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

Lawrence Andrews*, Carly Cardello, Jian Cui, Creg Workman, Dario Vignali. University of Pittsburgh, Pittsburgh, PA, USA

**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 IFNγ-stimulated genes up-regulated in PD1/LAG3-deficient pMEL. The IFNγ signaling pathway was further interrogated by retroviral transduced knockdown of IFNγR 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 IFNγ 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 IFNγ signaling was responsible for the phenotypes observed. Retroviral knockdown of IFNγR 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 IFNγR 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 IFNγ signaling. These results provide mechanistic insight for the success seen with the clinical development of anti-LAG3 agents in combination with anti-PD1.