Despite the unprecedented efficacy of checkpoint blockade (CPB) therapy in treating some cancers, a large number of patients still fail to respond to these interventions. Recent data indicate that the combination of CPB and neoantigen vaccines may prolong survival in some cancer patients.

Current approaches to neoantigen prioritization involve deep sequencing of tumor samples, followed by selection of epitopes based on prediction algorithms primarily examining MHC class I binding affinity and processivity. However, this strategy has proved challenging with the majority of candidates lacking immunogenic activity in patients.

ATLAS™ is a T cell antigen discovery platform in which putative suppressive antigens are presented as peptide epitopes in the context of their own MHC class I or II molecules.

We identified both MHC class I and II neoantigens inducing T cell cytokine responses in a non-small cell lung cancer (NSCLC) patient. Approximately 50% of MHC class I neoantigens were not predicted by multiple in silico methods; MHC class II neoantigens can not be effectively predicted using current in silico methods.

Methods: The ATLAS™ Platform

Figure 1. Lysteryolio O (cLLO) facilitates MHC class I presentation by MDDC

Figure 2. ATLAS™ technology workflow

Figure 3. Expression verification

Figure 4. Good alignment between replicate measurements for each cytokine

CD8+ T cell responses

Figure 5. Multiple neoantigens were identified through CD8+ T cell responses pre- and post-checkpoint blockade therapy

Results

Non-specifically expanded CD4+ or CD8+ T cells were screened in duplicate against autologous MDDC individually pulsed with E. coli expressing each of 195 or 201 somatic mutations, respectively. Clones that induced mean IFNγ or TNFα responses that were statistically different from background (Wilcoxon Rank Sum, p<0.05) and exceeded 3 standard deviations (SD) of the mean of the negative control NeoGreen clones (N=20) were considered antigens (indicated by horizontal dotted line).

Table 1. Mutations identified as neoantigens based on IFNγ and TNFα responses

CD4+ T cell responses

Figure 6. Increased breadth of CD4+ T cell IFNγ responses to potential neoantigens post checkpoint blockade therapy

Conclusions

ATLAS was able to detect neoantigen-specific immune responses in NSCLC patients both pre- and post-CPB therapy.

Based on ATLAS, not all T cell antigens are stimulatory; suppressive responses to T cell antigens were also observed; while these epitopes may emerge from predictive algorithms, their suppressive activity cannot be predicted.

CD4+ and CD8+ T cell antigens are mostly unique with only 3% of putative neoantigens eliciting responses from both T cell subsets

Breadth of CD4+ T cell responses based on IFNγ secretion increased upon checkpoint therapy

Current MHC class I epitope prediction algorithms have a high false positive rate, and missed a large number of relevant and inhibitory antigens.

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