ion torrent

Peripheral blood TCRB repertoire convergence and clonal expansion predict response to anti-CTLA-4 monotherapy for cancer

L Zhang[1], T Looney[2], D Topacio-Hall[2], G Lowman[2], D Oh[1], L Fong[1]

(1) University of California San Francisco (2) Thermo Fisher Scientific

University of California San Francisco

Abstract

Tumor antigen-driven selection may expand T cells having T cell receptors (TCRs) of shared antigen specificity but different amino acid or nucleotide sequence in a process known as TCR convergence. Efforts to evaluate the biomarker utility of TCR convergence through TCRB repertoire sequencing have been hampered by substitution sequencing errors, given that such errors may create artifacts resembling TCR convergence. Here we leveraged the low substitution error rate of the Ion Torrent platform to evaluate convergence as a predictive biomarker for response to anti-CTLA-4 monotherapy in a set of 22 individuals with cancer. For context, we compared convergence values obtained using this platform to those for the same samples interrogated with Illumina-based TCRB repertoire sequencing. Finally, we examined whether TCR convergence may be combined with measurements of clonal expansion to improve prediction of immunotherapy response.

Introduction

Chronic stimulation of T cells with tumor neoantigen may elicit convergent T cell responses. The frequency of convergent TCRs within a repertoire may provide an indication of the immunogenicity of a tumor and thus its sensitivity to checkpoint blockade therapy. Unlike biomarkers relying of the quantification of tumor genetic alterations, TCR convergence: (1) may detect T cell responses to tumor neoantigens beyond those arising from non-synonymous mutations; (2) avoids probabilistic models for prediction of immunogenicity; (3) is sequencing efficient, requiring ~1.5M reads per sample and (4) may be measured from the abundant genetic material within the buffy coat fraction of centrifuged peripheral blood to enable liquid biopsy applications.

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	✖	✔

Methods

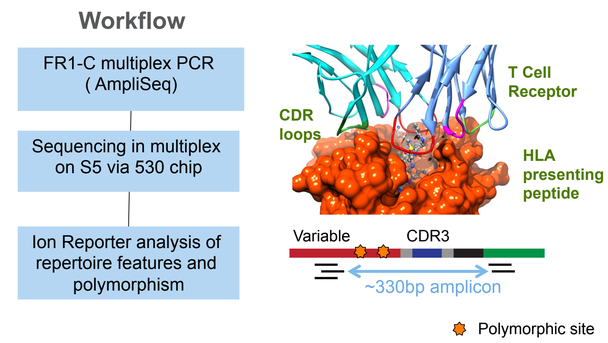

Figure 2. The Oncomine TCRB-LR assay utilizes AmpliSeq multiplex PCR primers to target the TCRβ Framework 1 and Constant regions, enabling both clonotyping and detection of TRBV gene polymorphism linked to adverse events during immunotherapy.

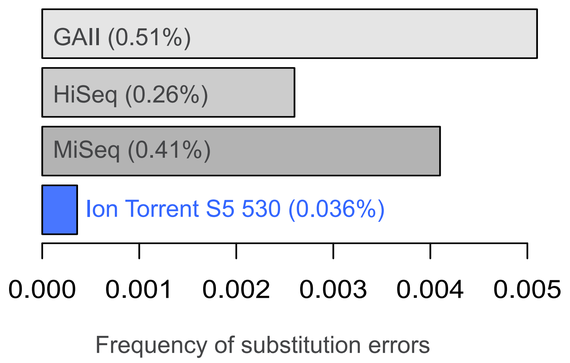

Figure 3. Substitution error rate for the Ion S5 530 chip and three common Illumina platforms. The substitution error rate for the Ion Torrent was obtained following sequencing of the ecoli dh1b genome and analyzed by Torrent Accuracy plugin as described in Looney et al (1). Illumina values are obtained from Schirmer et al (2). Substitution errors are a key challenge to the measurement of TCR convergence.

Variable	CDR3 AA	CDR3 NT	Freq
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGTCAGGCATACGAGCAGTAC	1.8E-3
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTGGGACAGGCCTACGAGCAGTAC	4.8E-4
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGGCAGGCCTACGAGCAGTAC	1E-4

Figure 4. Example of a convergent TCR group detected in an individual with melanoma. This group consists of three TCRβ clones that are identical in TCRβ amino acid space but have distinct CDR3 NT junctions owing to differences in non-templated bases at the V-D-J junction. Blue indicates bases contributed by the variable gene while yellow indicates bases contributed by the joining gene. Red arrows indicate positions where clones differ. Substitution sequencing errors and PCR errors can create artifacts that resemble convergent TCRs.

Results

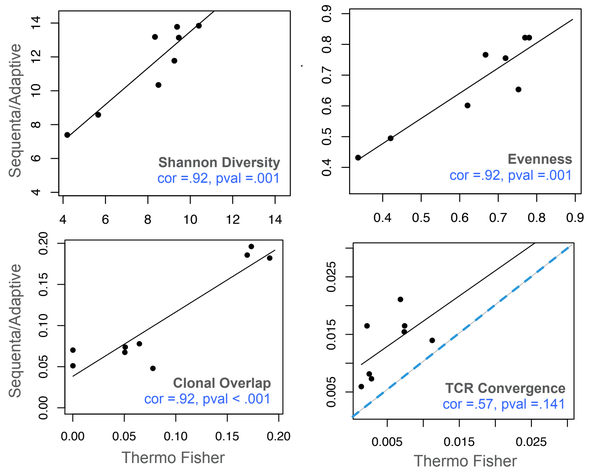

Figure 5: Comparative analysis of repertoire features in samples analyzed via Ion Torrent and Illumina-based assays. Eight peripheral blood leukocyte (PBL) samples derived from three donors were analyzed using the Oncomine TCRB-LR assay (Ion Torrent, X-axis) or Sequenta/Adaptive Biotechnologies TCRB assay (Illumina, Y-axis). Pearson's correlation coefficient was used to measure the consistency of two platforms with respect to clone diversity (Shannon entropy), overlap, evenness (normalized Shannon entropy) and TCR convergence. TCR convergence was calculated as the aggregate frequency of clones sharing an amino acid sequence with at least one other clone.

Category	Subdefinition	Responder	Non-responder
Cancer Type	Prostate	2	4
	Melanoma	7	6
	Adenocarcinoma	2	0
	Not Indicated	0	1
	Total	11	11
Repertoire Features	Clones Detected	32916 (5168-56231)	30015 (5894-58222)
	TCR Convergence	0.022 (.006- .092)	.008 (.002-.019)
	Evenness	.760 (.624 - .945)	.867 (.673-.945)

Table 1. Cancer type and repertoire features for 22 individuals receiving CTLA-4 monotherapy. Each individual was profiled via the Oncomine TCRB-LR Assay at a single baseline timepoint using 25ng of PBL total RNA. Repertoire feature values indicate the average and range for responders and non-responders.

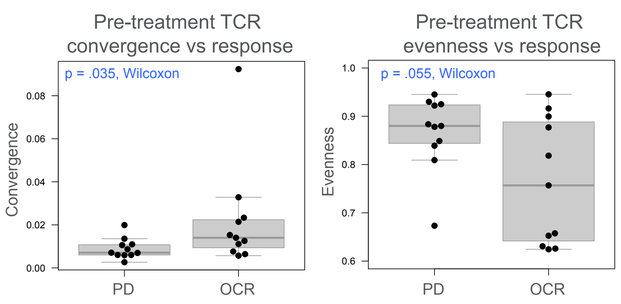

Figure 6. TCR evenness and convergence values for responders (N=11) and non-responders (N=11). TCR evenness is calculated as the normalized Shannon entropy of clone frequencies. Convergent TCR frequency was calculated as described above. All cancer types were included in the analysis.

Results

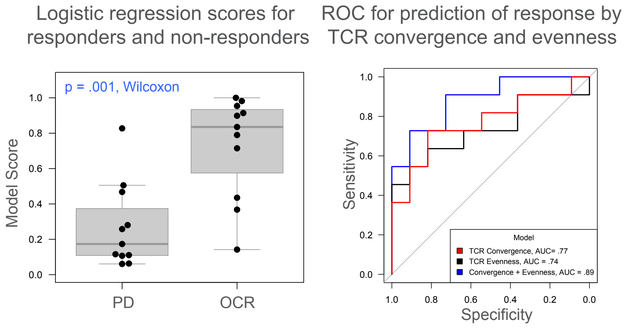

Figure 7. Prediction of immunotherapy outcome via TCR evenness and convergence. A logistic regression classifier (R caret package) was trained using TCR evenness and convergence as features to predict response to immunotherapy. (A) Model scoring of responders and non-responders. (B) Receiver operator characteristic curves derived from the use of evenness, convergence, or the combination of evenness and convergence to predict immunotherapy response. The combination of TCR evenness and convergence improves prediction of response (AUC = .89).

Conclusions

- We found measurements of TCR evenness, diversity and clonal overlap to be consistent between Ion Torrent and Illumina based TCR sequencing assays.
- By contrast, TCR convergence values were not significantly correlated, with all 8 samples showing higher convergence in the Illumina-derived dataset. Given that substitution sequencing errors may give rise to artifacts resembling convergence, these findings support the notion that the Ion Torrent may be well suited to the measurement of TCR convergence.
- Analysis of baseline PBL from 22 individuals receiving CTLA-4 blockade revealed that convergence and evenness values independently predicted response and could be combined to improve the accuracy of a logistic regression classifier (AUC = .89).
- TCR convergence may serve as a predictive biomarker for a wide range of cancers, including those where tumor mutation burden is not predictive of response.

References

1. Looney et al. Haplotype Analysis of the TRB Locus by TCRB Repertoire Sequencing (2018). bioRxiv 406157
2. Schirmer et al. Illumina error profiles: resolving fine-scale variation in metagenomic sequencing data (2016). BMC Bioinformatics

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