fator de transcriação com maior valor diferencial de expressão entre os listados. O HNF4α é descrito como um fator de transcrição que regula a expressão de vários genes hepáticos, podendo atuar no desenvolvimento do fígado, rim e intestinos e é expresso em tecido hematopoiético. As células HUDEP-2 foram tratadas com Decitabina 50 nM e coletadas após 72 horas. Os níveis de HbF foram mensurados por citometria de fluxo em triplicas biológicas. A porcentagem de células positivas para HbF em HUDEP-2, tratada com Decitabina, foi de 12,27 $\pm$ 0,7% n = 3, enquanto nas células controle 1,0 $\pm$ 0,06% n = 3, p < 0,0001. Estes resultados corroboram o aumento da expressão observado nas células CD34$^+$. As células HUDEP-2 foram nucleofectedas com Cas9 *high fidelity* (104 pmol), complexo crRNA: tracrRNA (120 pmol) e 1uM de gRNA HNF4α usando o kit de células humanas CD34$^+$ no dispositivo AMAXA Nucleofector 4D (Lonza). 48 horas após a nucleofecção, as células HUDEP-2 editadas foram submetidas à seleção de clones e expandidas por aproximadamente 28 dias. O DNA genômico dos clones foi sequenciado, e quatro clones -HNF4α-HUDEP-2 foram encontrados com *indels* no quarto éxon do gene HNF4α. Esses clones foram expandidos em cultura com um controle, e os níveis de HbF foram quantificados com anticorpo anti-HbF por citometria de fluxo. Os níveis de HbF destes quatro clones foram de 8,2 $\pm$ 2,4%, o nível do controle foi de 0,9 $\pm$ 0,02% p < 0,0258. Os clones editados, embora não homogeneamente, expressaram significativamente mais HbF do que o controle. Nossos resultados sugerem que o HNF4α pode desempenhar um papel importante na ativação do gene da gama globina em células HUDEP-2. Adicionalmente, o aumento na expressão dos genes TCF7L2 e HNF1α são sugestivos de um possível papel na regulação de HbF. Esses dados são importantes, pois indicam novos possíveis alvos para terapia gênica em hemoglobinopatias.

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ENERGIZE AND ENERGIZE-T: TWO PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDIES OF MITAPIVAT IN ADULTS WITH NON–TRANSFUSION-DEPENDENT OR TRANSFUSION-DEPENDENT ALPHA- OR BETA-THALASSEMIA

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Objectives: Thalassemias are characterized by imbalanced globin-chain production resulting in excess α- or β-globin precipitation, hemolytic anemia, and ineffective erythropoiesis. Adenosine triphosphate (ATP) levels are reduced in thalassemic red blood cells (RBCs), despite increased energy demands. Mitapivat is an oral activator of RBC pyruvate kinase (PKR), a glycolytic enzyme that regulates ATP production. In a phase 2 study of patients (pts) with α- or β-non–transfusion-dependent thalassemia (NTDT), twice-daily (BID) dosing with mitapivat increased hemoglobin (Hb) levels by ≥1.0 g/dL in 80% of pts, supporting the broadening of mitapivat's development in thalassemia. The study designs of ENERGIZE (2021-000211-23) and ENERGIZE-T (2021-000212-34), two phase 3 trials to assess the efficacy and safety of mitapivat in adults with α- or β-NTDT or transfusion-dependent thalassemia (TDT), respectively, are reported here. **Methods:** Both studies are phase 3, multicenter, randomized, double-blind, placebo-controlled trials. In ENERGIZE, approximately 171 eligible adults with NTDT will be randomized (2:1) to receive 100 mg mitapivat BID or placebo for 24 weeks (wks). Upon completion, eligible pts can transition to a 5-year, open-label extension. Key inclusion criteria: documented diagnosis of thalassemia (β-thalassemia $\pm\alpha$-globin mutations, Hb E β-thalassemia, or α-thalassemia [Hb H disease]), Hb concentration ≤10.0 g/dL, and NTDT defined as ≤5 RBC units during the 24-wk period before randomization and no RBC transfusion ≤8 wks prior. The primary endpoint is an Hb response defined as a ≥1.0 g/dL increase in average Hb concentration from Wk 12 through 24 compared with baseline (BL). Secondary endpoints include pt-reported outcomes, changes in Hb, markers of hemolysis and erythropoiesis, and safety. In ENERGIZE-T, approximately 240 eligible adults with TDT will be randomized (2:1) to receive 100 mg mitapivat BID or placebo for 48 wks. Upon completion, eligible pts can transition to a 5-year, open-label extension. Key inclusion criteria: documented diagnosis of thalassemia (same genotypes as detailed for the ENERGIZE study), and TDT defined as 6–20 RBC units transfused and no transfusion-free period ≥6 wks during the 24 wks before randomization. The primary endpoint is a transfusion reduction response, defined as a ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-wk period through Wk 48 compared with BL. Secondary endpoints include additional measures of transfusion burden, changes in iron markers, and safety. **Results:** Not yet available. **Discussion:** ENERGIZE and ENERGIZE-T are the first pivotal studies to assess a potential treatment across a broad spectrum of pts with thalassemia (ie, pts with TDT and NTDT; α- and β-thalassemias). **Conclusions:** ENERGIZE and ENERGIZE-T will evaluate the efficacy and safety of mitapivat, a novel, first-in-class oral activator of PKR. Both studies will start enrollment in 2021.

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