Background
Adoptive cell transfer using tumor-infiltrating lymphocytes (TIL) has shown clinical benefits in metastatic melanoma as well as other solid tumor types [1]. Liposarcoma is a soft tissue sarcoma with less than 40% TIL expansion success rate without current selection methods and a 60% success rate with selection based on at least 300 CD3+ T cells detected in the fresh tumor sample using flow cytometry (Fig. 1). Successful expanding (marked as E) was defined as 40X10^6 TIL expanded based upon threshold needed for clinical REP and treatment. CD73 has been correlated with poor patient prognosis and immunosuppression in the tumor microenvironment (TME) [2]. We hypothesized that high expression of adenosine pathway-associated markers, such as CD73, would result in a paucity of immune infiltration including TIL and therefore influence TIL expansion success.

Results

**Gating strategy workflow for flow cytometry**

![](Fig3.png)

**Evaluation of the phenotypes of expanded vs non expanded (E vs NE) TIL samples**

We observed no significant differences in immune infiltrate or lymphocyte subtype percentages prior to expansion between expanding and non-expanding liposarcoma tumor samples (Fig. 4A). Interestingly, CD8 cells exhibit higher CD73 expression on expanded TIL samples than on non-expanded TIL samples (Fig. 4B).

Additionally, we saw that CD73 expression in CD45 cells (tumor cells) shows no significant difference between the analyzed groups (Fig. 5). Samples from the expanded group also had higher Lag3 expression on CD4+ cells but not on CD8+ cells (Fig. 6). All the other explored molecules, including PD-1 and 41BB, showed no significant differences (Fig. 7).

Conclusions

- Immune cell subtype population percentages do not differ between expanded and non-expanded groups which indicates that subtype populations do not affect TIL expansion (Fig. 4A).
- PD-1 and 41BB receptor expression levels on lymphocytes were not significant, indicating that the TIL 3.0 method is stimulating both groups similarly (Fig. 7).
- The role of CD73 expression in the TME may be ambiguous (Fig. 4). Conversely to previous studies, we demonstrated that higher expression of CD73 in TME or tumor cells (CD45) may not affect the subsequent expansion of TIL in liposarcoma and potentially promotes CD8+ T cell proliferation indicating that CD73 is not completely an immunosuppressive molecule (Fig. 4B).
- Higher Lag3 on CD4+ TIL in the expanded group indicates that Lag3 is not behaving as a marker of T cell exhaustion as seen in previous studies [4] (Fig. 6).

Materials and Methods

**Flow cytometry**

![Flow cytometry](Fig2.png)

**Fig. 2: Methodology of tumor sample processing.** TIL expansion was attempted using the ‘TIL 3.0 MDACC method’ from a total of 18 surgically resected liposarcoma cases [3]. Briefly, 1-3 mm^3 pieces were plated in a 96-well with media containing OKT3 (anti-CD3), agonistic anti-IL2, and IL-2. Every 3-4 days, media with IL-2 was refreshed. After 21 days, the cells are harvested and counted. Expanded (E) was defined as 40 x 10^6 TIL expanded based upon thresholds needed for clinical REP and treatment. Phenotypic analysis of the TIL populations prior to expansion were performed using flow cytometry. Statistical analysis was performed in GraphPad Prism (two means t-test Mann-Whitney).

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References