EMPIRIC PROFILING OF PERIPHERAL T CELL RECALL RESPONSES TO TUMOR MUTANOMES VERSUS IN SILICO PREDICTIONS IN NSCLC PATIENTS UNDERGOING PEMBROLIZUMAB TREATMENT ± CHEMOTHERAPY

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Background Effective immune checkpoint blockade (ICB) treatment is dependent on T-cell recognition of patient-specific mutations (neoantigens). Empirical identification of neoantigens ex vivo has revealed shortcomings of in silico predictions.¹ To better understand the impact of ICB treatment on T cell responses and differences between in silico and in vitro methods, neoantigen-specific T cell responses were evaluated in patients with non-small cell lung cancer undergoing first-line therapy with pembrolizumab ± chemotherapy.

Methods Tumor and whole blood samples were collected from 14 patients prior to and after immunotherapy; seven each in monotherapy and combination therapy cohorts. The ex vivo ATLAS™ platform was used to profile neoantigen-specific T-cell responses. Patient-specific tumor mutations identified by next-generation sequencing (NGS) were expressed individually as ATLAS clones, processed patient-specific autologous antigen presenting cells, and presented to their T cells in vitro. ATLAS-verified antigens were compared with epitope predictions made using algorithms.

Results On average, 150 (range 37–339) non-synonymous mutations were identified. Pre-treatment, ATLAS identified T cell responses to a median of 15% (9–25%) of mutations, with nearly equal proportions of neoantigens (8%, 5–15%) and Inhibigens™, targets of suppressive T cell responses (8%, 3–13%). The combination therapy cohort had more confirmed neoantigens (46, 20–103) than the monotherapy cohort (7, 6–79). After treatment, the median ratio of CD4:CD8 T cells doubled in the monotherapy but not combination cohort (1.2 to 2.4 v. 1.6 to 1.3). Upon non-specific stimulation, T cells from patients on combination therapy expanded poorly relative to monotherapy (24 v. 65-fold, $p = 0.014$); no significant differences were observed pre-treatment (22 v. 18-fold, $p = 0.1578$). Post-treatment, the median number of CD8 neoantigens increased in the combination therapy cohort (11 to 15) but in monotherapy were mostly unchanged (6 to 7). Across timepoints, 36% of ATLAS-identified responses overlapped. In silico analysis resulted in 1,895 predicted epitopes among 961 total mutations; among those, 30% were confirmed with ATLAS, although nearly half were Inhibigens, which could not be predicted. Moreover, 50% of confirmed neoantigens were missed by in silico prediction.

Conclusions Monotherapy and combination therapy had differential effects on CD4:CD8 T cell ratios and their non-specific expansion. A greater proportion of neoantigens was identified than previously reported in studies employing in silico predictions prior to empirical verification.² Overlap between confirmed antigens and in silico prediction was observed, but in silico prediction continued to have a large false negative rate and could not characterize Inhibigens.

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