A pilot study of subcutaneous decitabine in β-thalassemia intermedia

Nancy F. Olivieri,1 Yogen Saunthararajah,2 Vivek Thayalasuthan,1 Janet Kwiatkowski,3 Russell E. Ware,4 Frans A. Kuypers,5 Hae-Young Kim,6 Felicia L. Trachtenberg,6 and Elliott P. Vichinsky,5 for the Thalassemia Clinical Research Network

1Hemoglobinopathy Research, University Health Network, University of Toronto, Toronto, ON; 2Cleveland Clinic, Cleveland, OH; 3Children’s Hospital of Philadelphia, Philadelphia, PA; 4St Jude Children’s Research Hospital, Memphis, TN; 5Hematology/Oncology, Children’s Hospital and Research Center Oakland, Oakland, CA; and 6New England Research Institutes, Watertown, MA

Ineffective erythropoiesis, the hallmark of β-thalassemia, is a result of α/non-α globin chain imbalance.1 One strategy to redress globin-chain imbalance is to induce γ-globin gene (HBG) expression. Repression of HBG in adult erythroid cells involves DNA methylation and other epigenetic changes. Therefore, the cytosine analog decitabine, which can deplete DNA methyltransferase 1 (DNMT1), can potentially activate HBG. In 5 patients with β-thalassemia intermedia, a dose and schedule of decitabine intended to deplete DNMT1 without causing significant cytotoxicity (0.2 mg/kg subcutaneous 2 times per week for 12 weeks) increased total hemoglobin from 7.88 ± 0.88 g/dL to 9.04 ± 0.77 g/dL (P = .004) and absolute fetal hemoglobin from 3.64 ± 1.13 g/dL to 4.29 ± 1.13 g/dL (P = .003). Significant favorable changes also occurred in indices of hemolysis and red blood cell densitometry. Consistent with a noncytotoxic, differentiation altering mechanism of action, the major side effect was an asymptomatic increase in platelet counts without erythrocyte micronucleus or VDJ recombination assay evidence of genotoxicity. This study was registered at www.clinicaltrials.gov as #NCT00661726.

(Blood. 2011;118(10):2708-2711)

Methods

Six patients (Table 1; supplemental Table 1) were enrolled into this pilot (phase Ia) study. Decitabine 0.2 mg/kg (reconstituted in 5 mL water or saline for injection) was administered subcutaneously daily on the same 2 consecutive days each week for 12 weeks. One patient withdrew from study after week 2 because of fatigue requiring transfusion. Two patients were treated for 12 weeks, 1 patient for 11 weeks, and 2 patients for 8 weeks. Various treatment durations were the result of treatment-induced platelet count increases to >1000 × 10^9/L, requiring therapy interruption per protocol. In addition to standard clinical and laboratory evaluations, measurements of RBC deformability, density, and phosphatidylserine exposure, and micronucleus and VDJ recombination assays, were performed using previously described methods.7,9 HbF was measured by cation exchange high performance liquid chromatography. The protocol was approved by the Thalassemia Clinical Research Network Data and Safety Monitoring Board and by the ethical review boards of all participating Thalassemia Clinical Research Network institutions. Written informed consent was obtained from all the participants in accordance with the Declaration of Helsinki.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2011 by The American Society of Hematology
The primary efficacy endpoint was an increase in total Hb of 1.5 g/dL or more from baseline to peak (baseline = mean from 3 pretreatment weekly samples; peak = highest level in biweekly samples taken between weeks 2-12 inclusive). Linear regression after adjusting for time duration was used to test the mean difference between baseline and follow-up values (peak, nadir, 12 weeks; SAS Version 9.2, SAS Institute; and R2.11.1 http://www.r-project.org). P values <.05 were considered statistically significant. The study was powered (n = 8, although final n = 6) to have 90% probability of observing at least 1 participant achieving the primary endpoint for true efficacy rates as low as 25% (highly detailed in supplemental Methods).

**Results and discussion**

The primary outcome, an increase in Hb of ≥1.5 g/dL above baseline, was achieved in 2 of 5 evaluable patients. In the group overall, Hb increased from baseline 7.88 ± 0.88 g/dL to peak 9.04 ± 0.77 g/dL (P = .004; Table 1). Absolute HbF increased from 3.64 ± 1.13 g/dL to 4.29 ± 1.13 g/dL (P = .003). Trends for increasing Hb and HbF were noted in all 5 patients during treatment (Table 1; Figure 1). Suggesting decreased hemolysis and more effective erythropoiesis, indirect bilirubin declined from baseline 3.2 ± 1.0 mg/dL to nadir 2.2 ± 0.8 mg/dL (P = .045; Table 1; Figure 1). There was a nonsignificant trend for decreasing serum lactate dehydrogenase, from 479.4 ± 125.8 U/L to 362.8 ± 100.4 U/L (P = .083; Table 1; Figure 1). There was a significant decrease in the absolute reticulocyte count from 157.0 ± 14.5 × 10^9/L to 123.0 ± 13.2 × 10^9/L (P = .039; Table 1; supplemental Figure 1). There was a nonsignificant trend for decreasing erythropoietin levels from 147.4 ± 39.7 mIU/mL to 103.6 ± 27.8 mIU/mL (P = .177). There was a significant shift toward normalization of RBC Hb concentration (P = .022; supplemental Table 2; supplemental Figure 2). There was a statistically nonsignificant decrease in RBC phosphatidylserine exposure (supplemental Table 2; supplemental Figure 2). There was no significant change in maximum RBC deformability (supplemental Table 2; supplemental Figure 2).

Platelet counts increased from 585.2 ± 90.6 × 10^9/L to 940.2 ± 184.3 × 10^9/L (P = .007; Table 1; supplemental Figure 1). Platelet increases were the only adverse event definitely related to therapy. Baseline platelet counts in these patients were already elevated. No clinical events were associated with these increases. In the only nonsplenectomized patient, platelet increases were minimal (from 233 to 296 × 10^9/L). There was a nonsignificant downward trend in neutrophils, from 6.51 ± 1.20 × 10^9/L to 3.36 ± 0.63 × 10^9/L (P = .069; Table 1; supplemental Figure 1). Quantitative in vitro assays for mutagenicity, specifically enumeration of illegitimate VDJ recombination events, and micronuclei within early reticulocytes did not identify significant changes between baseline and 24-week values (supplemental Figure 2).

Decitabine-induced shifts in differentiation include increased erythropoiesis and megakaryopoiesis. Therefore, increases in platelet counts were expected, and the interruption in study drug administration was protocol mandated procedures. Because patients with thalassemia intermedia, especially those after splenectomy, have a higher risk for venous and arterial thrombosis, this side effect is a concern. Although it is intuitive to expect an association between platelet count and thrombosis risk, this has not been evident in a number of studies, whereas qualitative RBC defects (or
qualitative platelet defects in myeloproliferative disease) probably have a role (supplemental References). Notably, in a sickle cell disease study using a similar regimen of decitabine, improvement in multiple indices of RBC pathology was accompanied by improvement in multiple markers of coagulation pathway activity, despite platelet count increases to $>800 \times 10^3$/L. Thus, it is possible that decitabine-induced improvements in RBC phenotype could reduce thrombophilia despite concurrent platelet count increases. To answer this question in β-thalassemia, coagulation pathway evaluation should be a component of future studies.

For life-long disease modification, pharmacologic induction of HbF must have an excellent safety profile. One concern with hydroxyurea or nucleoside analog therapy is the possibility of mutagenesis and hence carcinogenesis. Unlike some other cytosine analogues, for example cytarabine or gemcitabine, the sugar moiety of decitabine is unmodified. Therefore, at low concentrations, decitabine does not terminate DNA chain elongation and can deplete DNMT1 without causing significant DNA damage or cytotoxicity, both in vitro and in vivo. In addition to decreased mutagenic risk, this noncytotoxic, differentiation-altering mechanism of action, the prominent side effect was an increase in the platelet count, without significant changes in the erythrocyte micronucleus and VDJ recombination assays for genotoxicity. Accordingly, this treatment approach is mechanistically distinct from antimetabolite-based therapy with hydroxyurea, the current standard agent used for HbF induction. However, conclusions from this small, pilot study are limited. Therefore, the potential of this approach requires further clinical evaluation.

Acknowledgments

This work was supported by the National Institutes of Health, National Heart, Lung, and Blood Institute (NHLBI, cooperative agreement U01 HL065238).

Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NHLBI.

Authorship

Conflict-of-interest disclosure: The authors declare no competing financial interests.

This is publication number 21 of the Thalassemia Clinical Research Network. A list of Thalassemia Clinical Research Network member institutions and staff appears in the supplemental Appendix.

Correspondence: Yogen Saunthararajah, 9500 Euclid Ave, R40, Cleveland, OH 44195; e-mail: saunthy@ecf.org.

References