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

Joshua Ni[1], Tamara Griffiths[2], Marie-Andree Forget[3], Donastas Sakellariou-Thompson[2], Chantale Bernatchez[2]

[1]Department of Biomedical Engineering, Johns Hopkins University
[2]Biologics Development, University of Texas MD Anderson Cancer Center
[3]Melanoma Medical Oncology, University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background

TIL Therapy as a Promising Immunotherapy for Solid Tumors

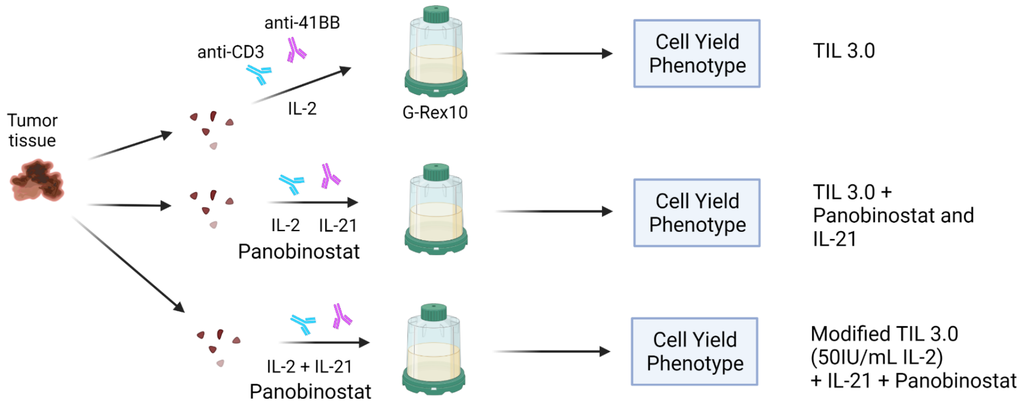
- Tumor-infiltrating lymphocytes (TIL) help generate an antitumor response
  - Already recognize and target tumor cells[1]
  - Diverse product against a broad variety of tumor-antigens
- ***>30%*** Melanoma patients respond to TIL therapy post-checkpoint inhibitors therapy
  - Improvement in response can be achieved through engineering and expanding TIL *ex vivo*

Altering TIL Phenotypes Can Improve Overall Clinical Effectiveness

- Persistence of infused T cells (specifically $CD8^+$) correlate with increased response rate[2]
  - 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}[2]
- 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

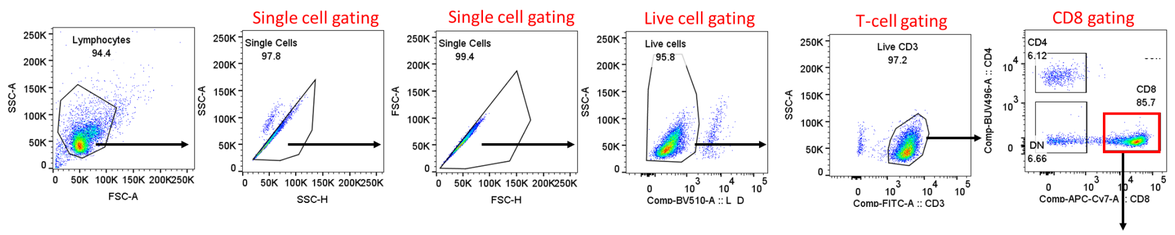
TIL Pre-Rep Expansion Protocol Setup



- 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

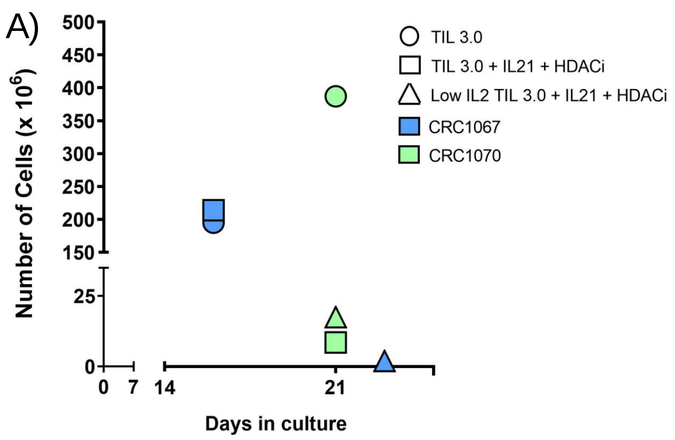
- CD3, CD4 & CD8 to identify T-cell populations
- CD62L, CD127, CD27, & CD28 expression to identify TIL differentiation status
- PD1 and Lag3 used to study T cell activation/exhaustion



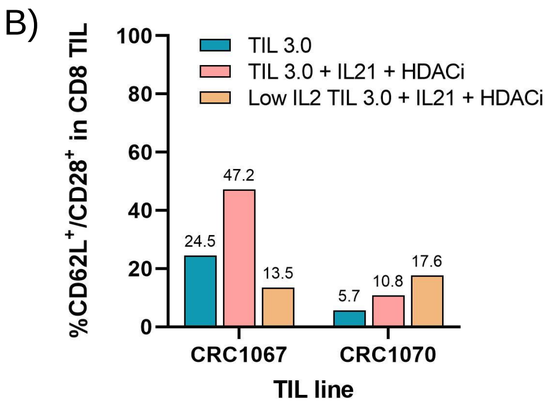
Representative gating strategy for flow cytometry assessment of markers of interest on $CD8^+$TIL. Cells were initially gated by size/granularity, then single cells, live cells, $CD3^+$T cells, and finally $CD8^+$TIL. Co-expression of CD62L & CD28 and expression of single markers were evaluated.

Results

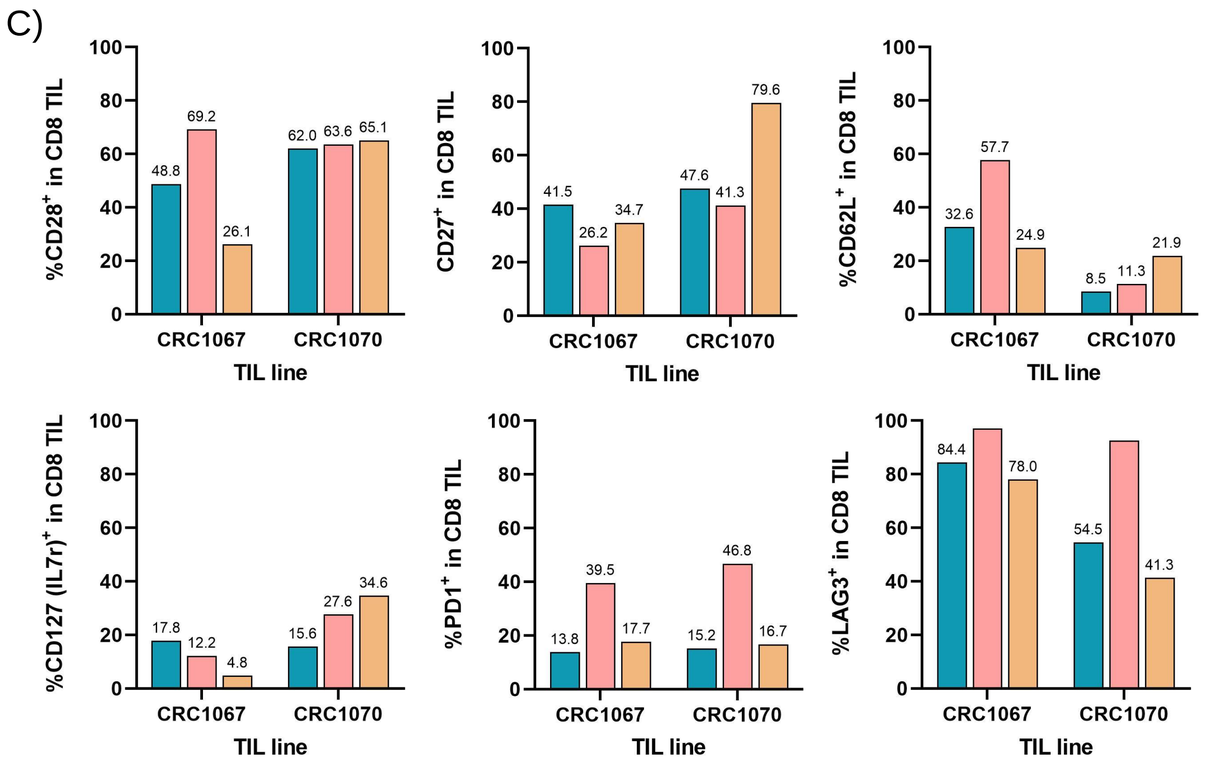
Expansion of TIL Cultures & Expression of Markers to Determine Phenotypes in $CD8^+$ TIL



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 patients are needed to confirm these observations

References

1. Qin SS, Melucci AD, Chacon AC, Prieto PA. Adoptive T Cell Therapy for Solid Tumors: Pathway to Personalized Standard of Care. Cells. 2021;10(4):808. Published 2021 Apr 5. doi:10.3390/cells10040808he a
2. Wang J, Hasan F, Frey AC, et al. Histone Deacetylase Inhibitors and IL21 Cooperate to Reprogram Human Effector CD8+ T Cells to Memory T Cells. Cancer Immunol Res. 2020;8(6):794-805. doi:10.1158/2326-6066.CIR-19-0619