Supplementary Materials for

Improved overall survival with T cell clonotype stability following anti-CTLA-4 antibody treatment in cancer patients

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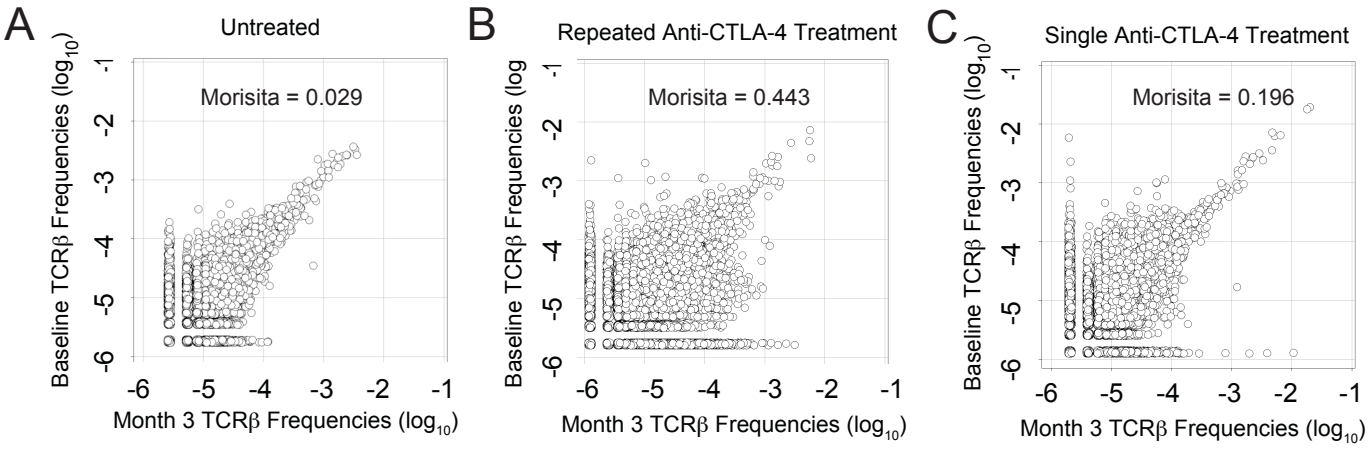
Supplementary Methods

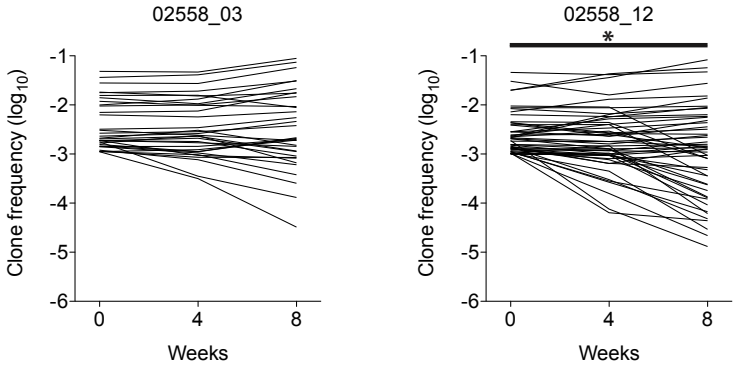
DESeq modification for identification of differentially abundant clonotypes

Identification of differentially abundant clonotypes in pairwise experiments was accomplished using a modified DESeq R package. The DESeq R package was developed for identification of differentially expressed genes in RNA-Seq experiments. Immune receptor repertoire data differs from typical gene expression data. For example, repertoire data tends to be heavily skewed towards rare species, with large numbers of clonotypes appearing only a few times, and many clonotypes appearing in only one of the two experiments due to sampling effects. Two modifications were made to the code to accommodate the specific case of repertoire analysis. First, normalization was performed using only clonotypes that were present in both experiments, and had at least five counts in at least one experiment. The normalization factor was calculated as the mean count ratio of these clonotypes. Second, for each experiment the dispersion model used to identify significant clonotypes was replaced by a dispersion model calculated as the median of dispersion curves from six normal, untreated sample pairs separated by one month, the same interval as the study sample pairs. This modification served to account for normal variation in the repertoire over time, and to compensate for the lack of replicates in the experimental design.



Supplementary Figure 1. Clonotype frequencies over time after a single treatment with anti-CTLA-4.

TCRβ clonotype frequencies plots are depicted for an untreated healthy individual (A), a patient treated with anti-CTLA-4 antibody (B), and a patient treated with a single dose of ipilimumab. Each point on the scatter plots represents a single clonotype with normalized $\log_{10}$ clone count graphed at baseline (x-axis) and after 3 months (y-axis). Morisita's distance was quantified for each patient.



Supplementary Figure 2. Clonotype kinetics in additional patients with improved survival. clonotype frequencies that exist at ≥ 10^{-3} at baseline for two additional patients with the longest survival are presented over time. *p = 0.038 were calculated using a two-sided Mann Whitney test.