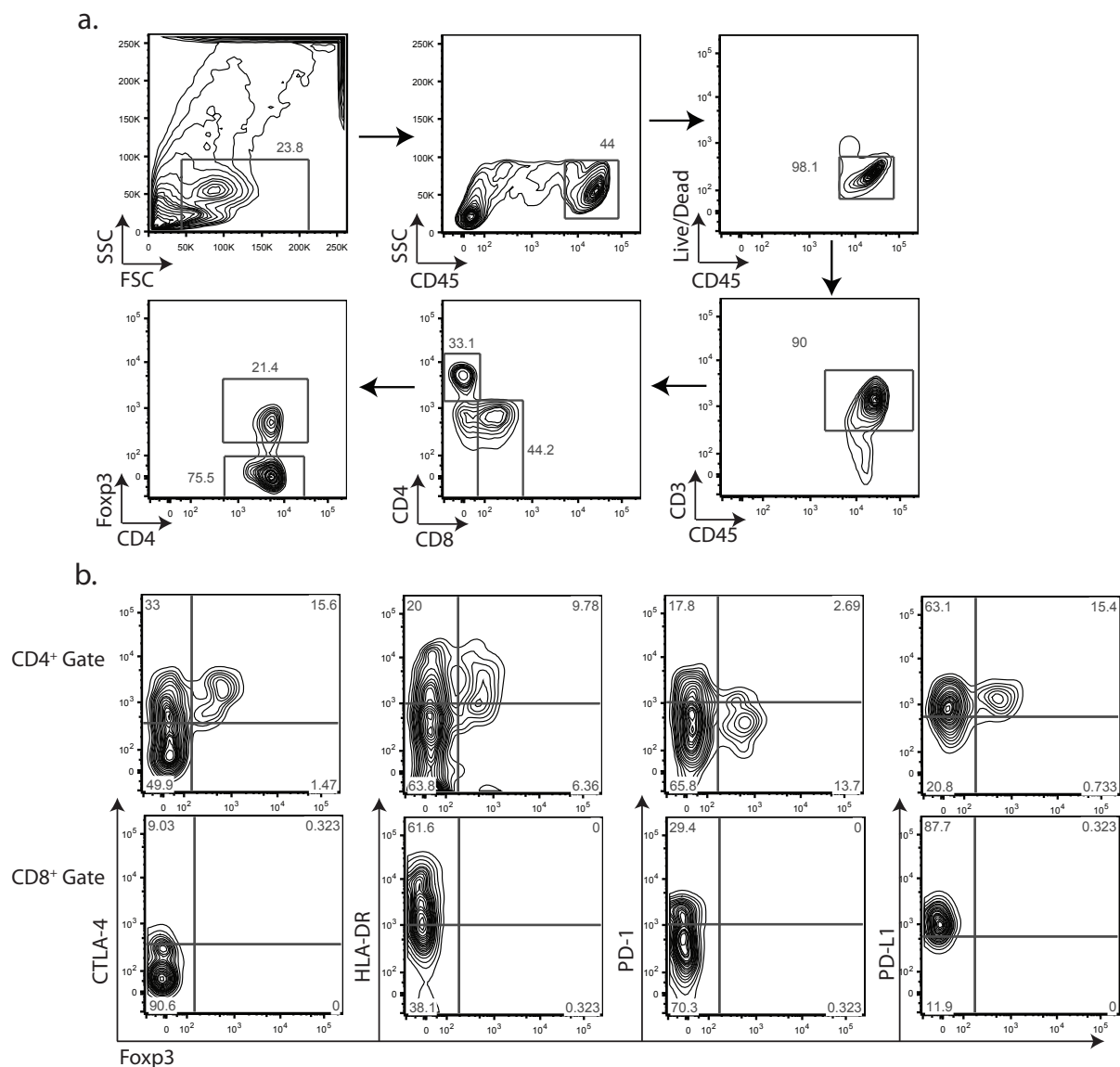
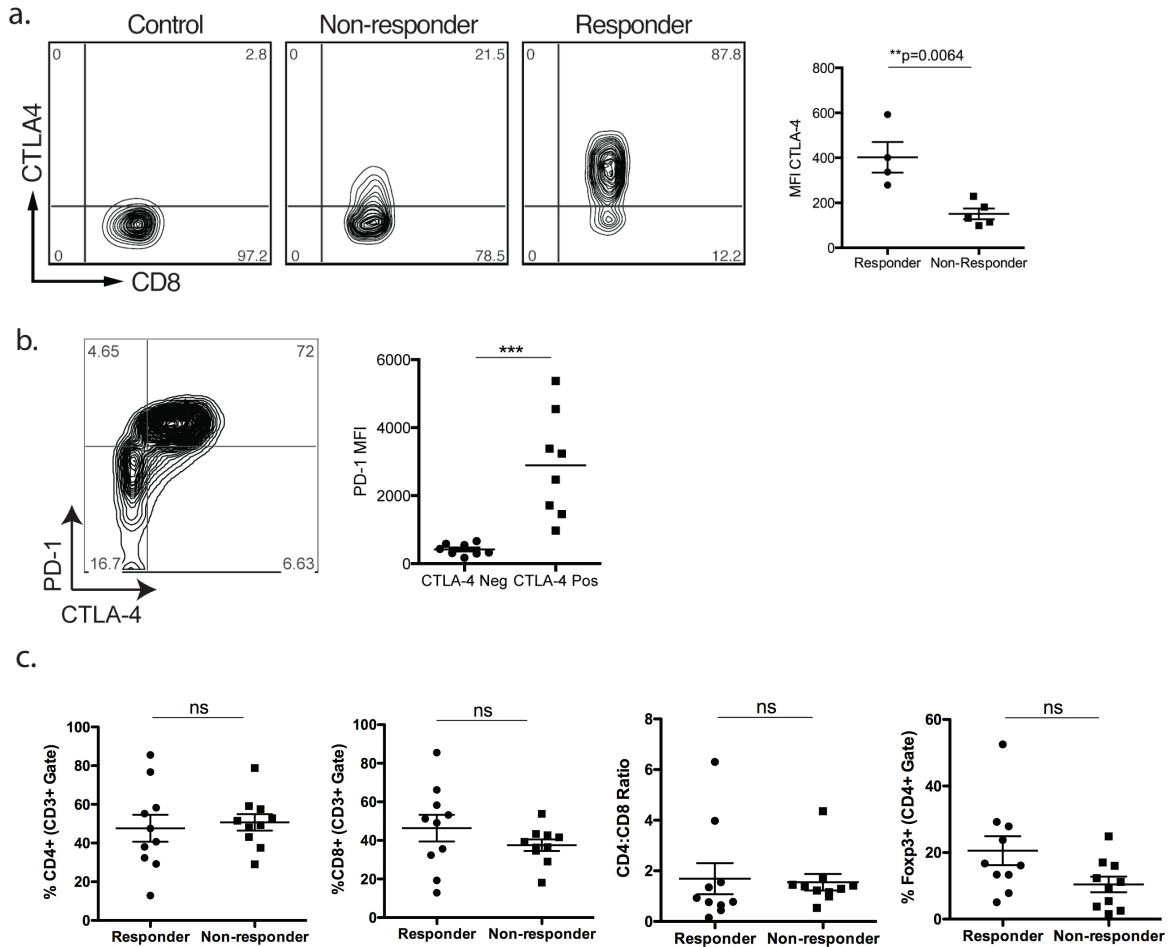


Supplemental Figures

Supplemental Figure 1



Supplemental Figure 2

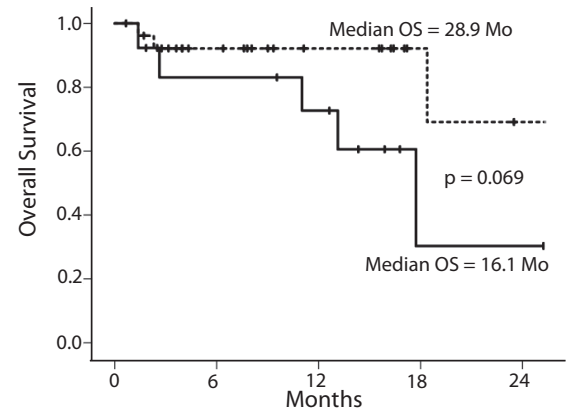


Supplemental Figure 2. Characterization of CTLA-4 and PD-1 expression on tumor-infiltrating CTLs and correlation with clinical response to anti-PD-1 therapy. (a)

Representative flow cytometric plots and flow cytometric quantification of CTLA-4 expression on tumor-infiltrating CD8⁺ CTLs in patients that responded or did not respond to anti-PD-1 therapy.

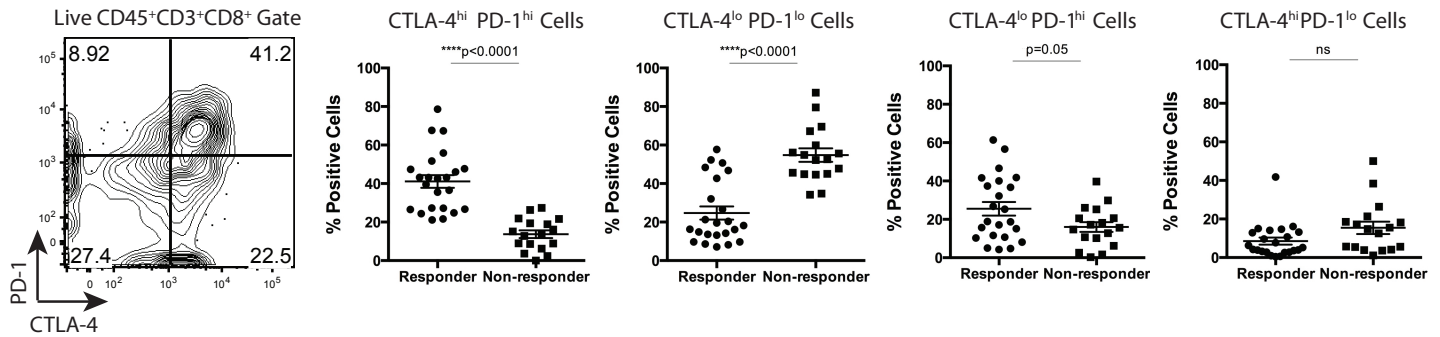
(b) Representative flow cytometric plots and flow cytometric quantification of PD-1 expression on CTLA-4^{neg} and CTLA-4^{pos} tumor-infiltrating CD8⁺ CTLs. (c) Flow cytometric quantification of CD4⁺ T cells, CD8⁺ T cells, the CD4:CD8 ratio, and regulatory T cells (Foxp3⁺ cells) in patients that responded or did not respond to anti-PD-1 therapy. All flow cytometric data was obtained from metastatic tumors prior to beginning treatment with anti-PD-1. Plots in (a) and (b) are pre-gated on live CD45⁺CD3⁺CD8⁺ cells. Symbols represent individual patients. MFI, mean fluorescence intensity. Lines represent mean values and bars represent standard error of the mean. p-values obtained by unpaired Student's *t* test. ns, non-significant.

Supplemental Figure 3



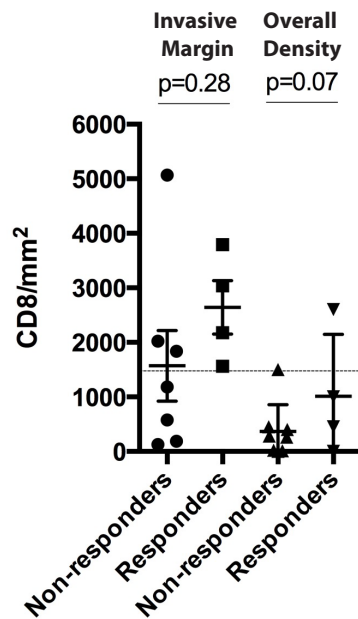
Supplemental Figure 3. Relative abundance of CTLA-4^{high}PD-1^{high} CTLs correlates with overall survival. Overall survival (OS) in patients that had $\geq 20\%$ (dotted line) or $\leq 20\%$ (solid line) tumor-infiltrating CTLA-4^{high}PD-1^{high} CTLs. Statistical significance was determined by Log-Rank test. (n=40).

Supplemental Figure 4



Supplemental Figure 4. Combined expression of CTLA-4 and PD-1 on tumor-infiltrating CD8⁺ T cells strongly correlates with clinical response to anti-PD-1 therapy. Representative flow cytometric plot of tumor-infiltrating CD8⁺ T cells dividing cells into CTLA-4^{high}PD-1^{high} double positive cells, CTLA-4^{low}PD-1^{high} single positive cells, CTLA-4^{high}PD-1^{low} single positive cells, and CTLA-4^{low}PD-1^{low} double negative cells. Scatter plots represent clinical response data based on the percentage of these 4 populations present within tumors prior to beginning anti-PD-1 monotherapy. Plot is pre-gated on live CD45⁺CD3⁺CD8⁺ cells. Symbols represent individual patients. Lines represent mean values and bars represent standard error of the mean. p-values obtained by unpaired Student's *t* test. ns, non-significant. n=40 patients.

Supplemental Figure 5



Supplemental Figure 5. Immunohistochemical analysis of tumor-infiltrating CD8+ T cells. Metastatic melanoma tumor samples were evaluated from 21 patients using the method described by Tumei *et. al.* (Nature 2011) for abundance of cells at the tumor invasive margin and overall cell density. Of 21 samples, 8 specimens (38.1%) were not able to be evaluated because the invasion margin could not be determined. Of the 13 evaluable remaining samples, 4 patients were responders and 7 were non-responders and 2 patients were lost to follow-up. Dashed line represents 1500 CD8 T cells/mm² at the invasive margin used to calculate predictive values. Lines represent mean values and bars represent standard error of the mean.