POSTER # 11

Remarkably Stable Tumor-Infiltrating Lymphocytes (TIL) For Infusion Phenotype Following Cryopreservation

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Abstract

Background

Lion Biotechnologies focuses on the development to commercialization of cancer immunotherapies based on tumor-infiltrating lymphocytes (TILs). Cryopreservation of TILs allows the final cell product to be shipped in a safe manner with less temporal constraints[1]. Clinical studies using cryopreserved TIL have not been conducted so far. Freezing and thawing of the cells may cause phenotypic changes such as loss of cell surface receptors[2]. Here, we tested fresh versus frozen/thawed TIL samples and evaluated the expression of individual phenotypic markers.

Results

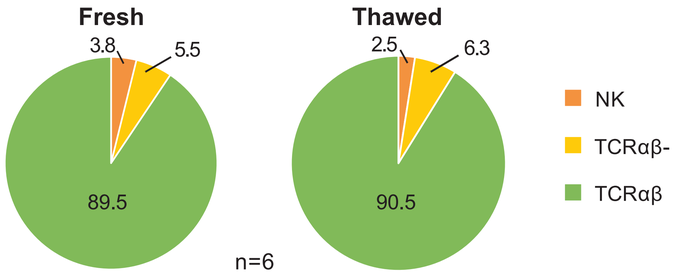
No significant differences in CD4, CD8, NK, TCRαβ expression, or memory markers comparing fresh versus thawed TIL were observed. The activation status of TIL as defined by HLA-DR, CD38, and CD69 expression was maintained while regulatory molecules LAG-3 and TIM-3 demonstrated a slight decrease in expression. In addition, the viability of both the fresh and thawed product was greater than 86%.

Methods

- TILs were obtained by culturing melanoma tumor fragments in IL-2 (6000 IU/ml) from six different individuals.
- Rapid Expansion Protocol (REP) cells were initiated using irradiated allogeneic PBMC feeder cells with OKT3 and IL-2 in a GREX-100 flask for 11-14 days.
- Cultured cells were cryopreserved in 5% DMSO.
- Flow cytometric evaluation of fresh and thawed TIL following rest for 1 to 2 hours in growth medium was performed using four panels consisting of lineage, differentiation, activation, and regulatory markers.

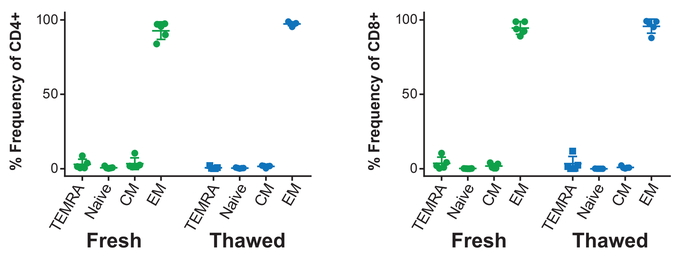
Results

Figure 1. Composition of fresh vs. thawed TIL



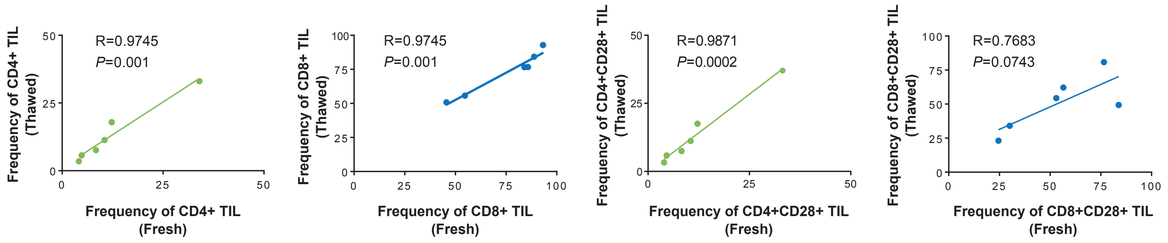
TILs were stained for TCRαβ and CD56 to define T-cell and NK populations. The data shown are averages of 6 individual TILs.

Figure 2. Memory phenotype is defined by CD45RA and CCR7 Expression



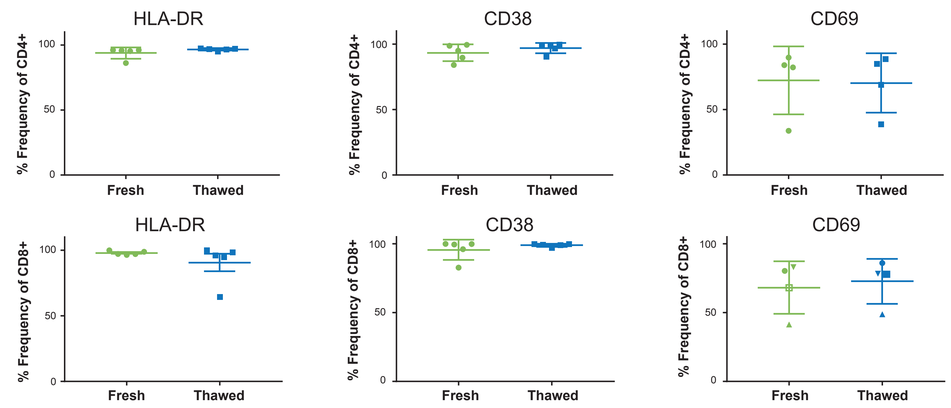
CD4 and CD8 TIL are mainly Effector Memory (EM). This remains the same in the thawed TIL. Each point is one sample analyzed. No significant difference is found in a Wilcoxon Matched-Pairs Signed Rank Test.

Figure 3. Pearson's correlation of CD4, CD8, CD4+CD28+, and CD8+CD28+ frequency between fresh and thawed TIL



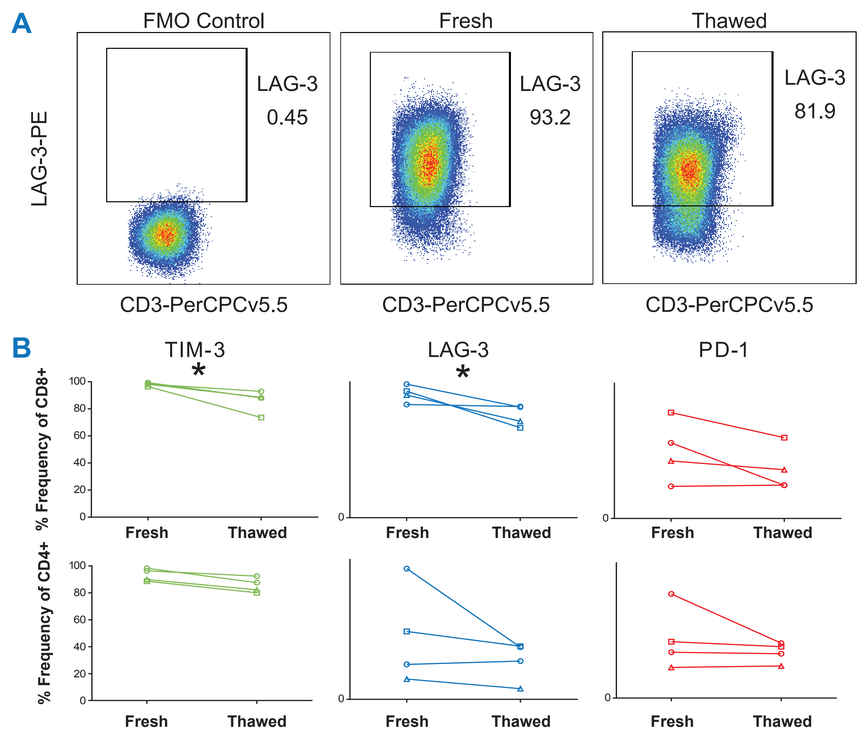
Cells were stained with above markers. Each point represents one individual with the fresh value on the x axis and the thawed value on the y axis. The fit line was drawn using linear regression analysis.

Figure 4. Comparable activation markers on fresh and thawed TILs



No significant difference in activation status of fresh vs. thawed TIL was found using a Wilcoxon Matched-Pairs Rank Test. Each point represents one sample analyzed and is shown as mean +/- SEM.

Figure 5. Maintenance of LAG-3 staining following cryopreservation and thaw



A) LAG-3 staining of CD8 TIL. B) % frequency of regulatory molecules of the CD4 and CD8 populations on fresh and thawed TIL. CD8+TIM-3+ and CD8+LAG-3+ thawed TIL have a lower % than fresh TIL. Mann-Whitney Statistical Test.

Conclusions

- Cryopreservation did not affect the measured phenotypic characteristics of TIL, with the exception of modest changes in some regulatory molecules.
- We are investigating the possibility of using cryopreserved TIL in a clinical setting.

References

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2. Sadeghi A, Ullenhag G, Wagenius G, Tötterman TH, Eriksson F: Rapid expansion of T cells: Effects of culture and cryopreservation and importance of short-term cell recovery. *Acta Oncol.* 2013, 52:978-86.

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