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TRBV polymorphism predicts adverse events during checkpoint blockade immunotherapy

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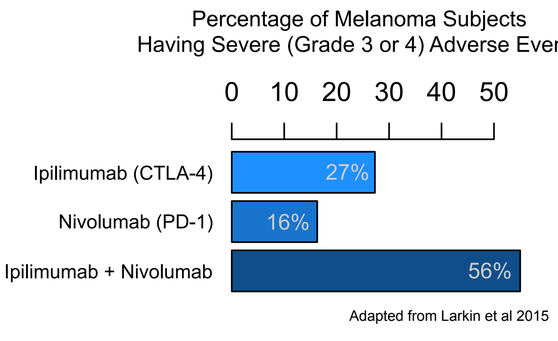
Abstract

Identifying predictive biomarkers for immune related adverse events (IRAEs) during checkpoint blockade immunotherapy (CPI) is a key objective of current immuno-oncology research. Polymorphism within the TCRB variable gene (TRBV) has been implicated in autoimmune disease and may be mechanistically linked to IRAEs [1]. Efforts to evaluate TRBV polymorphism by traditional approaches such as whole genome sequencing (WGS) have been hampered by the repetitive nature of the TCRB locus and incomplete genome assembly. Here we present a novel long-amplicon TCRB repertoire sequencing approach to evaluate the link between TRBV polymorphism and adverse events in 55 Caucasians receiving CPI for cancer.

Introduction

Three lines of reasoning support the notion that germline encoded TRBV polymorphism could be a key determinant of IRAEs. First, the TCR locus is repetitive and structurally complex, impeding the measurement of variation by traditional short read WGS or microarray based methods; second, single amino acid substitutions within the framework or CDR 1 and 2 regions of the rearranged TCRB chain are know to significantly alter TCR affinity for HLA; and third, adverse events during CPI may manifest as acute versions of chronic autoimmune diseases that have been separately linked to TRBV polymorphism. Identifying predictive biomarkers for IRAEs may be critical for combination and neoadjuvant use of CPI for cancer.

Figure 1. Percentage of Melanoma Subjects Having Severe (Grade 3 or higher) Adverse Events. Adaptive from Larkin et al. 2015



Predictive biomarkers for IRAEs could enable:

- Personalized drug selection and dosing
- Expanded use of combination therapies

→Safer and more effective immunotherapy

Methods

Workflow

- FR1-C multiplex PCR from 25ng PBL cDNA
- NGS library templating and sequencing
- Annotate rearrangements by alignment to IMGT
- Identify and eliminate indel and PCR errors
- Report VDJ rearrangements
- Identify non-IMGT alleles and construct allele profiles
- K-means clustering to identify haplotype groups
- Associate TRBV haplotypes with phenotypes of interest

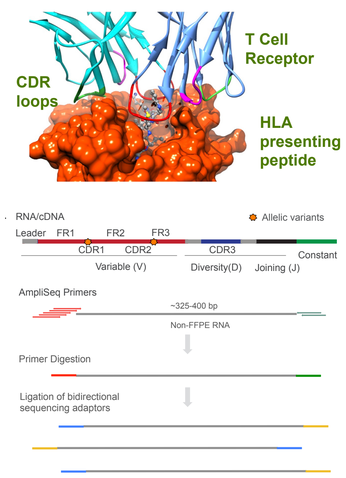


Figure 2 Overview of Workflow. The Oncomine TCRB-LR assay utilizes AmpliSeq multiplex PCR primers to target the TCRβ Framework 1 and Constant regions, enabling clonotyping and detection of TRBV gene polymorphism linked to adverse events during immunotherapy.

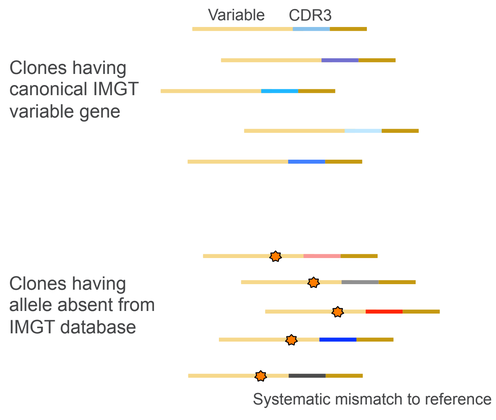


Figure 3. Strategy for identification of non-IMGT variable gene alleles. Bone fide novel alleles will present as systematic mismatches to IMGT across a plurality of clones, each possessing a distinct CDR3 nucleotide sequence.

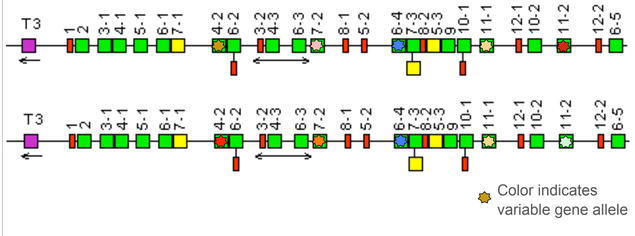


Figure 4. Cartoon of the TRB locus for two distinct haplotypes. The TRB locus contains tandemly arranged polymorphic variable genes. The Oncomine TCRB-LR assay allow one to detect all of the variable gene alleles present in a repertoire. This data can be used to infer the haplotype of the TRB locus.

Results

Category	Subdefinition	Overall	Grade 1-2 AE	Grade 3-4 AE	P-Value
	Number of Individuals	55	44	11	NA
Age					
	Median (range)	65 (34-84)	64 (42-84)	67 (34-77)	0.97
Sex					
	M	34	27	7	1
	F	21	17	4	
Ethnicity					
	Caucasian	54	44	10	0.20
	Unknown	1	0	1	
Cancer					
	Melanoma	26	19	7	0.73
	Adenocarcinoma	15	12	3	
	Urothelial Carcinoma	5	4	1	
	Renal Carcinoma	1	1	0	
	Squamous cell carcinoma	7	7	0	
	Unknown	1	1	0	
Treatment					
	Ipilimumab (CTLA-4)	25	18	7	0.18
	Nivolumab (PD-1)	18	17	1	
	Pembrolizumab (PD-1)	12	9	3	
Response					
	PD	14	11	3	0.77
	SD	9	8	1	
	PR	7	7	0	
	CR	3	3	0	
	Unknown	22	15	7	
Repertoire Features					
	Reported reads per sample (thousands)	552 (94-1718)	567 (94-1247)	533 (159-1718)	0.65
	Clones Detected (thousands)	32 (5-70)	32 (5-70)	30 (14-62)	0.64
	Clone Size Evenness	.86 (.46-.96)	.84 (.56-.96)	.88 (.46-.94)	0.87

Figure 5: Sample annotations and repertoire features for study cohort consisting of 55 Caucasians who developed adverse events grades 1-4 following CPI monotherapy. Samples derive from the Roswell Park Cancer Research Institute. Libraries were prepared and sequenced by OmniSeq using 25ng cDNA derived from peripheral blood leukocyte total RNA.

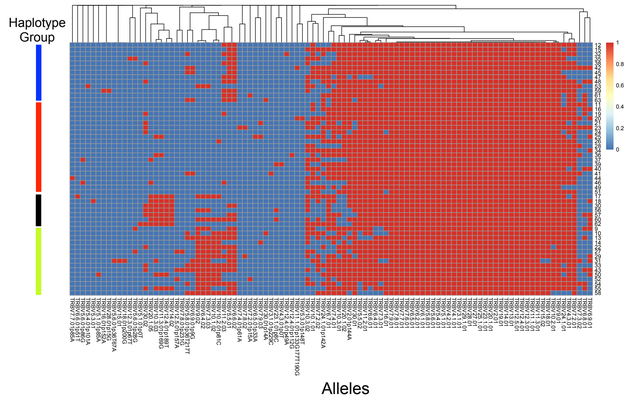


Figure 6. Heatmap of TRBV allele profiles for study cohort. TCRB repertoires were used to construct variable gene allele profiles for each individual. The sets of alleles detected in each individual are displayed in heatmap form, where each row represents a different individual and each column a different variable gene allele. Red tiles indicate that an allele was detected in an individual while blue tile indicate allele absence. Columns are arranged via hierarchical clustering, while rows are arranged according to haplotype group classification produced by k-means clustering.

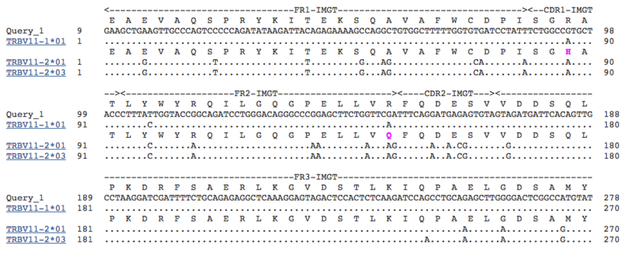
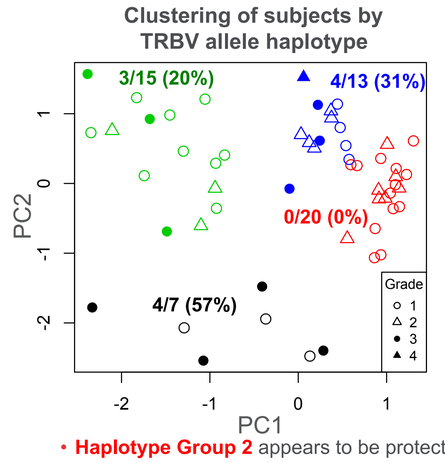


Figure 7. Example of a non-synonymous IMGT variant. IgBLAST alignment of an allele having two amino acid substitutions compared to the best matching IMGT allele. This particular allele was detected in our samples and the Lym1K database derived from 1000 genomes data. Detection of non-IMGT alleles improves the resolution of uncommon haplotypes.

Results



- **Haplotype Group 2** appears to be protected from severe adverse events.

Haplotype Group	Grade 1 or 2 AE	Grade 3 or 4 AE
1	9	4
2	20	0
3	3	4
4	12	3

p = .0024, Fisher's Exact Test

Figure 8. Principal component analysis of TRBV allele profiles was used to subdivide samples into four haplotype groups. Each point represents a different individual, while symbol indicates grade of adverse event. Haplotype group 2, comprising ~33% of this cohort, appears to be protected against severe adverse events following CPI.

Conclusions

- Long amplicon TCRB repertoire sequencing may be used to perform haplotype analysis of the repetitive and structurally complex TRB locus.
- We detected four major haplotype groups in a cohort consisting of 55 Caucasians.
- Members of haplotype group 2 showed no severe events during immunotherapy.
- Expanding the cohort size would reveal additional haplotypes and improve prediction of adverse events.
- TRBV polymorphism is germline encoded and therefore may serve as a true predictive biomarker for IRAEs following CPI for cancer.

References

1. Looney et al. Haplotype Analysis of the TRB Locus by TCRB Repertoire Sequencing (2018). bioRxiv 406157
2. Ye et al. IgBLAST: an immunoglobulin variable domain sequence analysis tool (2013). Nucleic Acid Research W34-40.

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