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 TRC-B 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 measurement of variation by traditional short read WGS or microarray based methods; second, single amino acid substitutions within the framework or CDR1 and 2 regions of the rearranged TCRB chain are known 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.

Methods

Workflow

- FRT-LC 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.

Results

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 8: Principal component analysis of TRBV allele haplotypes 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 TRBV 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.

Predictive biomarkers for IRAEs could enable:
- Personalized drug selection and dosing
- Expanded use of combination therapies
  ➔ Safer and more effective immunotherapy

References