EXPANSION OF TUMOR INFILTRATING IMMUNE CELLS FROM ADULT SOFT TISSUE SARCOMAS WITH GAMMA-CHAIN CYTOKINES

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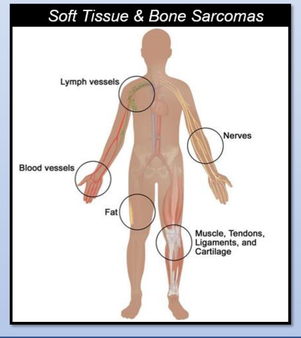
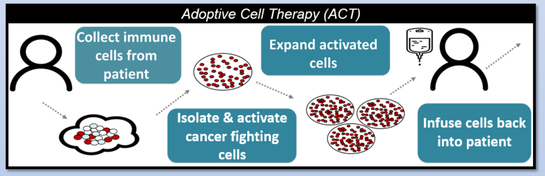
INTRODUCTION

Sarcoma is a group of rare soft tissue (E.g. blood and lymph vessels, nerves, and adipose tissues) and bone tumors with over 50 distinct subtypes. Survival rate ranges widely due to the lack of efficacious treatments. Immunotherapy, such as adoptive cell therapy (ACT), has drawn great interest due to its minimal toxicities. In ACT, tumor infiltrating lymphocytes (TILs) are isolated from patients, expanded, and autologously reinfused back. Clinical response to ACT varies across patients and subtypes, and further research is necessary to improve outcomes. We recently observed the presence of TILs in primary Undifferentiated Pleomorphic Sarcoma (UPS) and Myxofibrosarcoma (MFS) tumors and found that tumor's PD-L1 overexpression is correlated with better clinical outcome in UPS but not MFS.[1] The Th1 inflammatory pathway was identified to be highly activated in the former subtype, which may explain the better clinical outcome. These results illustrate the differences where TILs may play a critical role.





OBJECTIVES

We hypothesize that there are phenotypic and functional differences between TILs of UPS and MFS primary tumors that may be related to clinical outcome. Sarcoma TILs are rare and challenging to culture which impedes their studies. We first aim to robustly expand sarcoma TILs to sufficient numbers. In the future, we aim to characterize UPS and MFS TIL populations' lineage markers and cytokine profiles.

METHODS

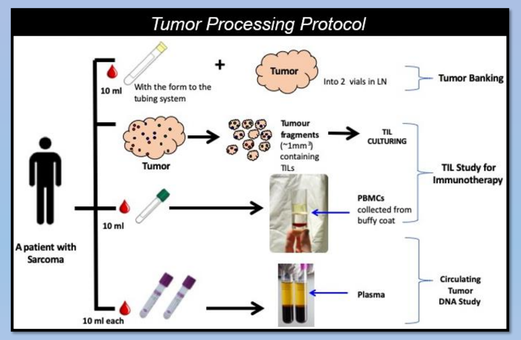
- TILs are being expanded and cultured from 5 UPS and 5 MFS primary tumors with various PD-L1 levels.

1) Tumor Fragment TIL Expansion

- Primary tumor fragments seeded on 24-well plates at 1 fragment/well.
- 2mL per well of complete media (CM)
  - IMDM
  - 6000 IU/mL human recombinant IL-2
  - 10% Human Serum
- At week 1, fast and slow growing TILs are determined by confluency, isolated, and cultured separately.
- Incubated at 37°C and 5% CO_2 for 3-4 weeks.
- Average ~1x10^6 cells.

2) Rapid Expansion Protocol (REP)

- To further expand TILs, treat the initially expanded TIL populations with anti-CD3/ anti-CD28 coated magnetic Dynabeads beads at a 1:1 ratio.
- Incubate for 3-4 weeks.



RESULTS

Sarcoma's exhibited sparse TIL counts, despite 4 weeks in CM cultures, may be due to its lower immune infiltration compared to other tumors, such as melanoma. Of the 4 MFS cases processed to date, 15 TIL populations were derived and cultured (Figure 1). Majority of TIL cultures obtained less than 1x10^6 cells after 4 weeks in high IL-2 CM. Only 6 out of 15 TIL cultures obtained ≥1x10^6 cells and are considered high initial cell count-populations. While 9 out of 15 TIL cultures obtained <1x10^6 cells and are considered low initial cell count-populations. REP successfully expanded 14 out of 15 TIL populations and obtained between 7.8 to 268.0 x10^6 cells (Figures 1&2). Negative controls (-) were allocated only for populations with sufficient cell count. Out of all gamma chain cytokines evaluated, only IL-2 and IL-15 sustained T cell growth. Whereas IL7 and IL21 does not. IL-12 also did not support T cell growth despite literature's report on its benefits in culturing PBMCs. Due to IL-2's known side effects of exhaustion and activation induced cell death on T cell culturing, future investigation of IL-15 for ACT culturing should be explored.

TABLES & FIGURES

Figure 1. Tumor Fragment Method of Expansion: 15 TIL populations were derived and cultured form 4 MFS cases over 4 weeks. Populations were categorized based on their growth rates and labeled as "1" or "2" representing "fast" or "slow" growing TILs, respectively. A and B are replicates. TIL162-2 has no replicates.

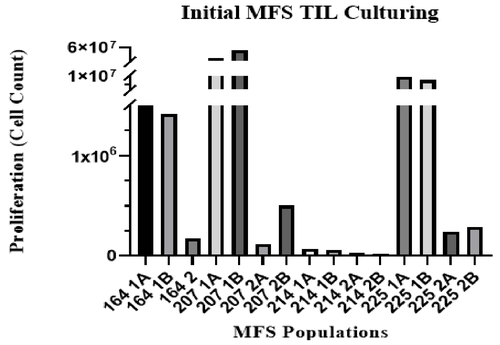


Figure 2. REP Method of Expansion: 15 TIL populations treated with CD3/CD28 coated magnetic beads for 3-4 weeks.

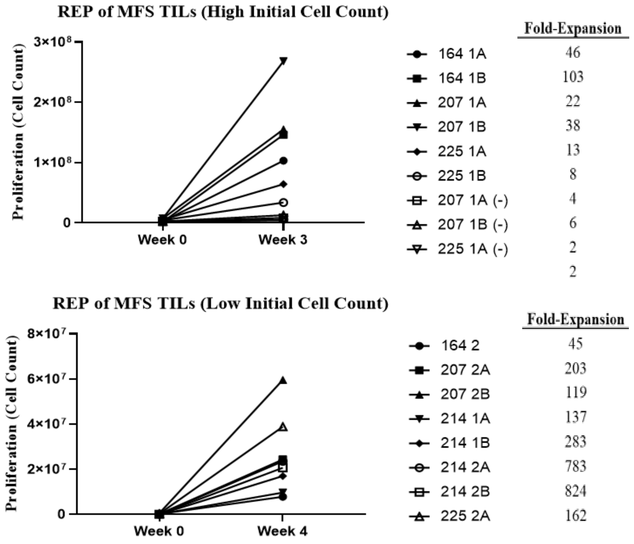
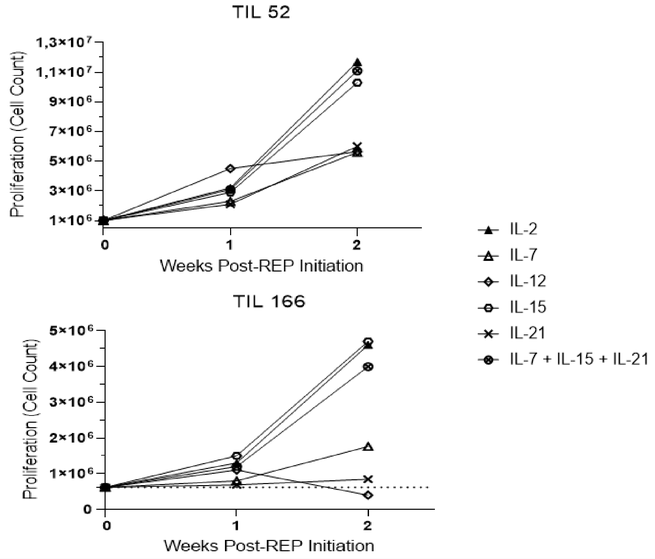


Figure 3. Gamma-chain cytokine treatments of UPS TILs with CD3/CD28 stimulation



CONCLUSION & FUTURE DIRECTIONS

Sarcoma infiltrates are difficult to culture and their roles remain largely unstudied. Our results demonstrate anti-CD3/ anti-CD28-mediated co-stimulation's capability in expanding most sarcoma TILs and established a robust method of expansion for downstream experimental analysis. Future investigation of lineage markers (CD3, CD4, CD8, T-bet, GATA3, FoxP3, CD56, CD11c, CD68, CD86, CD206), cytokine profiles (IL-4, IL-6, IL-8, IL-13, Angiogenin, IFN-gamma, TNF-alpha, TGF-Beta), and target specific cytotoxic functions aims to identify immunological differences between UPS and MFS. Understanding TILs and their relations with tumor's PD-L1 expression would allow clinicians to appropriately recognize sarcoma's subtype-specific tumor immune environments, design drugs to modulate this environment more favorably, and select the most desirable infiltrates for superior ACT.

References

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