**Evaluation of the Combination of HDACi and IL-21 in TIL Initial Phase of Expansion**

**Background**

**TIL Therapy as a Promising Immunotherapy for Solid Tumors**

- Tumor-infiltrating lymphocytes (TIL) help generate an antitumor response
- Already recognize and target tumor cells
- Diverse product against a broad variety of tumor-antigens
- >30% Melanoma patients respond to TIL therapy post-checkpoint inhibitors therapy
- Improvement in response can be achieved through engineering and expanding TIL

**Altering TIL Phenotypes Can Improve Overall Clinical Effectiveness**

- Persistence of infused T cells (specifically CD8+) correlate with increased response rate
- Central memory CD8+ T cells (T_CM), capable of long-term persistence, lesser differentiated status, characteristics most desirable for cell therapy
- Recently, the combined use of a histone deacetylation inhibitor (HDACi) and IL21 was shown to revert a differentiated effector memory T cell state (T_EM) into cells with attributes of T_CM
- Here we proposed that this new culture combination, paired with our new method of expanding TIL, (TIL 3.0) could produce greater number of CD8+TIL displaying T_CM features

**Methods**

**TIL Pre-Rep Expansion Protocol Setup**

- Adding IL-2, anti-CD3 & anti-4-1BB
- Tumor tissue
- G-Rex10
- IL-2, IL-21 Panobinostat
- IL-2 + IL-21 Panobinostat
- TIL 3.0 + Panobinostat and IL-21
- Modified TIL 3.0 (50IU/mL IL-2) + IL-21 + Panobinostat
- TIL 3.0 expansion method (high dose IL-2, anti-CD3 & anti-4-1BB) to produce strong CD8+TIL growth within 3 weeks
- Will be supplemented with HDACi (Panobinostat) + IL-21 + high or low-dose IL-2

**Flow Panel to Characterize Expanded TIL and gating strategy for analysis**

- CD3, CD4 & CD8 to identify T-cell populations
- CD62L, CD127, CD27, & CD28 expression to identify TIL differentiation status
- PD1 and Lag3 used to study T cell activation/exhaustion

**Results**

**Expansion of TIL Cultures & Expression of Markers to Determine Phenotypes in CD8+ TIL**

A) Graph depicting the numbers of TIL vs time of culturing before freeze for colorectal cancer-derived TIL following 3 types of expansions described in the Methods section.

B) Graph showing co-expression of CD62L/CD28 achieved in expanded TIL following culture under different conditions including/excluding HDACi + IL-21. This specific phenotype was previously reported as having T_CM attributes post-exposure to HDACi + IL-21.

C) Evaluation at the single marker level of the potential impact of exposure to HDACi + IL-21 during TIL 3.0 expansion on differentiation, activation and exhaustion status.

**Conclusions**

- Addition of HDACi + IL21 to the TIL 3.0 process successfully expanded TIL in 1 out of 2 cultures
- Successful culture showed increase in the percentage of CD62L+CD28+CD8+TIL compared to TIL 3.0
  - Indicative of more central memory-like phenotype
- LAG3 and PD1 were elevated compared to TIL 3.0
  - CD127 and CD27 expression decreased – suggests effector state
- More cultures from additional patient are needed to confirm these observations

**References**