1042 THE DUAL FUNCTION OF THE ADENOSINE PATHWAY IN THE LIPOSARCOMA TUMOR MICROENVIRONMENT

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Background Single agent anti-PD1 therapy has shown limited clinical benefit in liposarcoma, but combination with anti-CTLA-4 demonstrated a higher response rate.[1-3] In the MDACC study NCT02815995, combining anti-CTLA4 and anti-PDL1 across multiple soft-tissue sarcomas increased CD73, PD-1, and OX40 expression in T cells early on treatment. The adenosine pathway, which includes CD73 and the A2A receptor, induces immunosuppression[4,5] and protects from antitumoral responses.[6, 7] This pathway is related to immune therapy resistance[8-11], metastasis, and poor prognosis.[12-15] Conversely, CD73+ T cells have long-lived memory and higher proliferation and survival, suggesting a dual role of the pathway.[16-18] We hypothesized that adenosine would negatively correlate with TIL expansion and function.

Methods Surgically resected liposarcoma samples (n=41), are divided for fresh tissue phenotyping by flow cytometry, TIL expansion, and quantification of tissue adenosine, cytokines, and adipokines. The phenotypic analysis included: CD73, A2aR, PD-1, Tim3, CTLA-4, LAG3, OX40, ICOS and 41BB markers. TIL 3.0 expansion protocol[19] was performed, and expanded TIL were assessed for cytotoxicity by IFN-g production and metabolic state (ROS, mitochondrial activity) by flow cytometry.

Results We found high heterogeneity in the frequency of CD45+ cells (0-88.3%), CD4+ and CD8+ TIL infiltrate, and expression of PD-1 (0.66-59.60% and 0.99-76.6% respectively), CD73 (CD4+ 0- 40.97%, CD8+ 0-34.04%), and A2AR (mean CD4+ 0-0.6%, CD8+ 0-17.96%), with no co-expression of CD73 and A2AR. Interestingly adenosine concentration was highly variable (0-84.05 nmol/g tissue) but did not correlate with TIL phenotype or tissue cytokines. Tumors were rich in growth factors and chemokines but lacked a pro-inflammatory profile. The success of TIL expansion was 50% (n=24 cases) in samples with a count of 300 CD3+ events in the fresh tissue by flow analysis. The frequency of CD8 +CD73+ TIL was significantly higher in cases with successful TIL expansion (p=0.041). The expanded TIL phenotype showed CD73 expression (range: CD4+ 0.67-29 % and CD8 + 0.85-13.9%) and upregulation of A2AR (range: 0.2-15.40% and 1.44-87.6%). IFN-g production was related to intrinsic metabolic profiles by mitochondrial mass and ROS production (n=8).

Conclusions A heterogeneous adenosine landscape was found in liposarcoma. A2AR but not CD73 correlated with mitochondrial dysfunction and reduced cytotoxicity. Conversely CD73+ TIL were more likely to expand. Our results indicate a dichotomy in two key members in the adenosine pathway in liposarcoma and suggest that CD73 might play a positive role in TIL function.

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Ethics Approval This study was written and conducted in accordance with the principles from the Declaration of Helsinki. Written informed consent was provided by all study participants or their legal representatives. The study was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board.

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