THE IMMUNE LANDSCAPE OF PRIMARY AND RECURRENT ADOLESCENT AND YOUNG ADULT BONE SARCOMAS

¹Anthony Cillo*, ¹Elina Mukherjee, ²Jessica Daley, ¹Sayali Onkar, ¹Xiang Li, ¹Dongyan Liu, ¹Dario Vignali, ¹Tullia Bruno, ¹Kelly Bailey. ¹University of Pittsburgh, Pittsburgh, PA, United States; ²UPMC Children's Hospital, Pittsburgh, PA, United States

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.653>

Background Pediatric patients with metastatic or recurrent bone sarcomas have poor survival, necessitating new therapies. Studies of immunobiology in pediatric bone sarcomas have focused on analysis of samples from surgical local control, when patients are receiving immunosuppressive chemotherapy, with little data available from relapse. Here, we sought to leverage transcriptomic and imaging approaches in tandem to characterize the immune landscape of primary and recurrent Ewing sarcoma and osteosarcoma and to identify new therapeutic avenues for these patient populations.

Methods Single-cell RNAseq (scRNAseq; 10X Genomics) was performed on sorted CD45+ cells from paired peripheral mononuclear cells (PBMC) and tumor infiltrating leukocytes (TIL) from freshly resected bone tumor specimens in the setting of pre-treatment or >6 months post-chemotherapy in the setting of disease relapse. Multiplexed immunofluorescence (mIF) analysis for CD45, DAPI, CD4, CD8, CD68, CD20, and FOXP3 was also performed on FFPE tissue from the same tumors. CIBERSORTx was used in conjunction with TARGET-OS bulk RNAseq from osteosarcoma tumors to infer cell type frequencies. Expression of ligands and receptors in primary versus relapsed disease was assessed from scRNAseq using CellTalker.

Results We analyzed a total of 29,993 cells from 20 donors (4 paired PBMC/TIL from osteosarcoma, 4 paired samples from Ewing sarcoma, 4 healthy donor PBMC) by scRNAseq. A total of 5 TIL samples were from primary disease sites and 3 were from metastatic sites. We identified major immune populations by canonical expression profiles and used transcriptional profiles from TIL to derive a signature matrix for CIBERSORTx. In 88 osteosarcoma samples from TARGET-OS, we found that higher frequencies of CD14+CD16+ macrophages were associated with better survival (HR:0.28, $p = 0.01$). scRNAseq from our cohort revealed expression of CXCL12, CCL7, and CCL3L1 by CD14+CD16+ macrophages, suggesting this macrophage population may drive tumor immune infiltration. In both Ewing sarcoma and osteosarcoma, mIF revealed greater numbers of tumor-infiltrating immune cells in the setting of relapse versus primary tumors. scRNAseq analysis revealed higher levels of interferon-gamma expressing CD8+ T cells and CD4+ regulatory T cells in relapsed versus primary disease, suggesting that recurrent tumors may be more immunogenic.

Conclusions Although pediatric bone sarcomas are typically considered “immunologically cold”, our transcriptomic and imaging approaches revealed a role for a myeloid cell subset in overall survival and increased immune infiltration and T cell activation in recurrent disease. These data suggest specific immunotherapeutic avenues should be tailored to both primary and recurrent disease to improve outcomes in pediatric bone sarcoma.

Ethics Approval Human specimens were collected with written informed consent under the IRB approved STUDY19030108 and the IRB approved Musculoskeletal Oncology Biobank and Tumor Registry.