INTRODUCTION

Polymorphism of deoxygenated hemoglobin S (HbS) leads to red blood cell (RBC) sickling and vaso-occlusion.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only approved curative option available for sickle cell disease (SCD).

Objective biomarkers are needed to assess disease status, population-scale sickle kinetics and to monitor the pharmacodynamic impact of Hb-modifying and curative therapies.

Functional Fluidics has developed a dynamic sickling assay (DSA) that uses an enzymatic O2 scavenging system providing tight control over the rate and depth of induced-hemolysis.

AIM

Assess real-time RBC sickling kinetics in sickle cell trait (SCT) and SCD patients with variable distributions of HbA, HbS and HbF, and compare pre- and post-HSCT.

METHOD

Dynamic Sickling Assay (Figure 1)

- Hypoxia is induced through a timed protocatechuate-3,4-dioxygenase enzymatic reaction (PCD-PCA).
- The fraction of sickled cells is determined by time-lapse imaging, analyzed with AI technology and reported as a function of time.

- Measured parameters include:
  - mPoS≥95%: time to reach 5% induced sickling (min).
  - mPoS≥50%: time to reach 50% induced sickling (min).
  - Rate of Sickling: highest induced sickling rate (% min).
  - Maximum Sickling: highest percentage (%).
  - AUC at 10 min: the area under the curve during 10min of DSA (% minute).

Sample collection

- SCT and SCD subjects, with and without chronic exchange transfusions were enrolled in the Institute for Regenerative and Cellular Medicine (IRCM) RBC study under the IRCM health initiative protocol (FF-RBC-003v5).
- SCD subjects were enrolled in NIH haploidentical HSCT protocol 17-H-0069. Blood samples from 4-10 planned subjects were collected pre- and 3, 6, and 12 months after HSCT.

RESULTS

SICKLING KINETICS IMPROVE AS HbS% DECREASES AND HbF% INCREASES

- SCT subjects with similar HbS% exhibit different sickling kinetics upon exposure to the same hypoxic stress due to intracellular RBC distribution of HbA and HbS.
- Transfusion SCD samples show an improvement in sickling kinetics as HbS% decreases approaching a significant correlation with AUC10.
- Statistically significant correlations are observed with increasing HbF percentages on 4 out of 5 sickling parameters.
- Compared to pre-transplant values, post-HSCT samples exhibit significant improvement in: mPoS≥5%, rate of sickling, maximum sickling and AUC10.

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CONCLUSIONS

- SCT subjects with similar HbS% exhibit different sickling kinetics upon exposure to the same hypoxic stress due to intracellular RBC distribution of HbA and HbS.
- Transfusion SCD samples show an improvement in sickling kinetics as HbS% decreases approaching a significant correlation with AUC10.
- Statistically significant correlations are observed with increasing HbF percentages on 4 out of 5 sickling parameters.
- Compared to pre-transplant values, post-HSCT samples exhibit significant improvement in: mPoS≥5%, rate of sickling, maximum sickling and AUC10.
- DSA can differentiate samples based on Hb distribution and can be used to assess sickling kinetics pre- and post-HSCT modifying and curative therapies, such as hematopoietic stem cell (HSC).

REFERENCES


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