Integrated Editing and Manufacturing Process Design for BEAM-101, an Autologous CD34+ HSPC Therapy for Sickle Cell Disease, Results in Robust Process Yield and Increased HbF Induction

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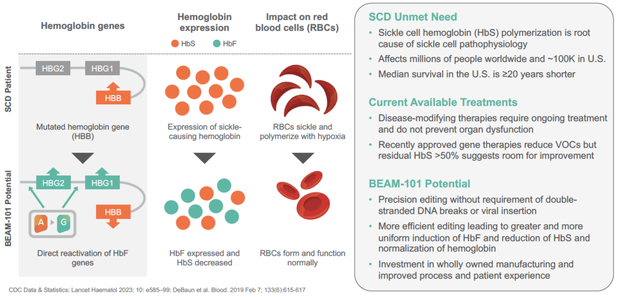
1. Introduction

- The development of autologous cell therapies for sickle cell disease (SCD) is inherently complex and requires integration of both product and process design.
- This integration includes not only the development of CD34+ cell isolation and editing manufacturing processes that have the potential to improve yield, reduce the number of mobilization cycles, and shorten the processing duration but also careful selection of gene editing methodology that maximizes the desired therapeutic outcomes.
- Currently, re-expression of fetal hemoglobin (HbF) can be achieved through distinct mechanisms of action including base editing of HBG1/2, Cas9-nuclease editing of HBG1/2, or Cas9-nuclease disruption of BCL11A expression.
- A comprehensive approach to therapeutic and process design that optimizes the induction of HbF and optimizes manufacturing process automation has the potential to improve product quality and the overall therapeutic experience for patients.

2. CD34+ HSCT Therapy for Sickle Cell Disease

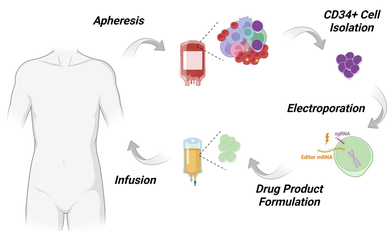
- BEAM-101: Designed to be best-in-class genetic medicine for SCD



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

3. Objective

- The aims of this study were 1) to evaluate base-editing and nuclease-editing methodologies to both increase re-expression of HbF and minimize cell damage and repair pathway activation 2) to evaluate the capability of an automated process to manufacture high drug product quality for use in the BEACON Phase 1/2 clinical trial (NCT05456880).

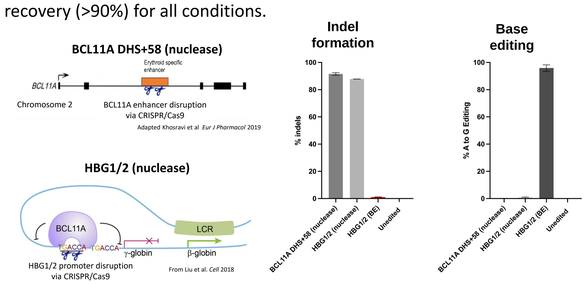


4. Study Methods

- Nuclease-based disruption of the erythroid enhancer in the BCL11A gene was assessed at research scale. Indel formation was measured by NGS and expression of HbF was determined by high-performance liquid chromatography conducted on in vitro erythroid differentiated cells derived from ex vivo edited CD34s. Results are reported as Mean ± Standard Deviation and shown as error bars in all plots.
- GMP clinical manufacturing was performed for 149 HPSC Isolations and 29 editing runs. An automated cell counter was used for cell count and viability, a flow cytometer for CD34+%, and next generation sequencing (NGS) for base editing rate. The data analysis exclude the clinical runs with known deviations. Results are reported as median, range (min – max).

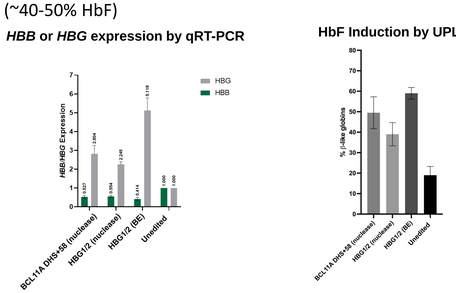
5. High efficiency editing of *HBG1/2* and *BCL11A* locus

- Cas9 nuclease disruption of the *BCL11A* erythroid enhancer region (DHS+58) and *HBG1/2* promoter was assessed at research scale and compared to base editing (BE) of *HBG1/2* promoter via electroporation of mRNA of nuclease or base editor enzymes and sgRNA.
- High efficiency nuclease (>85% indels) and BE (>90% editing, <1% indels) rates were achieved in hCD34s with high viability (>95%) and high cell recovery (>90%) for all conditions.



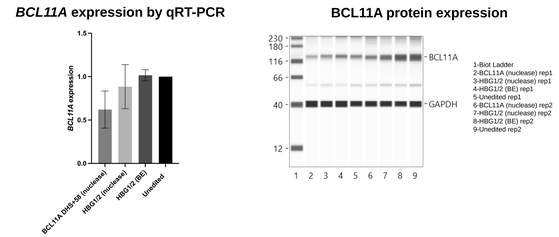
6. Higher HbF induction with BE of HBG1/2 promoter

- BE of *HBG1/2* had 5x-fold higher induction of *HBG* expression, compared to ~2.8x fold by nuclease-based disruption of erythroid enhancer of *BCL11A* by qRT-PCR in in vitro erythroid differentiated (IVED) cells (d14)
- BE of mobilized PB hCD34s led to higher HbF induction by Ultra-High Performance Liquid Chromatography (UHPLC) for BE (60% HbF) vs nuclease-based (~40-50% HbF)



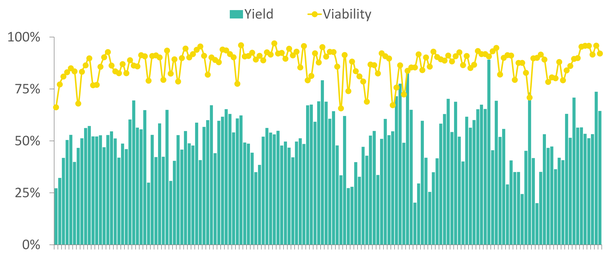
7. Nuclease erythroid enhancer disruption partially knocked down BCL11A

- Nuclease based disruption (>85% indels) reduced *BCL11A* expression in IVED cells (d14) ~50% by qRT-PCR
- BCL11A protein was only partially reduced despite high levels of indel formation
- Lower HbF induction may be due to incomplete BCL11A knockout in erythroid cells, or possibly due to variable knockout efficiency of random insertions and deletions resulting from double strand break repair



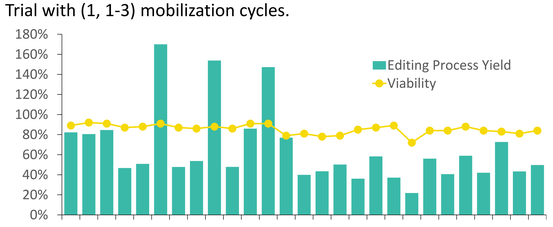
8. CD34+ Isolation Yield, Viability and Purity

- Robust clinical isolation process performance was achieved in 149 clinical manufacturing batches with the yield (52.3%, 20.0% - 89.1%), viability (89.3%, 65.7% - 97.0%), and purity (85.6%, 52.8% - 98.6%).



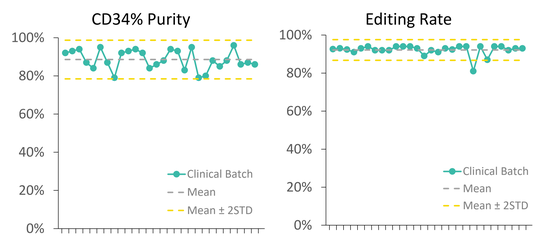
9. Robust Editing Process Yield and Viability

- Consistent Editing Process yield (50.9%, 21.8% - 170.0%) and Drug Product Viability (86%, 72% - 92%) profiles were achieved in the GMP clinical runs for SCD patients.
- Editing process yield was consistent with quiescent cell culture with no evidence of multiple population doublings characteristic of differentiation, suggesting maintenance of pluripotency for BEAM-101 DP.
- BEAM-101 Dose Target was met for 100% of patients (29 of 29) on the Beacon Trial with (1, 1-3) mobilization cycles.



10. Consistent Drug Product Quality

- The BEAM-101 drug product manufactured exhibited consistently high CD34+ purity (88%, 79% - 96%) and editing rate (93%, 81% - 94%).



11. Conclusions

- BEAM-101, a CRISPR base-edited therapeutic for SCD, has been developed through the integration of process development with optimal selection of gene editing methodology.
- Robust process yield, viability, and editing rates have been demonstrated while the number of mobilization cycles has been reduced.
- Likewise, the selection of base editing the HBG1/2 promoter yielded superior desired fetal hemoglobin induction compared to a nuclease editing approach. The resulting process and therapeutic design delivered consistent drug product quality and potentially improved patient experience.

- More information on BEACON clinical results for BEAM-101 — **See Poster PF1151**
- More information on BEACON biomarkers for BEAM-101 — **See Poster PF1155**

12. Acknowledgement

Beam Headquarters

Beam Manufacturing

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