PD1 AND LAG3 SYNERGIZE ON CD8+ T CELLS TO HINDER IFN-γ-DEPENDENT ANTI-TUMOR IMMUNITY

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Background
Overcoming immune-mediated resistance to PD1 blockade remains a challenge, however enhanced efficacy has now been demonstrated in metastatic melanoma patients with a combinatorial regimen of nivolumab and relatlimab, an anti-LAG3 targeting antibody recently FDA approved. Despite this success, little is known about how PD1 and LAG3 synergize to hinder anti-tumor immunity, particularly on CD8+ T cells, which is the dominant LAG3-expressing intratumoral population signifying highly dysfunctional exhausted T (T$_{EX}$) cells regulated by the transcription factor TOX.

Methods
To understand the cellular and mechanistic basis for PD1/LAG3 synergy, conditional knock-in mice “surgically dissect” Pdcd1 and/or Lag3 floxed alleles restricted to CD8+ T cells expressing E8ICre.GFP. These mice were crossed with the pMEL transgene to assess PD1 and/or LAG3-sufficient or deficient gp100-specific CD8+ T cell populations adoptively transferred into mice harboring a B16-gp100-overexpressing tumor. Single-cell RNAseq analysis was performed, identifying both effector and IFN-γ-stimulated genes up-regulated in PD1/LAG3-deficient pMEL. The IFN-γ signaling pathway was further interrogated by retroviral transduced knockdown of IFN-γR in pMEL.

Results
While little therapeutic benefit was observed with a prophylactic adoptive transfer (AT; d-1) of wild-type CD8+ pMEL cells into mice, there was reduced B16-gp100 tumor growth in mice receiving PD1-deficient CD8+ pMEL cells, which was further enhanced in mice receiving PD1/LAG3-deficient CD8+ pMEL cells with long-term tumor-free survival. Likewise, therapeutic AT (d6) of PD1/LAG3-deficient CD8+ pMEL cells showed initial therapeutic benefit, with enhanced survival, that was not demonstrated with AT of PD1 or LAG3-deficient, or wild-type, counterparts.

PD1/LAG3-deficient CD8+ T cells were shown to be more proliferative and more functional, with increased IFN-γ and GzmB observed by flow cytometry. Interestingly, PD1 and LAG3 were demonstrated to synergize together to modulate the T$_{EX}$ program by restraining the expression of TOX and promoting a more terminally-exhausted phenotype defined by expression of TIM3 and Ly108.

As this population was shown to be transcriptionally distinct by transcriptomic analysis, we interrogated whether enhanced IFN-γ signaling was responsible for the phenotypes observed. Retroviral knockdown of IFN-γR reversed the therapeutic benefit observed with AT of PD1/LAG3-deficient pMEL. In addition, other IFN-responsive genes upregulated in PD1/LAG3-deficient pMEL, such as CCL5, were reduced with IFN-γR knockdown.

Conclusions
Overall PD1 and LAG3 limit antitumor immune effects as PD1/LAG3-deficient pMEL AT resulted in reduced tumor growth and enhanced survival due to increased functionality, dependent on IFN-γ signaling. These results provide mechanistic insight for the success seen with the clinical development of anti-LAG3 agents in combination with anti-PD1.