

Elevated TCRB repertoire convergence and clonal expansion in the NSCLC tumor microenvironment of responders to anti-PD-1 monotherapy

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ABSTRACT

There is an outstanding need to identify predictive biomarkers for response to anti-PD-1 monotherapy for NSCLC. Here we used the Oncomine TCRB-SR assay to investigate the predictive value of TCRB evenness and convergence within the pretreatment tumor microenvironment of 45 individuals with NSCLC. For context, we compared the predictive value of these features with tumor mutation burden and PD-L1 IHC values from the same tumors.

INTRODUCTION -- Definition of TCR Convergence and Evenness

TCR Evenness is a measure of the similarity of clone frequencies in a TCR repertoire. It is also referred to as the normalized Shannon Entropy and is equivalent to 1 - "clonality". Evenness nearing 1 indicates that all clones are found at similar frequencies in a sample.

Convergent TCRs are identical in amino acid space but different in nucleotide space (Figure 1). They represent instances where T cells independently underwent VDJ recombination and proliferated in response to a common antigen. In the context of cancer, TCR convergence has been proposed to serve as an indicator of the immunogenicity of a tumor and thus its sensitivity to checkpoint blockade therapy (1,2,3). Importantly, TCR convergence has been proposed to arise in response to a broad range of tumor associated antigens (Table 1), including those derived from chromatin dysregulation and viral infection.

Figure 1. Example of a convergent TCR group. These three clones are identical in amino acid space (same variable gene and CDR3 AA) but differ at a nucleotide level, indicating they arose separately and independently proliferated in response to antigen.

Variable	CDR3 AA	CDR3 NT	Frequency
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGTCAGGCA TACGAGCAGTAC	1.8E-3
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTGGACAGGC TACGAGCAGTAC	4.8E-4
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGGCAGGC TACGAGCAGTAC	9.9E-05

Table 1. Types of antigens measured by tumor mutation burden and TCR convergence.

Antigen Source	Tumor Mutation Burden	TCR Convergence
Non-Synonymous Mutations	✓	✓
Aberrant Post-Translational Modifications	✗	✓
Ectopic Gene Expression	✗	✓
Splicing Defects	✗	✓
Autoantigens	✗	✓
Virus-Derived Antigens	✗	✓

INTRODUCTION – Assay Design and Workflow

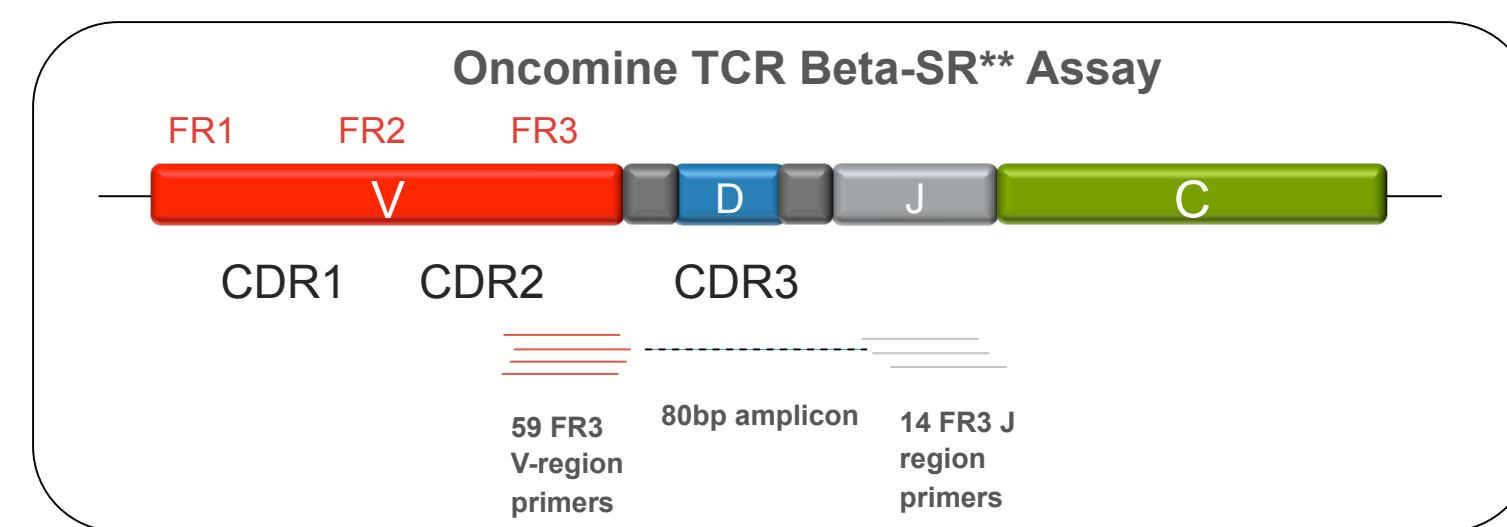
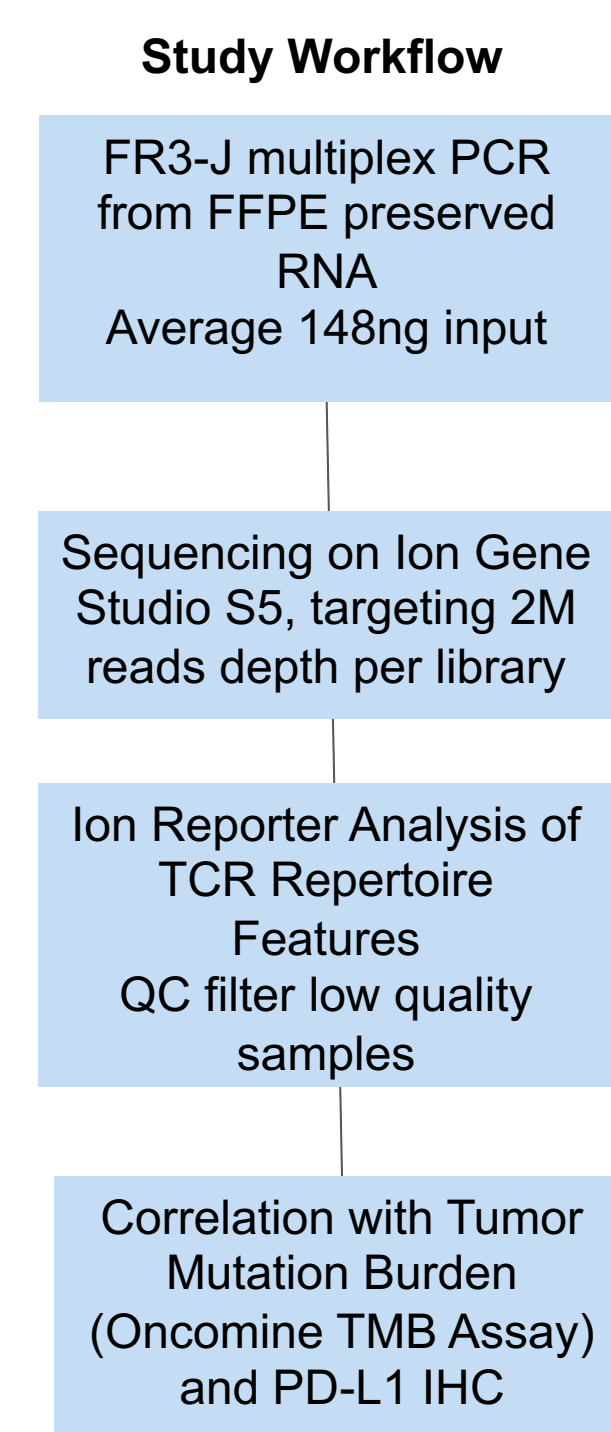


Figure 2. Assay Design The Oncomine TCRB-SR assay utilizes framework 3 (FR3) and joining gene (J) primers to amplify the CDR3 region of the TCRB chain (~80bp amplicon) from fresh or FFPE-preserved RNA or DNA input. **SR = Short Read

METHODS – Study Overview

Figure 3. Experimental Study. Total RNA from pretreatment FFPE biopsies of individuals receiving anti PD-1 blockade for NSCLC. Approximately 150 ng RNA was used for TCRB sequencing and analysis via the Oncomine TCRB-SR assay and Ion Reporter. TCR evenness and clonality were compared to Tumor Mutation Burden values for the same samples (via Oncomine Tumor Mutation Burden Assay) and tumor PD-L1 IHC via the Ventana PS263 antibody.

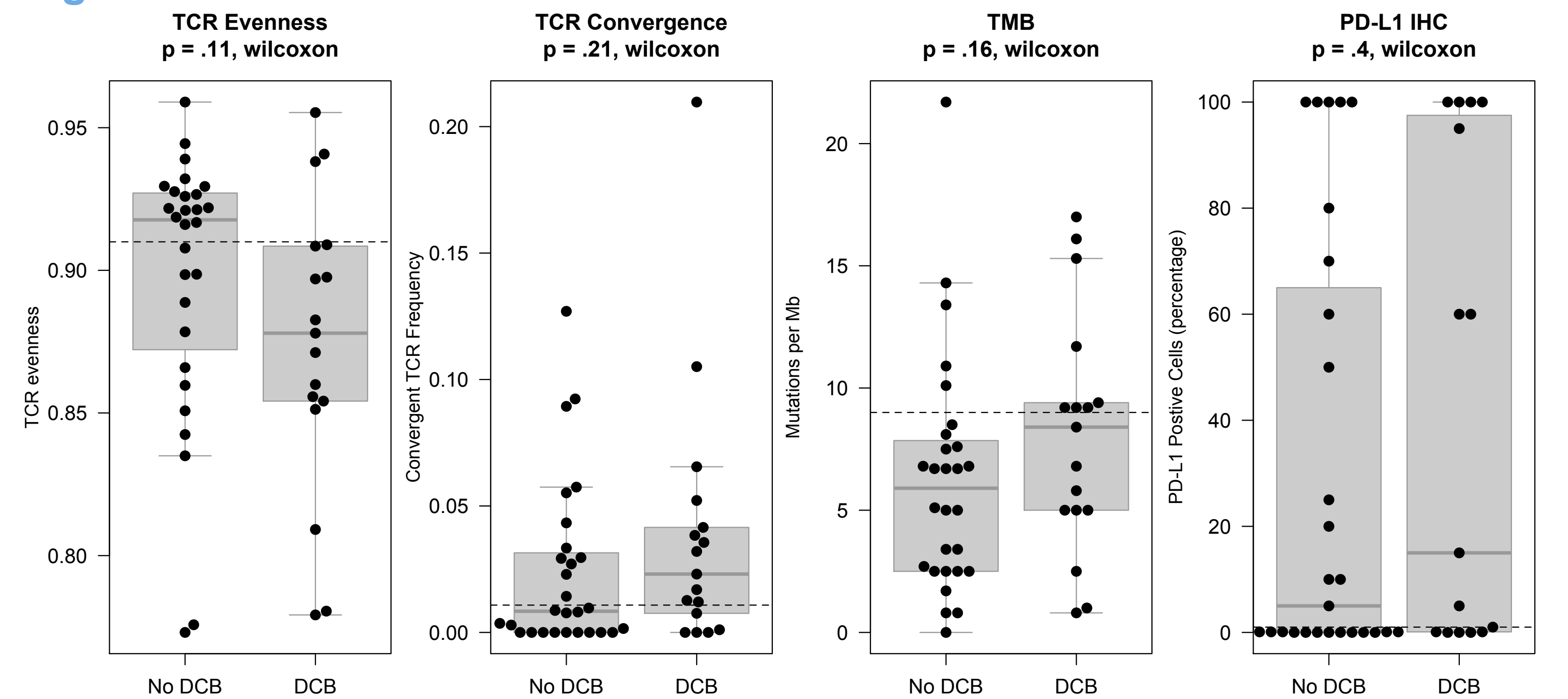


Results – Summary Sequencing Metrics for anti-PD-1 NSCLC Cohort

	No DCB*	DCB*
Number of Samples	28	17
RNA Input (ng)	119 (14-250)	203 (28-250)
Sequencing Depth (millions of reads)	2.85 (0.1-2.81)	2.55 (0.37-3.48)
Percentage Productive Reads	59 (38-76)	61 (32-76)
Clones Detected	2840 (61-19300)	2329 (200-6831)

*DCB: Durable Clinical Benefit. Table indicates average and range for select features.

RESULTS – Elevated TCRB Clonal Expansion and Convergence in Baseline NSCLC Tumor of Responders to PD-1 Blockade. Figure 4



Dashed lines indicate optimal cutoff based on Youden's J method. IHC via Ventana SP263. Note: PD-L1 IHC available for only 42 samples.

Figure 5

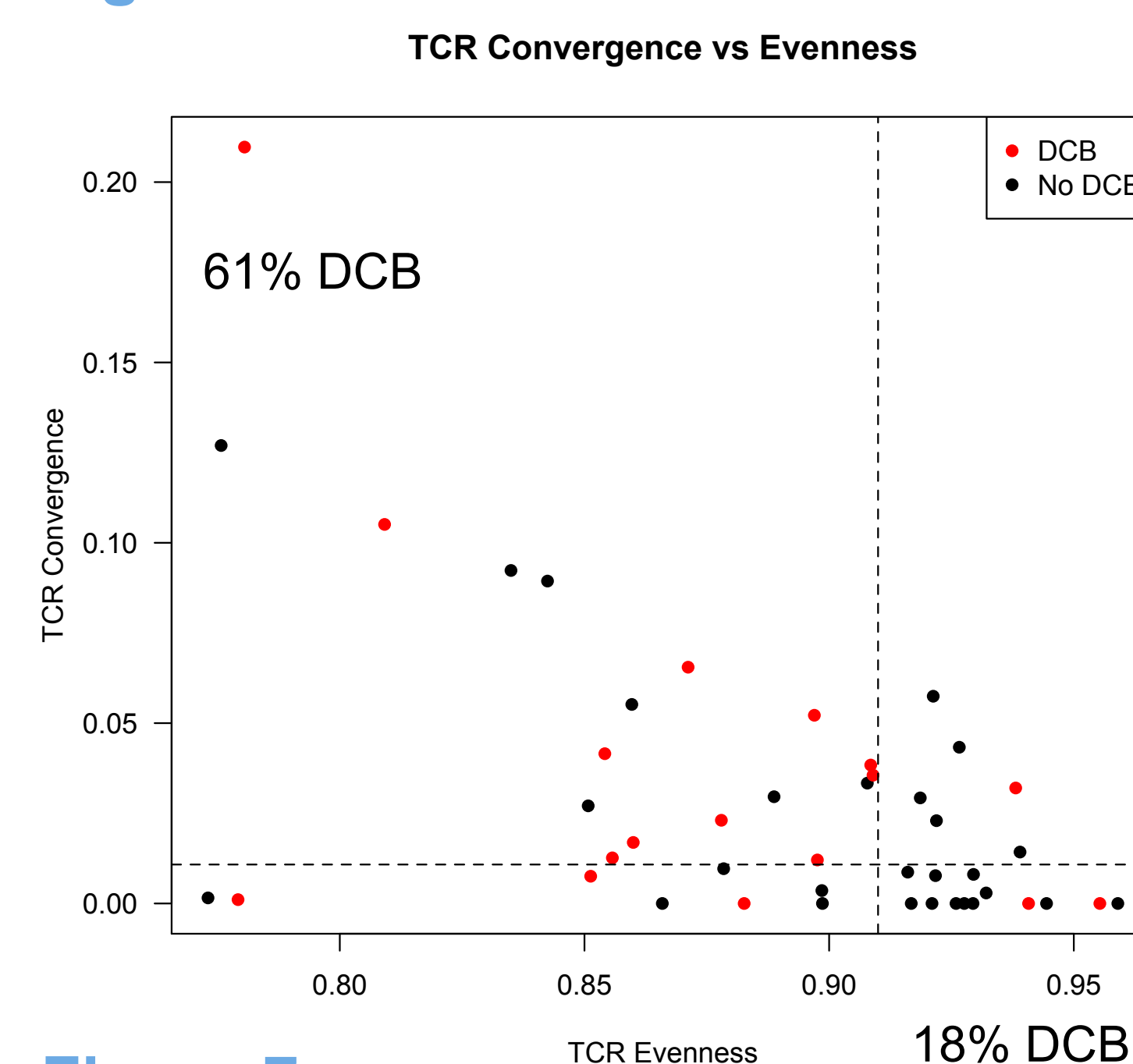


Figure 7

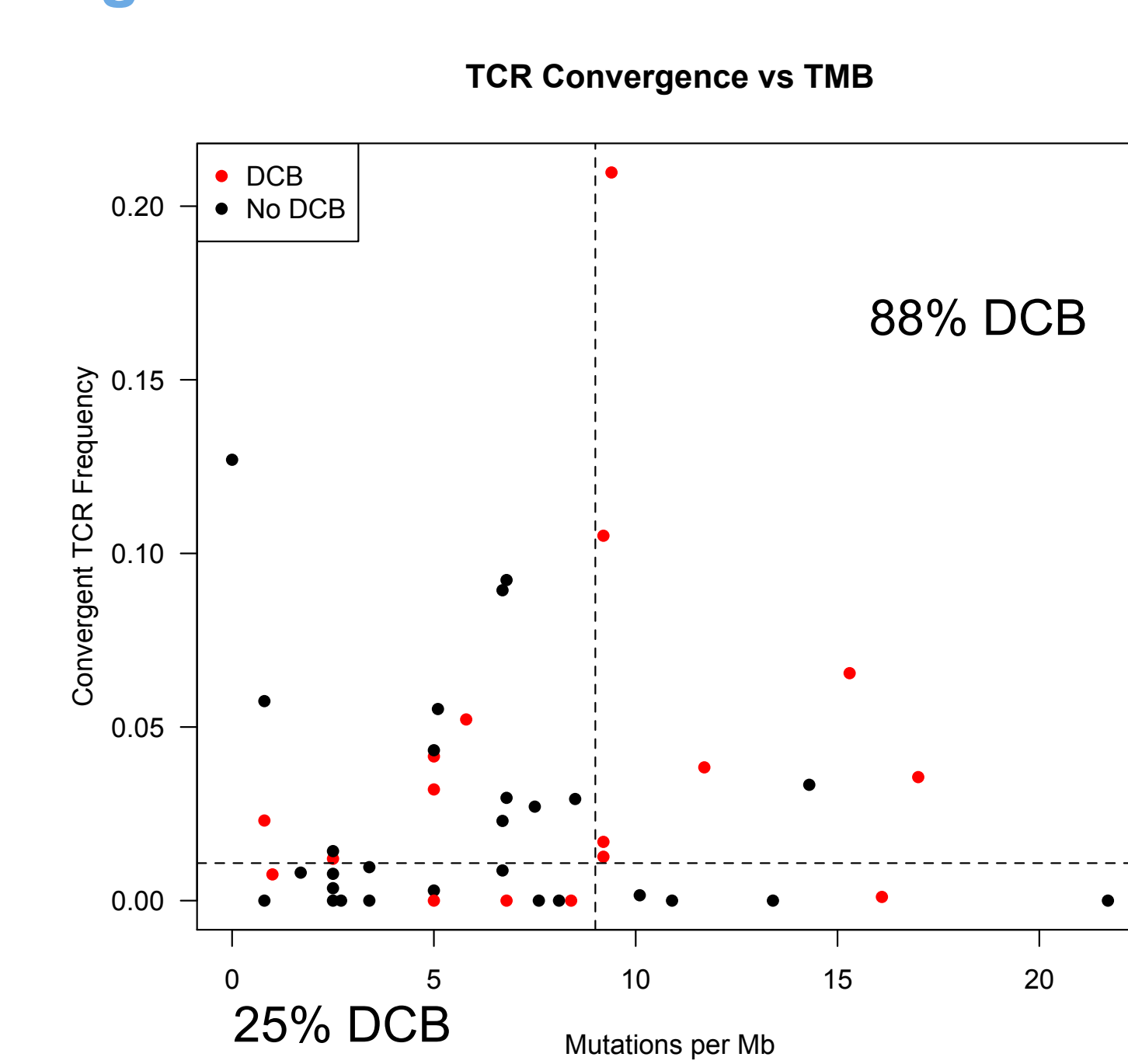
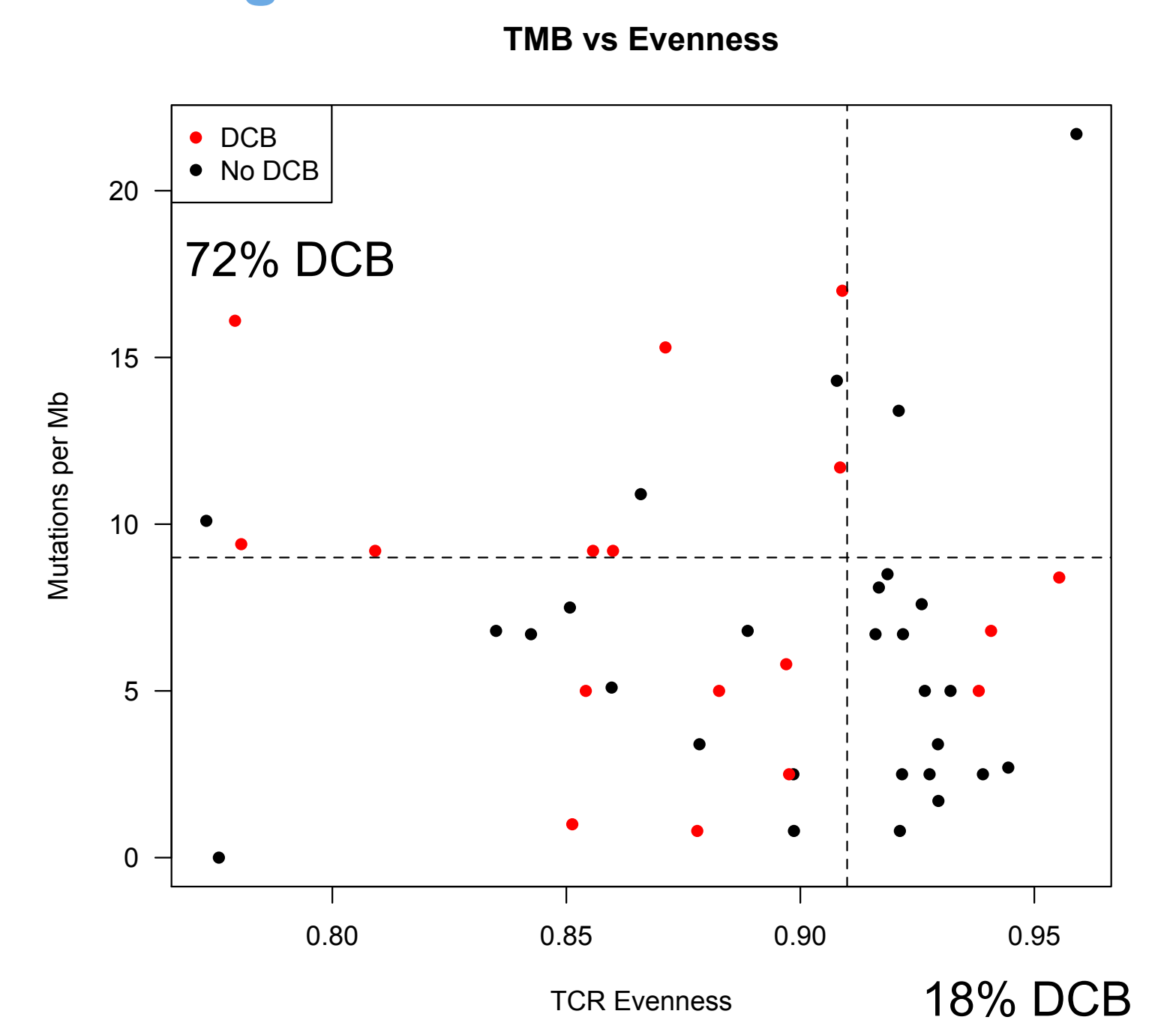


Figure 4 Boxplot of TCR, TMB and PD-L1 IHC values as a function of response. P-values obtained by one-sided Wilcoxon test. **Figures 5-7** Correlation of convergence vs evenness, evenness vs TMB, and convergence vs TMB. Percentages indicate response rate among individuals who appear to be responders by both metrics or neither metric.

Figure 6



CONCLUSIONS

- We identify reduced T cell evenness and elevated TCR convergence as features of the pretreatment NSCLC tumor microenvironment of responders to anti-PD-1 blockade.
- TCR-based features outperformed tumor mutation burden and PD-L1 IHC staining as predictors of response in this dataset.

REFERENCES

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