Expansion of Tumor-Infiltrating Lymphocytes (TIL) Using Static Bag for the Clinical Manufacturing Rapid Expansion Protocol (REP) Process

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Introduction

Background

- Lymphocar (LN-149) and LN-145, adoptive cell therapies using autologous tumor infiltrating lymphocytes (TIL), have demonstrated encouraging efficacy with acceptable safety in a variety of tumor types.

- The Tumilloque Gen 2 clinical manufacturing process uses gas-permeable rapid expansion bioreactors (G-Rex®, Wilson Wolf, Sent In., MN) for T-cell expansion.

- Static gas-permeable cell culture bags (EXP-Pak®, ChemoMedical, Waltham, MA) are alternative bioreactors that have been used for clinical manufacturing of T-cells.

- T-cell product characteristics were compared after expansion at small- and full-scale using G-Rex bioreactors and EXP-Pak bags.

Methods

Study Objectives

- To determine the feasibility of using static gas-permeable cell culture bags to expand TIL for the clinical manufacturing rapid expansion protocol (REP).

- To characterize the final harvested product for the following quality attributes:
  1. Dose: Cell count and % viability
  2. Identity: % CD3+CD8+, % CD25+ and % CD38+.
  4. Phenotype: Memory, activation, exhaustion, and maturity status
  5. Reduction OXIDATION (REDOX): T-cell proliferation capacity, metabolic products, apoptosis, cell-cycle analysis, and mitochondrial function.
  6. T-cell Receptor (TCR) Clonotypes: Unique CDR3 counts and frequency distribution of the TIL product.

Proposed TIL Manufacturing Process Using EXP-Pak Bags

Figure 1. Gen 2 TIL Manufacturing Process and Experiment Design

Results

Figure 2. Viable Cell Dose, Purity, Identity, and Potency of the TIL Product

Figure 3. TIL Purity, Identity, Memory, and Differentiation

Discussion

- The final harvested TIL product met the release criteria for cell dose, purity (% viability), identity (%CD3+CD8+), and potency (IFN-γ release) in the supernatant.

- All of the harvested TIL products met the release criteria for viable cell dose, purity (% viability), identity (%CD4+CD5+), and potency (IFN-γ release).

- No difference was observed in activation and exhaustion status TIL between the G-Rex and EXP-Pak conditions.

Figure 5. Cellular REDOX State was Measured by Antibody, Cell Cycle, Proliferation, Metabolic By-Product, and Glucose Uptake

Figure 6. Mitochondrial Function of TIL

- In both G-Rex and EXP-Pak conditions, low contaminating non-T cells (B cells, monocytes) infected with human herpesvirus 8- or CMV-positive; immune populations were observed.

- T-cell proliferation was determined using student’s unpaired t-test (*, p-value) compared between the G-Rex and EXP-Pak conditions.

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- No difference was observed in activation and exhaustion status TIL between the G-Rex and EXP-Pak conditions.

- The final T-cell product generated in EXP-Pak bags did not differ in growth, functional viability, or phenotype compared to TIL manufactured in G-Rex flasks.

- Frequency distribution of the top 20 clones was similar between G-Rex and EXP-Pak conditions.

Conclusions

- The final T-cell product generated in EXP-Pak bags did not differ in growth, functional viability, or phenotype compared to TIL manufactured in G-Rex flasks.

- T-cell redox state and mitochondrial function were comparable between both conditions.

- TCR Vβ clonal diversity, sequence, and frequency of all comparability samples showed a high degree of similarity between both conditions.

- These data support further evaluation of EXP-Pak or similar static gas-permeable cell culture bags for potential use in clinical and commercial TIL cell therapy manufacturing applications.

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