

579-C REINVIGORATION OF PROGENITOR-EXHAUSTED CD8 T CELLS BY ANTI-CTLA-4 CONTRIBUTES TO THE SUSTAINED ACTIVITY OF COMBINATION CHECKPOINT BLOCKADE

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Background Combination checkpoint blockade with anti-PD-1 and anti-CTLA-4 antibodies has shown promising efficacy in melanoma. However, the underlying mechanism in humans remains unclear. A better understanding of the cellular and molecular mechanisms of α PD-1 and α CTLA-4 individually, and in combination, will guide the development of safer and more effective combination immunotherapy strategies.

Methods We performed multimodal (scRNA + TCR + epitope) analysis across time in 32 stage IV melanoma patients treated with anti-PD-1, anti-CTLA-4, or anti-PD-1 + anti-CTLA-4 (combination) therapy. In order to understand the effect of checkpoint blockade on T cells at a single-clone resolution, we developed a novel algorithm *Cyclone* to track temporal clonal dynamics and their underlying cell states.

Results Anti-CTLA-4 induced more durable immune responses than anti-PD-1, whereas combination therapy mobilized greater and more sustained immune responses. Using *Cyclone*, we identified 6 clonotypic trajectories with distinct temporal patterns. These analyses revealed that checkpoint blockade induced waves of immune responses composed of distinct clonotypes that peaked at different timepoints. Combination therapy generated clonal effector and exhausted CD8 T cells (T_{EX}) responses that peaked at 6–9 weeks after treatment. Focused analyses of T_{EX} in additional cohorts identified that anti-CTLA-4 induced robust expansion and proliferation of progenitor T_{EX} , which synergized with anti-PD-1 to generate a large and durable reinvigoration of T_{EX} . Immune profiling of a cohort of patients that first received anti-CTLA-4, followed by anti-PD-1 revealed that these enhanced progenitor responses were largely due to anti-CTLA-4 therapy. The induction of progenitor T_{EX} by anti-CTLA-4 were independently validated using samples collected from the Checkmate 238 clinical trial of adjuvant nivolumab versus ipilimumab in resectable melanoma.

Conclusions First, durable immune responses represent waves of immune responses generated by different T cell clones. Second, progenitor T_{EX} induced by CTLA-4 blockade may contribute to durable immune responses through self-renewal and replenishing of the T_{EX} pool.

Ethics Approval Patients were consented for blood collection under the University of Pennsylvania Abramson Cancer Center's melanoma research program tissue collection protocol UPCC 08607, in accordance with the Institutional Review Board. For specimens from Checkmate 238, PBMC were

obtained following informed consent under an IRB-approved protocol at NYU.

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