Emigrant Tumor Infiltrating Lymphocytes (TIL) Profoundly Differ from Remnant T-cells

Michelle R. Simpson-Abelson, Christopher Mosychuk, Maria Fardis, and Michael T. Lotze

LION BIOTECHNOLOGIES
LEADERSHIP & INNOVATION IN ONCOLOGY
999 Skyway Road, STE 150, San Carlos, CA 94070
For more information, please contact
Michelle.Abelson@lionbio.com

ABSTRACT

Adoptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) provides up to 56% objective response rates and a complete response in 24% of patients with metastatic melanoma.[1] The process of generating TIL from resected tumor involves morcellating the tumor into 1-3 mm^3 fragments and expanding TIL in the presence of Interleukin 2 (IL-2) in a pre-Rapid Expansion Protocol (pre-REP). During the 'pre-REP', tumor-resident immune cells emigrate (eTIL) and proliferate. The length of the pre-REP typically varies between 11-21 days, depending on cell growth. Residual tumor fragments (remnants) are discarded and the expanded eTIL are subjected to a Rapid Expansion Protocol (REP) with irradiated PBMC feeders, anti-CD3 and IL-2. Viable cells remaining in the tumor remnants (rTIL) following the pre-REP were investigated to assess their function and phenotype. We evaluated and compared the rTIL and eTIL in melanoma, breast, renal, pancreatic, lung and colorectal tumors (n=9). Tumor rTIL are consistently phenotypically distinct from eTIL, as determined by differential expression of various markers (**Table 1**). The fundamental differences in rTIL were: Increased CD69$^+$ (7 fold MFI in CD4$^+$) (p<.001); diminished LAG3 (2 fold MFI in CD8) (p<.05); TIM3 (3 and 2 fold MFI in CD8 and CD4 respectively) (p<.05/.01); CD154 (3 fold MFI in CD4) (p<.01); and CD56 (5%) (p<.05). A REP of rTIL and eTIL resulted in comparable expansion. The phenotypic signature of TIL was sustained post-REP with fidelity of the individual expression of LAG3, Tim3, and CD28. These studies have identified notable differences in the biology of cell populations in terms of tissue-resident T cells and the signals associated with emigration and retention. These data provide additional insights on the individual TIL populations that could be utilized for adoptive T-cell therapy in patients and raise important questions about the nature of tissue-resident T cells in sites of chronic inflammation such as tumor.

[1]Goff, et al. Randomized, Prospective Evaluation Comparing Intensity of Lymphodepletion Before Adoptive Transfer of Tumor-Infiltrating Lymphocytes for Patients With Metastatic Melanoma. *J Clin Oncol.* 2016 Jul 10;34(20):2389-97.

Generating TIL for adoptive T-cell therapy

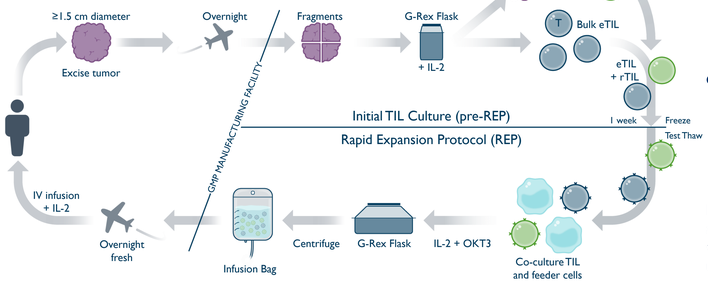

Figure 1. The tumor is excised from the patient and transported to the GMP Manufacturing facility. Upon arrival the tumor is fragmented and placed in G-Rex flasks with IL-2 for TIL expansion (pre-REP expansion). eTIL are cells that emigrant out of the tumor in response to IL-2. The rTIL are tumor retained cells that are isolated from a enzymatic digestion of tumor remnants. The eTIL and rTIL are cultured with feeders and OKT3 for REP expansion.

RESULTS

rTIL have reduced NK cells and phenotypically resemble a tissue-resident memory T cell

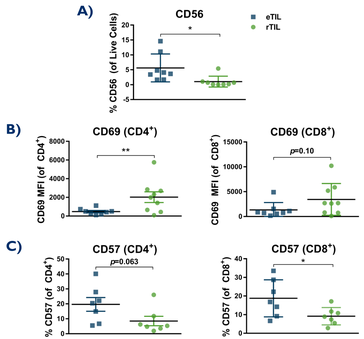

Figure 2. A) CD56, CD4$^+$ and CD8$^+$ T cells in the eTIL and rTIL for **B)** CD69 and **C)** CD57 were assessed via flow cytometry (n=9). P values represent the difference between rTIL and eTIL using student's unpaired T test; *p<0.05, **p<0.01. P values approaching significance are also indicated above.

Tumor resident remnant T-cells are phenotypically distinct from emigrating T-cells

Marker Expression	LAG3 CD8/ CD4 MFI	Tim3 CD8/ CD4 MFI	PD-1 CD8/ CD4 %	CD69 CD8/ CD4 MFI	CD154 CD8/ CD4 MFI	CD28 CD8/ CD4 MFI	CD57 CD8/ CD4 %	CD56 (%)
eTIL	507/ 144	2832/ 1756	36.95/ 47	1320/ 1543	1498/ 3751	1163/ 5036	18.76/ 19.6	5.615
rTIL	209/ 106	877/ 742	42.8/ 48	3437/ 223.4	1034/ 1167	458.3/ 2795	9.16/ 8.5	1.027
*P-values (CD8/ CD4)	0.05/ 0.21	0.05/ 0.01	0.38/ 0.89	0.11/ 0.001	0.55/ 0.01	0.05/ 0.11	0.05/ 0.06	0.05

Table 1. eTIL/ rTIL pairs derived from melanoma, breast, renal, pancreatic, lung and colorectal tumors (n=9) were assessed phenotypically, using flow cytometry post pre-REP. *P-values represent the difference between rTIL and eTIL using student's unpaired T test.

rTIL demonstrate a less exhausted phenotype compared to eTIL

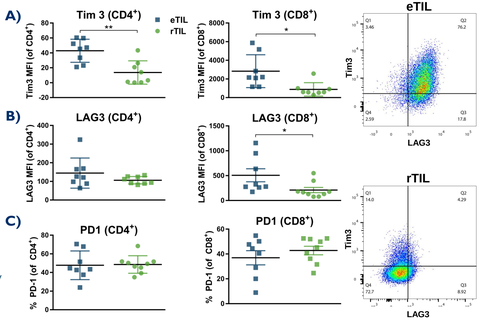

Figure 3. The CD4$^+$ and CD8$^+$ T cells in the eTIL and rTIL were assessed for **A)** Tim3, **B)** LAG3, **C)** PD-1 via flow cytometry (n=9). p values represent the difference between rTIL and eTIL using student's unpaired T test; *p<0.05, **p<0.01.

rTIL have greater metabolic capacity than eTIL

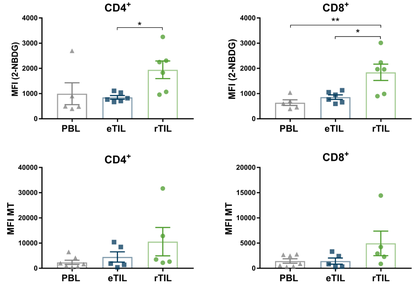

Figure 4. CD4$^+$ and CD8$^+$ normal donor peripheral blood lymphocytes (PBL) and pre-REP eTIL and rTIL were stained with **A)** 2-NBDG to assess glucose uptake and **B)** mitotracker to assess mitochondria mass. The cells were evaluated using flow cytometry (n=5-7). P values represent the difference between the rTIL and eTIL using student's unpaired T test; *p<0.05, **p<0.01.

Enhanced production of IFNγ in CD4$^+$ T cells of rTIL

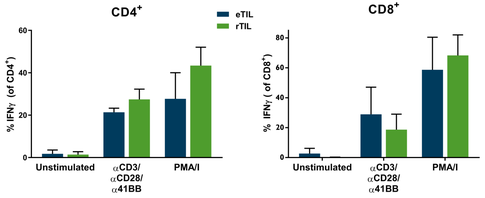

Figure 5. eTIL and rTIL were stimulated with αCD3/ α CD28/ α 41BB beads with Brefeldin overnight or PMA/Ionomycin for 4-5 hours. IFNγ in the CD4$^+$ and CD8$^+$ cells were assessed by intracellular flow cytometric analysis (n=3).

rTIL expand and remain phenotypically distinct from eTIL during the REP

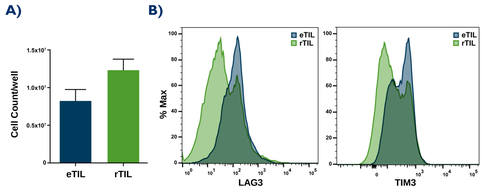

Figure 6. eTIL and rTIL were subjected to a rapid expansion protocol (REP) with irradiated PBMC feeders, anti-CD3 (OKT3) and IL-2 for 14 days. Viability and cells counts were assessed in duplicate (n=4). LAG3$^+$ and TIM3 were assessed by flow cytometry.

SUMMARY

- Viable cells (both T-cells and other immune cells) can be isolated from tumor remnants post pre-REP cultures (11-21d).
- Emigrant (eTIL) and remnant T-cells (rTIL) are phenotypically distinct.
- rTIL are more indicative of a resident memory T-cell, and have reduced expression of exhaustion markers (i.e., LAG3$^+$, TIM3), compared to eTIL.
- rTIL have enhanced metabolic capacity and IFNγ production, compared to eTIL.
- rTIL can be expanded during the REP using OKT3 and feeders, and retain a robust phenotypic signature similar to the pre-REP, but differential expression pattern compared to eTIL.
- Experiments are currently investigating the pre-clinical and clinical implications of rTIL for adoptive T-cell therapy.