Immune Profiling of Tumor-Infiltrating Lymphocytes in Patients With Soft Tissue Sarcomas

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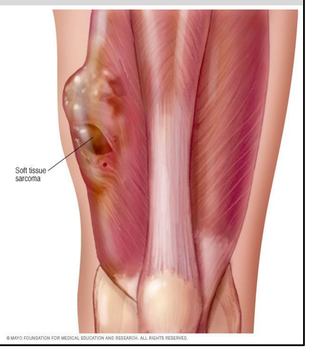
Background

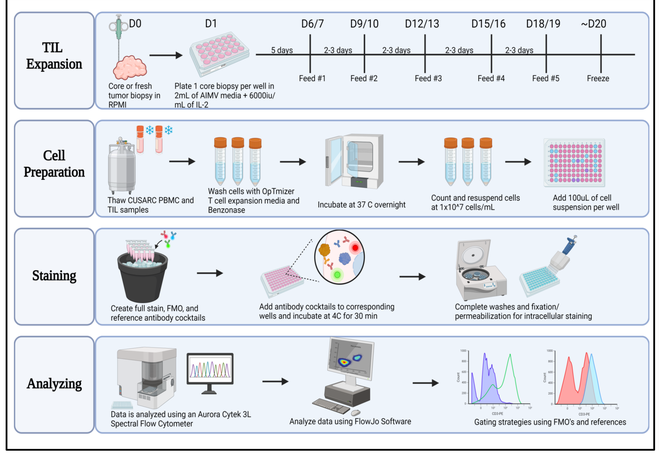
- Sarcomas are rare cancers of bone and soft tissue with over 100 subtypes[1]
- Sarcomas make up only 1% of adult cancers and 15% of pediatric cancers
- Despite conventional chemotherapies, patients with metastatic disease generally have a poor prognosis, with median overall survival of 12-18 months
- Immunotherapy can produce dramatic responses in a subgroup of sarcoma patients
- Little is known about how the quantity, subtypes, or function of TILs in sarcoma patients affect immunotherapy response
- CUSARC samples are blood and tumor tissue obtained from patients enrolled on an IRB approved tumor banking protocol

Objective

Our goal is to analyze previously collected TILs from sarcoma patients undergoing routine surgeries in comparison with matched peripheral blood mononuclear cells (PBMCs) to understand the phenotypes and function of TILs and form the foundation for future immunotherapy strategies. We hypothesize that we will see unique phenotypic profiles in TILs compared to PBMCs

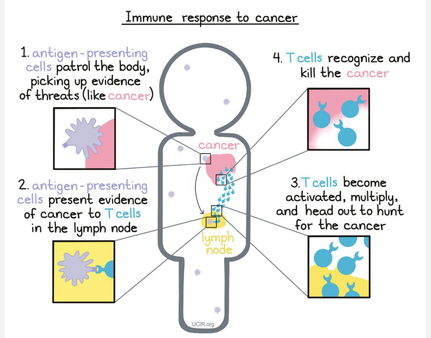


Methods

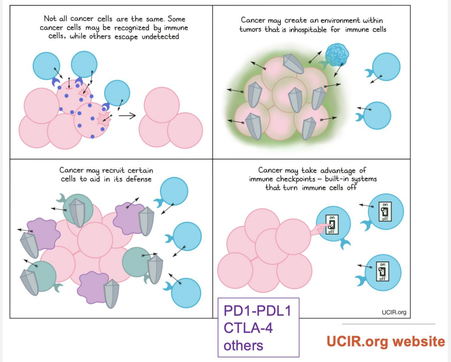


Impact

A) Immune Response to Cancer



B) Why does the Immune System Fail to Kill off Cancer



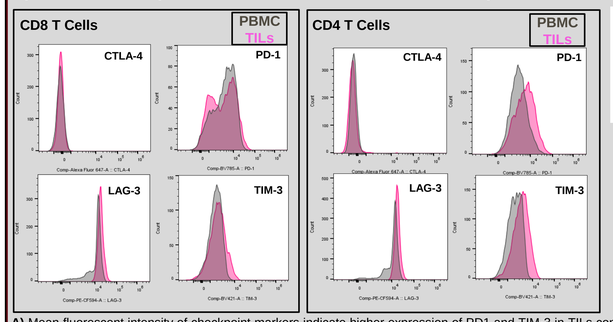
C) CUSARC Clinical Trial Demographics

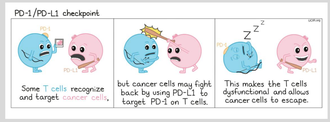
Characteristic	N=23 enrolled patients (%)
Gender	
Male	13 (56.5)
Female	10 (43.5)
Age	
Median	65 (range 30- 77)
Race/Ethnicity	
White or Caucasian	22 (95.7)
Hispanic or Latino	3 (13.0)
Native Hawaiian or Pacific Islander	1 (4.3)
Grade	
Unknown	4 (17.4)
I	2 (8.7)
II	8 (34.8)
III	9 (39.1)
Sarcoma Type	
Liposarcoma	8 (34.8)
Dedifferentiated Liposarcoma (DDLS)	7 (30.4)
Liposarcoma, Myxoid	1 (4.3)
Leiomyosarcoma (LMS)	4 (17.4)
Visceral/Vascular Leiomyosarcoma	3 (13.0)
Soft Tissue Leiomyosarcoma	1 (4.3)
GI Stromal Tumor	5 (21.7)
Other	6 (26.1)
Chondrosarcoma, extraskeletal myxoid	1 (4.3)
Osteosarcoma NOS	1 (4.3)
Rhabdomyosarcoma, pleomorphic	1 (4.3)
Myxofibrosarcoma	1 (4.3)
Synovial Sarcoma	1 (4.3)
Endometrial Stromal Cell Sarcoma	1 (4.3)
Disease Stage	
Metastatic	5 (21.7)
Locally Advanced	18 (78.3)
Prior Treatments	
Surgery	7 (30.4)
Radiation	4 (17.4)
Chemotherapy	7 (30.4)
Targeted Therapy	5 (21.7)
Immunotherapy	2 (8.7)

Results

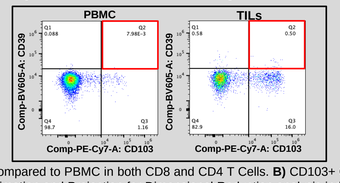
CUSARC63: 70-year-old white male with pleomorphic rhabdomyosarcoma on humerus

A) Immune Checkpoint Markers Mean Fluorescent Intensity

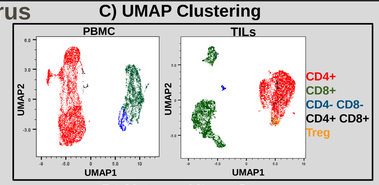




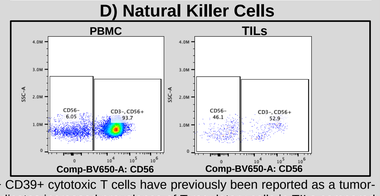
B) Tissue Resident Memory Cells



C) UMAP Clustering



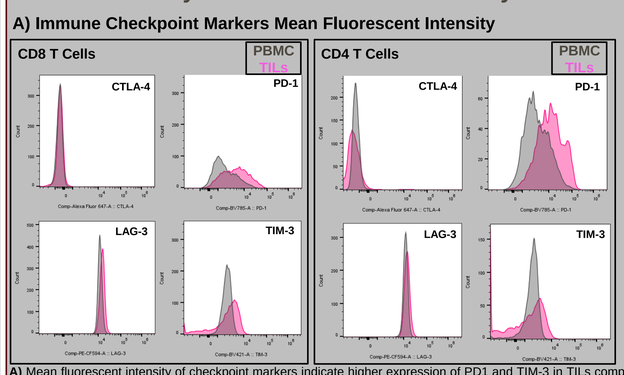
D) Natural Killer Cells

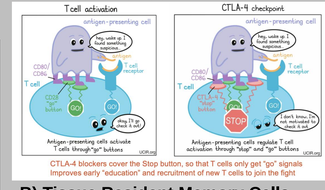


A) Mean fluorescent intensity of checkpoint markers indicate higher expression of PD1 and TIM-3 in TILs compared to PBMC in both CD8 and CD4 T Cells. **B)** CD103+ CD39+ cytotoxic T cells have previously been reported as a tumor-specific TIL phenotype. Trend towards increased CD103+ CD39+ CD8 T Cells. **C)** Uniform Manifold Approximation and Projection for Dimensional Reduction analysis indicates increased prevalence of T-regulatory cells in TILs compared to PBMCs. **D)** Greater presence of natural killer cells in PBMC as compared to TILs.

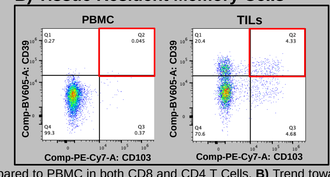
CUSARC79: 60-year-old white female with myxofibrosarcoma on forearm

A) Immune Checkpoint Markers Mean Fluorescent Intensity

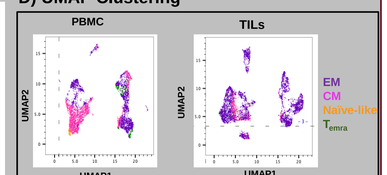




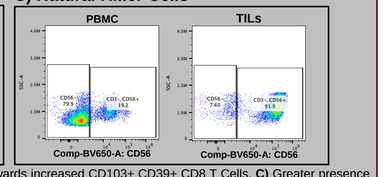
B) Tissue Resident Memory Cells



D) UMAP Clustering



C) Natural Killer Cells



A) Mean fluorescent intensity of checkpoint markers indicate higher expression of PD1 and TIM-3 in TILs compared to PBMC in both CD8 and CD4 T Cells. **B)** Trend towards increased CD103+ CD39+ CD8 T Cells. **C)** Greater presence of natural killer cells in TILs as compared to PBMCs. D) UMAP analysis indicates increased prevalence of central memory (CD27+ CD45RA-) CD4 T cells in PBMCs compared to TILs.

Conclusion

- We optimized flow cytometry panel to profile T cells
- Higher PD-1 and TIM-3 expression in TILs vs. PBMCs in both CD8 and CD4 T cells
- Natural Killer cell abundance is variable in PBMCs compared to TILs among patients
- CD103+ CD39+ Cytotoxic T cells are more abundant in TILs

Future Directions

- Expand our pilot methodology to profile TILs and PBMCs in 26 sarcoma patients
- Correlate flow cytometry data with clinical data including subtypes, treatments, and overall survival
- Profile patients who have received doxorubicin plus checkpoint inhibitors to compare immune infiltration in PBMC vs. TILs and correlate with response to treatment

References

- Ferrari et al, Pediatr Blood Cancer, 2011. **2.** Moreno Tellez et al., Surg. Oncol. Clin. N. Am., 2022. **3.** Tap et al., JAMA, 2020.
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Acknowledgments

Sumra Chaudhry was supported by a Diversity in Cancer Research (DICR) Post Baccalaureate Grant DICR POST-BACC-23-1158368-01-DPBACC from the American Cancer Society Center for Diversity in Cancer Research Training with additional support from the University of Colorado Comprehensive Cancer Center (grant # P30CA046934).