Improvement in Red Blood Cell Physiology in Children With Sickle Cell Anemia Receiving Voxelotor

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INTRODUCTION

- Sickle hemoglobin (HbS) under conditions of deoxygenation polymerizes to cause sickling of red blood cells (RBCs). Oxygenated HbS does not polymerize; thus, increasing oxygen affinity to HbS is a therapeutic strategy for sickle cell disease (SCD).[1]
- Voxelotor (GBT440) is a first-in-class, small molecule that increases the affinity of hemoglobin for oxygen, inhibits polymerization, and reduces sickling, with a subsequent increase in the half-life of RBCs.[2,3]
- In the phase 3 HOPE trial, the use of voxelotor in patients with SCD caused a significant reduction in markers of hemolysis and anemia. We hypothesized that, given this mechanism of action, we would observe improvements in RBC physiology in patients receiving voxelotor.[2]
- In this study, an ektacytometer laser optical rotational red cell analyzer (Lorrca; RR Mechatronics, NL) was used to analyze deformability of RBCs using a defined value of shear stress with an increasing osmotic gradient (Osmoscan) as well as a newer technology to subject these cells to gradual deoxygenation (Oxygenscan). Lorrca and Hemox Analyzer (TCS Scientific Corp.) informed the understanding of RBC physiology in children receiving voxelotor.[4,5]

STUDY DESIGN

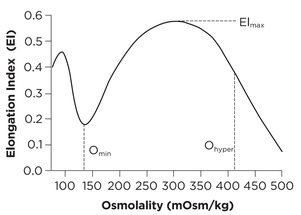
- This pilot study was conducted at Emory University/Children's Healthcare of Atlanta as an investigator-initiated ancillary study to the institutional review board–approved GBT440-007 clinical trial, which evaluated multiple doses of voxelotor at equivalent exposure to adults as 1500 mg/day, based on body weight (NCT02850406).
- Samples from children aged 4 to 11 with SCD (HbSS) who received voxelotor were analyzed before and 12 weeks after starting therapy.
- All participants continued their stable, optimal hydroxyurea dose during treatment with voxelotor.

METHODS

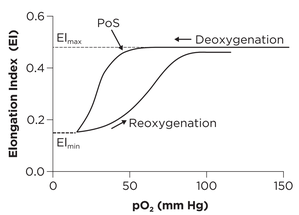
- Blood collected in K2/EDTA was suspended in polyvinylpyrrolidone buffer for analysis. Deformability of RBCs in Osmoscan (**Figure 1A**) was performed at a shear stress of 30 Pa and varying osmolality gradients (0-600 mOsm/kg). Oxygenscan (**Figure 1B**) was similarly performed under controlled deoxygenation using nitrogen.
- Evaluated parameters were[5-7]:
  - **O_{min}**: value of the hypotonic osmolality, where 50% of the cells hemolyze in a classic osmotic fragility assay; provides information on the initial surface area to volume ratio.
  - **EI_{max}**: maximal deformability or elongation index; informs RBC cytoskeletal mechanics.
  - **O_{hyper}**: osmolality corresponding to 50% of the EI_{max}; provides information regarding the cytoplasmic viscosity (MCHC).
  - **PoS**: point of sickling, or point on the curve during deoxygenation when sickling begins.
  - **EI_{min}**: corresponds to when sickle RBCs can least elongate.
- Oxygen dissociation curves were obtained using a HemOx Analyzer, and complete blood count parameters were determined on a clinical laboratory hematology analyzer (ADVIA, Siemens). Data were analyzed with Prism using a paired *t* test.

Figure 1. Representative Curves Generated by:

A. Osmoscan With Varying Osmolality Gradients[5]



B. Oxygenscan Under Controlled Deoxygenation[7]



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RESULTS

Table 1. Marked Increases in Hemoglobin Were Observed in Pediatric Patients at Weeks 12 and 24

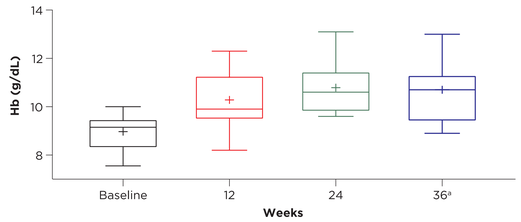
Patient	Age, years	Baseline HbF, %	Baseline Hb, g/dL	Hb change 0-12 weeks, g/dL[a]	Hb change 0-24 weeks, g/dL[b]
1	7	18.5	9.0	−0.8 (8.2)	1.5 (10.4)
2	5	19.2	9.1	2.7 (11.8)	2.2 (11.3)
3	7	23.1	10.0	2.3 (12.3)	3.1 (13.1)
4	4	14.5	9.2	0.4 (9.6)	0.8 (10.0)
5	7	23.6	9.5	0.2 (9.6)	0.4 (9.8)
6	9	28.4	9.4	1.7 (11.1)	1.8 (11.2)
7	8	4.5	8.2	3.0 (11.1)	3.8 (11.9)
8	8	21.8	7.6	2.0 (9.5)	2.1 (9.6)
9	7	29.4	8.4	1.9 (10.2)	2.5 (10.8)
10	10	7.3	9.6	−0.2 (9.4)	0.3 (9.8)

[a]Values in parentheses indicate Hb (g/dL) at week 12. [b]Values in parentheses indicate Hb (g/dL) at week 24.

Table 2. Anemia and Hemolysis Markers Were Improved From Baseline at Weeks 12 and 24

Parameter	Mean change (range) from baseline at week 12 (n=10)	Mean change (range) from baseline at week 24 (n=10)
Hb, g/dL	1.3 (−0.8 to 3.0)	1.8 (0.3 to 3.8)
Reticulocytes, % change	−15.6 (−42.6 to 30.7)	−16.0 (−53.6 to 24.3)
Indirect bilirubin, % change	−51.8 (−70.4 to −24.1)	−53.9 (−76.0 to −25.9)
LDH, % change	−2.3 (−17.9 to 28.4)	−16.0 (−35.1 to 7.8)

Figure 2. Hemoglobin Increased From Baseline at Weeks 12, 24, and 36

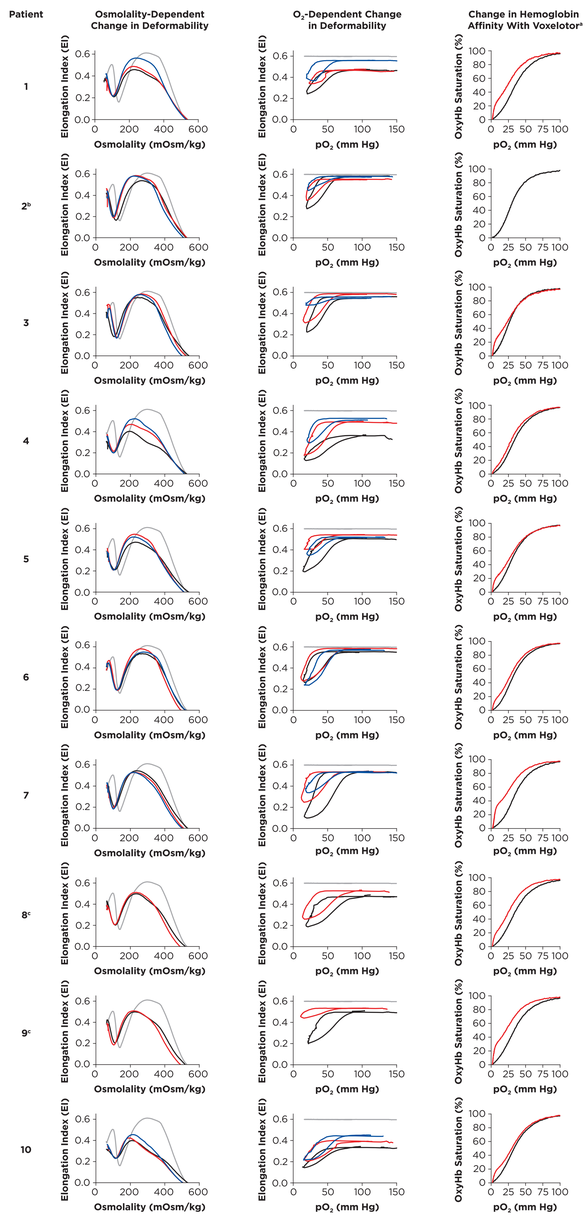


[a]Data for week 36 include 7 out of 10 patients.
Mean for each box-and-whisker plot is shown by '+'.

Table 3. Significant Improvements in Ektacytometry, p50, and p20 Were Observed Under Shear Stress and Deoxygenation

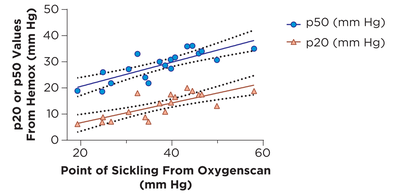
	n	Pre-treatment (mean ± SEM)	Week 12 (mean ± SEM)	*P* value
EI_{max} (Osmoscan)	10	0.49 ± 0.02	0.51 ± 0.01	0.0147
O_{hyper} (Osmoscan)	10	412.7 ± 2.5	398.6 ± 5.5	0.0052
O_{min} (Osmoscan)	10	112.6 ± 1.9	101 ± 2.6	0.0321
PoS (Oxygenscan)	10	44.1 ± 2.2	30.1 ± 2.0	0.0001
EI_{min} (Oxygenscan)	10	0.19 ± 0.02	0.28 ± 0.03	0.0079
EI_{max} (Oxygenscan)	10	0.49 ± 0.03	0.52 ± 0.02	0.0347
p50	9	32.8 ± 0.9	24.1 ± 1.4	0.0011
p20	9	17.2 ± 0.7	9.0 ± 0.9	0.0001

Figure 3. Osmoscan, Oxygenscan, and p50 Curves at Baseline, Week 12, and Week 36



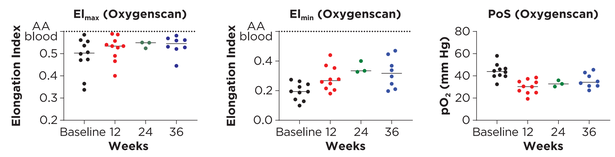
[a]36-week data are not presented, as most curves closely overlaid with 12-week data. [b]Data for change in Hb affinity with voxelotor for Patient 2 are not available. [c]36-week data for Patients 8 and 9 are not available.

Figure 4. Point of Sickling Decreases as the Hb-O_2 Affinity Increases



- The Oxygenscan curve indicates that deformability of SS RBCs is dependent on the oxygen tension.
- The above correlations (n=19, R^2=0.65 and 0.59 for p50 and p20, respectively) show that as the Hb-O_2 affinity increases, the PoS decreases (**Figure 4**).
- Previously, we have shown that the p20 decreases as the RBC concentration of voxelotor increases in blood from patients taking voxelotor.[1,8]
- Therefore, as the RBC concentration of voxelotor increases, we expect the PoS to decrease and the deformability of RBCs under low oxygen tensions to improve.

Figure 5. Sustained Improvements in RBC Deformability Were Observed for Children on Voxelotor Treatment



- For children on voxelotor, there was an improvement in RBC deformability in the Oxygenscan as captured by an increase in EI_{max} and EI_{min} and a decrease in the PoS (**Figure 5**).

SUMMARY

- This is the first time that the effect of voxelotor therapy in children aged 4 to 11 has been reported, and shows marked improvements in anemia and hemolysis after 12 and 24 weeks of treatment.
- In this small cohort of children with HbSS, there was:
  - Improved RBC deformability, suggested by an increase in EI_{max} in Osmoscan.
  - Improvements in EI_{max} and EI_{min} with a decrease in PoS during deoxygenation in Oxygenscan, which theoretically supports voxelotor's ability to inhibit HbS polymerization, improve RBC deformability, and subsequently reduce sickling and hemolysis.
- This study is currently ongoing with periodic clinical and laboratory analyses collected at various time points for longitudinal data.

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Abbreviations: Hb, hemoglobin; HbAA, normal adult hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin; HbSS, homozygous SCD; LDH, lactate dehydrogenase; MCHC, mean cell hemoglobin concentration; p20, partial pressure to achieve 20% saturation; p50, partial pressure to achieve 50% saturation; pO_2, partial pressure of oxygen; RBC, red blood cell; SCD, sickle cell disease; SEM, standard error of the mean.

Disclosures: Satheesh Chonat: Agios, Alexion, honoraria. **Earl Fields, Hannah Baratz, Amanda Watt:** nothing to disclose. **Mira Pochron, Sandy Dixon, Margaret Tonda, Joshua Lehrer-Graiwer:** Global Blood Therapeutics, employees and equity ownership; **Clark Brown:** Global Blood Therapeutics, trial investigator; Novartis, Pfizer, research support; Global Blood Therapeutics, Imara, consultant. **David Archer:** Global Blood Therapeutics, consultant and research funding; AstraZeneca, research funding.

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