Integrating CD8 and CD4 effector neo-epitope content with regulatory T cell epitope exclusion is a superior prognostic biomarker for bladder cancer patient compared to their tumor mutation burden

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Background:
We hypothesized that neo-epitope-based prediction using an advanced in silico T cell epitope screening system (Ancer™) may better identify patients with improved prognosis than tumor mutation burden. Analysis of genomic data derived from the muscle-invasive bladder cancer (BLCA) cohort of The Cancer Genome Atlas (TCGA) database for CD4, CD8, *and* Treg neo-epitopes was performed to determine whether Ancer™ would improve prognostic stratification compared to tumor mutational burden (TMB).

Methods:
BLCA patient mutanomes (n=412) were retrieved from the TCGA and evaluated with Ancer™, an innovative and automated neo-epitope screening platform that combines proprietary machine learning-based HLA I and HLA II neo-epitope identification tools with removal of inhibitory regulatory T cell epitopes for neo-epitope ranking and personalized cancer vaccine design. BLCA patients were separated based on median TMB or neo-epitope burdens. We investigated the effect of integrating both CD8 and CD4 neo-epitope burdens as most mutanome pipelines exclusively focus on the identification of Class I neo-epitopes. Overall survival was analyzed using the Kaplan-Meier method and differences analyzed by log-rank testing.

Results:
Compared to low TMB, high TMB was significantly associated with improved survival ($p = 0.0001$, difference of 38.5 months in median survival, Figure 1). Improved differentiation of median survival times was obtained when separating patients based on their Class I neo-epitope content, as estimated by Ancer™ ($p < 0.0001$, difference of 59.8 months in median survival). Adding Class II neo-epitope burden further increased separation of OS times, showcased by a 69.6-month increase in median survival for BLCA patients with both high CD8 and high CD4 neo-epitope contents compared to other patients ($p = 0.0001$). Since we discovered that Class II neo-epitopes can induce inhibitory responses, we further evaluated whether the screening of these detrimental sequences could improve our analysis. Upon identifying Class II neo-epitopes likely to induce T effector (Teff) responses, we found that the median survival of patients with high CD8 and high CD4 Teff contents was extended by nearly 4 months to 73.4 months compared, to the remainder of the cohort ($p < 0.0001$, Figure 2).

Conclusions:
Our analysis suggests that optimal host-immune recognition of CD8+, CD4+, and Treg epitopes plays a key role in cancer survival. While defining CD8 neo-epitope burden enhanced associations with OS, the inclusion of CD4 Teff neo-epitope burden substantially helped identify long-term survivors. These results suggest that defining the number of true neo-epitopes using Ancer™ may represent a novel prognostic or predictive biomarker.