Molecular characterization of immune-related severe adverse events (irSAE)

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Immune checkpoint inhibitors (ICIs) have made a profound impact on the treatment of a variety of cancers. However, toxicities can occur that cause significant morbidity and/or mortality.

CIs can cause unique autoimmune toxicities, resulting in inflammation of numerous organ systems, in some cases fatal. The molecular underpinnings of these toxicities have not been extensively explored.

We recently reported a small case series of two patients with myocarditis resulting in death arising following combination ICI therapy (Johnson et al, NEJM, 2016).

High lymphocytic infiltration, coupled with PD-L1 expression was present in the affected myocardium and skeletal muscle. Common T cell clones were identified between the affected tissue and tumor, and abnormal expression of muscle-specific transcripts was identified in the associated tumor, suggesting release of peripheral tolerance to tumor-expressed self-antigens.

Objectives
1. To characterize the molecular features of ICI-AI, including severe refractory immune-related colitis, myocarditis (MC), and encephalitis following ICI treatment.
2. To determine the similarities and differences in immune components between ICI-AI colitis and inflammatory bowel disease.
3. To evaluate TCR clonality in ICI-AI diseased specimens.

Hypotheses
We hypothesize that molecular analysis of ICI-AI tissues will identify causal factors in the etiology of these toxicities, and how to better predict, prevent, and treat them.

Methods
1. To characterize the molecular features of ICI-AI, we collected healthy and afflicted tissue from a series of cancer patients with immune-related colitis, myocarditis (MC), and encephalopathy following ICI treatment.
2. We performed:
   1. Standard immunohistochemistry,
   2. RNA sequencing for >2000 immune-related genes (HTG EdgeSEQ)
   3. TCRβ sequencing by ImmunoSEQ (Adaptive Biotechnology) to evaluate T cell clonality in diseased specimens.
   4. Digital spatial profiling for 20 immune-related protein biomarkers performed in conjunction with NanoString.

Fig. 1
Expansion of shared TCR sequences in diseased specimen of ICI-induced myocarditis patient (Johnson et al, NEJM)
1. **Process:** FFPE tissue slide incubated with a cocktail of oligo conjugated antibodies
2. **View:** Regions of interest (ROIs) are identified with visible light-based imaging
3. **Profile:** Selected ROIs are chosen for high-resolution multiplex profiling, and oligos from the selected region are released upon exposure to UV light
4. **Collection:** Photocleaved oligos are then collected via a microcapillary tube and stored in a microplate well
5. **Digital counting:** Photocleaved oligos from the spatially-resolved ROIs in the microplate are hybridized to 4-color, 6-spot optical barcodes, enabling up to ~1 million digital counts of the protein targets (distributed across all targets) in a single ROI using standard NanoString nCounter® instruments

We performed nanoString DSP (technology and example described above) on 8 ICI-induced colitis (ICI-C) samples, 4 inflammatory bowel disease resections (IBD; primarily Crohn's) and 2 “normal” colon (N) samples. Twenty-nine antibodies were used for protein detection across 10 regions of interest (ROI) in each sample. Heat map representation (averages of 10 ROIs; *p<0.05 for comparison of IBD vs. ICI-C) is on the lower left, and individual markers are plotted to show intra and inter-sample heterogeneity on the lower right.
RNAseq analysis of immune-related genes shows commonality in autoimmune and ICI-induced colitis

We performed HTG EdgeSEQ on 7 ICI-induced colitis (Colitis/ICI-C) samples, 2 inflammatory bowel disease resections (Crohn’s) and 2 ‘normal’ colon (Colon- NL/NML) samples. 3 matched (pre-ICI) melanomas were also included as well as diseased and non-diseased brain specimens from a fatal encephalitis case resulting from anti-PD-1 treatment. RNA sequencing targeted >2000 immunerelated transcripts. A) Ward’s distance measure was used for hierarchical clustering of all samples across all 2000+ genes. B) Heatmap representation (row-scaled) showing similarities and dissimilarities among samples. Crohn’s and ICI-C samples tend to cluster together. C) PCA analysis of data shows similar patterns of commonality and the transcriptome level between ICI-colitis and Crohn’s/IBD.

Common T cell clones observed among colitis specimens

We performed TCRβ sequencing from ICI-C biopsies, matched melanomas (pre-treatment), and unmatched Crohn’s disease colon resections. Common TCR clones were clearly detected in melanomas before ICI therapy, and in biopsies of severe refractory ICI-C specimens (after ICI therapy; A). We also detected shared TCR sequences among Crohn’s and ICI-C samples B). Shared clones were also relatively prevalent across Crohn’s patients C), but were substantially rarer across ICI-C patients D).
High degree of clonality and inflammation in a unique case of fatal encephalitis after PD-1 treatment

A recent patient treated with anti-PD-1 at our institution for metastatic melanoma presented with encephalitis secondary to ICI therapy that ultimately proved fatal. We performed IHC for immune biomarkers in healthy and inflamed brain tissue from the case. We also evaluated these tissues by RNAseq (shown to the left). Finally, we evaluated T cell clonality by TCRβ sequencing. We detected a high degree of both CD8 and CD4 cells in the brain microenvironment that localized to sites of inflammation. Interestingly, the immune profile by RNAseq of this tissue was more similar to a melanoma profile than the colonic (ICI-AI or Crohn's) environment. We also detected a substantial degree of clonality (~20% of T cells in the inflamed brain), suggesting a unique antigen target.

Conclusions

• Many similarities in terms of protein and mRNA expression patterns can be identified across auto-immune and ICI-induced colitis specimens.
• A tendency toward greater T cell infiltrate and reduced B cell infiltrate was identified in ICI-C specimens.
• Shared T cell clones were detected in all sample types, suggesting the possibility of TCR-mediated autoimmunity and commonalities. However, the antigen specificity of these clones is not known and HLA haplotyping has not yet been performed.
• Continued studies of differentially expressed cell types and gene expression markers may help better understand the disease process in ICI-C.
• A unique case of fatal encephalitis identified an extremely high degree of TCR clonality in the heavily inflamed portion of the brain at autopsy. Efforts to identify the target antigen, as well in cases of myocarditis and refractory colitis are underway.

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