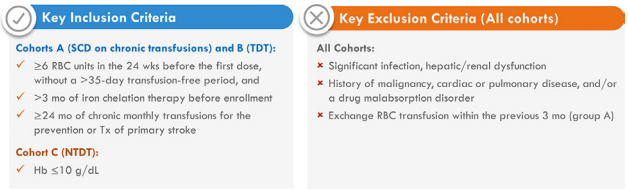
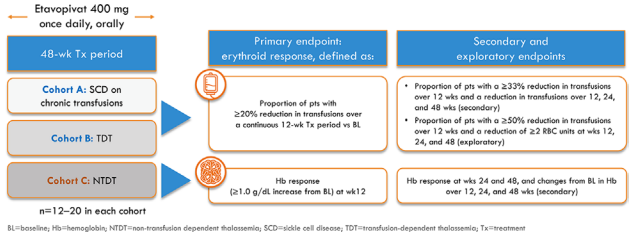
Transfusions received during the study (every ~3-5 wks) will be recorded and include Hb values before and ≥15 min after transfusion, transfusion dates, number of RBC units, volume of packed RBCs and hematocrit of the transfused unit (if available). If a patient has an increase ≥1.0 g/dL in pre-transfusion Hb vs baseline, the investigator may delay transfusion 1 wk or reduce the number of RBC units transfused. RBC exchange transfusions may also be performed in patients with SCD.
Primary, secondary and exploratory endpoints are outlined in Figure 2. The following additional endpoints will be assessed in all cohorts: change from baseline in quality of life (using SF-36 and PROMIS); change from baseline in serum ferritin levels at 12, 24 and 48 wks; liver iron at 48 wks; 2,3-DPG and ATP; pharmacokinetics; and safety. Primary endpoints will be analyzed using a 1-sided test at α=0.025.
Results: Results are not yet available for this trial in progress. Planned enrollment includes ≤20 patients (aged 12-65 y) in each of the 3 cohorts (Figure 2).
Conclusion: Etavopivat is a novel, investigational, once-daily, selective PKR activator with potential to improve RBC health and lifespan. This Phase 2 study will assess the safety of etavopivat and its impact on Hb levels and transfusion burden in patients (aged 12-65 y) with SCD or thalassemia.
Brown et al, Blood 2021.
Kalfa et al, Blood 2021.



Key eligibility criteria for patients 12–65 y of age.



Study Design.

R. BROWN declares a conflict of interest:
Consultancy, Expert: Global Blood Therapeutics; Novo Nordisk
Research support/Scientific studies: Doris Duke Foundation, Global Blood Therapeutics, Forma, Imara, Novartis
C. TRENOR declares a conflict of interest:
Consultancy, Expert: employment - Forma Therapeutics
K. WOOD declares a conflict of interest:
Stock shareholder: Forma Therapeutics Inc.
Other: Employee of Forma Therapeutics Inc.

P-056 SICKLE CELL DISEASE: DIAGNOTIC CHALLENGES AND FUTURE PERSPECTIVE IN NICARAGUA

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Purpose: Nicaragua is a developing country located in Central America. The country has a multiethnic population, with Mestizos, American Indian, and black being the most predominant ethnic groups. Generally, there is a lack of national screening programs as well as a research center dedicated to the study of hemoglobinopathies. Commonly, solubility is used as screening test. Other Diagnostic methods such as electrophoresis or HPLC are restricted to a very small number of patient due to their high cost and availability. Therefore, Sickle cell disease (SCD) is diagnosed on the basis of the patient's clinical examination, history, and routine hematologic analysis. Altogether, the deficient facilities, expertise, and economical limitations contribute to the unknown prevalence of this pathology. Recently, great efforts have been made to carry out screening studies in different parts of the country. Overall more than 800 were tested by using cellulose acetate electrophoresis (CAE). The aims of this study are to estimate the prevalence of homozygotes and heterozygotes as well as analyze the accuracy of the clinical diagnostic. Additionally, to foreseen the SCD distribution statistical analysis will be performed
Materials and methods: Hemoglobin lysate from peripheral blood was obtained and cellulose acetate electrophoresis was carried out to define the hemoglobin phenotype and to confirm the clinical diagnostic. Descriptive statistics and probabilistic graphical model (Markov influence diagrams) were used to calculate the SCD prevalence and to foresee SCD distribution, respectively.
Results: In the first group, 150 blood samples from patients with a clinical diagnosis of SCD were analyzed over a 2-year-long study period. Sixty-five patients (43%) with the hemoglobin phenotype of SCD were confirmed. However, 85 patients (57%) had an SCT or normal phenotype. In the second group, 450 blood samples from healthy individuals were screened, 21 (4.6%) were positive for SCT, and the other 429 (95.4%) were normal
Conclusion: Although significant advances have been made in the diagnostic and prevention of SCD, in low-income countries such as Nicaragua, there is a great need for resources including newborn screening and counseling programs, specialized and equipped laboratories, and training for healthcare professionals. In this context, epidemiological studies, for instance, the one here presented are vital to assess the current status of the disease and can provide guidance for future clinical care of the patient with SCD
The authors do not declare any conflict of interest

P-057 GENETIC VARIABILITY AND DISEASE SEVERITY IN A COHORT OF ANGOLAN SICKLE CELL DISEASE PATIENTS

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Purpose: Sickle Cell Anaemia (SCA) is an inherited autosomal and lethal blood disorder caused by a mutation in the HBB gene that promotes haemoglobin (Hb) polymerization and consequent sickling of red blood cells (RBCs) in hypoxia. Regardless of being a monogenic disease, SCA has a remarkably high clinical heterogeneity in its phenotypic expression. Several factors have been shown to modulate the clinical manifestations of SCA, namely genetic markers such as α-thalassaemia and β-globin cluster haplotypes, that can modulate biological parameters like the degree of haemolytic anaemia or the levels of foetal haemoglobin (HbF).
Materials and methods: Our study incorporates 112 patients of all ages and genders that are followed in the SCA consultation at Clínica Girassol and obstetrics consultation at Maternidade Lucrécia Paim, in Luanda. All patients or their legal caretakers, in the case of minor patients, signed an informed consent form. Clinical, biochemical, and haematological data were collected, as well as the patient's clinical history including the age at diagnosis, the number of hospitalizations, blood transfusions and strokes, among others Additionally, samples of peripherical blood were used for genetic analysis. All samples were genotyped for the HbS mutation by PCR-RFLP to confirm the SCA diagnosis. Moreover, four SNPs were genotyped in the β-cluster to determine the HbS haplotype by RT-PCR (rs968857, rs10128556) and PCR-RFLP (rs28440105, rs3834466). The presence of the 3.7kb deletion of the α-globin gene was determined by Gap-PCR
Results: A total of 112 SCA patients, including 76 females (67,9%) and 32 males (28,6%), were studied with ages ranging from 1 to 67 years old (mean of 18,8±12,1). All the samples were homozygous for the HbS mutation. The observed frequency of homozygous for 3.7kb α-thalassaemia deletion was 14%, with an allelic frequency of 35% in this sample. The data was consistent with the Hardy-Weinberg equilibrium ($\chi 2$=0.823, p=0.364) with the wild-type homozygous genotype being 44% and the heterozygous genotype being 42%. Most of the patients have the CAR/CAR haplotype (86 patients, 76,8%), and all the patients have at least one CAR allele. We observed an apparent differential survival of SCA patients that co-inherit the α-thalassaemia

deletion, and with the homozygous CAR haplotype. We observed apparent higher mortality for homozygous with the normal alpha gene and for heterozygous, as well as an increase in 3.7kb α-thalassaemia deletion frequency in the individuals above ten years old. This effect was more evident when comparing the under-10 and over-10 years classes. Moreover, a clear decrease in the frequency of CAR homozygotes was observed in older age classes. This differential survival should be taken into account during patient management since the first ages of diagnosis. Analysing the haematological data, we observed also that the presence of the α-thalassaemia deletion reflects on the red blood cell count (p-value < 0.001) and on the haemoglobin level (p-value = 0.029).
Conclusion: We confirm the positive impact of co-inheritance of 3.7kb α-thalassaemia deletion in SCA patients, particularly in the clinical severity and survival. The genetic polymorphisms studied can be powerful prognostic markers.
The authors do not declare any conflict of interest

P-058 INFLUENCE OF HEME OXYGENASE-I GENE PROMOTER POLYMORPHISM ON THE VASO OCCLUSIVE CRISIS OF SICKLE CELL ANEMIA PATIENTS OF EASTERN INDIA

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Purpose: Intravascular hemolysis in sickle cell anemia (SCA) leads to release of heme into the circulation and oxidative stress predisposing to vaso occlusive crisis (VOC). Heme is metabolized by the Heme Oxygenase (HO) enzyme resulting in decrease in oxidative stress. The HO-1 expression is altered by HMOX-1 gene promoter polymorphism. We investigated the role of three HMOX-1 gene polymorphisms [two single nucleotide polymorphisms (SNP), 19(G>C)(rs2071747) and -413A>T(rs2071746), and one dinucleotide (GT)n length polymorphisms (rs3074372)] on VOC in SCA patients of eastern India.
Materials and methods: A total of 170 SCA cases (80 mild and 90 severe phenotypes) and 101 healthy controls were considered for this study. Those without incidence of a single pain episode or VOC episode were considered as 'mild phenotype' of SCA while those who had three or more acute pain episodes in the last twelve months were considered as 'severe phenotype'. Genotyping of HMOX-1 (19 G>C) polymorphism was done by ARMS-PCR. Whereas, -413A>T polymorphism and (GT)n repeats were done by DNA sequencing. In HMOX-1 gene promoter, the (GT)n repeats were categorized into three types: first, (GT)n≤27 is noted as 'Small (S)' repeats; second, (GT)n=28-32 as 'Medium (M)' repeats and third, (GT)n≥33 as 'Large (L)' repeats. We categorized repeats into two groups: group-I contains S/S and S/M; whereas, group-II contains L/L, L/M, M/M, and S/L. Genotypes and allele frequency were calculated and compared between cases and controls using SNPstarts. Fisher's exact test, odds ratio (ORs) and 95% confidence intervals (95% CIs) were calculated by using GraphPad Prism v5.0. The p-value of <0.05 was considered statistically significant.
Results: On analysis of HMOX-1 (19G>C) polymorphism (rs2071747) between the mild and severe phenotype of VOC, no significant (p>0.05) variation was found in the allele and genotype distribution. Whereas, in HMOX-1 (-413 A>T) polymorphism, a significantly higher mutant allele (T) (p=0.0185) and genotype were found [AA vs TT (p=0.011) and AA vs AT+TT (p=0.02)] in severe phenotype. However, there is no difference among AA vs AT (p=0.56). On (GT)n repeat analysis, it was found that small repeats were associated with the mild phenotype and large repeats with that of severe phenotype in SCA {Gp-I vs Gp-II [p=0.0014; OR(95%CI)- 3.143 (1.538-6.422)]}. The difference was significant when comparison of severe phenotype SCA was done with controls for (GT)n repeats with larger repeats being commoner in severe phenotype.
Conclusion: No difference was noted between mild and severe SCA phenotypes HMOX-1 (19G>C) polymorphism. The prevalence of the small repeat (GT)n was found to be significantly higher in the mild SCA phenotypes and controls. Higher incidence of mutant allele and genotype of HMOX-1(-413T) polymorphism and larger (GT)n repeats in the promoter region of the HMOX-1 gene were associated with the severe phenotypes. Both have a negative effect on the HO-1 enzyme activity leading to increased VOC in severe SCA- phenotype. The capacity of HO-1 enzyme activity appears to be overwhelmed by heme-induced oxidative stress in severe phenotypes and the affected patients suffer more VOC compared to mild SCA phenotypes and controls.
The authors do not declare any conflict of interest

P-059 TRIAL IN PROGRESS: THE RANDOMIZED, DOUBLE-BLIND, MULTICENTER, PLACEBO-CONTROLLED PHASE 3 RESOLVE TRIAL INVESTIGATING THE EFFICACY OF VOXELOTOR WITH STANDARD OF CARE IN THE RESOLUTION OF LEG ULCERS IN PATIENTS WITH SICKLE CELL DISEASE

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Purpose: Sickle cell disease (SCD) is a heritable blood disorder caused by a point mutation in the β-globin gene. Polymerization of deoxygenated mutated hemoglobin is the major molecular process in SCD pathogenesis that leads to red blood cell sickling and hemolysis. In addition to the major complications of SCD—hemolytic anemia, inflammation, and vascular occlusion—patients often experience secondary complications such as stroke, organ damage, shortened lifespan, and painful and debilitating skin ulcers. The prevalence of leg ulcers among people with SCD is likely underestimated, given the lack of registries and large prospective studies examining this complication. Current estimates of leg ulcers vary widely by geographic region: 43% in Brazil, 30% in Jamaica, 27% in Nigeria, 19% in Ghana, 13% in Sierra Leone, 8% in Saudi Arabia, and 1%-5% in the US. Among Americans with SCD, an estimated 14%-18% may develop leg ulcers. Voxelotor, a sickle hemoglobin polymerization inhibitor, is approved in the US for the treatment of SCD in adults and pediatric patients aged ≥4 years and in the EU for the treatment of hemolytic anemia due to SCD in adults and pediatric patients aged ≥12 years as a monotherapy or in combination with hydroxycarbamide. In a post hoc analysis of voxelotor-treated patients from the phase 3 HOPE study (NCT03036813), leg ulcers resolved within 24 weeks in 10 out of 14 patients, and leg ulcers improved or resolved in 13 out of 14 patients by week 72.
Materials and methods: RESOLVE is an ongoing phase 3, randomized, double-blind, placebo-controlled, multicenter trial investigating the efficacy of voxelotor with standard of care (SOC) in the resolution of leg ulcers in patients with SCD. Target enrollment is 80 patients from Nigeria, Kenya, and Brazil with a confirmed diagnosis of SCD (HbSS or HbSβ0 genotype), aged ≥12 years, and with ≥1 cutaneous ulcer on the lower extremity (leg, ankle, or dorsum of foot) that meets the following criteria: ≥2 weeks' and <6 months' duration at screening and >2 cm2 in area before randomization. After a 2-week run-in period, participants are randomized 1:1 to receive once-daily oral voxelotor 1500 mg or placebo in addition to SOC for 12 weeks. After the randomized treatment period, all participants receive open-label voxelotor 1500 mg plus SOC for 12 weeks (Figure).
Results: The primary objective of the study is to assess the efficacy of voxelotor plus SOC compared with placebo plus SOC on leg ulcer healing, measured by the proportion of patients with resolution of target ulcer(s) in each treatment group by week 12. Key secondary endpoints include days to resolution of target ulcer(s) up to week 12, change from baseline in total surface area of target ulcer(s) at week 12, and incidence of new ulcers by week 12.
Conclusion: Results from this study will further guide clinicians and patients regarding the clinical use of voxelotor for the treatment of leg ulcers in patients with SCD. Participants will have the option to enroll in a separate open-label extension study after the 24-week treatment period.