STUDY OBJECTIVES

- To determine whether TIL with therapeutic potential might be isolated and cultured from a random subset of patients with lymphoma.

- To compare the characteristics of NK-derived TIL, which are typically derived from melanoma tumors, with which clinical data support objective clinical responses.

METHODS

- Flow cytometry: TIL were stained for expression of co-stimulatory and costimulatory molecules, HLA class I and II, and markers of activation.

- ELISPOT and TEMRA (CCR7 and CD45RA) ELISPOT and TEMRA (CCR7 and CD45RA) were used as differentiation panel (CD8, CD4, CD103, CD45RA, CD45RO, CD27, and CD28). The most common T cell subsets were: Naive (CCR7 and CD45RA), Effector Memory (CD45RO and CD27), and Central Memory (CD45RO and CD27). A subset of CD45RO TIL were assessed for phenotypic and functional characteristics to those of melanoma TIL, suggesting similar therapeutic potential.

- Immunohistochemistry: TIL were stained using standard phenotype and functional antibodies and counted using a microscope.

- Gene expression profile of lymphoma and melanoma TIL was assessed by NanoString. Heat map is representative of fold change in the expression of a single gene in lymphoma TIL compared to melanoma TIL, suggesting a higher expression of IL-1A and IL-8, and inactivation of IFN-gamma in lymphoma TIL.

RESULTS

Figure 2. Proportions of CD4 Naive and CD4, CD8 TEMRA TIL Subsets (Suggestive of Homeostatic, Multi-Targeted & Anti-Target Potential) Were Higher for Lymphoma vs Melanoma TIL

Figure 3. A Higher Proportion of CD18+CD4 TIL Subsets Was Observed in Lymphoma vs Melanoma TIL Suggesting Higher Proliferative Potential of Lymphoma TIL

Figure 4. Lymphoma and Melanoma TIL Produced Similar Levels of IFN-γ Suggesting Similar Cytolytic Potential

Figure 5. IFN-γ Production by TIL following stimulation with melanoma tumor (CD2, CD8, CD83, CD137) was assessed using (A) ELISPOT and (B) ELISA. ELISPOT data is shown as IFN-γ producing cells per 10^6 TIL (B) (inset levels in supernatants from TIL cultures (E) (inset) measured by ELISA is shown in logarithmic scale. P values were calculated using two-tailed Mann-Whitney test (unpaired).

Figure 6. Lymphoma TIL Exhibit Higher Proportion of CD18+ and CD28+ TIL, Suggestive of Skepting Towards a Th17 Phenotype

CONCLUSIONS

- These preliminary data demonstrate the feasibility of isolation and expansion of TIL from lymphoma.

- TIL were expanded successfully from 37 NHL tumors including:
  - 1 mantle cell lymphoma
  - 3 follicular lymphomas
  - 2 diffuse large B cell lymphomas

- Phenotypic analysis of TIL showed a higher proportion of naive and CD8+ TIL in lymphoma TIL, suggesting similar therapeutic potential.

- Taken together these data demonstrate the feasibility of isolation and culture of TIL from lymphoma and suggest lymphoma-derived TIL share similar phenotypic and functional characteristics to those of melanoma-derived TIL that have proven effective in the treatment of melanoma.

- These data suggest lymphoma-derived TIL may represent therapeutic candidates worthy of further clinical evaluation against a range of NHL targets.