directly correlated with absolute number of reticulocytes (n=18,r=0.69, P< 0.001). As would be expected we were able to link mitochondria-retention with hemolysis. In addition when we investigated energy metabolism we made the novel finding that SCD RBC have increase oxygen consumption rate (OCR). Mitochondrial-RBC retention and markers of hemolysis (serum bilirubin, and LDH) were compared by using Pearson's correlation coefficient analysis. The total serum bilirubin was directly associated with the percentage of mitochondria-containing RBCs in SCD patients' blood samples (r=0.31, n=41, p<0.04). A subgroup analysis showed a strong association between bilirubin and mitochondria-retaining RBCs in SCD patients who were not taking Hydroxyurea (r=0.55, n=17, P<0.02). There was a less significant association of percentage of mitochondria-containing RBCs in SCD with serum LDH (r=0.02, n=40, P<0.2) when all patients were analyzed, however, a strong association was seen with serum LDH and mitochondria retaining RBCs in SCD patients who were not taking Hydroxyurea (r=0.67, n=17, P<0.002). Lactate dehydrogenase (LDH) is a known biomarker for hemolysis-and associated disease complications and early mortality in patients with SCD. Percentage of mitochondria retaining RBCs also directly correlated with absolute number of reticulocytes (n=18,r=0.69, P< 0.001).We next investigated the mitochondrial RBCs energy metabolism in SCD as compared to controls in both human and mice. RBCs obtained from Townes SCD or control mice were seeded in an Agilent Seahorse XF 24-well plate using the Seahorse XF Base Medium with supplements and we monitored the OCR and extracellular acidification rate (ECAR), in real time by Seahorse XFe-extracellular flux analyzer. OCR is significantly higher in sickle cell RBCs of both human and mice origin compared to control. OCR was reduced to normal levels with oligomycin which is a known blocker of mitochondrial respiration. The mechanism responsible for mitochondrial retention in SCD is unknown. We further investigated that stress erythropoiesis is responsible for abnormal mitochondrial retention in erythrocytes. Terminal erythrocyte precursors and reticulocyte from bone marrow and spleen and peripheral blood had ROS and mitochondrial content by FACS2. Phlebotomized showed similar increased in erythrocyte mitochondrial retention and similar pattern of precursor erythrocyte in bone marrow to SCD mice precursors.

Conclusion: Our data suggest a novel pathophysiology of stress erythropoiesis in SCD.

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Novel therapies, gene therapies, bone marrow transplant and emerging diagnostics

P104 BASE EDITING REPAIRS THE HBE MUTATION RESTORING THE PRODUCTION OF NORMAL GLOBIN CHAINS IN SEVERE HBE/β-THALASSEMIA PATIENT HEMATOPOIETIC STEM AND ERYTHROID CELLS

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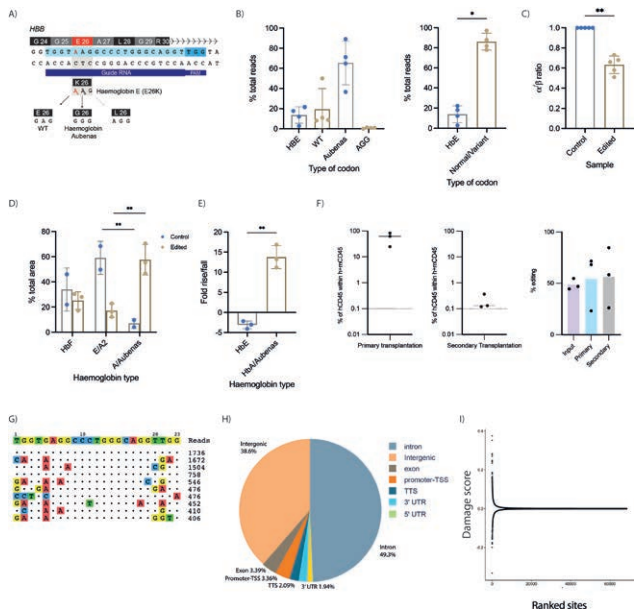
Aims: HbE/β-thalassemia is the commonest form of severe β-thalassemia, and comprises approximately 50% of all cases worldwide[1]. HbE/β-thalassemia is caused by the HbE codon 26 G>A mutation on one allele and any β0-thalassemia mutation on the other. There is a reduction in β-globin production, resulting in a relative excess in α-globin chains that leads to ineffective erythropoiesis. Importantly, individuals with a mutation on one, but not two, alleles have β-thalassemia trait, a carrier state with a normal phenotype shared by 1.5% of the world's population[2]. Recent gene therapy and gene editing approaches have been developed to treat β-thalassemia but do not directly repair the causative mutation *in-situ*. Gene replacement approaches rely on lentiviral vector-based sequence insertion or homology directed repair (HDR). HbF induction strategies rely on non-homologous end joining targeting of enhancers *in-trans*. These approaches, whilst variably successful, are associated with potential safety concerns.

Methods: Adenine base editors (ABEs) circumvent these problems by directly repairing pathogenic variants *in-situ* through deamination. ABEs catalyse A-T to G-C transitions. Conversion of the HbE codon to WT through base editing is an attractive strategy to recapitulate the phenotypically normal β-thalassemia trait state without potentially harmful double-strand breaks or random vector insertions. ABEs are able to convert the HbE codon (AAG) to wild-type (GAG), but also to GGG or AGG (Fig A). GGG at codon 26 is found in a naturally occurring haemoglobin, Hb Aubenas[3]. Heterozygotes have normal red cell indices and are phenotypically normal. We electroporated the latest generation of ABE8 editors[4] as mRNA into 3 different severe HbE/β-thalassemia donor HSPCs with sgRNAs targeting the HbE codon.

Results: The mean conversion from the HbE codon to a normal or normal variant in unselected cells was 86.2 (SD±8.1%, Fig B). The indel rate from inadvertent on-target Cas9 cleavage was below 0.5%. Edited cells did not show any perturbations in erythroid differentiation as assessed by Immunophenotyping and cellular morphology. In differentiated erythroid cells, RT-qPCR showed a mean fall in the α/β mRNA ratio to 0.65±0.08 (unedited patient cells normalised to 1, n=5, Figure C), indicating a reduction in the excess α-globin gene expression. Protein analysis by CE-HPLC showed a 3.6-fold reduction in HbE levels (SD±1.3) and a 13.5-fold increase in HbA/Hb Aubenas (SD±2.4, Fig D & E).

In serial NSG-murine xenotransplantation experiments, base edited cells were found to persist in secondary transplants, showing editing is possible in long-term HSCs (mean editing efficiency 34.5%, Fig F). Potential off-target effects were assessed *in-vitro* by CIRCLE-seq[5]; most candidate sites were in intergenic and intronic regions (Fig G & H). The top 250 sites were sequenced using deep targeted NGS. Only 5 sites showed OT deaminations at low levels in patient cells (mean 1.5%). We developed a machine learning model to assess potential OT-effects on chromatin accessibility, at all candidate sites in 49 different blood cell types[6]. Only 17 potential edits were predicted to result in a significant change in chromatin state (Fig I). 3 of these were in the top 250 sites sequenced previously, and none showed deamination *in-vivo*.

Conclusion: Together these data provide robust evidence for base editing being used as an effective and safe therapeutic strategy for HbE/β-thalassemia.



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P105 BLOOD MICROVESICLES AS BIOMARKERS TO PREDICT VASO-OCCLUSIVE CRISIS IN SICKLE CELL ANEMIA

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Background/Aims: Sickle cell anemia (SCA) is a monogenetic disorder caused by a mutation that results an abnormal hemoglobin (HbS) with a susceptibility to polymerize and deform erythrocytes which leads to complications such as Haemolysis, chronic infections and vaso-occlusive and pain crises (Williams et Thein, 2018). The polymerization of HbS inside red blood cells leads to complex interactions with the cell membrane and other molecules which triggers the apoptosis of different blood cells and the release of microvesicles (MVs) (Mahfoudhi *et al*, 2012). MVs are phospholipid microparticles that are derived from the cytoplasmic membrane of cells submitted to stress conditions that result in apoptosis or activation. The generation of MVs in SCA could serve as a potential biomarker to predict serious cardiovascular complications (Olatunya *et al*, 2019). Therefore, our study suggests the research of MVs as potential cellular biomarkers and the implementation of a new strategy of innovative predictive diagnosis in order to avoid the serious complications of sickle cell anemia.
Methods: Clinically diagnosed homozygous SCA patients from the Tunis national bone marrow transplant center and healthy donors were sampled for hematological and cellular assays. Flow cytometry was performed in to quantify apoptotic MVs derived from erythrocytes, platelets and endothelial cells using specific fluorescent antibodies and dyes.
Results and Discussion: Our results showed a statistically significant increase in the number of apoptotic MVs which suggests a high apoptosis rate in patients' cells comparing to healthy donors. We also found that MVs derived from erythrocytes, platelets and endothelial cells were clearly elevated in SCA patients which suggests their potential contribution in thrombotic risk and chronic hemolytic anemia. Our findings suggest then that microvesicles can be considered as hemolytic biomarkers for a predictive diagnosis so that disease complications could be avoided.

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P106 EARLY PREVENTIVE DIAGNOSIS OF HEMOLYTIC ANEMIA IN SICKLE CELL PATIENTS BY DETECTING THE TRIGGERING OF ERYPTOSIS.

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Background/Aims: Sickle cell disease (SCD) also known as sickle cell anemia is one of the most worldwide-disseminated hereditary hemoglobinopathies. It is caused by a single amino acid substitution at the sixth residue of β globin gene (Glu6Val), which results in an abnormal hemoglobin called hemoglobin S (HbS). The acceleration of HbS polymerization induces rigid and dysfunctional erythrocytes that play a central role in acute and chronic clinical manifestations of SCD(Conran *et al.*,2009). Vaso-occlusion and hemolytic anemia are the hallmarks of SCD.
We aim to explore the cellular environment of red blood cells to explain the physiopathology of SCD. In fact, the life span of circulating erythrocytes in healthy individuals vary from 100 to 120 days. In SCD, red blood cells undergo a form of cell death, namely, eryptosis before they reach their full life span. Eryptosis is triggered by a wide variety of factors as hyperosmolarity, oxidative stress and energy depletion. It is characterized by the presence of membrane blebbing, cell shrinkage, and phosphatidylserine (PS) exposure (Lang *et al.*,2012). In this study, we will explore the mechanism of triggering of eryptosis in Sickle cell disease.
Methods: Following clinical diagnosis, 50 homozygous SCD patients and 30 healthy donors were identified for hematological and cellular assays. Flow cytometry was performed in order to determine the viability parameters of erythrocytes. The morphology of red blood cells and the externalization of phosphatidylserine was detected by labeling red blood cells with Annexine V. Moreover, we had identified intracellular calcium concentration and ceramide level by labeling erythrocytes with Fluo3-am and anti-ceramide antibodies. Finally, we had quantified reactive oxygen species (ROS) by labeling red blood cells with CM-H2DCFDA.
Results and Discussion: Eryptosis in sickle cell patients is accelerated. In fact, PS (+) red blood cells are more present in patients than in healthy subjects. Therefore, eryptosis is triggered by oxidative stress, which stimulates the increase of calcium activity and subsequent externalization of PS and red blood cells shrinkage in sickle cell patients. However, the pathway of ceramide can also be considered a potential stimulating factor of eryptosis in SCD. Eryptosis ensures healthy erythrocyte quantity in circulation, whereas excessive eryptosis is the cause of acute anemia and may contribute to vaso-occlusive crisis in SCD patients.

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Clinical and epidemiological studies

P107 A PHASE 2/3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF MITAPIVAT IN PATIENTS WITH SICKLE CELL DISEASE

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Background: The key pathology in sickle cell disease (SCD), a life-threatening, hereditary hemoglobin (Hb) disorder, is red blood cell (RBC) sickling due to polymerization of deoxygenated sickle Hb (HbS), which can be exacerbated by increased levels of the glycolytic metabolite 2,3-diphosphoglycerate (2,3-DPG), and decreased ATP.[1–3] Sickled RBCs are rigid, not deformable, and fragile, resulting in vaso-occlusion triggering pain and chronic hemolysis.[4–6] SCD treatment options are limited, with an unmet need for safe and effective therapies to improve anemia and reduce pain. Mitapivat is an oral, activator of RBC pyruvate kinase (PKR), a key glycolytic enzyme. PKR activation decreases 2,3-DPG and increases ATP, which may reduce HbS polymerization, RBC sickling, and hemolysis in SCD.[3,7–9] Data from the phase (ph) 1 National Institutes of Health multiple ascending dose study of up to 100 mg mitapivat twice daily (BID) in SCD (NCT04000165) showed that mitapivat was safe and tolerable, increased ATP and decreased 2,3-DPG in a dose-dependent manner, and improved anemia and hemolysis.[8,10]
Aims: To report the study design of RISE UP (NCT05031780, EudraCT: 2021-001674-34), a ph 2/3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat in patients (pts) with SCD.
Methods: Eligible: pts aged ≥16 yrs with documented SCD (HbSS, HbSC, HbSβ^0/HbSβ$^+$ thalassemia, other SCD variants), 2–10 sickle cell pain crises (SCPCs; acute pain needing medical contact, acute chest syndrome, priapism, hepatic or splenic sequestration) in the prior 12 months, and Hb 5.5–10.5 g/dL. If taking hydroxyurea (HU), the dose must be stable for ≥90 days before starting study drug. Ineligible: pts receiving regularly scheduled blood transfusions, with severe kidney disease or hepatobiliary disorders, currently receiving SCD therapies (excluding HU) or who have received gene therapy, bone marrow or stem cell transplantation.
In the double-blind ph 2 part, 69 pts will be randomized (1:1:1) to 50 mg or 100 mg mitapivat, or placebo BID for 12 weeks (wks). The primary objective of ph 2 is to determine the recommended ph 3 mitapivat dose by evaluating anemia and safety vs placebo via the following endpoints: Hb response (≥1.0 g/dL increase in average Hb concentration over Wks