A POPULATION OF ECTOENZYME EXPRESSING T-CELLS WITH AN IMMUNOSUPPRESSIVE PHENOTYPE ARE ASSOCIATED WITH CHECKPOINT IMMUNOTHERAPY RESISTANCE IN METASTATIC MELANOMA PATIENTS

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Background Therapies targeting T-cell checkpoints have resulted in anti-tumor responses leading to FDA approval of multiple immunotherapies for metastatic melanoma and an expanding list of other malignancies. Despite having unprecedented efficacy, PD1 and CTLA4 antagonist antibodies still fail to benefit many patients. Critically, an understanding of mechanisms of resistance to immunotherapies is lacking. We recently identified and validated a novel population of peripheral blood T-cells predictive of resistance in nivolumab (aPD1) treated metastatic melanoma patients. This population is defined by the marker set CD3+CD4+CD127-GARP-CD38+CD39+. Based on the co-expression of CD38 and CD39, we have termed the population ectoenzyme expressing T-cells (Teee).

Methods We utilized high-dimension flow cytometry to assess Teee frequencies in adjuvant immunotherapy treated metastatic melanoma patient peripheral blood samples. To characterize the phenotype of Teee we utilized flow cytometry and CITE-Seq on melanoma patient tumor specimens. We also evaluated the presence and phenotype of Teee in several syngeneic murine tumor models.

Results We found that Teee were of low frequency in demographic matched healthy donors and increased in both resected (p=0.018) and active disease metastatic melanoma patients (p=0.003). Circulating Teee frequencies positively correlated with frequencies of Tregs (R²=0.4367, p<0.0001), MDSCs (R²=0.2706, p=0.0004) and M2-like monocytes (R²=0.1514, p=0.0109). Increases in circulating Teee were associated with relapse in resected melanoma patients treated with adjuvant combination ipilimumab (aCTLA4) and nivolumab (p=0.0213). Human tumors showed high frequencies of Teee in tumor infiltrate. We also demonstrated the existence of this population in MC38 and YUMM mouse tumor models. Intratumor frequencies of Teee positively correlated with tumor volume (R²=0.96, p=0.0006) and inversely correlated with overall immune infiltrate (R²=0.3198, p=0.0280). Our characterization of this population showed an enhanced adenosine generating phenotype (i.e. CD73high, CD26low), a terminal exhaustion phenotype (i.e. TOXhigh, TCF1low), expression of inhibitory receptors (e.g. CTLA4, TIM3) and ligands (e.g. PD1L, B7-H4), and expression of immunosuppressive cytokines (e.g. IL-8, TGFβ). Supporting a mechanistic relationship to resistance, Teee suppressed autologous T-cell proliferation (p=0.0278) and inflammatory function.

Conclusions We have identified a novel population of ectoenzyme expressing T-cells associated with immunotherapy resistance in metastatic melanoma patients. This population of cells had phenotypes associated with immune suppression and suppressed autologous T-cells in vitro. Collectively, our data support evaluation of targeting Teee to augment the efficacy of immunotherapy.

Ethics Approval Study was approved by IRB at CU Anschutz and NYU Langone.