Robust autologous CD34+ HSPC manufacturing with a closed and automated process optimized for patients with sickle cell disease

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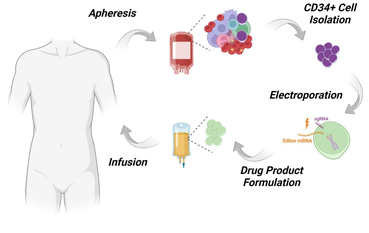
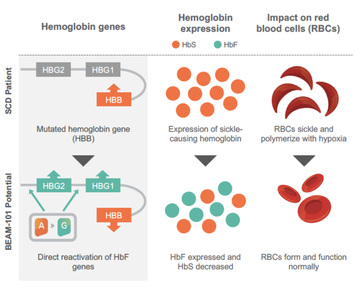
1. Introduction

- The manufacture of autologous cell therapies and genetic medicines, particularly for sickle cell disease (SCD), face significant challenges in the mobilization, collection, and processing of CD34+ hematopoietic stem and progenitor cells (HSPCs).
- The resulting variability in mobilized leukapheresis starting material necessitates a versatile manufacturing process.
- Key technologies to improve process performance include spinning membrane filtration for platelet reduction, blood filters to mitigate cell clumping, and counter-flow centrifugation
- These technologies have the potential to accommodate a wide range of batch sizes, increase process yield, and reduce the number of manufacturing runs per patient.



2. CD34+ HSCT Therapy for Sickle Cell Disease

- BEAM-101: Designed to be potential best-in-class genetic medicine for SCD



SCD Unmet Need
- Sickle cell hemoglobin (HbS) polymerization is root cause of sickle cell pathophysiology
- Affects millions of people worldwide and ~100K in U.S.
- Median survival in the U.S. is ≥20 years shorter

Current Available Treatments
- Disease-modifying therapies require ongoing treatment and do not prevent organ dysfunction
- Recently approved gene therapies reduce VOCs but residual HbS >50% suggests room for improvement

BEAM-101 Potential
- Precision editing without requirement of double-stranded DNA breaks or viral insertion
- More efficient editing leading to greater and more uniform induction of HbF and reduction of HbS and normalization of hemoglobin
- Investment in wholly owned manufacturing and improved process and patient experience

CDC Data & Statistics; Lancet Haematol 2023; 10: e585–99; DeBaun et al. Blood. 2019 Feb 7; 133(6):615-617

3. Objective

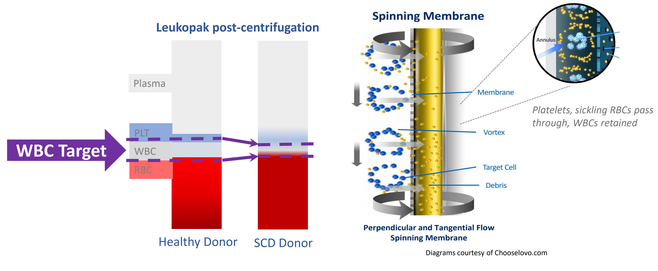
- The aim of this study was to develop a closed and automated CD34+ HSPC process for manufacturing of cell therapies modified by base-editing which generate a high frequency of productive edits and without creating double strand breaks. The process must have the flexibility for a wide range of patients and variable starting cell numbers while maintaining robust cell yield and high drug product quality and be suitable for use in a Phase 1/2 clinical trial (NCT05456880).

CD34+ HSPC Isolation

CD34+ HSPC Editing

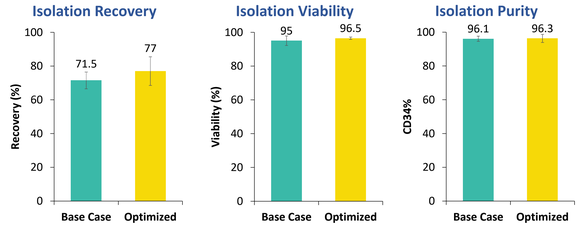
4. Spinning Membrane Filtration Improves WBC Recovery

- Establishing a clean interface via centrifugation between the platelet, WBC, and RBC layers in SCD starting material can be difficult
- Spinning membrane filtration provides an automated method to remove platelets and some RBCs while recovering nearly <u>100% of the target WBCs</u>
- Customization of automation protocol results in high viability throughout processing regardless of incoming material source (i.e. healthy donor, sickle cell trait donor, or sickle disease patient)



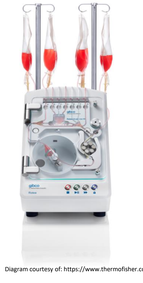
5. CD34+ Isolation Capacity Optimization

- Wide variability in starting material WBC content and purity necessitates capacity versatility while maintaining robust performance
- Optimization of bead incubation and loading parameters enables a 2.5-fold increase in starting material cell loading capacity with equivalent process metrics



6. Counter-flow Centrifugation Automates Formulation

- Implementation of counter-flow centrifugation increased process capacity and flexibility
- Three-dimensional washing of cells removed spent media, debris, and other undesirable components
- Automation of cell wash and concentration eliminated yield and contamination risk of centrifugation-based manual wash and resuspension steps



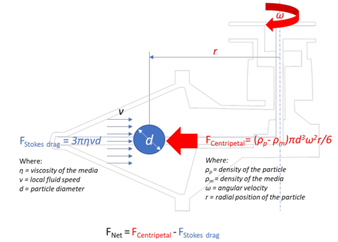
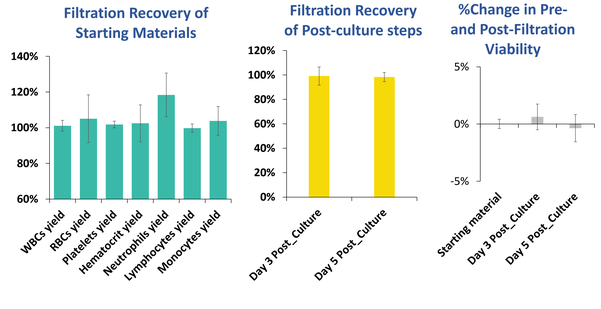


Diagram courtesy of: https://www.thermofisher.com/us/en/home/clinical/cell-gene-therapy/cell-therapy/cell-therapy-manufacturing-solutions/rotea-counterflow-centrifugation-system

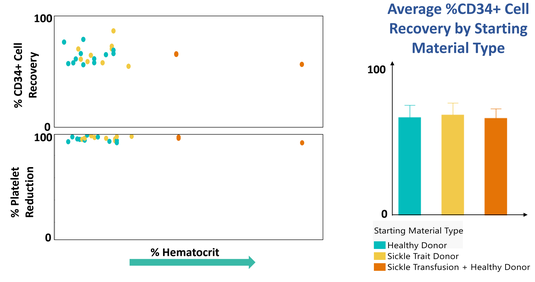
7. Blood Filters Mitigate Clumping

- SCD CD34+ HSPCs may form clumps during processing that can clog automated unit operations and negatively impact product quality
- Implementation of blood filters to remove debris from starting material and post-culture steps mitigates this risk without introducing cell loss



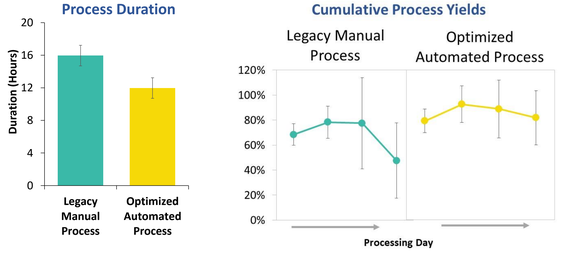
8. Surrogate SCD Starting Material Process Development

- SCD derived starting material cannot be used for process development
- Sickle cell trait and healthy donor leukapheresis spiked with sickle cell disease exchange transfusion RBCs can be a suitable surrogate starting material to evaluate process robustness



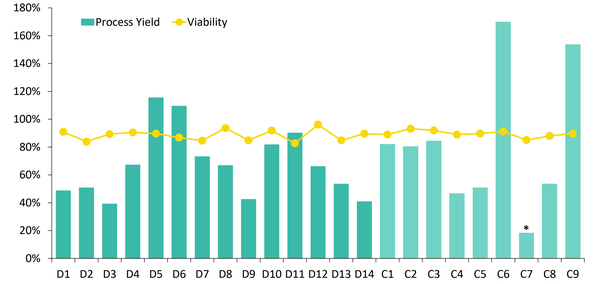
9. Automation Improves Manufacturing Execution

- Increased cumulative yield process for automated vs. legacy manual process
- Reduced overall process duration for automated process
- Increased process capacity by 3x in all parameters of incoming starting material
- Reduced contamination risk with closed processing
- Decreased operator variability using automated unit operations



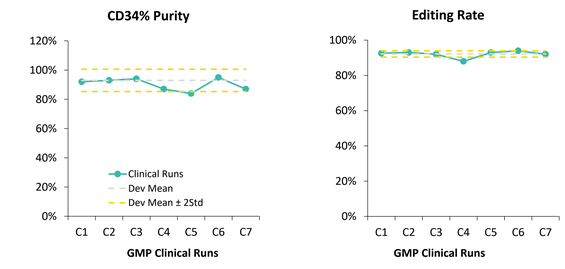
10. Robust Process Yield and Viability

- Process yield and drug product viability are consistent between development healthy donor runs and SCD patient GMP clinical runs and support clinical requirements



11. Automated Process Consistent Drug Product Quality

- The optimized closed and automated BEAM-101 process produces drug product with consistently high CD34+ purity and editing rate



N = 14 Developmental Runs used to establish the Dev Mean and Std

12. Conclusions

- The BEAM-101 manufacturing process has accommodated a wide range of patient starting material variability and generated consistent drug product yield, viability and quality
- Automated processing technologies including spinning membrane filtration, optimized CD34 isolation, blood filters, and counterflow centrifugation provided robust process performance while maintaining the batch size flexibility necessary to support the range of scales required by an autologous SCD patient population
- Surrogate starting material leveraging SCD RBC spiking of health donor leukapheresis collections enabled evaluation of manufacturing process robustness during development
- The closed and automated BEAM-101 manufacturing process mitigates risk of contamination and manufacturing execution variability. It is anticipated that this could contribute to an improved patient treatment experience and potential clinical benefit
- High yielding manufacturing processes such as the one described here have the potential to minimize the number of leukapheresis collections needed to meet the dose target for individuals with SCD