

## INTEGRATED IMMUNOGENOMIC AND DIGITAL HISTOPATHOLOGICAL ANALYSIS REVEALS CORRELATION OF IMMUNE LANDSCAPE WITH THE MOLECULAR SUBTYPES IN LEIOMYOSARCOMA

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**Background** Leiomyosarcoma (LMS) is one of the most common types of soft tissue sarcoma arising from the smooth muscle cells. Despite the current treatment regimens, many LMS patients experience progression and poor survival and might benefit from Immune Checkpoint Blockade (ICB). Yet LMS tumors have responded mildly to ICB. Deep immunogenomic profiling of LMS tumors might help in improving ICB outcomes by deconstructing the immune landscape to discover biomarkers of response and identify novel targets which might potentiate ICB outcomes in LMS.

**Methods** LMS tumors from The Cancer Genome Atlas (TCGA) Sarcoma cohort were classified into molecular subtypes based on previously identified gene signatures.<sup>1</sup> We then examined the relationship of the subtypes with expression of various immune related genes. We comprehensively profiled the LMS immune landscape by performing Immunohistochemistry (IHC) (n = 65) for CD3, CD8, PDL1, CD163, TIM3, CD20, CD21 and CD31 on archived formalin fixed paraffin embedded blocks and multi-parameter flow cytometry (n = 22) on single cell suspensions isolated from LMS tumors resected at Johns Hopkins Hospital (JHH). We used HALO Digital Pathology Suite for further analysis.

**Results** LMS tumors from the TCGA cohort were classified into 4 major subtypes which associated with expression levels of the immune genes and survival outcomes. Based on the tissue slides available for the TCGA data, we observed presence of lymphoid aggregates predominantly in one subtype. IHC from the JHH cohort showed higher density of CD163 and PDL1 cells as compared to lymphoid cells and were associated with poor survival. We also observed aggregates of CD3 and CD8 cells and Tertiary Lymphoid Structures (TLS), as seen by CD21, CD20, CD3 IHC in a subset of specimens. Upon merging the different IHC for each specimen (n = 52) we saw a marked colocalization of PDL1 with the lymphoid aggregates hinting at the possibility of Interferon Gamma induced PDL1 expression. We noted high expression of PDL1 and TIM3 on myeloid cells which was correlated with poor survival outcomes.

**Conclusions** Deep immune profiling of the LMS specimens suggests that the TIM3 expressing myeloid cells could be a potential target to augment immunotherapy in LMS. Through spatial analysis, we observed a higher density of PDL1 cells around lymphoid aggregates as compared to the rest of the tumor pointing to evidence of immune related activity. Coupling our discoveries vis-à-vis the immune landscape to the molecular subtypes of LMS will be crucial to identify patients who will most benefit from ICB.

### REFERENCE

1. ML Hemming, et al, *Mol Cancer Res* 2020;**18**(9):1302–1314

**Ethics Approval** This study was conducted in accordance with the ethical principles and was approved by the Johns Hopkins Institutional Review Board and all samples were obtained in accordance with the Health Insurance and Accountability Act.

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