[f] *Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (Hemorio), Rio de Janeiro, RJ, Brazil*
[g] *Unidade de Onco-hematologia, Instituto da Criança, Instituto de Tratamento do Câncer Infantil (ITACI), Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (HCFMUSP), São Paulo, SP, Brazil*
[h] *Departamento de Ciência da Computação (DCC), Universidade de São Paulo (USP), São Paulo, SP, Brazil*
[i] *Fundação Pró-Sangue Hemocentro de São Paulo, São Paulo, SP, Brazil*
[j] *Vitalant Research Institute, San Francisco, United States*
[k] *UCSF Benioff Children's Hospitals, Oakland, United States*
[l] *Department of Laboratory Medicine, University of California San Francisco (UCSF), San Francisco, United States*

Aim: Chronic kidney disease (CKD) has a significant impact on sickle cell disease (SCD) morbidity and mortality. Early identification of individuals at highest risk of developing CKD may allow therapeutic intervention to prevent worse outcomes. This study aimed to evaluate the prevalence and risk factor for CKD among adults with SCD in Brazil. **Methods:** Participants in the REDS-III multicenter SCD cohort with more severe genotypes [sickle cell anemia (SCA) = hemoglobin (Hb) SS, Hb Sβ0-thalassemia, Hb Sβ+-thalassemia with mutations associated with severe phenotype] aged ≥18 years at time of enrollment with at least two serum creatinine values were analyzed. Participants were enrolled between 2013-2015 and were prospectively followed and monitored for the occurrence of clinical complication, including death, for up to three years. The estimated glomerular filtration rate (eGFR) was calculated using the JSCCS-GFR Equation. The CKD stage was defined according to the K/DOQI. For participants with multiple eGFR measures, we use the two most recent values to define CKD; for those who presented different CKD stages, we used the mean value to define the outcome. Because albuminuria status was not known in all participants, those with CKD stage 2 or higher were compared to those with eGFR≥90. The association among the characteristics and the presence of CKD stage 2 or higher were assessed using binary logistic regression model. **Results:** 497 (65.7%) participants were receiving hydroxyurea (HU) therapy, 105 (13.9%) were treated with chronic blood transfusion (CBT), and 69 (9.1%) were under both therapies. Among the 756 participants, 569 (75.3%) had eGFR≥90, 175 (23.1%) had stage-2, six (0.79%) had stage-3, and six (0.79%) had ESRD. Participants with CKD stage 2 or higher were significantly older and predominantly male when compared with those with eGFR≥90. There was no association between CKD stage 2 or higher and HU or CBT. Participants with CKD stage 2 or higher had a 2.3 (95%CI: 1.4–3.7) times higher odds for death when compared to those with eGFR≥90. Male sex (OR: 34.7; 95%CI: 20.3–62.5), higher age (OR: 1.03; 95%CI: 1.01–1.05), higher diastolic blood pressure (OR: 1.03; 95%CI: 1.002–1.05), lower Hb (OR: 0.704; 95%CI: 0.59–0.83), and lower reticulocytes (OR: 0.91; 95%CI: 0.86–0.96) levels were significantly associated with stage 2 or higher in the final multivariate model. **Discussion:** We observed a higher odds for mortality in participants with CKD stage 2 or higher when compared to those with eGFR≥90. This highlights the importance of early detection and implementation of preventative therapies. We also observed a significant independent association between lower levels of Hb and reticulocytes with CKD stage 2 or higher. These data might reflect a state of hypoproliferative anemia in participants with CKD stage 2 or higher likely due to deficiency of erythropoietin. In this study, CKD stage 2 or higher was more prevalent in men than in women, and male sex was the strongest independent predictor of CKD stage 2 or higher in the final model. This finding might be used to target individuals at highest risk for CKD in prevention campaigns. **Conclusion:** CKD stage 2 or higher was observed in one-quarter of adults with SCA. Older age, male sex, higher diastolic blood pressure, lower hemoglobin, and lower reticulocytes levels were significantly associated with occurrence of CKD stage 2 or higher, which, in turn, significantly increased the risk of mortality.

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DETECÇÃO DE GENES ASSOCIADOS AO INCREMENTO NA PRODUÇÃO DE HBF POR UM NOVO MÉTODO "IN VITRO"

IG Sousa [a], FP Albuquerque [a], PKM Martin [a], DM Albuquerque [a], C Lanaro [a], R Kuryta [b], Y Nakamura [c], FF Costa [a]

[a] *Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil*
[b] *Central Blood Institute*
[c] *RIKEN BioResource Research Center*

A anemia falciforme é uma doença hereditária recessiva causada por uma mutação em um único nucleotídeo no gene da beta globina. Esta mutação provoca uma alteração estrutural na qual o ácido glutâmico é substituído por uma valina acarretando na falcização das hemácias quando desoxigenadas. Alguns fármacos com ação hipometilante são capazes de induzir a expressão de gama globina através da inibição da metilação no promotor desse gene. Porém, pouco se sabe a respeito desta regulação e sobre os genes envolvidos neste processo. Visando compreender esta regulação, células CD34$^+$, de indivíduos controle, foram tratadas com 1μM de Decitabina no 9°dia de cultura celular para indução da produção de Hemoglobina Fetal (HbF). Os níveis de expressão dos fatores de transcrição e modificadores da cromatina foram avaliados por meio da plataforma PCR Array (Qiagen^{TM} Germany). Nesse ensaio, foram evidenciados 24 fatores de transcrição diferencialmente expressos, dentre eles, o HNF4α (*Hepatocyte Nuclear Factor 4 Alpha*) regulado positivamente em 26,1 vezes, HNF1α (*Hepatocyte Nuclear Factor 1 Homeobox A*) 20,0 vezes e o TCF7L2 (*Transcription Factor 7 Like 2*) 5,9 vezes, em células tratadas com Decitabina comparadas ao controle. Selecionamos o gene HNF4α como um candidato para silenciamento utilizando a ferramenta CRISPR/Cas9 em células HUDEP-2 (*immortalized human erythroid progenitor*) por ser o

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

KH Kuo [a], DM Layton [b], H Al-Samkari [c], A Kattamis [d], S Sheth [e], A Taher [f], V Viprakasit [g], CX Chamberlain [h], L Czapla [h], S Gheuens [h], L Jiang [h], M Lynch [h], B Tong [h], K Uhlig [h], MD Cappellini [i]

[a] *Division of Hematology, University of Toronto, Toronto, Canada*
[b] *Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom*
[c] *Division of Hematology, Massachusetts General Hospital, Harvard Medical School, Boston, United States*
[d] *First Department of Pediatrics, National and Kapodistrian University of Athens BR, Athens, Greece*
[e] *Weill Cornell Medical College, New York, United States*
[f] *American University of Beirut, Beirut, Lebanon*
[g] *Siriraj-Thalassemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[h] *Agios Pharmaceuticals, Inc., Cambridge, United States*
[i] *University of Milan, Milan, Italy*

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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