Iterative Optimization of Hematopoietic Stem Cell Collection for Gene Therapy in Sickle Cell Disease

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Background

- Commercial SCD gene therapies require reliable autologous CD34+ collection.
- G-CSF is avoided because of vaso-occlusive risk; CXCR4 antagonists are used for mobilization.
- Even with adequate pCD34+ counts, leukapheresis may fail from interface instability, platelet clumping, and circuit clotting.

- Objective: describe iterative process optimization after an initially inadequate collection.

Case

Adult male with HbSS undergoing autologous collection for gene therapy

- Cycle 1: plerixafor after red blood cell exchange.
- Four procedures over 3 days collected 4.17 × 10^6 CD34+/kg.
- CE remained low at 31.2%-38.4%.
- Recurrent interface instability and clotting required interruptions and rescue heparin.
- Collected dose was inadequate for manufacturing.

Multimodal Optimization Strategy

RBC exchange q3w HbS ~30% → Hct + iron optimization → Motixafortide → Aspirin + therapeutic LMWH → Real-time interface tuning

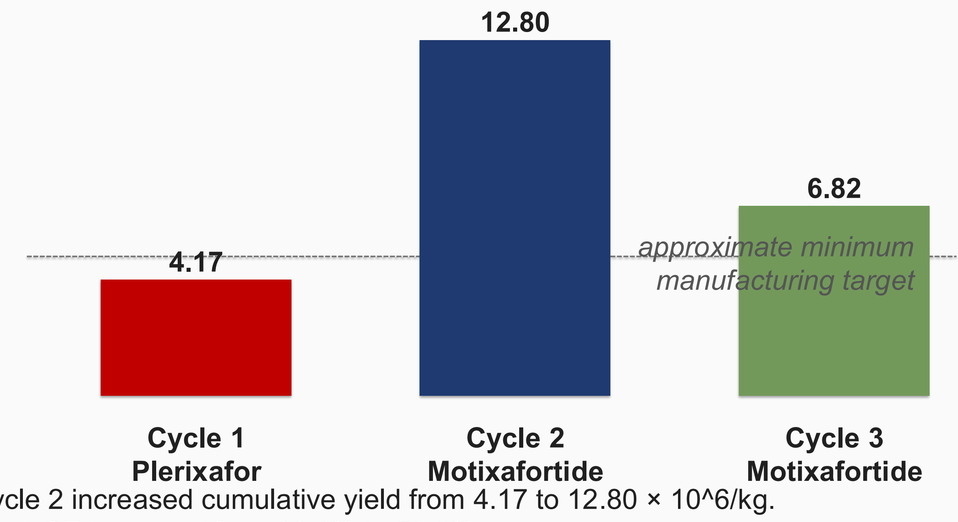
- ACD-A remained the circuit anticoagulant; systemic anticoagulation was individualized for recurrent thrombosis.
- Calcium/magnesium replacement and real-time apheresis review were standardized.
- Single-patient observation: not evidence for routine systemic anticoagulation.

Key Takeaways

- Mobilization success and collection success are related, but not equivalent.
- Hematologic preparation and interface management can determine manufacturing readiness.
- Product hematocrit, AC:WB ratio, and circuit stability should be reviewed in real time.
- This experience identifies testable strategies for prospective multicenter study.

Outcomes

Cumulative CD34+ yield (×10^6/kg)



- Cycle 2 increased cumulative yield from 4.17 to 12.80 × 10^6/kg.
- Peak CE improved from 38.4% to 58.2%.
- Cycle 3 reproduced collection: 6.82 × 10^6/kg in 2 days.

Collection Metrics

Cycle	Mobilizer	pCD34+ (cells/µL)	Peak CE (%)	Yield (×10^6/kg)
1	Plerixafor	19-38	38.4	**4.17**
2	Motixafortide	49-56	58.2	**12.80**
3	Motixafortide	47-50	51.6	**6.82**

- Cycle 1: instability, clotting, interruptions, rescue heparin.
- Cycle 2: best daily yield and peak collection efficiency.
- Cycle 3: trial of AC:WB 1:8 destabilized interface; 1:6 restored stability.

Conclusion

- Prospective studies should evaluate sustained HbS control, motixafortide-based mobilization, individualized anticoagulation, product hematocrit, and real-time interface metrics.
- Improved collection efficiency and manufacturing success while preserving safety in high-risk SCD patients.