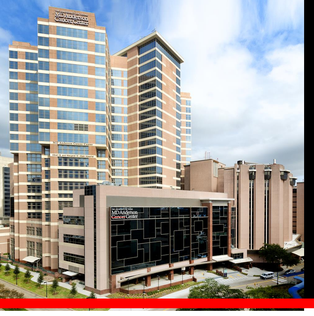

The two faces of CD73 in tumor-infiltrating lymphocytes expanded from Liposarcoma

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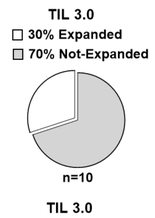
Background

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.

A. **Before selecting for >300 CD3$^+$ events**

Patient Account Number	Harvested TIL Cell Count (10^6 cells)	Number of CD3+ in Fresh Flow
319	7	22742
320	0	0
321	0	154
321	0	4017
322	0	56
323	126.9	333
324	0	274
326	141.7	51002
325	232	1743
327	0	263



B. **After selecting for >300 CD3$^+$ events**

Patient Account Number	Harvested TIL Cell Count (10^6 cells)	Number of CD3+ in Fresh Flow
319	7	22742
321	0	4017
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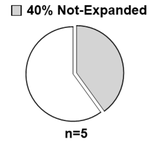

Fig. 1: Selection for at least 300 CD3$^+$ events in fresh flow increased success rates by 30%. CD3$^+$ events in representative liposarcoma samples (n=10, numbered using a de-identified accession number) were determined using FlowJo software. **(A)** Before selecting by CD3+ count, a low expansion (E) rate of 30% was observed as seen in the pie chart. **(B)** Samples with more than 300 CD3$^+$ events (319, 321, 323, 326 and 325) were selected (n=5) and a success rate improvement of 30% was observed. Events of less than 300 CD3$^+$ cells were marked in red.

Materials and Methods

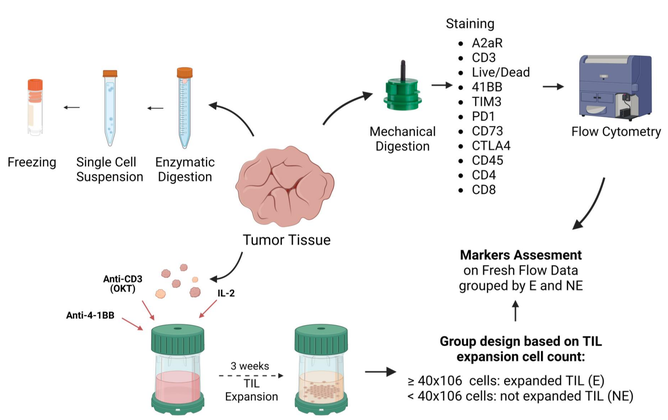

Fig. 2: Methodology of tumor sample processing. TIL expansion was attempted using the 'TIL 3.0 MDACC method' from a total of 18 surgically resected liposarcoma cases [3]. Briefly, five 1-3 mm^3 pieces were plated in a G-rex with media containing OKT3 (anti-CD3), agonistic anti-41BB, and IL-2. Every 3-4 days, media with IL-2 was refreshed. After 21 days, the cells are harvested and counted. Expanding (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).

Results

❖Gating strategy workflow for flow cytometry

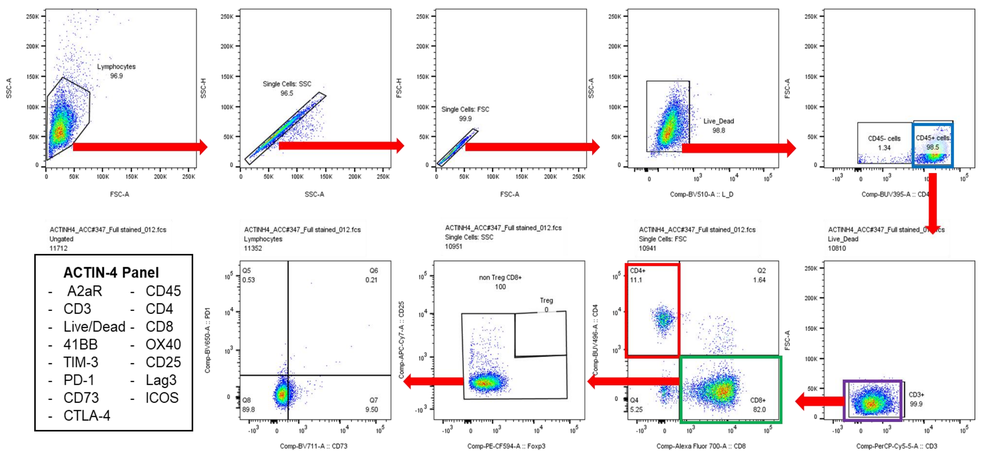

Fig. 3: Gating strategy used to analyze cell subtypes and measure markers expression. Flow cytometry gating strategies were established using FMO controls and analysis was performed using FlowJo 10.8.1. Gating strategy was used to analyze all receptors and markers in the ACTINH-4 panel. The flow chart walks through how PD-1 and CD73 expression was determined in CD8+ T cells.

❖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).**

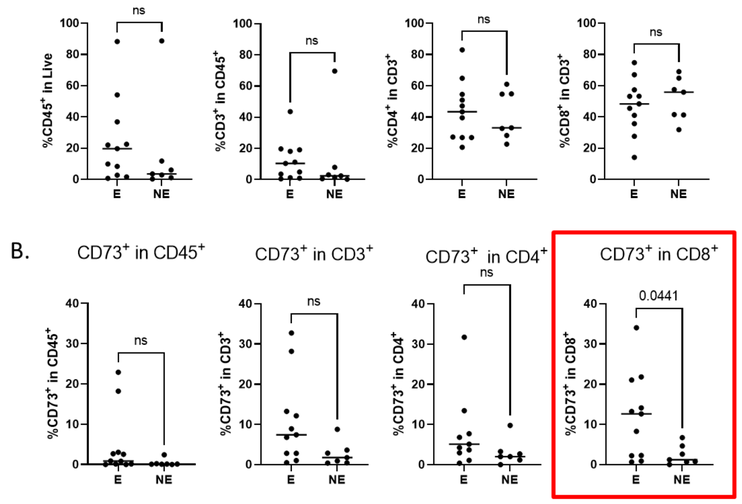

Fig. 4: Expression profiles of tumor microenvironment markers across analyzed liposarcoma groups. **(A)** Comparison of expression profiles of CD45+ (total immune cells in live population), CD3+ (T cells of CD45+ cells), CD4+ and CD8+ (T cells subpopulations of CD3+ cells) markers across analyzed groups. **(B)** Evaluation of CD73 expression on each immune cell subtype population across analyzed groups. Labels: E - expanded TIL, NE - non expanded TIL

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)**.

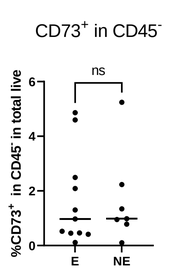

Fig. 5: CD73 expression on tumor cells in expanded and non-expanded groups.

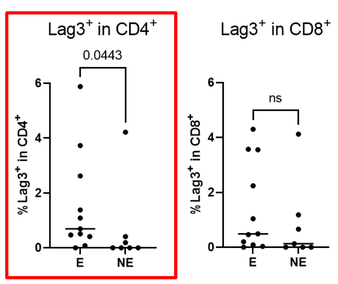

Fig. 6: Expression profiles of Lag3 on CD4$^+$ and CD8$^+$ T cell populations in expanded and non-expanded groups.

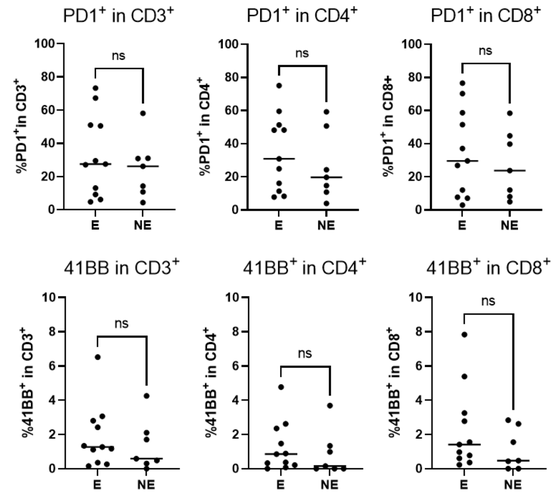

Fig. 7: PD-1 and 41BB expression profiles in lymphocyte subtypes across analyzed groups.

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 tumoral 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).**

Acknowledgments

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References

1. Forget MA, Haymaker C, *et al.,* Prospective Analysis of Adoptive TIL Therapy in Patients with Metastatic Melanoma: Response, Impact of Anti-CTLA4, and Biomarkers to Predict Clinical Outcome. Clin Cancer Res. 2018 Sep 15;24(18):4416-4428. doi: 10.1158/1078-0432.CCR-17-3649. Epub 2018 May 30. PMID: 29848573; PMCID: PMC6139043.

2. Tripathi A, Lin E, Xie W, *et al*., Prognostic significance and immune correlates of CD73 expression in renal cell carcinoma. *Journal for ImmunoTherapy of Cancer* 2020;**8:**e001467. doi: 10.1136/jitc-2020-001467

3. Tavera RJ, Forget MA, et al., Utilizing T-cell Activation Signals 1, 2, and 3 for Tumor-infiltrating Lymphocytes (TIL) Expansion: The Advantage Over the Sole Use of Interleukin-2 in Cutaneous and Uveal Melanoma. J Immunother. 2018 Nov/Dec;41(9):399-405. doi: 10.1097/CJI.0000000000000230. PMID: 29757889; PMCID: PMC6177302.

4. Shah, P., Forget M-A Frank ML, et al. Combined IL-2, agnostic CD3 and 4-1BB stimulation preserve clonotype hierarchy in propagated non-small cell lung cancer tumor-infiltrating lymphocytes. Journal for Immunotherapy of Cancer 2022; 10:e003082. doi10.1136/jitc-2021-003082