transfusion mediated immunomodulation and transmission of viruses, Hydroxyurea, and bone marrow stress due to chronic anemia. Interestingly, patients with sickle cell disease share these risk factors and have been also noted to exhibit an increased risk of malignancies, mostly of the hematologic type.[6] Aside from blood donor screening to decrease the risk of transmission of viruses such as HTLV-1, CMV, and Hepatitis C, optimization of iron chelation practices, and leukoreduction of the transfused blood, there are currently no specific recommendations as far as risk factor modification is concerned. There are no screening guidelines for hematologic malignancies in patients with thalassemia.

While the association between thalassemia and hematologic malignancies remains in its early stages, it will be important to continue to explore the field and conduct more large scale studies with long-term follow-up. It is also paramount to remain very attentive to new signs and symptoms patients with thalassemia exhibit that may indicate a hematologic malignancy, with the understanding that some of these findings may overlap with those of thalassemia, such as worsening anemia, fatigue, and splenomegaly.

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Genetic modulation of fetal hemoglobin in hydroxyurea-treated sickle cell anemia

To the Editor:

HbF levels are associated with haplotypes of the *HBB* gene cluster although the mechanisms accounting for this are largely unknown. Genome-wide association studies (GWAS) have revealed three quantitative trait loci (QTL), *HBG2* on chromosome 11p15, *HBS1L-MYB* (*HMIP*) intergenic region on chromosome 6q23 and *BCL11A* on chromosome 2p16, which account for 20%-50% of HbF variation in sickle cell anemia (SCA). The olfactory receptors genes might have a regulatory role in γ-globin gene expression.[1,2]

Hydroxyurea (HU) induces the production of HbF in SCA, providing a pharmacological therapeutic approach for ameliorating clinical complications.[3] Accordingly, we analyzed *HBB* haplotypes along with SNPs in HbF associated QTL to evaluate their role in regulating HbF in SCA treated with HU.

The study was conducted from 2013 to 2014 in 141 SCA patients, 42 on and 99 not on HU, who attended Sickle Cell Disease Reference Center in Itabuna, Bahia, Brazil. Mean age was 15.2 ± 11.1 years (median, 13 years) with 71 females. Laboratory variables were measured in patients without clinical manifestations of vaso-occlusive crisis and no transfusions in the preceding three months. The study was approved by the Research Board of the CPqGM-FIOCRUZ-Bahia-Brazil.

Hematological analyses were done on a Sysmex Count KX 21 N (Sysmex Corporation, Tokyo, Japan) and blood chemistries on a Cobas (Roche Diagnostics, Salt Lake City, Utah, USA). Hemoglobin fractions were quantified by high-performance liquid chromatography (HPLC) (BioRad, Hercules, CA, USA) at the Laboratory of Research in Anemia (LPA/UFBA) at the Universidade Federal da Bahia and Laboratory of Hematology, Genetic and Computational Biology (LHGB) at CPqGM-FIOCRUZ-Bahia-Brazil.

β^S-globin gene cluster haplotypes were ascertained by polymerase chain reaction (PCR) and restriction fragments length polymorphisms (RFLP). SNPs of *BCL11A* (rs6732518, C > T; rs766432, A > C), *HBS1L-MYB* interval (rs11759553, A > C; rs35959442, C > G), and *OR51B5/6* genes (rs4910755, A > C; rs7483122, C > T), corresponding to QTL on chromosomes 2, 6, and 11 respectively, were analyzed by Real-Time PCR (Applied Biosystems, Foster City, California, USA).

Variables for analysis were evaluated in means, medians and percentile. Quantitative variables were compared using the *t*-test for normal data, and Mann-Whitney for non-normal data. Differences in laboratory data associated with SNP genotypes and dose of HU (mg/kg/day) were determined by the Kruskal-Wallis test. Multivariate linear regression analyses were performed to estimate the likelihood of having HbF levels as outcome and a possible interaction with age, sex, HU use, CAR haplotype, and polymorphisms in genes related to HbF

TABLE 1 Laboratory characteristics of SCA patients taking and not taking hydroxyurea (HU)

Laboratory value	All patients *N* = 141 Median (25th-75th)	No HU use* *N* = 99 Median (25th-75th)	HU * *N* = 42 Median (25th-75th)	*P value**
RBC, $\times 10^{12}$/L	2.4 (2.2-2.8)	2.6 (2.3-2.8)	2.2 (2.0-2.6)	**.004**
Hemoglobin, g/dL	7.7 (7.2-8.7)	7.6 (7.2-8.5)	8.0 (7.3-9.2)	.206
Hematocrit, %	22.0 (20.0-25.0)	21.8 (20.0-24.4)	22.4 (19.6-25.2)	.757
MCV, fL	89.2 (83.6-96.3)	86.2 (81.4-90.9)	97.2 (90.7-103.4)	**<.001**
Total bilirubin, mg/dL	2.4 (1.6-3.5)	2.6 (1.7-3.8)	2.1 (1.5-3.0)	.084
Direct bilirubin, mg/dL	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.3-0.5)	.501
Indirect bilirubin, mg/dL	2.0 (1.2-3.0)	2.2 (1.2-3.2)	1.6 (1.1-2.5)	.072
LDH, U/L	999.0 (738.5-1586.5)	1094.0 (791.0-1710.0)	897.0 (677.2-1480.5)	.101
HbF, %	9.9 (5.7-14.6)	9.3 (5.2-14.1)	11.1 (9.0-15.4)	**.013**
HbF, g/dL	0.76 (0.43-1.10)	0.71 (0.40-1.09)	0.85 (0.69-1.20)	**.010**
HbS, %	85.4 (81.1-90.0)	86.7 (81.2-91.5)	83.6 (80.3-86.6)	**.007**
WBC, $\times 10^9$/L	12700.0 (9700.0-15600.0)	13900.0 (11,200.0-16100.0)	9700.0 (8050.0-12800.0)	**<.001**
Neutrophils, $\times 10^9$/L	5396.0 (4005.0-7153.0)	5760.0 (4081.0-7182.0)	4839.0 (3684.5-6705.7)	.102
Eosinophil count, $\times 10^9$/L	642.0 (249.0-1541.0)	696.0 (282.0-1807.0)	394.5 (146.0-738.0)	**.001**
Lymphocyte count, $\times 10^9$/L	4998.0 (3918.0-6726.5)	5560.0 (4350.0-7224.0)	4156.5 (3329.2-5085.0)	**<.001**
Monocyte count, $\times 10^9$/L	338.0 (200.0-611.5)	360.0 (220.0-688.0)	279.5 (156.7-517.5)	**.045**

RBC, red blood cell; MCV, mean cell volume; LDH, lactate dehydrogenase; WBC, white blood cell. *P*-value obtained using Mann-Whitney and **P*-value using t test. Bold values indicate significance at $P < .05$ between patients not using and using HU.

modulation. Data were analyzed using SPSS version 20.0 (IBM, New York, NY) and *P* values <.05 were considered significant.

Hematologic data from patients taking and not taking HU are shown in Table 1. The dose of HU was available in all HU treated patients; mean length of use was 13.4 ± 9.7 months (median 12 months; 2-50 months). Fifteen mg/kg/day was used in 47.6% of cases, followed by 26.2% using 25 mg/kg/day and 23.8% using 20 mg/kg/day; 1 patient received the maximum tolerated dose of 35 mg/kg/day and was excluded from the analysis. The dose of 25 mg/kg/day was associated with lower platelet count ($P = .03$) and mean platelet volume (MPV) ($P = .08$) (Supporting Information Table 1). Exposure to HU for more than 12 months was associated with lower reticulocyte counts ($P = .03$) and higher direct bilirubin level ($P = .02$). There was no correlation between HU dose and HbF response, as described previously.[4] This might be consequence of individualized pharmacodynamics and metabolism of HU.

Forty-five percent of chromosomes (127/282) were CAR haplotype, 47.9% (135/282) Benin (BEN) and 7.1% (20/282) were atypical haplotypes. Forty-one percent (58/141) of the patients were BEN/CAR compound heterozygotes; 24.8% BEN/BEN (35/141) homozygotes and 22.7% (32/141) CAR/CAR homozygotes. Patients with an atypical haplotype represented 5.0% (7/141) of BEN/Atypical and 3.5% (5/141) of CAR/Atypical haplotypes. The Atypical/Atypical haplotype was found in 2.8% (4/141). The CAR haplotype was associated with average HbF of 8.9% ($P = .01$).

SNP genotypes in *BCL11A* (rs766432, rs6732518), *HBS1L-MYB* (rs11759553, rs35959442) and *OR51B5/6* (rs4910755, rs7483122) were successfully assayed in 141 patients and are shown in Supporting Information Table 2. In patients on HU there was a significant association with homozygosity for the minor allele (C) of rs766432 in *BCL11A* with an increased hemoglobin concentration ($P = .03$), RBC count ($P = .01$), and hematocrit ($P = .02$). This same group had lower platelet count ($P = .05$) and direct bilirubin ($P = .04$) (Supporting Information Table 3).

Multivariate linear regression analysis models were used to investigate association of SNPs in genes related to HbF expression. We found statistical significance in an adjusted model that demonstrated the influence of HbF associated variables like gender, age, HU therapy, CAR haplotype and *BCL11A* rs766432; *HIMP* rs11759553; *OR51B5/6* rs7483122 ($R = .43$; $P < .001$). The model showed that association of independent variables likes HU use ($P = .008$), CAR haplotype ($P = .04$) and *HIMP* rs11759553 ($P = .007$) contributed significantly and independently to modulating HbF expression (Supporting Information Table 4).

We hypothesized that variation in known modulators of HbF gene expression would affect HbF response to HU. The CAR haplotype was associated with decreased HbF. In our patients, *BCL11A* rs6732518 C > T and rs766432 A > C had minor allele frequencies of 0.59 and 0.27. Homozygotes for the minor C allele of *BCL11A* rs766432 had a higher RBC count, hematocrit, hemoglobin concentration and HbF level, suggesting that in the presence of this allele patients treated with HU had less hemolysis that could contribute to clinical improvement. Our results

suggest that SCA patients homozygous for the minor allele of *BCL11A* rs766432 and who use HU had greater increases in HbF expression and corresponding improvement in hematological parameters.

The results of a study with polymorphisms in *BCL11A* and *OR51B5/6*, and *HBS1L-MYB* in 622 Brazilian sickle cell disease patients including patients with HbSC disease and SCA who were not taking HU were similar to the present study.[5] A study of Tanzanian SCA found SNPs in *HBS1L-MYB* and *BCL11A* associated with HbF.[6] The multivariate model shows that *HBS1L-MYB*, sex, HU treatment, and HbS gene haplotype modulated HbF expression. This was a cross-sectional study and a determination of the change in HbF in response to HU according to SNP genotype requires prospective studies.

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MMA performed the interview, collected the samples, analyzed the data and wrote the manuscript. RPS, CCG analyzed the data and co-wrote the manuscript. TCCF, FIN, RSQ, attended the patients. CVBF, SCMAY, RMO, JRDF reviewed the article. BAVC, CGB, JNM, MHS, and MSG analyzed the data, provided academic support and revised the article critically.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study received approval from the institutional review board of the Instituto Gonçalo Moniz at the Fundação Oswaldo Cruz (IGM-FIOCRUZ – Bahia - Brazil) (CAAE 08452913.9.0000.0040) and T32HL007501 from the National Heart Lung and Blood Institute, NIH (JNM).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

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Concomitant monitoring of *WT1* and *FLT3-ITD* expression in *FLT3-ITD* acute myeloid leukemia patients: which should we trust as a minimal residual disease marker?

To the Editor:

FLT3-ITD mutations (FMS-like tyrosine kinase 3) are the most common molecular alterations observed in Acute Myeloid Leukemia (AML). The presence of these mutations has an unfavorable prognostic significance and is the target of specific *FLT3-ITD* inhibitor drugs, currently in advanced stage clinical trials.[1,2] In recent years minimal residual disease (MRD) detection in AML has taken a very important prognostic role, becoming essential for the choice of the most appropriate consolidation strategy.[1–4] A recent study by Gaballa et al has shown how *FLT3-ITD* molecular status at the time of transplant is a key predictor of disease relapse and survival in patients with *FLT3-ITD* AML.[3]