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

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Introduction

Background

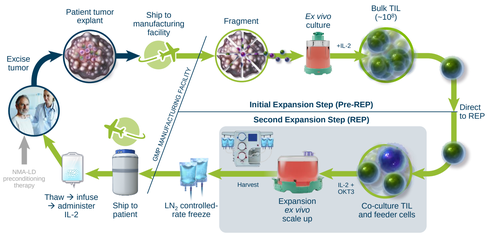
- Lifileucel (LN-144) and LN-145, adoptive cell therapies using autologous tumor-infiltrating lymphocytes (TIL), have demonstrated encouraging efficacy with acceptable safety in a variety of tumor types and settings[1–4]
- The lifileucel Gen 2 clinical manufacturing process uses gas-permeable rapid expansion bioreactors (G-Rex®, Wilson Wolf, Saint Paul, MN) for TIL expansion
- Static gas-permeable cell culture bags (EXP-Pak™, Charter Medical, Winston-Salem, NC) are alternate bioreactors that have been used for clinical manufacturing of T-cells
- TIL product characteristics were compared after expansion at small- and full-scale using G-Rex bioreactors and EXP-Pak bags

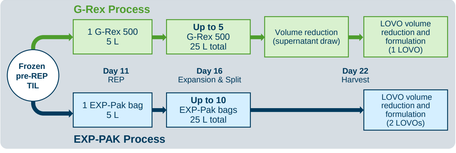
Study Objectives

- To determine the feasibility of using static gas-permeable cell culture bags to expand TIL for the clinical manufacturing rapid expansion protocol (REP) process
- To characterize the final harvested product for the following quality attributes:
  1. Dose: Cell count and % viability
  2. Identity: % $CD45^+CD3^+$ T-cells
  3. Potency: Ability to secrete IFNγ in response to stimulation with anti-CD3, -CD28, and -CD137
  4. Phenotype: Memory, activation, exhaustion, and impurity status
  5. Reduction/Oxidation (REDOX) Potential: TIL proliferation capacity, metabolic by-products, apoptosis, cell-cycle analysis, and mitochondrial function
  6. T-cell Receptor (TCR) Clonotypes: Unique CDR3 counts and shared clones of TCR repertoire

Proposed TIL Manufacturing Process Using EXP-Pak Bags

Figure 1. Gen 2 TIL Manufacturing Process and Experiment Design





Methods

Manufacturing

- Cryopreserved pre-REP TIL were cultured with OKT-3 and irradiated peripheral blood mononuclear cells in small-scale (G-Rex 5M flasks or EXP-50 bags) or full-scale (G-Rex 500MCS or EXP-5L bags) conditions using the same culture media formulation throughout the 22-day process (Figure 1)

Dose

- Final harvested TIL and in-process samples were assayed for total nucleated cells, total viable cells, and viability determined by acridine orange / DAPI counterstain using the NucleoCounter® NC-200™ (ChemoMetec, Lillerød, Denmark) automated cell counter

Identity

- Final harvested TIL products were sampled and assayed for identity by flow cytometry staining
- Percent T-cells was determined as the percentage of $CD45^+CD3^+$ (double positive) population of viable cells

Potency

- The ability of the harvested TIL product to secrete IFNγ upon reactivation was measured following coculture with antibody-coated beads (anti-CD3, -CD28, and -CD137; ThermoFisher, Waltham, MA)
- After 24 hours of co-culture, culture supernatants were harvested, frozen, thawed, and assayed by ELISA
- Quantikine® IFNγ ELISA kit (R&D Systems, Minneapolis, MN) was used to measure IFNγ in the supernatant

Phenotype

- Final harvested TIL products were thawed and assayed for extended phenotypic markers using two flow cytometry panels
- Multicolor flow cytometry was performed to characterize TIL purity, identity, memory subset, activation, and exhaustion status
- Data were acquired from stained sample products on the FACS Canto II™ (BD Biosciences, Franklin Lakes, NJ) and ZE5 (Bio-Rad, Hercules, CA) cell analyzers

Cellular REDOX State

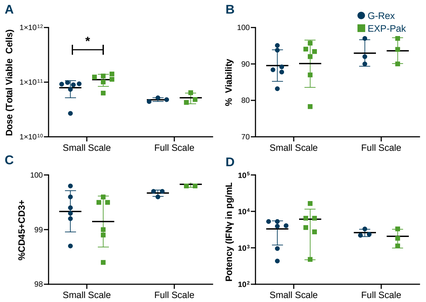
- Reactive oxygen species (ROS) are thought to have effects on T-cell function and proliferation; low concentrations of ROS in T-cells are a prerequisite for cell survival, and increased ROS accumulation can lead to apoptosis/necrosis[5,6]
- Final harvested TIL products were tested for apoptosis, cell cycle, proliferation, metabolic by-product, and mitochondrial function
  - Late-stage apoptosis was measured by staining the final TIL product using Annexin-V and 7AAD (7-aminoactinomycin D) antibody staining
  - Cell cycle stage was measured using FxCycle™ PI/RNase Staining Solution kit (ThermoFisher)
  - Cell proliferation was measured using MTT reagents on harvested TIL products that were rested and incubated with or without IL-2 for 6 days
  - Metabolic by-products (lactate) were measured in the spent media on days 11, 16, and 22
  - MitoSOX™ (mitochondrial morphology and reactive oxygen species, ThermoFisher), MitoTracker™ Green (dysfunctional mitochondria, ThermoFisher) or MitoTracker Red (functional mitochondria, ThermoFisher) were determined using flow cytometry

TCR Clonotypes

- Final harvested TIL products were sampled and mRNA was isolated for TCR V-beta deep sequencing performed at iRepertoire, Inc. (Huntsville, AL)

Results

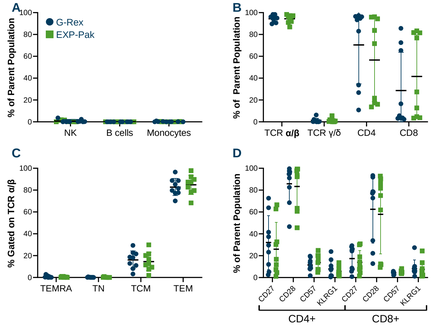
Figure 2. Viable Cell Dose, Purity, Identity, and Potency of the TIL Product



P-values represent the difference between G-Rex and EXP-Pak condition using student's unpaired T test (*, *P* <0.05). Mean ± SD are displayed.

- All of the harvested TIL products met the release criteria for viable cell dose, purity (% viability), identity (%$CD45^+CD3^+$), and potency (IFNγ release)

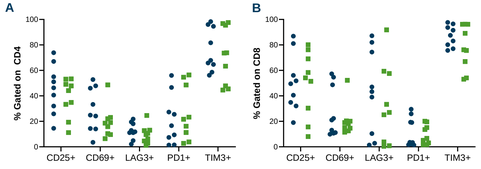
Figure 3. TIL Purity, Identity, Memory, and Differentiation



Memory subsets were identified based on the levels of CD45RA and CCR7. Mean ± SD are displayed. TEMRA=$CD45RA^+$ effector memory ($CD45RA^+$,$CCR7^-$), TN=naïve ($CD45RA^+$, $CCR7^+$), TCM=central memory ($CD45RA^-$, $CCR7^+$), TEM=effector memory ($CD45RA^-$, $CCR7^-$).

- In both G-Rex and EXP-Pak conditions, few contaminating non–T-cell (NK cells, B cells, and monocytes) immune populations were observed
- TIL generated in both conditions predominantly expressed TCR α/β with lesser TCR γ/δ
- The ratios of $CD4^+$ and $CD8^+$ populations were comparable in both conditions
- Majority of TIL displayed effector memory phenotype and exhibited less differentiated status in both conditions

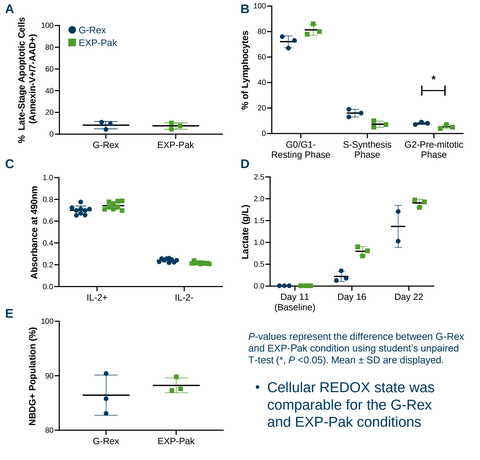
Figure 4. TIL Activation and Exhaustion Status



Statistical significance between G-Rex and EXP-Pak condition was determined using student's unpaired T test (*P* <0.05).

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

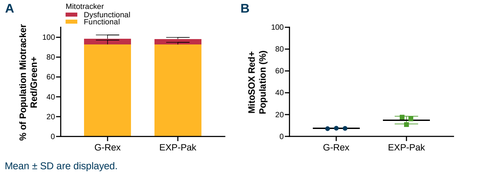
Figure 5. Cellular REDOX State was Measured by Apoptosis, Cell Cycle, Proliferation, Metabolic By-Product, and Glucose Uptake



P-values represent the difference between G-Rex and EXP-Pak condition using student's unpaired T-test (*, *P* <0.05). Mean ± SD are displayed.

- Cellular REDOX state was comparable for the G-Rex and EXP-Pak conditions

Figure 6. Mitochondrial Function of TIL



Mean ± SD are displayed.

- Mitochondrial function was comparable for TIL grown in G-Rex and EXP-Pak conditions

Figure 7. Summary of Final TIL Product TCR V-beta Clonotype and Frequency Distribution

A

			Number of Common uCDR3 (% Overlap)	
			D22 G-Rex	D22 EXP-Pak
Number of Common uCDR3 (% Overlap)	Run 1	D22 G-Rex	10584 (100)	5784 (97,* 91†)
		D22 EXP-Pak	-‡	20212 (100)
	Run 2	D22 G-Rex	11701 (100)	4607 (93,* 96†)
		D22 EXP-Pak	-‡	14934 (100)
	Run 3	D22 G-Rex	3792 (100)	2122 (99,* 99†)
		D22 EXP-Pak	-‡	4172 (100)

*Percentage of uCDR3 clones in G-Rex found in EXP-Pak condition.
†Percentage of uCDR3 clones in EXP-Pak found in G-Rex condition.
‡Not applicable.

B

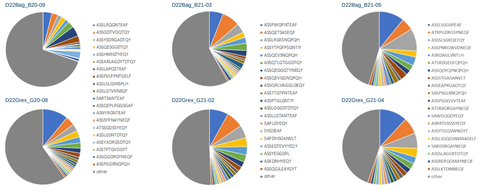


Figure shows frequencies of the most abundant 20 clones in each of the samples.

- 91% − 99% of TCR clones of TIL produced from EXP-Pak bags were present in TIL produced from G-Rex flasks
- Frequency distribution of the top 20 clones was similar between G-Rex and EXP-Pak conditions

Conclusions

- The final TIL product generated in EXP-Pak bags did not differ in growth, functionality, or phenotype compared with cells produced in G-Rex flasks
- TIL cellular REDOX state and mitochondrial function were comparable between both conditions
- TCR V-beta 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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Abbreviations
2-NBDG, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose; DAPI, 4′,6-diamidino-2-phenylindole; GMP, good manufacturing process; IFN, interferon; IL-2, interleukin-2; MHC, major histocompatibility complex; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NK, natural killer; REDOX, reduction/oxidation; REP, rapid expansion protocol; ROS, reactive oxygen species; TCM, central memory T-cells; TCR, T-cell receptor; TEM, effector memory T-cells; TEMRA, effector memory RA+ T-cells; TIL, tumor-infiltrating lymphocytes; TVC, total viable cells; uCDR3, unique CDR3.

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