VOLUMETRIC MUSCLE LOSS INJURY IMPACTS MURINE TUMOR GROWTH AND RESPONSE TO IMMUNE CHECKPOINT BLOCKADE THERAPY

Anna Ruta*, Jordan Garcia, Locke Davenport Huyer, Joselyn Mejias, Kavita Krishnan, Helen Nguyen, Elise Gray-Gaillard, Jin Han, Andrew Pardoll, Jennifer Elisseeff. Johns Hopkins University, Baltimore, MD, United States

**Background** Tumor resection and subsequent surgical reconstruction is a prevalent, often effective, treatment approach for many operable solid cancers. Unfortunately, post-operative surgical trauma and tissue injury induce major physiological stressors (i.e. acute inflammation and subsequent wound-healing) that can contribute to cancer progression, recurrence and metastatic spread. In this study, we investigate whether effectively treating distal tissue injuries with biologically-derived wound-healing therapeutics can help mitigate injury-induced accelerated tumor progression. Given the numerous clinical trials underway that couple surgical intervention with (neo)adjuvant immune checkpoint blockade (ICB) treatment, we further explore whether physiological stress due to tissue injury can impact tumor responsiveness to ICB therapy.

**Methods** A concurrent biological insult murine model was established in which a bilateral volumetric muscle loss (VML) injury, consisting of a 3x4mm excisional defect performed in the quadriceps muscles, is followed by subcutaneous inoculation of syngeneic cancer cells on the flank (CT26 colon carcinoma and B16F10 melanoma, 100,000 cells/mouse). Wound-healing was promoted by directly implanting a porcine-derived decellularized extracellular matrix (ECM) scaffold into the muscle injury site. The ICB treatment regimen consisted of either aPD1 (5mg/kg, RMP1-14) monotherapy or aPD1/aCTLA4 (5mg/kg, 9H10) combination therapy delivered via intraperitoneal injection for 4 total doses. Tumor size was measured every 2-3 days with predetermined survival criteria of tumor volume >1500mm³. The immunological landscape of tumors and draining lymph nodes was assessed via high-parameter flow cytometry, transcriptional analysis (RT-qPCR, bulk RNA sequencing) and immunofluorescence staining of paraffin-embedded tissue sections.

**Results** VML injury accelerated CT26 and B16F10 tumor growth in comparison to the non-injured control group. However, treatment of the VML injury site with pro-regenerative ECM scaffold slowed tumor progression to match the non-injured baseline. Tumors harvested from mice with ECM-treated muscle injuries displayed an elevated type 2 immune signature, marked by CD206+ M2 macrophages and interleukin (IL)-4 production by CD4+ T cells and myeloid cells. Lastly, mice with concurrent VML injury exhibited impaired response to ICB treatment measured by faster tumor growth and shortened overall survival.

**Conclusions** Physiological stress induced by tissue injury can impact tumor growth kinetics, immunological phenotype of tumor microenvironment, and responsiveness to ICB therapy. Treatment of injury site with wound-healing biomaterials may offer a novel approach to reducing exacerbated tumor outcomes. Further studies will help discern the immunological mechanisms that connect concurrent tissue injury and tumor insults.

**Ethics Approval** All animal studies were performed in accordance with approved Johns Hopkins University Animal Care and Use Committee protocol (Elisseeff, MO21M80).