Biomarkers Reveal Improved Red Cell Health Following Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

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Introduction

- Sickle cell disease (SCD), an autosomal recessive genetic disorder is caused by a single mutation in the *HBB* gene encoding hemoglobin subunit β causing hemoglobin polymerization, vaso-occlusion and endothelial dysfunction [1].
- Despite new pharmacological options, sustainable improvement in clinical outcomes and quality of life in SCD remain elusive.
- Allogeneic hematopoietic stem cell transplant (HSCT) is the only approved curative option available for SCD. HSCT efficacy is assessed by frequency of pain crises, hemoglobin (Hb) response, markers of hemolysis, and level of engraftment [2].
- Normalization of red blood cell (RBC) health is a fundamental goal of successful HSCT; however, normalization in response to HSCT has not been clearly established.
- Mechanical fragility (MF), flow adhesion of whole blood to vascular cell adhesion molecule (FA-WB-VCAM) and P-selectin (FA-WB-Psel) are standardized blood-based biomarkers to assess RBC membrane stability and adhesive properties respectively. Dynamic sickling assay (DSA) assesses the ability of RBCs to sickle under controlled hypoxia.
- **The objective of this study was to determine if MF, FA-WB-VCAM, FA-WB-Psel and DSA can monitor the normalization of RBC function in individuals with SCD post-HSCT.**

Proprietary Assays and Methods

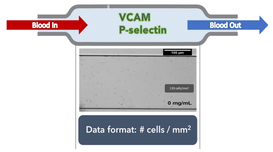

Figure 1. Flow Adhesion Assay

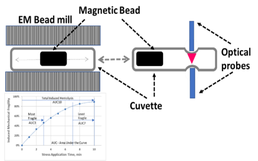

Figure 2. Mechanical Fragility Assay

Flow Adhesion Assay (Figure 1):

- Microfluidic channels are coated with vascular cell adhesion molecule-1 (VCAM-1) or P-selectin (Psel) for FA-WB-VCAM and FA-WB-Psel respectively.
- Whole blood is flowed at 1.0 dyne/cm² using a BioFlux 1000Z microfluidic well-plate system as previously described [3].
- Adherent cells are quantified and reported as cells/mm².

Mechanical Fragility (Figure 2)

- Measures RBC membrane stability by subjecting RBCs to external stress using an electromagnetic bead mill.
- Cell-free hemoglobin is assessed with fiberoptic spectrophotometry for non-invasive measurement of mechanically-induced RBC hemolysis.

Proprietary Assays and Methods (cont'd)

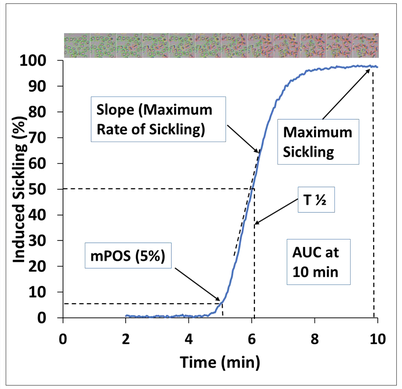

Figure 3. Dynamic Sickling Assay

Dynamic Sickling Assay (Figure 3)

- Hypoxia is induced through an enzymatic reaction; the fraction of sickled cells, determined by time-lapse imaging, is reported as a function of time and/or oxygen concentration.
- RBC subpopulations with: varying Hb subtypes (e.g.,HbA, HbS, HbF, etc), drug-altered Hb-oxygen affinity, or from products of gene-edited hematopoietic stem cells can influence the sickling profile.
- Reported parameters include: 1) morphological point of sickling (mPOS at 5%, 2) T ½: time to reach 50% induced sickling, 3) maximum rate of sickling 4) maximum sickling and 5) AUC at 10 minutes [4].

Sample collection

- SCD subjects were enrolled in NIH haploidentical HSCT protocol 17-H-0069. Blood samples from 3 of 10 planned subjects were collected twice before (baseline) and 3, 6, and 12 months after HSCT. Prior to HSCT, all SCD subjects experienced recurrent vaso-occlusive crises, chronic pain, and transfusion-associated iron overload.

Table 1: Patient clinical information

Patient	Timepoint	WBC (x10⁹/L)	Hb (g/dL)	Platelets (x10⁹/L)	ARC (x10⁹/L)	HbS %	HbA%	HbF%	LDH (U/L)	Total bilirubin (mg/dL)
1	BSL	5.2	10.1	198	174.6	67.6	0	30.2	324	2.6
	6M	4.2	11.9	353	87.2	35.2	57.5	3.8	208	0.4
	12M	4.1	13.5	317	100.7	35.6	59.4	1.5	234	0.4
2	BSL	5.6	9.2	216	314.8	33.7	58.5	5.3	526	2.1
	3M	25.5	9	252	155.7	31.1	65.2	0.3	392	0.2
	6M	8.1	12.9	174	102.6	38.4	58	0.2	742	0.2
3	BSL	11.6	8.2	269	293	83.2	0	13.4	503	11.5
	3M	13.9	10.6	144	29.5	38	58.2	0.6	929	1.3

Results

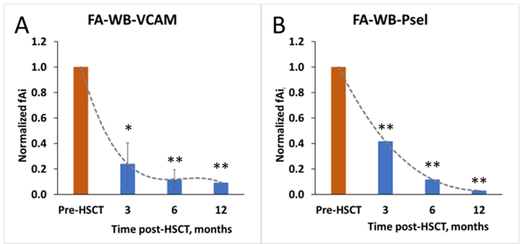

Figure 4 Whole blood flow adhesion on VCAM1 and P-selectin

- Averaged (n=3) pre-HSCT baselines for FA-WB-VCAM and FA-WB-Psel were 470±290 cells/mm² and 74±93 cells/mm², respectively.
- At 6 months, both FA-WB-VCAM and FA-WB-Psel decreased 95% to 25 cells/mm² and 79% to 22 cells/mm², respectively.
- All data normalized to pre-HSCT values were plotted against the time (Figures 4A 4B). Statistically significant decreases (*p<0.05, **p<0.01) are observed as early as 3 months post-HSCT.

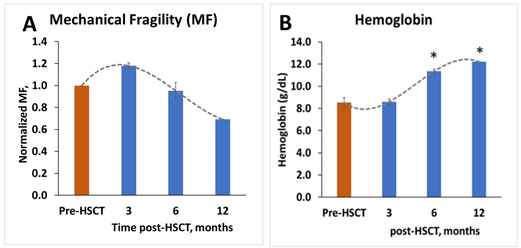

Figure 5 Mechanical Fragility and Hemoglobin

- The average MF index at 10 minutes (MFI10) decreased by 16% from pre-HSCT to 12 months post-HSCT (Figure 5A). The decrease did not reach statistical significance.
- Hemoglobin increased 37% from 8.5 to 11.4g/dL at 6 months and 43% to 12.2g/dL at 12 months post-HSCT (Figure 5B). Hb increases reach statistical significance (*p<0.05).

- *Pt. 1 pain medication-pre-HSCT: long acting morphine 30mg/12h, 6M post-HSCT:30mg/day, 12M:off long-acting morphine*

Results (cont'd)

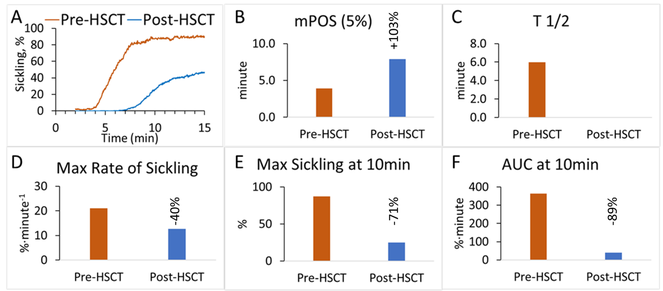

Figure 6: Dynamic sickling analysis

- Pre- and 3-month post-HSCT samples of Patient 3 were examined with dynamic sickling assay (DSA), results were plotted as a function of time in Figure 6A.
- Time to 5% sickling of the sample, known as morphological point of sickling (mPOS-5%) increased 103% from 3.9 to 7.9minutes (Figure 6B). 3-month post-HSCT sample didn't reach T ½ within 15 minutes of observation (Figure 6C).
- Maximum rate of sickling (Figure 6D), maximum sickling at 10 min (Figure 6E) and AUC at 10 minutes (Figure 6F) decreased 40%, 71% and 89%, respectively, at 3 months post-HSCT compared to pre-HSCT values.

Conclusions

- Significant changes in FA-WB-VCAM, FA-WB-Psel and DSA profiles were seen as early as 3 months post-HSCT while significant hemoglobin increases were only observed after 6 months post-HSCT.
- This data suggests that FA-WB-VCAM, FA-WB-Psel, DSA and MF may provide earlier insights into improvements in RBC health following HSCT compared to standard hematological labs.
- Longer follow-up is required to confirm clinical improvement although the reduction in pain and opioid consumption in one patient is encouraging.
- Future studies are needed to establish the relationship between RBC function biomarkers and long-term clinical outcomes.

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References

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4. Tarasev M, Gao X, Ferranti M, Goodrich R, Edenstrom A, Zaidi A, Hines, PC; Dynamic Sickling Assay: Using Enzymatically Induced Hypoxia to Assess Real-Time Sickling Scalable from Large Erythrocyte Populations to Individual Cells. *Blood* 2022; 140 (Supplement 1): 4976–4977