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SHORT REPORT

Voxelotor improves red blood cell functionality in children with sickle cell anaemia: An ancillary study of the HOPE-KIDS 1 trial

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Abstract

Introduction: Sickle haemoglobin (HbS) polymerisation perturbs red blood cell (RBC) rheology and drives sickle cell disease (SCD) pathophysiology. Voxelotor is an HbS polymerisation inhibitor that increases haemoglobin (Hb)–oxygen affinity. Methods/Results: In this 48-week, prospective, single-centre translational study, 10 children aged 4–11 years with SCD were treated with voxelotor. Improvements in RBC deformability were observed using osmotic/oxygen gradient ektacytometry, with increases in minimal and maximal elongation index and reductions in point of sickling. Increased Hb and reduced markers of haemolysis were also observed. Conclusion: These findings suggest that voxelotor treatment is associated with reduced RBC sickling and haemolysis in children with SCD.

KEYWORDS
deoxygenation, ektacytometry, red blood cell deformability, sickle cell disease, voxelotor

1 | INTRODUCTION

Sickle cell disease (SCD) is characterised by polymerisation of sickle haemoglobin (HbS) under conditions of deoxygenation, causing chronic anaemia and haemolysis and leading to severe morbidity and early mortality [1, 2]. Voxelotor is an HbS polymerisation inhibitor that targets the underlying cause of SCD pathology [3]. Preclinical studies indicate that voxelotor increases HbS–oxygen affinity and red blood cell (RBC) half-life, reduces RBC sickling, and improves RBC deformability and blood viscosity [4, 5]. The US Food and Drug Administration (FDA) approval of voxelotor in 2019 for patients with SCD aged ≥12 years was based on results from the pivotal phase 3 HOPE trial, in which voxelotor treatment led to rapid and sustained improvements in haemoglobin (Hb) and markers of haemolysis compared with placebo [6, 7]. In 2021, the FDA expanded the approved use of voxelotor for the treatment of SCD in children as young as 4 years. Additionally, voxelotor has received marketing authorisation in Great Britain, the European Union, Kuwait and Oman for patients aged ≥12 years, and in the United Arab Emirates for patients aged ≥4 years [8]. The aim of this study was to assess whether voxelotor therapy improves RBC functionality by increasing RBC deformability in parallel with potential improvements to Hb levels and Hb–oxygen affinity in a subgroup of paediatric patients participating in a phase 2 study of voxelotor for SCD.

2 | METHODS

2.1 | Patient population

HOPE-KIDS 1 (NCT02850406) is a phase 2a, open-label, multicentre clinical trial evaluating the pharmacokinetics, safety, tolerability

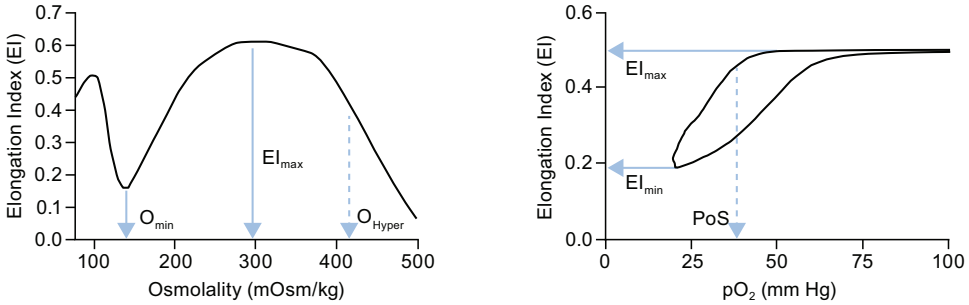

FIGURE 1 Osmoscan (left) and Oxygenscan (right). Representative Oxygenscan and Osmoscan curves obtained from Lorrca. Evaluated parameters are the following: O_{min}, value of the hypotonic osmolality, where 50% of the cells haemolyse in a classical osmotic fragility assay (provides information on the 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 on the cytoplasmic viscosity [mean corpuscular Hb concentration]); PoS, point of sickling, or point on the curve during deoxygenation when sickling begins; EI_{min}, corresponds to deformability of sickle RBCs at deoxygenation; pO_2, partial pressure of oxygen. Figures used with permission from RR Mechatronics.

and treatment effects of weight-based voxelotor dosing (Table S1) in paediatric patients with SCD (homozygous HbS [HbSS] or sickle beta zero thalassaemia [HbSβ^0]). This report is from a single-centre ancillary pilot study performed as part of the ongoing HOPE-KIDS 1 trial to assess changes in RBC functionality such as deformability in voxelotor-treated children with SCD [9]. Additional patient information can be found in the Supporting Information.

2.2 | Osmoscan and oxygenscan

Whole-blood samples from voxelotor-treated children aged 4–11 years with SCD were collected to measure the effects of deoxygenation and reoxygenation on RBC deformability under shear stress [10].

Osmoscan (osmotic gradient ektacytometry): Samples were analysed before treatment and at Weeks 12, 24, 36 and 48. An ektacytometry laser optical rotational red cell analyser (Lorrca; RR Mechatronics) was used to analyse RBC deformability at a shear stress of 30 Pa, under osmolality gradients ranging from 0 to 600 mOsm/kg. Three Osmoscan parameters were evaluated (Figure 1): O_{min}, the value of the hypotonic osmolality, when 50% of the cells haemolyse in a classic osmotic fragility assay, providing information on the initial surface area to volume ratio; maximal elongation index (EI_{max}), representing RBC deformability at normoxia; and O_{hyper}, the osmolality corresponding to 50% of the EI_{max}, providing information about cytoplasmic viscosity [8].

Oxygenscan (oxygen gradient ektacytometry): Deformability was investigated using similar conditions as in the Osmoscan assay, under controlled deoxygenation using Lorrca Oxygenscan. Three Oxygenscan parameters were evaluated (Figure 1): point of sickling (PoS) or the partial pressure of oxygen during deoxygenation at which sickling begins; EI_{max}; and minimal elongation index (EI_{min}), representing deformability of sickle RBCs under deoxygenation [10].

2.3 | Analyses

Oxygen dissociation curves were obtained using a Hemox Analyzer (TCS Scientific Corp) to examine changes in the binding affinity of oxygen to Hb. Whole blood collected in K2/EDTA was oxygenated with compressed air within the Hemox Analyzer, and the oxygen equilibrium curves were collected during deoxygenation, as previously described [11]. The partial pressures of oxygen at which Hb is 20% and 50% saturated with oxygen, p20 and p50, respectively, were obtained using a nonlinear regression analysis. Complete blood count parameters were determined on a clinical laboratory haematology analyser (ADVIA, Siemens). Data were analysed using GraphPad Prism software and a paired *t*-test. Complete methodology is in the Supporting Information.

3 | RESULTS AND DISCUSSION

3.1 | Voxelotor improved Hb and reduced markers of haemolysis

A total of 10 patients (median age: 7 years), with the HbSS genotype and a mean (range) baseline Hb of 90 g/L (76–100 g/L), were included (Table S2). The mean baseline Hb was comparable with that for the overall patient population in HOPE-KIDS 1 Part C [9]. At Week 24, 7 of 10 patients (70%) had an Hb response (defined as a greater than 10 g/L increase in Hb from baseline; Table S3) compared with 47% in the overall study population. Mean Hb exceeded 100 g/L at each treatment week assessed (Figure 2A); by Week 24, Hb exceeded 100 g/L in 6 out of 10 patients. Additionally, voxelotor reduced markers of haemolysis (reticulocytes, indirect bilirubin and lactate dehydrogenase) at Weeks 12, 24, 36 and 48 (Table 1). Similar to the trend in Hb response, reductions in markers of haemolysis at Week 24 were higher in this ancillary study than in the overall HOPE-KIDS 1 patient population [9].

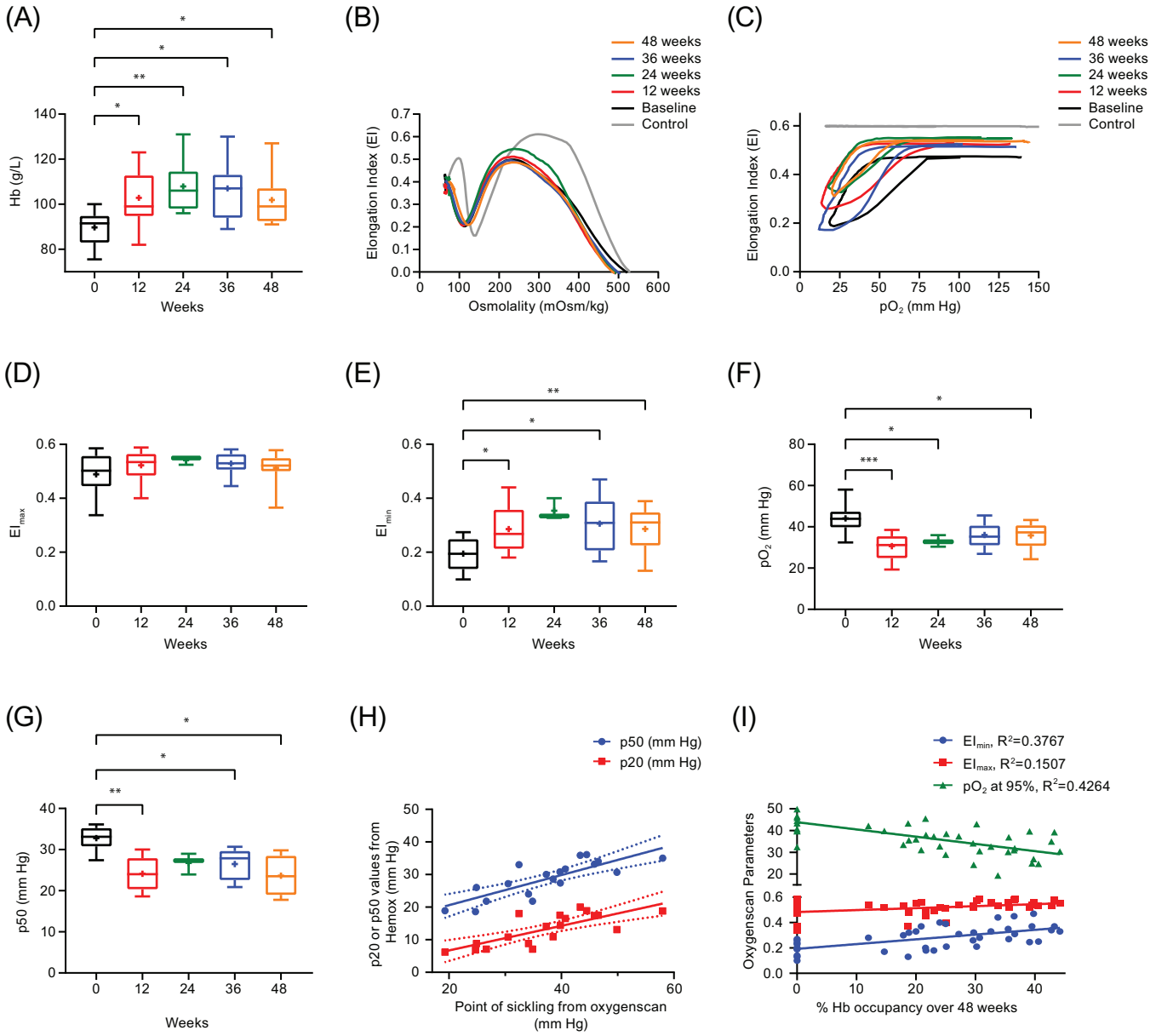

FIGURE 2 Hb and erythrocyte response in patients receiving voxelotor. (A) Hb increase from baseline at Weeks 12, 24, 36 and 48. (B and C) Representative (B) Osmoscan and (C) Oxygenscan curves obtained from Lorrca in patients at various time points on voxelotor. (D–F) Improvements in RBC deformability observed in Oxygenscan, as captured by increases in (D) EI_{max} and (E) EI_{min} and (F) a decrease in PoS. (G) Improvement of oxygen dissociation curves (partial pressure p50) while on voxelotor. (H) Measurement of PoS using Oxygenscan, correlated against oxygen dissociation curves (partial pressure p50 or p20) while on voxelotor, shows that as the p50 or p20 decreases, the PoS decreases. (I) Correlation of Oxygenscan parameters to the Hb occupancy, which was calculated as the ratio of the concentrations of voxelotor to Hb in blood. Mean for each box and whisker plot is shown by a '+'. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus baseline (mean ± SEM). EI_{max}, maximal elongation index; EI_{min}, minimal elongation index; Hb, haemoglobin; p20, partial pressure of oxygen at which Hb is 20% saturated with oxygen; p50, partial pressure of oxygen at which Hb is 50% saturated with oxygen; pO_2, partial pressure of oxygen; R^2, coefficient of determination.

3.2 | Voxelotor effects on RBC deformability

The primary aim of this pilot study was to assess whether voxelotor treatment improves RBC functionality by increasing RBC deformability in paediatric patients using ektacytometry, the gold standard for studying deformability of RBCs with membrane protein disorders [12]. Deformability was first assessed using a defined value of shear stress with an increasing osmotic gradient (Osmoscan). Improvement in RBC deformability, observed after voxelotor treatment, was observed in a representative Osmoscan curve from one patient (Figure 2B) and supported by the numerical increase in EI_{max} over 48 weeks compared with baseline values, with a significant change from baseline noted at Week 12 (Table 2). RBC deformability was also measured during controlled deoxygenation using Oxygenscan. Improvements in

TABLE 1 Mean change in haemoglobin and markers of haemolysis from baseline.

Parameter	Week 12	Week 24	Week 36	Week 48
Hb (g/L), change (range)	13 (−8 to 30)	18 (3 to 38)	17 (−7 to 44)	12 (−4 to 29)
Hb response > 10 g/L, % patients (*n*/*N*)	60 (6/10)	70 (7/10)	80 (8/10)	60 (6/10)
% Reticulocytes, % change (range)	−15.6 (−42.6 to 30.7)	−16.0 (−53.6 to 24.3)	−11.0 (−57.9 to 25.1)	−20.3 (−77.3 to 17.5)
Indirect bilirubin, % change (range)	−51.8 (−70.4 to −24.1)	−53.9 (−76.0 to −25.9)	−49.1 (−64.5 to −27.3)	−47.4 (−69.2 to −25.9)
LDH, % change (range)	−2.3 (−17.9 to 28.4)	−16.0 (−35.1 to 7.8)	−9.1 (−46.0 to 9.6)	2.0 (−24.6 to 94.8)

Abbreviations: Hb, haemoglobin; LDH, lactate dehydrogenase.

TABLE 2 Change in deformability and haemoglobin–oxygen affinity from baseline.

	Baseline (mean ± SEM)	Post-treatment (mean ± SEM) at Week 12	Post-treatment (mean ± SEM) at Week 24	Post-treatment (mean ± SEM) at Week 36	Post-treatment (mean ± SEM) at Week 48
EI_{max} (Osmoscan)	0.49 ± 0.02	**0.52 ± 0.02***	0.55 ± 0.01^{a}	0.53 ± 0.01	0.50 ± 0.02^{b}
O_{hyper} (Osmoscan)	412.0 ± 2.25	**397.3 ± 4.20****	387.0 ± 4.16^{a}	**393.8 ± 3.50*****	**394.8 ± 4.37**^{**,b}
O_{min} (Osmoscan)	112.9 ± 2.14	109.3 ± 2.81	107.0 ± 1.00^{a}	110.9 ± 2.50	114.5 ± 2.35^{b}
PoS (Oxygenscan)	44.1 ± 2.16	**30.6 ± 1.93*****	**33.0 ± 1.63**^{*,a}	36.1 ± 1.85	**35.9 ± 2.06**^{*,c}
EI_{min} (Oxygenscan)	0.19 ± 0.02	**0.29 ± 0.03***	0.35 ± 0.02^{a}	**0.31 ± 0.03***	**0.29 ± 0.03**^{**,c}
EI_{max} (Oxygenscan)	0.49 ± 0.03	0.52 ± 0.02	0.54 ± 0.01^{a}	0.53 ± 0.01	0.51 ± 0.02^{c}
p50	32.8 ± 0.85	**24.1 ± 1.38**^{**,c}	26.7 ± 1.48^{a}	**26.5 ± 1.73**^{*,d}	**25.1 ± 2.49**^{*,d}
p20	17.2 ± 0.66	**9.0 ± 0.85**^{***,c}	N/A	N/A	**8.9 ± 1.41**^{**,d}

Abbreviations: EI_{max}, maximal elongation index; EI_{min}, minimal elongation index; N/A, not applicable; O_{hyper}, osmolality corresponding to 50% of the EI_{max}, hypertonic region; O_{min}, osmolality at EI_{min}, hypotonic region; p20, partial pressure of oxygen at which Hb is 20% saturated with oxygen; p50, partial pressure of oxygen at which Hb is 50% saturated with oxygen; PoS, point of sickling; SEM, standard error of the mean.

$^{a}n = 3$, $^{b}n = 8$, $^{c}n = 9$, $^{d}n = 5$.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. baseline (mean ± SEM).

PoS (leftward shift) and deformability (upward shift in EI_{min} and EI_{max}) with voxelotor therapy were evident in a representative Oxygenscan curve from one patient, from baseline through Week 48 (Figure 2C). Furthermore, improvement in RBC deformability with voxelotor was sustained over 48 weeks, as indicated by the higher EI_{max} and EI_{min} values and concurrent reductions in the PoS in combined Oxygenscan data from all patients (Figure 2D–F). Individual Osmoscan and Oxygenscan curves from all patients are shown in Figure S1. Foetal Hb remained consistent at baseline and at Week 12 and was not correlated with EI_{max} (data not shown).

3.3 | Voxelotor increases oxygen affinity

Deformability of sickle RBCs is dependent on oxygen tension. At low oxygen tension, HbS undergoes polymerisation and triggers RBC sickling. Preclinical studies have demonstrated that voxelotor binds to the N-terminal valine of the alpha-haemoglobin chain, increases HbS–oxygen affinity, delays in vitro HbS polymerisation, and reduces RBC sickling. This is associated with reductions in p20 and p50 [4]. Furthermore, it was previously shown that p20 decreased as the RBC concentration of voxelotor increased in the blood of voxelotor-treated patients [13, 14]. In this study, a gradual and sustained leftward shift in the oxygen equilibrium in the presence of voxelotor was reflected by the significantly reduced p20 and p50 values at Weeks 12 and 48 compared with pretreatment values ($p < 0.05$; Table 2 and Figure 2G), demonstrating increased Hb–oxygen affinity after voxelotor treatment. Therefore, as the RBC concentration of voxelotor increased, we expected the PoS to decrease and the RBC deformability to improve under low oxygen tensions. A linear regression analysis of PoS and the partial pressure of oxygen (Figure 2H) showed that PoS positively correlated with p20 and p50 values, that is, a decrease in PoS correlated with a decrease in p20 or p50. Decreases in p20 and p50 values suggest an increase in Hb–oxygen affinity. Lastly, the percentage Hb occupancy, which correlates with percentage Hb modification [6, 14], was calculated as a molar ratio of voxelotor concentrations in RBCs to the estimated Hb concentrations, measured over 48 weeks (Table S4). The percentage Hb occupancy positively correlated with EI_{max} and EI_{min} and negatively correlated with PoS (Figure 2I).

Animal and clinical studies have demonstrated that voxelotor is an effective HbS polymerisation inhibitor [4, 6, 14], associated with specific modulation in red cell rheology under normoxic and deoxygenating conditions. As summarised in Table 2, substantial improve-

ment in RBC deformability was observed at Week 12 and sustained over 48 weeks of voxelotor treatment. Improved Oxygenscan curves and leftward-shifted oxygen dissociation curves further confirmed the ability of voxelotor to increase Hb–oxygen affinity.

In this ancillary study, voxelotor therapy in children with SCD was associated with improvements in RBC functionality through increased RBC deformability after 12 weeks of treatment. Also, voxelotor was well tolerated, with minimal grade 1 or 2 adverse events (Table S5). Limitations of this study are the small number of patients and the recruitment of all patients from a single centre that may not be representative of the larger patient population. Additionally, changes in clinical sequelae (e.g., vaso-occlusive crises) were not monitored. As RBC deformability in individuals with SCD is highest in the early years of life [15], these findings may not be generalisable to adults with SCD. Nonetheless, the collective findings demonstrate that voxelotor has the potential to be the first RBC rheology-modifying HbS polymerisation inhibitor for SCD management. RBC functionality improvements may lead to prolonged sickle RBC survival and oxygen delivery to tissues [4, 5].

AUTHOR CONTRIBUTIONS

Satheesh Chonat collected and analysed the data and wrote the manuscript. Earl Fields and Hannah Baratz performed all the analyses. Amanda Watt obtained consent from the patients and collected the samples. Mira Pochron, Sandy Dixon and Margaret Tonda analysed the data and reviewed the manuscript. Clark Brown and David Archer analysed and reviewed the data and provided critical revisions to the manuscript.

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CONFLICT OF INTEREST STATEMENT

Satheesh Chonat: consultant, research funding: Agios, Alexion, Daichi Sankyo, Forma Therapeutics, Genentech/Roche, Pfizer, Novartis, Novo Nordisk, Takeda Pharmaceuticals. Earl Fields, Hannah Baratz and Amanda Watt: nothing to disclose. Mira Pochron and Sandy Dixon: currently employed with Pfizer. Margaret Tonda: former employee of Pfizer. Clark Brown: employee: Pfizer; consultant: Imara, Novo Nordisk; research support: Novartis Pharmaceuticals, Imara, Forma Therapeutics. David Archer: consultant, research funding: Pfizer, Forma Therapeutics.

DATA AVAILABILITY STATEMENT

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions and exceptions, Pfizer may also provide access to the related individual de-identified participant data. See https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information.

CLINICAL TRIAL REGISTRATION

Clinical trial registration: NCT02850406.

ETHICS STATEMENT

This study was approved by the institutional review board at Emory University/Children's Healthcare of Atlanta.

PATIENT CONSENT STATEMENT

Written consent and age-appropriate assent were obtained from all patients enrolled in this study.

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REFERENCES

1. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. Nat Rev Dis Primers. 2018;4:18010.
2. Piccin A, Murphy C, Eakins E, Rondinelli MB, Daves M, Vecchiato C, et al. Insight into the complex pathophysiology of sickle cell anaemia and possible treatment. Eur J Haematol. 2019;102(4):319–30.
3. Salinas Cisneros G, Thein SL. Recent advances in the treatment of sickle cell disease. Front Physiol. 2020;11:435.
4. Oksenberg D, Dufu K, Patel MP, Chuang C, Li Z, Xu Q, et al. GBT 440 increases haemoglobin oxygen affinity, reduces sickling and prolongs RBC half-life in a murine model of sickle cell disease. Br J Haematol. 2016;175(1):141–53.
5. Dufu K, Patel M, Oksenberg D, Cabrales P. GBT440 improves red blood cell deformability and reduces viscosity of sickle cell blood under deoxygenated conditions. Clin Hemorheol Microcirc. 2018;70(1):95–105.
6. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381(6):509–19.
7. Howard J, Ataga K, Brown R, Achebe M, Nduba V, El-Beshlawy A, et al. Voxelotor in adolescents and adults with sickle cell disease (HOPE): long-term follow-up results of an international, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Haematol. 2021;8(5):e323–33.
8. Oxbryta. Prescribing information. South San Francisco, CA: Global Blood Therapeutics, Inc.; October 2022.
9. Estepp JH, Kalpatthi R, Woods G, Trompeter S, Liem RI, Sims K, et al. Safety and efficacy of voxelotor in pediatric patients with sickle cell disease aged 4 to 11 years. Pediatr Blood Cancer. 2022;69(8):e29716.
10. Rab MAE, van Oirschot BA, Bos J, Merkx TH, van Wesel ACW, Abdulmalik O, et al. Rapid and reproducible characterization of sickling during automated deoxygenation in sickle cell disease patients. Am J Hematol. 2019;94(5):575–84.
11. Guarnone R, Centenara E, Barosi G. Performance characteristics of Hemox-Analyzer for assessment of the hemoglobin dissociation curve. Haematologica. 1995;80(5):426–30.
12. Da Costa L, Suner L, Galimand J, Bonnel A, Pascreau T, Couque N, et al. Diagnostic tool for red blood cell membrane disorders: assessment of a new generation ektacytometer. Blood Cells Mol Dis. 2016;56(1):9–22.
13. Howard J, Hemmaway CJ, Telfer P, Layton DM, Porter J, Awogbade M, et al. A phase 1/2 ascending dose study and open-label extension study of voxelotor in patients with sickle cell disease. Blood. 2019;133(17):1865–75.

14. Hutchaleelaha A, Patel M, Washington C, Siu V, Allen E, Oksenberg D, et al. Pharmacokinetics and pharmacodynamics of voxelotor (GBT440) in healthy adults and patients with sickle cell disease. Br J Clin Pharmacol. 2019;85(6):1290–302.
15. Renoux C, Romana M, Joly P, Ferdinand S, Faes C, Lemonne N, et al. Effect of age on blood rheology in sickle cell anaemia and sickle cell haemoglobin C disease: a cross-sectional study. PLoS One. 2016;11(6):e0158182.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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