

### CONCURRENT NEOANTIGEN VACCINATION ENHANCES THE ANTITUMOR EFFECT OF DYSFUNCTIONAL T CELLS DURING CELL THERAPY

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**Background** Adoptive cell therapies (ACT) utilizing neoantigen-specific T cells represents a promising approach to treat metastatic solid tumors.<sup>1-3</sup> Multiple studies have demonstrated that antitumor tumor infiltrating lymphocytes (TIL) are highly enriched in dysfunctional T cell subsets defined by the expression of the marker CD39.<sup>4-6</sup> We previously showed that response to TIL-ACT administered to metastatic melanoma patients was mediated by a small pool of CD39- stem-like T cells, while ACT due to CD39+ CD69+ dysfunctional T cells do not mediate tumor regressions.<sup>6</sup> Here, we sought to evaluate if concurrent vaccination can enhance the poor antitumor response due to ACT containing largely dysfunctional T cells.

**Methods** We repeatedly stimulated Pmel murine T cells and subsequently isolated CD39+ CD69+ dysfunctional T cells (DP-Pmel), and less-dysfunctional CD39<sup>low</sup> T cells targeting the hgp100<sup>KVP</sup>-mutated neoepitope for ACT against large established vascularized murine tumor models.<sup>6, 7</sup> We investigated the synergistic effect of multiple neoantigen vaccine modalities during ACT using these two T cell subsets. We studied the mechanisms of vaccine-mediated rescue and investigated post-ACT phenotype and dynamics of transferred T cells. Lastly, we studied the phenotypic states of transferred TIL and tracked the persistence of GP100 targeting TIL clones in a human melanoma patient who responded to ACT only after receiving concurrent fowlpox GP100 vaccine.<sup>8</sup>

**Results** Intravenously administered vaccinia-virus expressing hgp100<sup>KVP</sup>-neoepitope (VACV<sup>KVP</sup>+DP-Pmel), or anti-CD40 with hgp100<sup>KVP</sup>-neopeptide administration ( $\alpha$ CD40<sup>KVP</sup>+DP-Pmel) rescued the poor antitumor effect of dysfunctional DP-Pmel in neoantigen tumor models (figure 1). Mechanistically, VACV<sup>KVP</sup>+DP-Pmel rescue was contingent on host antigen presentation and expression of neoepitope in the vaccine backbone. Vaccine rescue of DP-Pmel was impacted by host B7.1/B7.2 blockade while  $\alpha$ CD40<sup>KVP</sup>+DP-Pmel was unimpacted. Relative to DP-Pmel ACT alone, VACV<sup>KVP</sup>+DP-Pmel ACT that mediated tumor regression was associated with a significant persistence of transferred dysfunctional DP-Pmel T cells in spleen (12-fold), draining lymph nodes (3-fold), and in tumor (2.5-fold). In a human melanoma patient who only responded to TIL-ACT after concurrent GP100 vaccine administration, TIL infusion product contained > 93% CD39+ dysfunctional

antitumor GP100-TIL with an increased persistence of transferred GP100 TIL clones only after concurrent fowlpox-GP100 vaccine administration, suggesting the antitumor rescue effect of vaccine on largely dysfunctional TIL infusion rather than denovo vaccine induced T cell responses.

**Conclusions** ACT using terminally dysfunctional antitumor T cells is rescuable by a neoantigen vaccine in murine models and may be relevant to ACT targeting human neoantigens. Mechanisms behind the rescue of effect of neoantigen vaccine during dysfunctional T cell ACT is ongoing.

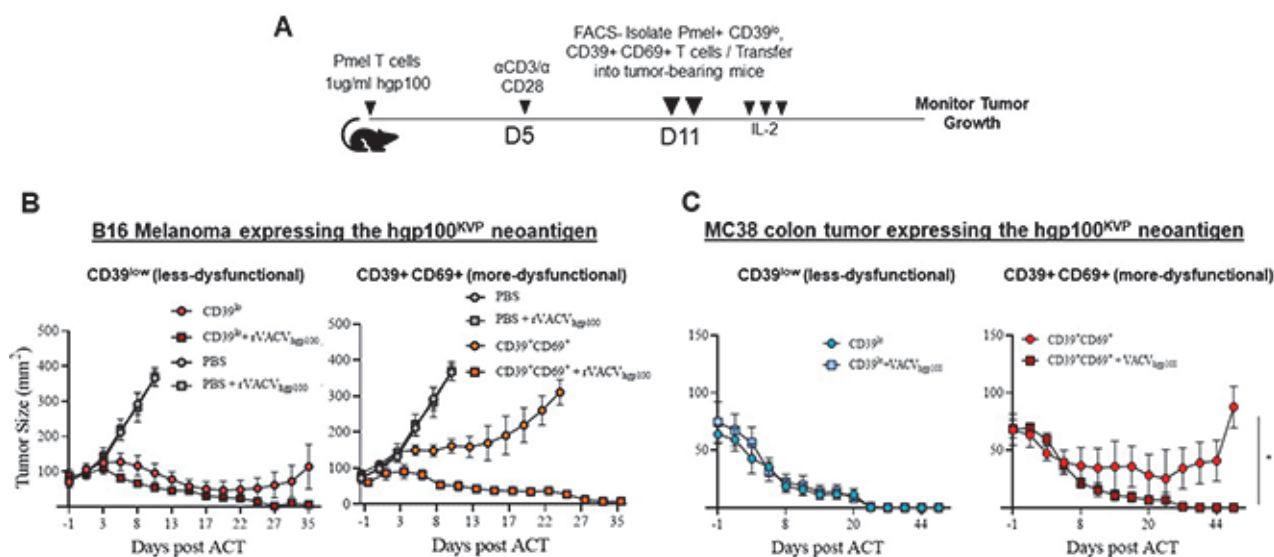
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**Ethics Approval** All mice experiments utilizing tumors were implanted on mice was approved under the Institutional Review Board, NIH, animal protocol number SB 194. Human TIL samples used in this study were obtained from patients enrolled on NCT00068003 and NCT00001823 NIH clinical protocols, both approved by the institution review board of the National Cancer Institute. Informed consent was obtained and documented in accordance with the Declaration of Helsinki.

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**Abstract 369 Figure 1** (A) Schema for ACT using terminally differentiated dysfunctional Pmel TCR transgenic T cells. Splenocytes from Pmel TCR transgenic mice were stimulated twice in vitro, expanded, and CD39<sup>+</sup> CD69<sup>+</sup> dysfunctional T cells and CD39<sup>lo</sup> less-dysfunctional T cells are isolated and immediately transferred to large tumor bearing mice (>50mm<sup>2</sup>). Effect of concurrent vaccine administration (vaccinia virus expressing the hgp100KVP-neoepitope) on less-dysfunctional CD39<sup>lo</sup> T cells and dysfunctional DP-Pmel on B16-melanoma tumor with neoantigen (B) and MC38 colon tumor with neoantigen (C). PBS- untreated, and PBS + VACV<sub>hgp100</sub> vaccine alone controls are shown.

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