We recently reported a small case series of two patients with immune checkpoint inhibitors (ICIs) causing 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 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 clonality and analogous T cell populations 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 antigens.

**Background**

- Immune checkpoint inhibitors (ICIs) have made a profound impact on the treatment of a variety of cancers. However, toxicities can occur that cause significant mortality and morbidity.
- Clp 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 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 clonality and analogous T cell populations 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 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 affected 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. TCR sequencing by ImmuneSEQ (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.

**Results**

We performed RNAseq analysis of immune-related gene expression markers shows commonality in autoimmune and ICI-induced colitis.

**Conclusions**

- Many similarities in terms of protein and mRNA expression patterns can be identified across auto-immune and ICI-induced colitis.
- 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-mediating 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 clonality (>20% T cells in the inflamed brain), suggesting a unique antigen target.

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