

TCRB chain convergence in chronic cytomegalovirus infection and cancer: insights from a novel potential immune repertoire biomarker

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ABSTRACT

Human cytomegalovirus (CMV) is a common immune-evasive herpes family virus leading to lifelong asymptomatic infection in 50 to 80% of humans. Current research evaluating the use of TCR sequencing to predict response to immunotherapy has focused on measurements of T cell clonal expansion and TCR convergence (2,3,4) as potential predictive biomarkers for response. Given that CMV infection has been reported to elicit large clonal proliferations of CMV reactive T cells (1), and is a source of chronic antigen stimulation, we hypothesized that CMV infection might alter T cell repertoire features in a manner relevant to the potential biomarker use of TCR sequencing. Here we sought to identify features of CMV infection using TCRB profiling of peripheral blood (PBL) total RNA. We identify reduced T cell evenness and elevated TCR convergence as features of chronic CMV infection.

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. 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 (2,3,4 and Table 2). Importantly, TCR convergence has been proposed to arise in response to a broad range of tumor associated antigens (Table 1), including those derived from viral infection.

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 – Recent Reports Correlating TCR Convergence with Immunotherapy Response

Study Lead and Publication	Cancer and Therapy	Correlation between TCR Convergence and Response
Walt Storkus UPMC SITC 2018 (2)	Dendritic cell vaccine for advanced melanoma	++
Larry Fong UCSF SITC 2018 (3)	Anti CTLA-4 for multiple cancers	+
Philip Jermann Univ of Basel EACR 2019	Anti PD-1 for NSCLC	+

† Indicates significant positive correlation between pretreatment TCR convergence and response

METHODS – Study Overview

Figure 2. Experimental Study.

Total RNA from PBL was obtained from 35 healthy blood donors of known CMV status, then used for TCRB sequencing via the Oncomine TCRB-LR assay (amplicon spanning CDR1, 2 and 3) and the Oncomine TCRB-SR assay (CDR3 only). Libraries were sequenced using the Ion Gene Studio S5, then analyzed via Ion Reporter. Data were used to identify TCRB repertoire features correlated with CMV status and compare repertoire features across the two assays.

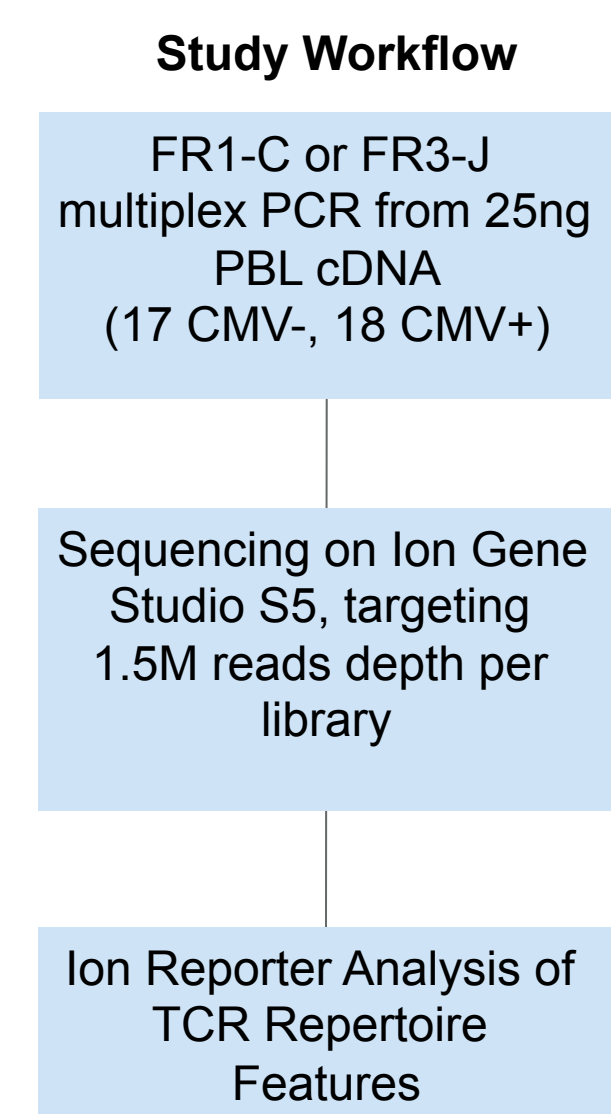
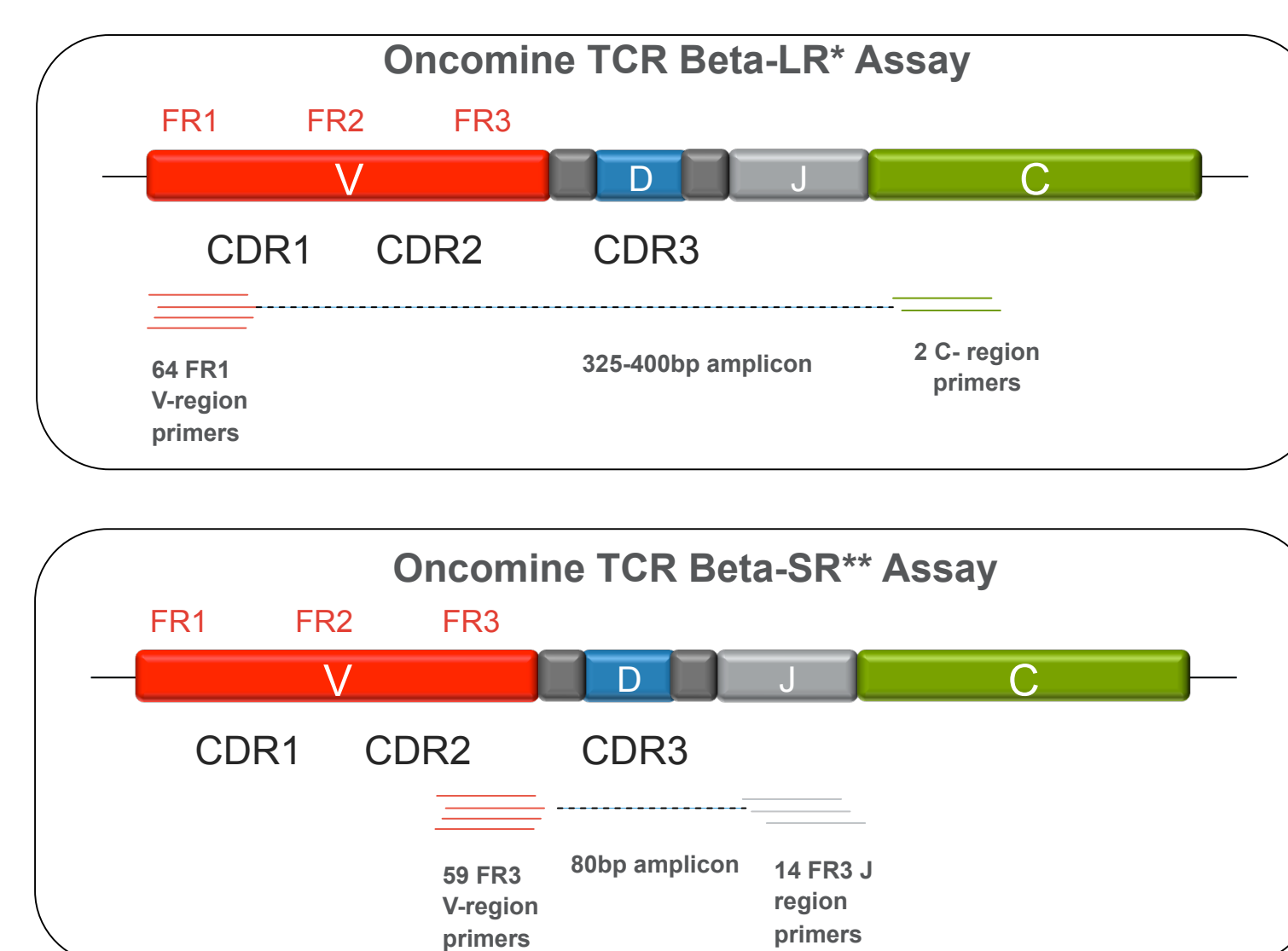


Figure 3. Assay Design. The Oncomine TCRB-LR assay utilizes AmpliSeq multiplex framework 1 (FR1) and constant gene primer to amplify all three CDR domains of the rearranged TCRB chain (~330bp amplicon) from non-FFPE RNA. The Oncomine TCRB-SR assay utilizes framework 3 (FR3) and joining gene (J) primers to amplify the CDR3 region of the TCRB chain (~80bp amplicon) from fresh or FFPE-preserved RNA or DNA input.



RESULTS – Elevated TCR Clonal Expansion and Convergence in CMV+ Donors

Figure 5. TCR evenness is reduced in CMV+ donors. Evenness values were lower in CMV+ subjects profiled using the TCRB-LR and SR assays, suggesting that CMV infection elicits T cell clonal expansion in asymptomatic subjects (one-sided student's t-test).

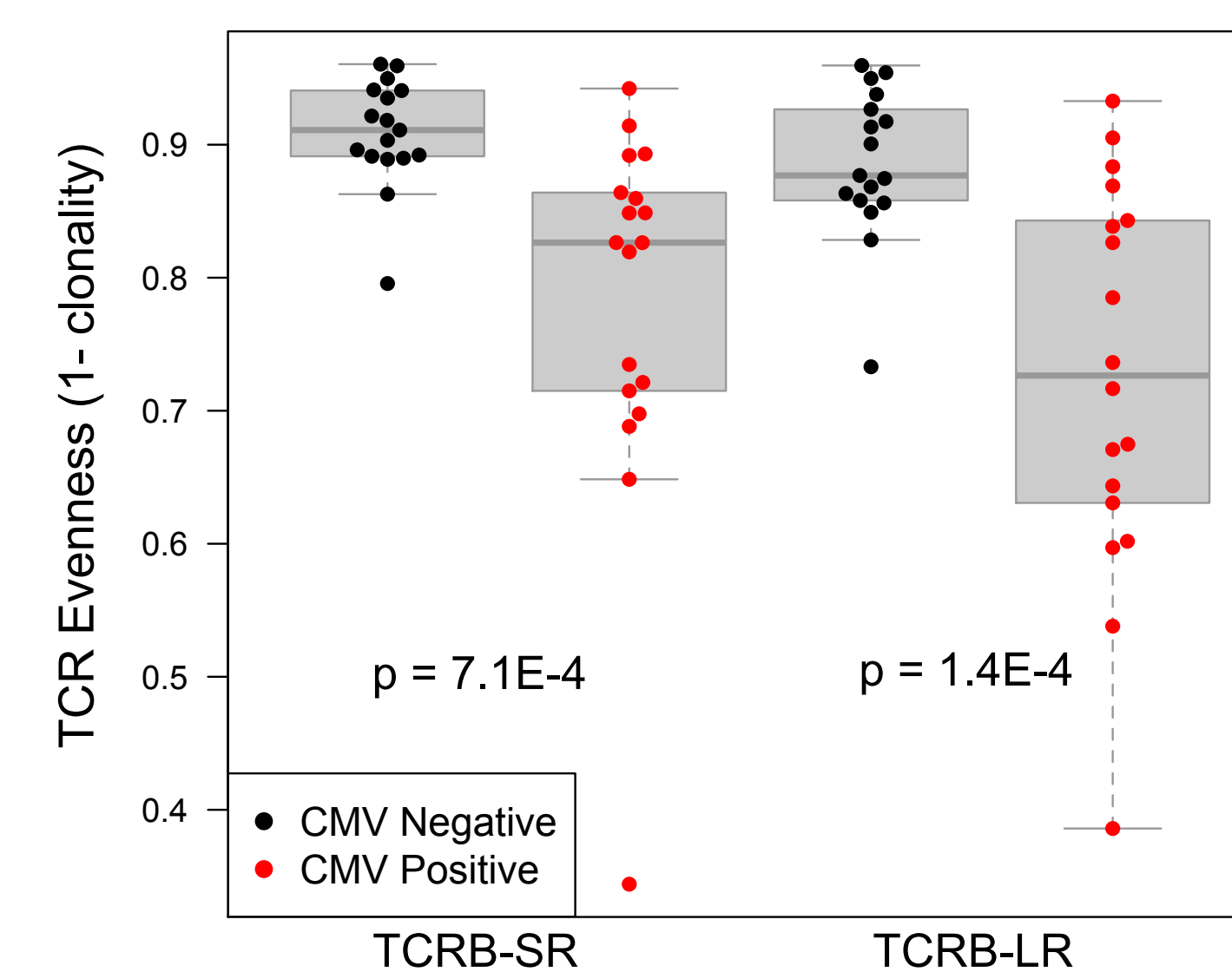


Figure 6. TCR convergence is elevated in CMV+ donors. The aggregate frequency of convergent TCRs was higher in CMV+ subjects profiled using the TCRB-LR and SR assays, suggesting that T cell responses to chronic CMV infection give rise to convergent TCR responses (one-sided student's t-test).

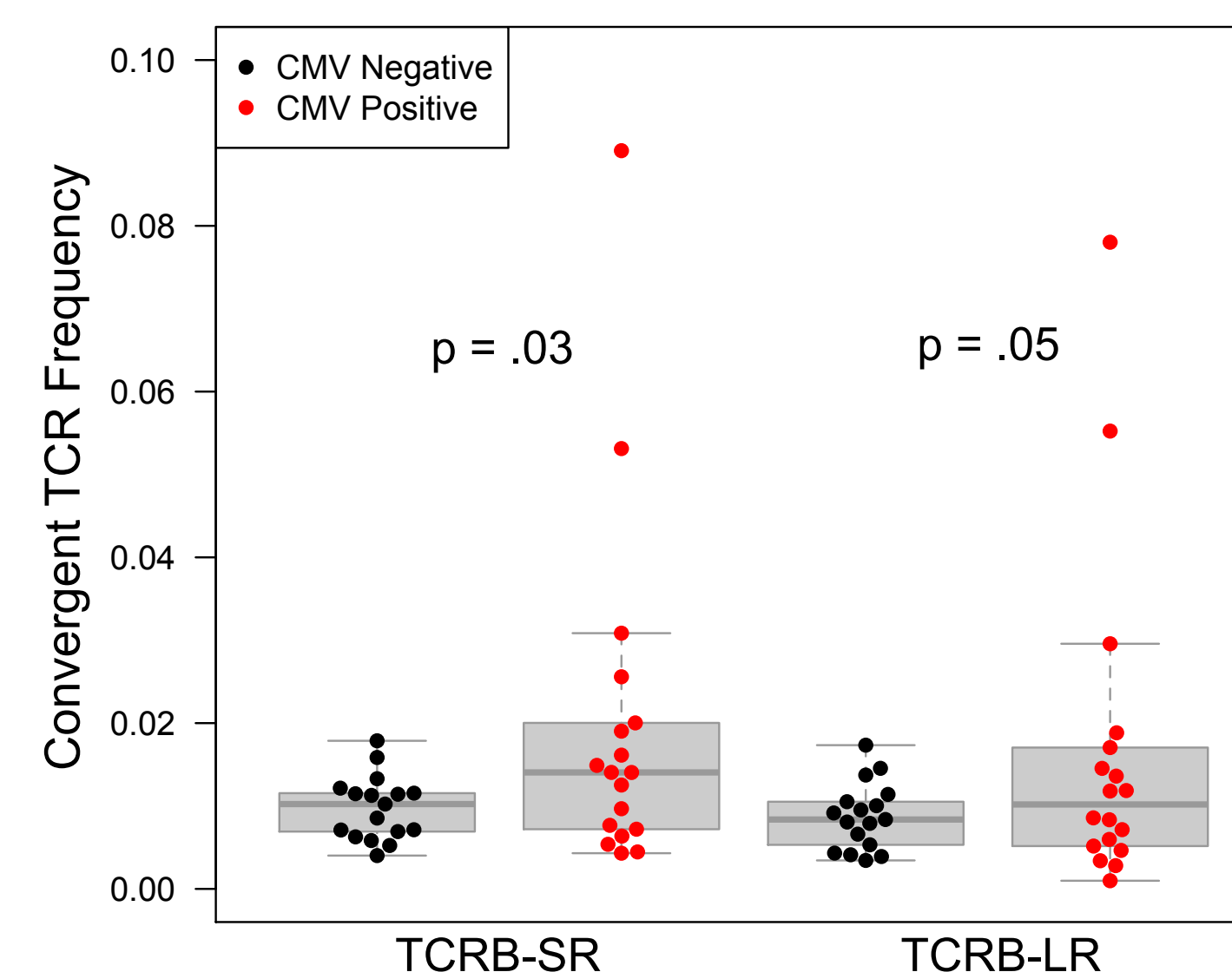
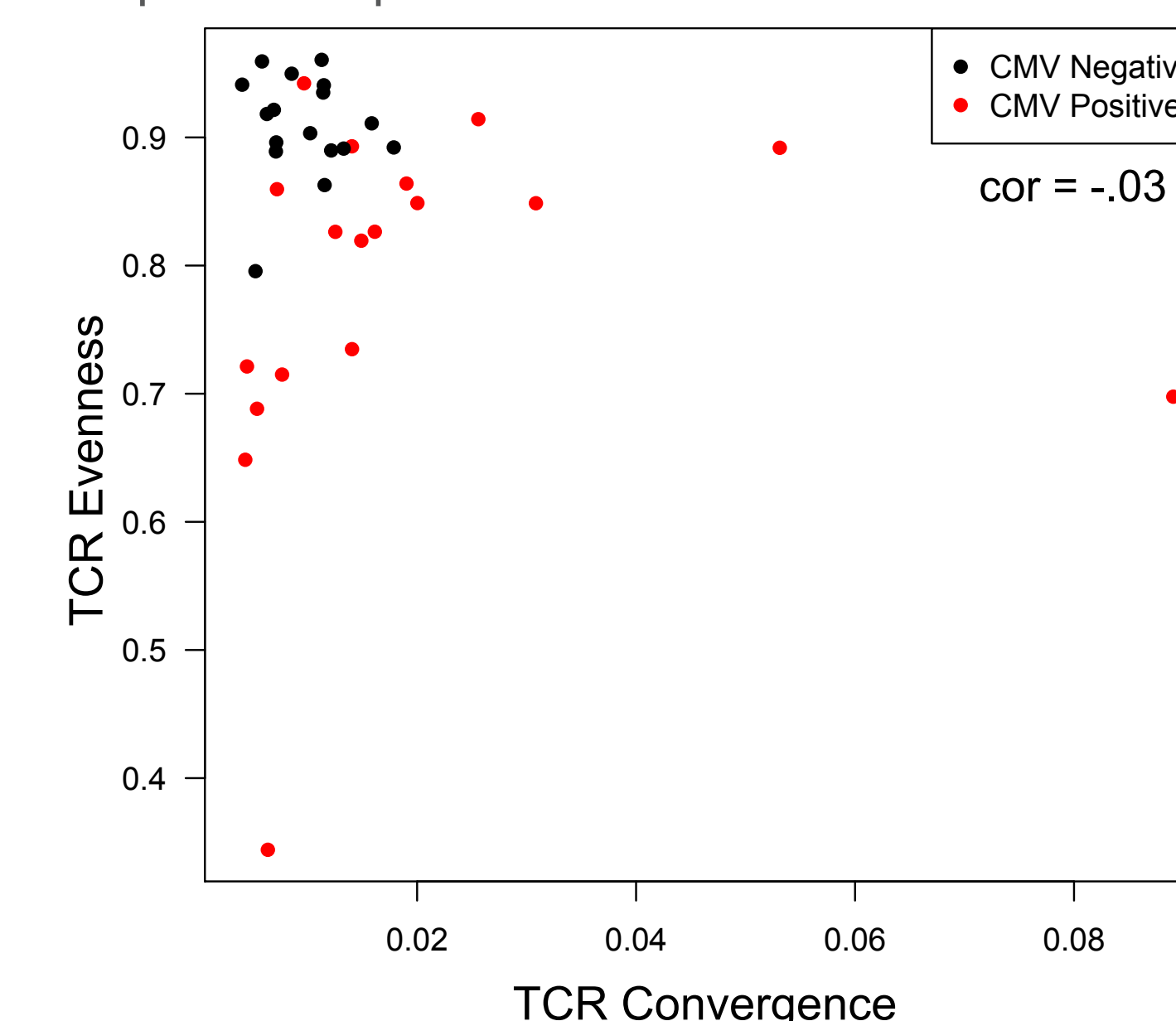
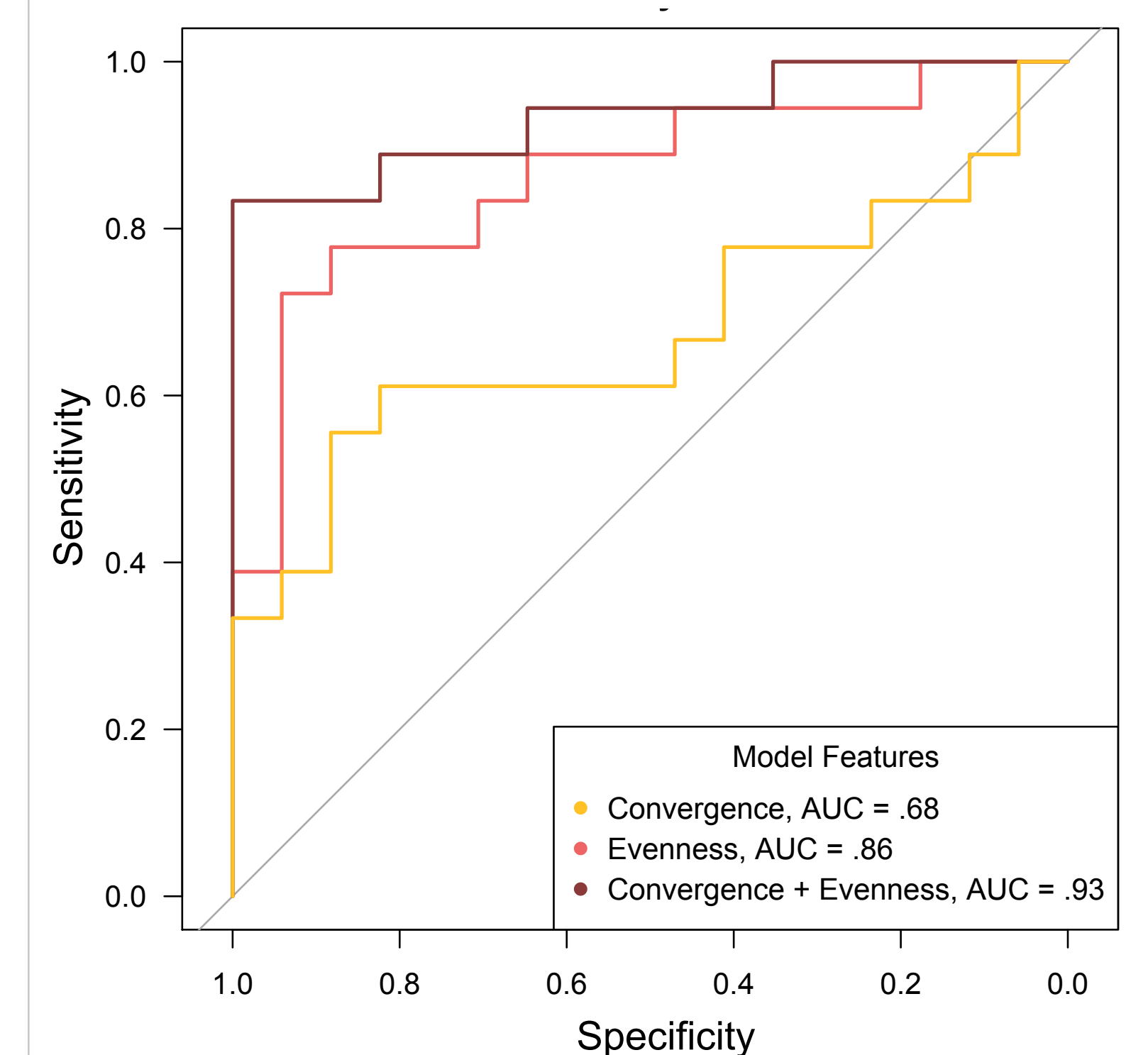


Figure 7. TCR convergence and evenness are uncorrelated. Correlation was evaluated using Spearman test (TCRB-SR data shown). The lack of correlation suggests these features may be combined to improve the prediction of CMV status.



RESULTS – Combination of TCR Convergence and Evenness Improves Prediction of CMV Status

Figure 8. ROC curve for the prediction of CMV status using TCR convergence and evenness. ROC curves are presented for each feature alone, and for a logistic regression classifier (R caret package) trained using TCR evenness and convergence as features to predict response to immunotherapy. The combination of features improves the prediction of CMV status (AUC = .93)



CONCLUSIONS

- We identify reduced T cell evenness and elevated TCR convergence as features of chronic CMV infection.
- CMV infection appears to significantly alter the T cell repertoire, suggesting that CMV status may be required for proper interpretation of T cell clonal expansion and TCR convergence in the context of immunotherapy for cancer.

REFERENCES

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