THE UNIVERSITY OF CHICAGO
Center for Global Health

Innovative Protocols for Improved HLA Typing and Engraftment Monitoring in Sickle Cell Disease Management

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Background

- Sickle cell disease (SCD) has high morbidity and mortality in Tanzania

- Hematopoietic stem cell transplantation (HSCT) is the only curative treatment but often results in complications like graft-versus-host disease (GVHD) due to poor donor matching and/or monitoring of chimerism rates

- Our study focused on developing standardized pre- and post-transplant protocols for HLA typing and chimerism detection at Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es Salaam, Tanzania

Objectives

1. Enhance donor selection through HLA typing, focusing on optimizing polymerase chain reaction (PCR) protocols to reduce errors and increase efficiency.
2. Strengthen post-transplant monitoring by introducing flow cytometry-based detection of erythroid chimerism.
3. Identify locally adaptable strategies to improve long-term patient outcomes.

Methods

- We conducted a comprehensive review of the existing HLA typing protocols at MUHAS through collaboration with laboratory technicians, sickle cell specialists, hospital administrators, and patients.

- In parallel, we performed an extensive literature review of pre- and post-transplant methods across multiple databases

Results

Pre-Transplant Donor Matching
Alternatives to proposed HLA sequencing:

Method	Advantages	Disadvantages
Whole-Genome Sequencing (WGS)	Comprehensive coverage of entire genome, detects structural variations, novel allele discovery	High cost, data complexity, less targeted for HLA regions
Whole-Exome Sequencing (WES)	Targeted and cost-effective for coding regions, suitable for clinical settings	Limited to exons, coverage gaps, significant data analysis required
Hybrid Capture-Based Methods	High specificity, comprehensive HLA coverage, customizable	Higher cost and complexity, technical challenges, robust data analysis needed

Post-Transplant Engraftment Monitoring
Alternative for Detecting Chimerism:

Method	Advantages	Disadvantages
WBC Chimerism	Established, highly sensitive (~1-5% sensitivity)	Limited in assessing engraftment as RBCs engraft non-linearly with white blood cells (WBCs)
RBC-specific flow cytometry	Specifically assesses red blood cell (RBC) engraftment, able to detect both nucleated RBC precursors + mature RBCs	Can be confounded by antigens on transfused RBCs (would take 4 months post-transfusion to accurately detect chimerism)

- The proposed use of high- fidelity enzymes such as repliQa HiFi ToughMix decreases PCR error rates and processing times by 2-3 times thereby enhancing donor matching in HSCT.

- The use of flow cytometry to measure levels of the CD71+ reticulocytes enhances early prediction of donor RBC rejection

Discussion

Pre-transplant Improvements:

- RepliQa HiFi ToughMix offers significant advantages over the current enzyme (DreamTaq), including 90x higher fidelity, 2.5x faster processing, and the ability to amplify longer sequences (up to 24 kb)
- RepliQa is approximately four times more expensive than DreamTaq ($1,500 vs. $350 for 500 reactions), which may pose challenges in resource-constrained settings

Post-transplant Monitoring:

- Flow cytometry is more effective than current methods for early detection of donor RBC rejection
- MUHAS is equipped with two clinical flow cytometers (DxFLEX and BC FACSCanto)
- Current challenges include the need for highly trained staff and antigen-specific knowledge of recently transfused RBCs

Conclusion

Ongoing challenges in Africa include limited resources for pre- and post-transplant protocols, with expensive high-fidelity PCR and advanced flow cytometry techniques. However, the proposed interventions pointed at both pre- and post- transplant care have the potential to improve donor matching and early detection of engraftment success to bolster patient outcomes

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