Phenotypic and Functional Characterization of Tumor Infiltrating Lymphocytes (TIL) Grown from Non-Hodgkin Lymphoma Tumors – Implications for the Development of Novel Therapies for Lymphoma

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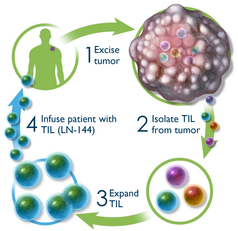
BACKGROUND

- The use of adoptive cellular therapies (ACT) for the treatment of patients with cancer is warranted based on their potential to attenuate or reverse disease progression.
- ACT using tumor infiltrating lymphocytes (TIL) has demonstrated impressive objective response rates (ORR) of 56%, including 24% complete responses (CR) in metastatic melanoma patients, and is currently being explored in other solid tumors *(Goff SL et al., J Clin Oncol 2016)*.
- There is a significant need for the development of effective cellular immunotherapies with the potential to target tumors with high mutational load or those lacking a clearly defined stable tumor antigen (Ag).
- In this regard, TIL exhibit the key attributes of polyclonality which ensures availability of a broad array of tumor Ags for immune recognition and elimination.
- This study presents preliminary data on the isolation and culture of TIL from hematologic malignancies as a prelude for potential clinical evaluation in patients who remain in need of alternative therapies.

Figure 1. TIL Therapy Process



- EXTRACTION: Patient's TIL are removed from the suppressive tumor microenvironment (after surgical resection of a lesion)
- EXPANSION: TIL expanded exponentially in culture with IL-2 to yield 10^9 – 1.5 x 10^{11} TIL, before infusing them into the patient
- PREPARATION: Patient receives NMA-LD (non-myeloablative lymphodepletion with cyclophosphamide: 60 mg/kg, IV x 2 doses and fludarabine: 25 mg/m^2 x 5 doses) to eliminate potentially suppressive cells within the tumor microenvironment, drive homeostatic proliferation, and maximize engraftment and potency of TIL therapy
- INFUSION: Patient is infused with their expanded TIL (LN-144) and high-dose of IL-2 (600,000 IU/kg for up to 6 doses) to promote activation, proliferation, and anti-tumor cytolytic activity of TIL

STUDY OBJECTIVES

- To determine whether TIL with therapeutic potential might be isolated and cultured from a range of Non-Hodgkin's Lymphomas (NHL) tumors.
- To compare the characteristics of NHL-derived TIL with those previously derived from melanoma tumors, for which clinical data supports objective clinical responses.

METHODS

- **Flow cytometry:** TIL were analyzed for markers of differentiation. Anti-CD56, anti-TCR$\alpha\beta$ anti-CD3, anti-CD4, anti-CD8, anti-CD27 and anti-CD28 antibodies were used as differentiation panel 1 (DF1). Anti-CD3, anti-CD4, anti-CD8, anti-CD38, anti-HLA-DR, anti-CCR7 and anti-CD45RA antibodies were used as differentiation panel 2 (DF2). DF2 panel was used to identify the following T cell subsets: Naïve (CCR7$^+$ CD45RA$^+$); Central Memory- CM (CCR7$^+$ CD45RA$^-$); Effector Memory-EM (CCR7$^-$ CD45RA$^-$) and Terminally Differentiated Effector Memory RA$^+$ - TEMRA (CCR7$^-$ CD45RA$^+$).
- **Immunoassays:** IFN-γ production by TIL was measured following stimulation with mAb–coated Dynabeads (CD3, CD28, and CD137), using ELIspot (Immunspot CTL) and enumerated using Immunospot S6 entry analyzer, and also by ELISA using DuoSet ELISA kit (R&D systems) following manufacturers instructions.
- **Bioluminescent Redirected Lysis Assay (BRLA):** Lytic potential of TIL was determined using BRLA. P815 cells transduced with lentiviral vector encoding eGFP and firefly luciferase were used as target cells. TIL and target cells were cocultured for 4 hrs/24 hrs in the presence of OKT3. Luciferin was then added and cells were incubated for 5 mins. Bioluminescence was measured using a luminometer. Percent survival and percent cytotoxicity were calculated using following formulas: % Survival = (experimental survival-minimum)/(maximum signal – minimum signal) x 100; % cytotoxicity = 100 – (% Survival). Lytic potential of TIL was expressed as lytic unit, LU_{50}, which represents 50% cytotoxicity of target cells induced by effector cells.
- **Autologous and allogeneic tumor killing assay:** TIL were mixed with autologous lymphoma cells or allogeneic melanoma cell lines (526 melanoma cell line) at different effector cell-to-target-cell ratio (E:T ratio) (10:1, 20:1, 50:1, 100:1). Tumor cells were labeled with CellTrace Violet dye (ThermoFisher) prior to coculture. After 24 hrs, cells were stained with 7-AAD to determine cell death. Proportion of tumor cells killed by TIL were represented as 7-AAD positive tumor cells that were gated on CellTrace Violet dye vs CD19 (for lymphoma tumor cells) and CellTrace Violet vs MCSP (for melanoma tumor cells).
- **Gene expression analysis using NanoString nCounter system:** nCounter GX Human Immunology V2 panel (NanoString, Seattle, WA) was used. 100ng total RNA was assayed per manufacturer's instructions. Data were normalized by scaling with geometric mean of the built-in control gene probes for each sample.

RESULTS

Figure 2. Proportions of CD4 Naïve and CD4, CD8 TEMRA TIL Subsets (Suggestive of Higher Proliferative & Anti-Tumor Potential) Were Higher for Lymphoma vs Melanoma TIL

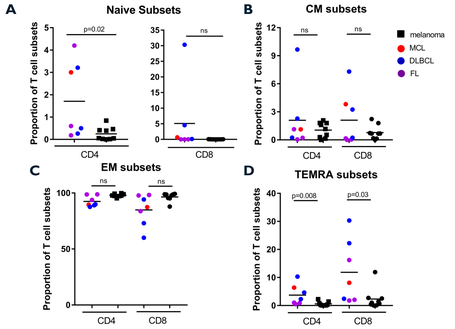


Figure 2. Lymphoma and melanoma TIL were stained using standard phenotype panel DF2 (Materials and Methods). Data shown represents different subpopulations of total CD4 and CD8 T cells in TIL. P values were calculated using two-tailed Mann-Whitney test (unpaired). Mean proportion of cell subsets is presented by horizontal bars.

Abbreviations: CM, central memory; EM, effector memory; TEMRA, Terminally differentiated effector memory.

Figure 3. A Higher Proportion of CD28$^+$CD4$^+$ TIL Subsets Was Observed in Lymphoma vs Melanoma TIL Suggesting Higher Proliferative Potential of Lymphoma TIL

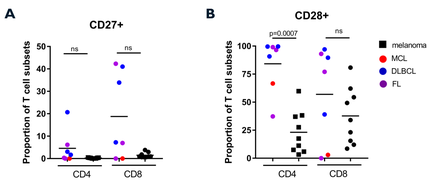


Figure 3. Lymphoma and melanoma TIL were stained using standard phenotype panels DF1 (Materials and Methods). Data shown represents different CD27 and CD28 subpopulations of total CD4 and CD8 T cells in TIL, and indicates higher proportion of costimulatory molecule-CD28 expressing CD4 T cells in lymphoma TIL. P values were calculated using two-tailed Mann-Whitney test (unpaired).

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

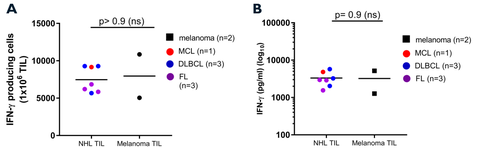


Figure 4. Comparable IFN-γ production by TIL following stimulation with dynabeads (CD3,CD28, CD137). IFN-γ was assessed by **(A)** ELISPOT and **(B)** ELISA. **A)** ELISPOT data is shown as IFN-γ producing cells per 10^6 TIL. **B)** IFN-γ levels in supernatants from TIL cultures (5x10^5 TIL/well) measured by ELISA is shown in logarithmic scale. P values were calculated using two-tailed Mann-Whitney test (unpaired).

Figure 5. Lymphoma and Melanoma: Derived TIL Exhibit Similar Lytic Activity

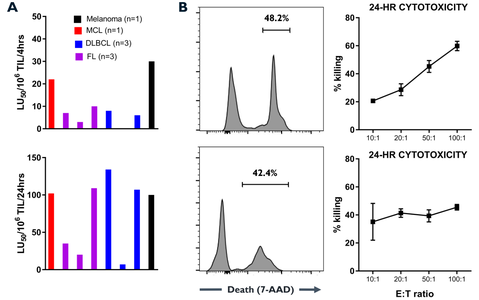


Figure 5. A) Data represents 50% lysis (LU_{50}) of target cells normalized to 10^6 TIL at 4 hrs (upper panel) and 24 hrs (lower panel) in co-culture (Effector cells: TIL, Target cells: GFP$^+$P815 cells) **B) Left Upper panel:** Cytolytic activity of melanoma TIL against allogeneic 526 target cells. **B) Left Lower panel:** cytolytic activity of lymphoma TIL against autologous tumor cells: determined by 7-AAD uptake. Histograms show % of dead cells in co-cultures with 50:1 E:T ratio. **Right Panel:** Graphs representative of percent killing of target cells induced by melanoma TIL (upper right) and lymphoma TIL (lower right) at different E:T ratio.

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Figure 6. Lymphoma TIL Exhibit Higher Expression of IL-17A and RORC Suggestive of Skewing Towards a Th17 Phenotype

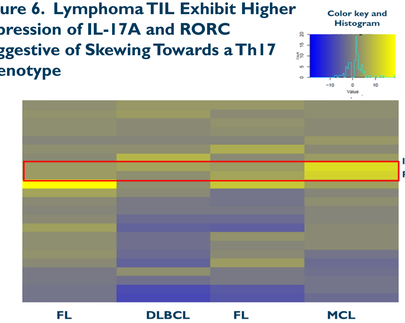

Figure 6. Gene expression profile of lymphoma and melanoma TIL was determined by 579 plex nCounter GX Human Immunology V2 CSO panel from NanoString. Heat map is representative of fold change in the expression of a particular set of genes in lymphoma TIL compared to melanoma TIL, and suggests a higher expression of IL-17A and RORC from lymphoma-derived TIL vs melanoma-derived TIL.

Abbreviations: FL3: follicular lymphoma TIL 3; DLBCL: diffuse large B-cell lymphoma TIL; FL1: follicular lymphoma TIL1; MCL: mantle cell lymphoma TIL.

CONCLUSIONS

- These preliminary data demonstrate the feasibility of isolation and expansion of TIL from lymphomas.
- TIL were expanded successfully from 7/7 NHL tumors including:
  - 1 mantle cell lymphoma,
  - 3 follicular lymphomas,
  - 3 diffuse large B cell lymphomas
- Phenotypic analysis of lymphoma TIL showed a relative increase in the proportion of naïve and CD4$^+$ CD28$^+$ T cell subsets, that are characteristics of cells in earlier development and with higher proliferative capacity.
- Functional analysis demonstrated similar lytic activity of lymphoma and melanoma TIL, suggesting similar therapeutic potential.
- Taken together, these data demonstrate the feasibility of isolation and culture of TIL from lymphomas and suggests 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 potential therapeutic candidates worthy of further clinical evaluation against a range of NHL targets.