**Abstract #2591**

**Evidence for selective silencing of MHC-binding neoepitopes to avoid immune surveillance**

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**Background**

Overall response rates to immune checkpoint inhibition (ICI) are <50% even in Tumor Mutation Burden (TMB)-high patients (e.g. Checkmate-227), suggesting other mechanisms of immune escape exist beyond checkpoint inhibition. At least 18% of somatic-specific exonic DNA variants are not expressed into mRNA (Rafizabadi, 2018), yet the selection criteria for which variants to silence remains unclear. We sought to determine if Immunogenicity of variants factors Into their suppression

**Methods**

1. 14B clinical cases with paired tumor/normal whole-exome (>150x coverage) and whole-transcriptome (200kx10^6 reads) were available from the NantHealth database
2. TMB was calculated by counting somatic-specific non-synonymous exonic mutations. High-TMB was defined as >200 exonic mutations as in Ribizil et al, 2015
3. All possible 9-mer neoepitopes resulting from SNV or INDEL variants were generated and assessed for immunogenicity by NetMHC-4.0. For each variant, the neoepitope with the highest predicted affinity was analyzed further
4. Neoepitopes were designated as non-expressed if fewer than 2 RNA reads supported the generating variant
5. Immune-cell infiltration was estimated using RNA deconvolution on known immune cell marker genes (Binda et al, 2013)

**Results**

- TMB is highly correlated with neoantigen load (Fig 1 & Table 1). For each variant, the neoepitope with the highest predicted affinity was analyzed further
- Diverse neoepitopes were predicted to contribute to a silencing phenomenon (Fig 2).
- Silencing of potential neoepitopes was most prominent in high-TMB patients. Over 90% of patients had a non-expressed neoepitope predicted to be a strong binder (Fig 3).
- High-TMB patients almost all express at least one high-affinity neoepitope (Fig 4).
- Silencing of potential neoepitopes was most prominent in 19% of patients with high inferred immune infiltration but low PD1L expression (N = 261, OR = 1.37, p = 2.0x10^-16).
- TMB and neoantigen load are highly similar biomarkers.

**Conclusions:**

We observe significant preferential silencing of MHC-binding neoepitopes. Specifically, when tumor infiltrating immune cells are activated, silencing neoepitopes may be an alternative to checkpoint expression for avoiding an immune cascade. Patients with TILs and silenced neoepitopes may benefit from epigenetic priming therapy prior to ICI therapy.

**Figure 1.** Checkpoint description. Aggregated demographics statistics for 14B clinical cases with paired tumor/normal whole-exome (>150x coverage) and whole-transcriptome (200kx10^6 reads) were available from the NantHealth database. Specific variants are grouped as known immune cell marker (left), unknown immunomodulator (center), or unknown (right).

**Figure 2.** Analysis of expression targets using RNA deconvolution on known immune cell marker genes (Binda et al, 2013). Variants were considered non-expressed if fewer than 2 RNA reads were supported.

**Figure 3.** Immunogenicity of predicted neoantigen load for 14B clinical cases with paired tumor/normal whole-exome (>150x coverage) and whole-transcriptome (200kx10^6 reads) were available from the NantHealth database. Specific variants were considered as non-expressed if fewer than 1 RNA reads were supported. Amino acid (AA) changes were analyzed individually.

**Figure 4.** Proposed immune evasion mechanisms. Treatment of silencing in immune-activated low PD1L patients suggests novel silencing mechanism as an alternative to increased expression. Additional experiments and clinical cases are needed to assess silencing neoepitope expression.