Smart Selection of Leukapheresis Starting Material and Continuous Process Improvement for iPBMC Manufacturing

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Introduction

Tumor-infiltrating leukocytes (TILs) are a promising autologous immunotherapeutic due to prior antigenic stimulation and ability to navigate tumor microenvironment (TMEs)[1]. The manufacturing process is highly dependent on feeder cells, irradiated peripheral blood mononuclear cells (iPBMCs), processed from leukapheresis starting material (i.e., leukopaks; **Fig. 1**). Quality control testing is needed to ensure patient safety; iPBMCs can contribute to graft vs. host (GvH) disease if co-transfused, and thus, quality control assays are employed to ensure irradiation status and non-proliferation of each iPBMC product. The primary challenges addressed in these research studies are to 1) identify critical material attributes (CMAs) to pre-screen starting material and mitigate manufacturing failures, 2) bring down manufacturing costs and increase safety without compromising product quality by modulating critical process parameters and materials, and 3) implement a product characterization strategy to improve iPBMC strength.

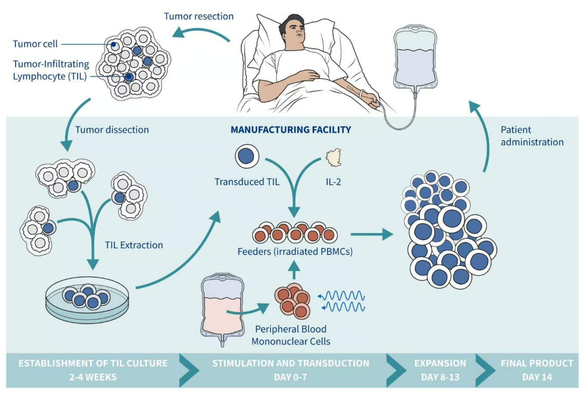

Figure 1: Vein-to-vein Lifecyle for TIL Therapies. A biopsy procedure removes a section of tumor containing TILs (e.g., melanoma tumors[2]), which are then fragmented and isolated in vitro based on their neoantigen reactivity, initial outgrowth, and other factors. Rapid expansion protocols are employed to coculture TILs with iPBMCs in the presence of IL-2 and activation molecules to reach a viable dose concentration. Irradiation is sufficient to interfere with cell growth cycles, which can be confirmed using quality control assays (not pictured). Expanded TILs are formulated as a final product and administered to patients who have received lymphodepleting chemotherapy. The entire process occurs over 6+ weeks for drug manufacturing.

Materials & Methods

- Strategic Blood Bank Collection Center partners are qualified for donor recruitment per 21 CFR Part 630, apheresis collections, and temperature-controlled distribution (**Fig. 1A**)**.**
  - Donor characterization database is continuously compiled **(Fig. 2B-D**).
- Leukapheresis starting material (i.e., Leukopaks) is manufactured to cryopreserved iPBMC feeder cells using a Ficoll-based manual isolation to remove impurities and a controlled-rate freezer.
  - Aim 1: Historical data analysis of automated cell counters and hematological analyzers, critical process parameters (CPPs) identified critical material attributes (CMAs) for leukopaks.
  - Aim 2: Optimization studies to a) increase the exposure of iPBMCs in cryosolution and b) like-for-like analysis of an alternative cryosolution utilized this manufacturing process with n=3 independent leukopaks.
- Manufacturing is performed in ISO5 biosafety cabinets with ISO7 background.
- Final product is evaluated for a) sterility via USP <71> compendial method and b) custom non-proliferation analytical test method in the G-Rex platform.
  - AIM 3: Investigated the role of deep characterization parameters of iPBMCs and the strength of supporting non-irradiated PBMCs expansion (i.e., TIL proxy).

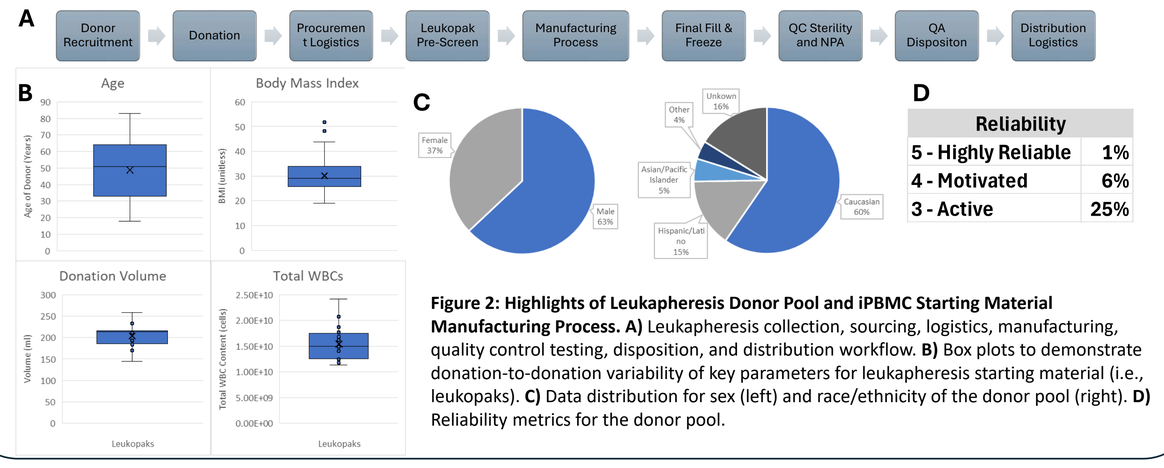

Figure 2: Highlights of Leukapheresis Donor Pool and iPBMC Starting Material Manufacturing Process. A) Leukapheresis collection, sourcing, logistics, manufacturing, quality control testing, disposition, and distribution workflow. **B)** Box plots to demonstrate donation-to-donation variability of key parameters for leukapheresis starting material (i.e., leukopaks). **C)** Data distribution for sex (left) and race/ethnicity of the donor pool (right). **D)** Reliability metrics for the donor pool.

Process Optimization Results

White blood cell (WBC) percent recovery and viability are CPPs measured using pre-screen leukopak and post-Ficoll isolated samples. Trending data yielded a subset of manufactured processes with low recovery; an analysis led to two observations: 1) an impurity level for 'X' above 20% reduced recovery below 40%, and 2) visual observation of clots reduced recovery to an average of 60%. Thus, CMAs were empirically defined for impurity 'X' <20% and no severe clotting (**Fig. 1A-B**)**.** Additionally, the CPP of ≤30-minute exposure to cryosolution, designed to support product quality, was compromising yield and personnel safety (due to final fill with syringe/needles). Experimental data demonstrated that an increase in cryosolution exposure time up to 120 minutes before the controlled-rate freezer start did not compromise product quality, as assessed by post-thaw recovery (target concentration of 1e8 cells/ml and viability of >90%; **Fig. 3C-D**); thus, a 90-minute exposure time was implemented. An effectiveness check was performed using analysis of manufacturing data between Aug 2024 and Aug 2025. Viability is not compromised between pre-screen and post-Ficoll samples (CPP lower limit of ≥70%; **Fig. 3E**). RBC and PLT impurities are reduced by 0.5- and 0.3-fold difference (**Fig. 3F**). Importantly, CPP stability is observed for WBC recovery (**Fig. 3G**) and mononuclear cell (MNC) purity (**Fig. 3H**). Thus, CMAs and increased exposure limits improved the yield of high-quality iPBMC products for downstream TIL manufacturing.

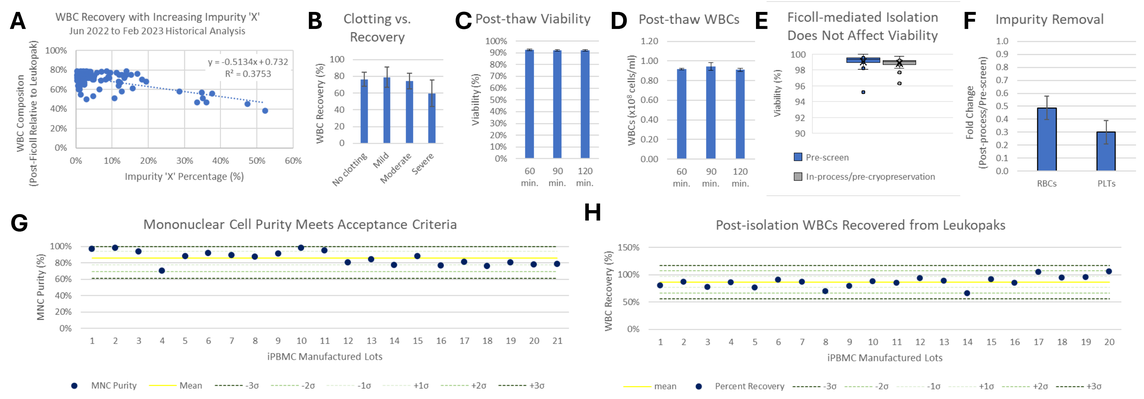

Figure 3: Optimization Strategy and Resulting Trends in Product Quality. A) WBC recovery relative to impurity 'X' using a scatterplot with linear trendline analysis or **B)** qualitative clotting using bar charts (independent variables reflect leukopaks; historical data compiled from June 2022 – Feb 2023). **C)** Post-thaw viability and **D)** WBC concentration for controlled exposure to cryosolution at 60-, 90-, and 120-minutes prior to freeze cycle. **E)** Current manufacturing process: Cell viability measured using an automated cell counter (AO, DAPI fluorescent imaging) for leukopaks and isolated iPBMCs using a manual Ficoll-based isolation, **F)** Efficiency of manual Ficoll-based isolation in reducing contaminants (i.e., red blood cells [RBCs], platelets [PLTs]), **G)** control trending for critical process parameters of WBC recovery, and **H)** MNC purity.

Continuous Improvement Results

The cryopreservation solution, as a critical material, was evaluated against an experimental like-for-like item. No significant impact on critical quality attributes (i.e., cell health and post-thaw recovery) was observed in this continuous improvement exercise across n=3 independent leukopaks processed and cryopreserved (**Fig. 4A-B**). The iPBMCs showed a slight increase in proliferation of an MNC (>2000-fold increase) and both declined in monoculture by >60% **(Fig. 4C-D**)**.** This process re-development is part of our quality commitment and efforts to bring affordable products, as shown by prospective cost savings of ~58% for 100 products manufactured (**Fig. 1E**). Increasing affordability of advanced therapies will support patient access to life-saving treatment.

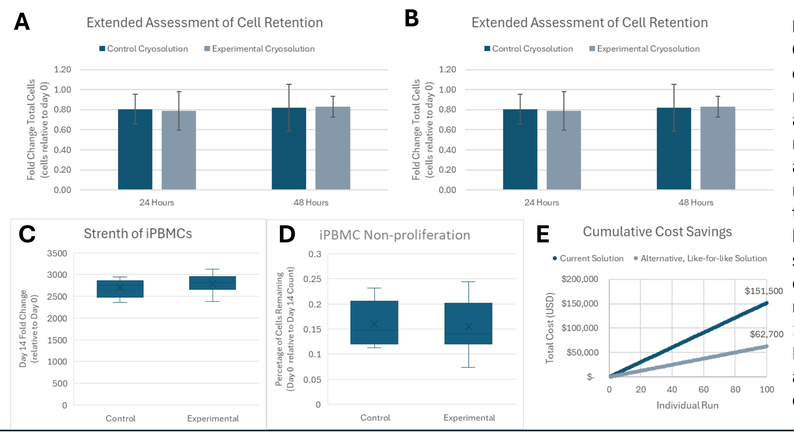

Figure 4: Like-for-like Analysis and Qualification of Alternative, Cost-effective Cryosolution: A) Cell health measured on days 0 (i.e., post-thaw) and at 24- and 48-hour checkpoints reported as percent viability using an automated cell counter. **B)** Cell retention at 24- and 48-hour timepoints to show consistency of long-term recovery. **C)** Assessing the strength of iPBMC to support MNC expansion and **D)** safety of iPBMCs measured via a non-proliferation over 14 days of culture using cell counts. **E)** Projected cost savings to switch to an alternative cryosolution without compromising product quality.

Quality Control Metrics

We developed a custom non-proliferation assay to define the optimal starting concentration for measuring iPBMC replication incompetence and MNC fold expansion by titrating iPBMC ratios in monoculture and coculture with non-irradiated MNCs. Empirically defined starting concentrations were then employed to meet the Phase I release strategy acceptance criteria: a <60% decrease in iPBMCs and >1-fold MNC expansion. Historical data confirmed stability, showing post-thaw viability is consistently ≥75 (lower acceptance limit), iPBMC replication incompetence is <0.4-fold, and MNC fold expansion is >1-fold (**Fig. 5A-C**). Lot-to-lot variability was observed in the MNC expansion metric, and ongoing optimization is focused on refining test method parameters to improve this stability. Nonetheless, the assay's primary purpose is to assess the safety metrics of iPBMCs for their subsequent use in TIL manufacturing. Next, we tested the hypothesis that high-performing iPBMC products support superior MNC fold expansion. iPBMC performance is measured using a proprietary algorithm that assesses demographics, metabolic fitness, and polyfunctionality to stratify performance levels correlating with clinical efficacy. An analysis of metabolic fitness and the Polyfunctional Strength Index (PSI) was performed on n=4 iPBMC products **(Fig. 5D-E).** Critically, PSI correlated significantly with increased MNC expansion (R^2 = 0.69; **Fig. 5F**), suggesting that iPBMCs can be proactively down-selected based on their capacity to support TIL expansion protocols.

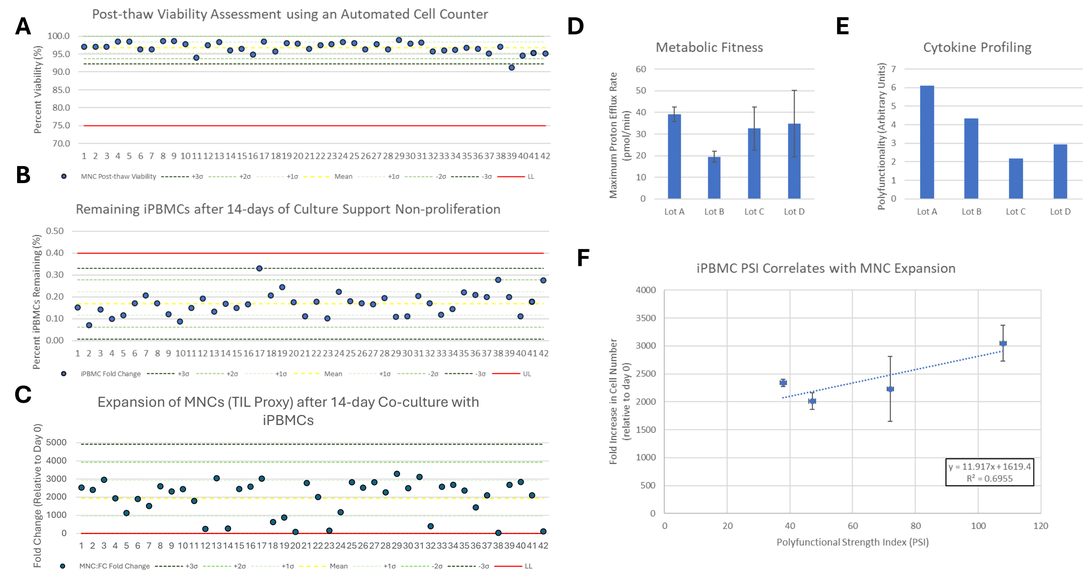

Figure 5: Implementation of a Non-proliferation Assay and Donor-selection Strategies to Improve iPBMC Strength. A) Post-thaw viability, measuring using an automated cell, stability trending (lower limit = 75%), **B)** percent remaining iPBMCs after 14-day culture (upper limit = 40%), and **C)** fold increase of MNCs (i.e., proxy for TILs) after 14 days of culture. **D)** Metabolic fitness measured using a T-cell activation assay and **E)** PSI measured using the manufacturer's proprietary algorithm using 32-secreted cytokine measurements at a single cell resolution for n=4 iPBMC lots. **F)** Scatterplot with linear trend analysis for MNC fold expansion and PSI.

Conclusions

In summary, our work utilized continuous monitoring data to define key CMAs, specifically establishing a limit of <20% impurity 'X' and disqualifying severely clotted leukopaks from further manufacturing. We optimized the cryopreservation process by safely extending cryosolution exposure time to enhance product yield and validated a cost-effective, like-for-like cryosolution alternative to mitigate supply chain logistical risks. Crucially, we developed a custom non-proliferation assay which, when combined with proprietary E-score algorithms, demonstrated that selecting 'stronger' iPBMCs significantly reduces the number of starting cells required to achieve a high-yield TIL drug products.

Future efforts will focus on sustained monitoring of iPBMC parameters, the evaluation of new critical material alternatives to reduce cost and improve quality, and expanding the iPBMC characterization analysis. Additionally, automated approaches (+/- Ficoll) are being explored to increase throughput. These efforts are designed to ultimately improve patient access and reduce the high-cost burden associated with complex TIL manufacturing.

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[1]Tas L, Jedema I, Haanen JBAG. Novel strategies to improve efficacy of treatment with tumor-infiltrating lymphocytes (TILs) for patients with solid cancers. Curr Opin Oncol. 2023 Mar 1;35(2):107-113. doi: 10.1097/CCO.0000000000000925. Epub 2023 Jan 27. PMID: 36607824; PMCID: PMC9894146.
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