Recent molecular studies of fetal hemoglobin (HbF) regulation have reinvigorated the field and shown promise for the development of clinical HbF inducers to be used in patients with β-thalassemia and sickle cell disease. However, while numerous promising inducers of HbF have been studied in the past in β-thalassemia patient populations, with limited success in some cases, no universally effective agents have been found. Here we examine the clinical studies of such inducers in an attempt to systematically review the field. We examine trials of agents, including 5-azacytidine, hydroxyurea, and short-chain fatty acids. This review highlights the heterogeneity of clinical studies done on these agents, including both the patient populations examined and the study endpoints. By examining the published studies of these agents, we hope to provide a resource that will be valuable for the design of future studies of HbF inducers in β-thalassemia patient populations. (Blood. 2013;121(12):2199-2212)

**Introduction**

Increased production of fetal hemoglobin (HbF) can ameliorate the severity of both β-thalassemia and sickle cell disease (SCD), the major disorders of β-hemoglobin. The defective production of the β-globin molecule in patients with β-thalassemia can be compensated for by an increase in the production of the β-like globin molecule, γ-globin, which pairs together with α-globin chains to form HbF. The increased γ-globin production decreases the α/β-chain imbalance that is a hallmark of β-thalassemia. As
a result, there is improvement in the ineffective erythropoiesis seen in the disease, decreased hemolysis, and increased total hemoglobin levels from the improved survival of red cells containing higher levels of HbF. The earliest clinical observations suggesting that this was the case came from patients with rare forms of β-thalassemia, particularly those with deletions that result in hereditary persistence of fetal hemoglobin, who express high levels of HbF and have a relatively benign clinical course. Additionally, infants with β-thalassemia only become symptomatic following the decrease in HbF production as the normal developmental fetal-to-adult hemoglobin switch occurs. More-recent clinical studies have substantiated the quantitative ameliorating effect of increased HbF production on the clinical course in a variety of patients with β-thalassemia.

These observations have prompted a 3-decade search for inducers of HbF that can therapeutically recapitulate what occurs in β-thalassemia patients who have naturally higher levels of HbF. These efforts started almost immediately after the cloning of the globin genes and began after the initial recognition that DNA methylation occurred at the silenced γ-globin genes. While these initial efforts have subsequently generated a large body of evidence in SCD that led to the successful use of HbF inducers, as exemplified by the widespread use of hydroxyurea, experience in patients with β-thalassemia remains much more limited.

Here we review all clinical studies evaluating HbF inducers that have been performed in β-thalassemia patients. We attempt to be simultaneously comprehensive and succinct in our analysis of the available evidence. Of note, there have been no large randomized clinical trials performed to date in this area of clinical investigation. This review serves a dual purpose. First, these studies have never been comprehensively reviewed, and therefore we have written this review to serve as a resource for those interested in examining the literature on clinical efforts to induce HbF in β-thalassemia. Second, recent basic science studies have identified extremely promising molecular regulators of HbF, including BCL11A, MYB, and KLF1. While no therapeutics currently exist to target these molecules or related pathways, it is likely that further work prompted by these molecular findings may lead to the discovery of promising candidates for clinical HbF inducers in the near future. Additionally, epigenetic modifier partners of these transcription factors, such as histone deacetylases (HDACs), already have inhibitors available in clinical use and thus may represent more-immediate targets for HbF induction trials. However, given the heterogeneous clinical studies of HbF inducers that have been performed to date, the best approaches to carry out such studies remains enigmatic. By carefully revisiting clinical studies that have been performed to date, we hope to provide a resource for all such future clinical studies in β-thalassemia patient populations.

The earliest attempts to induce fetal hemoglobin: DNA methylation inhibition

Soon after the initial cloning of the globin genes in the late 1970s, it became apparent that DNA methylation was present at the silenced γ-globin genes in adult erythroid cells, but was absent from the active γ-globin genes in fetal erythroid cells. Around this time, the mechanism of action of the DNA methylation inhibitor 5-azacytidine was being elucidated. This led DeSimone et al to examine whether 5-azacytidine could induce HbF in phlebotomized primates. The results were stunning and led Ley et al to test 5-azacytidine in a patient with severe β-thalassemia. After 7 days of therapy, γ-globin synthesis increased 7-fold, normalizing the globin chain imbalance and leading to an increase in total hemoglobin level from 80 to 108 g/L. Due to concerns and debates about the safety of this agent (cytotoxicity, mutagenicity, immuno- and myelosupression, activation of latent viruses), subsequent use was limited to severe cases for whom conventional therapy was unfeasible. Dunbar et al reported a remarkable improvement in total hemoglobin from 62 to 92 g/L in an alloimmunized β-thalassemia patient after 5 days of 5-azacytidine therapy. Similar findings of increases in total hemoglobin level of approximately 30 g/L, transfusion independence, and improvement in cardiac function were described in a report on 3 patients with end-stage β-thalassemia. Myelotoxicities requiring dose modifications were the main concern. Similar reports from patients with SCD confirmed the rapid and favorable effects on HbF production and the hematological outcomes. Of note, a 2008 study suggested that 5-azacytidine induction of HbF was not the result of global DNA demethylation or changes in differentiation kinetics, but rather may be due to a localized demethylation of the γ-promoter, although other results suggest that posttranscriptional regulation could also play an important role.

Decitabine (5-aza-2′-deoxycytidine) was also shown to demethylate and reactivate the expression of the γ-globin gene. At low concentrations, it has a favorable safety profile without causing significant DNA damage or cytotoxicity. Small studies in SCD suggest that decitabine can substantially increase HbF and total hemoglobin in the majority of patients treated. A pilot study in 2011 showed that subcutaneous decitabine given at 0.2 mg/kg 2 times per week for 12 weeks increased total hemoglobin from 78.8 to 90.4 g/L (2 patients had elevations >15 g/L), and it increased absolute fetal hemoglobin from 36.4 to 42.9 g/L in 5 patients with β-thalassemia intermedia. Favorable changes in red blood cell indices were also noted. Treatment was well tolerated, with the main adverse event being an elevation in platelet counts.

Hydroxyurea therapy in β-thalassemia: the largest body of evidence

Hydroxyurea (or hydroxycarbamide) is a cytotoxic, antimetabolic, and antineoplastic agent known for its use in the management of patients with myeloproliferative disorders and human immunodeficiency virus infection, where the drug acts as a potent inhibitor of ribonucleotide reductase, an enzyme required for DNA synthesis and repair. After being identified as a potent HbF inducer, hydroxyurea became 1 of the key therapeutic agents for the management of patients with SCD. However, the exact mechanisms by which hydroxyurea induces HbF production are not fully understood. A cytotoxic effect resulting in stress erythropoiesis, with increased HbF levels occurring as a result, is most commonly proposed. More-complex effects involving the production of nitric oxide and the soluble guanylyl cyclase and cyclic guanosine monophosphate–dependent protein kinase pathway gene have been proposed as being responsible for this activity. Hydroxyurea therapy exerts a 2- to 9-fold increase in γ-mRNA expression in β-thalassemia patients, leading to improvement in the α/non–α-chain imbalance and more-effective erythropoiesis. There is good correlation between in vitro γ-mRNA fold increase and the in vivo HbF fold increase; however, increases in HbF level did not always correlate with increases in total hemoglobin level in clinical studies, as described in subsequent sections. This may be best explained by findings from earlier studies showing increases in the
α/β but not the α/γ biosynthetic ratio in β-thalassemia patients receiving hydroxyurea.51,52 Thus, in addition to its known effects in stimulating γ-globin production, hydroxyurea may have a more general role in augmenting globin synthesis, including β-globin in some patients who maintain the capacity to express normal β-globin chains.51

Evidence from patients with SCD suggests that the benefits of hydroxyurea may not be limited to the increase in HbF levels, because improved erythrocyte morphology and deformability, a lowering of circulating leukocyte and reticuloocyte counts, a reduction in hemolysis, and a potentially local release of nitric oxide also appear to contribute to improved clinical outcomes.53 Similar studies in patients with β-thalassemia are limited. Hydroxyurea therapy is not associated with considerable or steady effects on erythrocyte deformability in β-thalassemia,54 which may explain the reduced response to the drug in some patients.55 However, in splenectomized patients with hemoglobin E/β-thalassemia, there is evidence that hydroxyurea diminishes phosphatidylserine externalization on the red cell,56 an observation previously established in patients with SCD.56 Whether this is attributed to HbF induction and an associated decrease in α-globin aggregates remains to be elucidated.54 Regardless, phosphatidylserine membrane exposure is not only associated with reduced red cell survival but also with increased thrombin generation, leading to hypercoagulability and subsequent morbidity in patients with β-thalassemia.57

Hematological outcomes

After early case reports documented hematological improvements in β-thalassemia patients treated with hydroxyurea,51,58-64 several studies evaluated the efficacy and safety of the drug in this patient population (Table 1).44,49,50,65-85 These primarily included small single-arm trials or retrospective cohort studies. Reported elevations in HbF level from baseline showed substantial variability, ranging between 1% and 90% and averaging 20%. Such increases in α/β ratios were generally higher than those reported in SCD trials,33,53 although patients with β-thalassemia recruited in the available studies have higher baseline HbF levels. An association between the degree of HbF level increase and improved hematological outcomes was noted in several studies,70,73 while others failed to document such an association.65,66,82 In fact, some reports noted a reduction in HbF level, especially at low doses,86 despite observed hematological responses,51,72 further supporting the idea that the effects of hydroxyurea in β-thalassemia patients could extend beyond HbF induction.

In the available studies, two main hematological outcomes were commonly evaluated, depending on patients’ transfusion status before hydroxyurea therapy. In transfusion-dependent phenotypes (β-thalassemia major, severe β-thalassemia intermedia, or severe hemoglobin E/β-thalassemia), patients were considered responders to therapy if they became transfusion independent. The approximate response rate ranged between 30% and 70% in β-thalassemia major patients and between 60% and 100% in β-thalassemia intermedia patients, although the latter group was less frequently transfused before hydroxyurea therapy. The response rate in patients with hemoglobin E/β-thalassemia was around 50%. The evaluation of reported elevations in total hemoglobin level in this transfusion-dependent group of patients is challenging considering the variable time of baseline measurement with respect to transfusion therapy. However, responding patients usually had higher increases in total hemoglobin level than nonresponding patients did. Partial response, usually defined as a decrease in transfusion requirements, ranged between 15% and 50% (Table 1).

Although these findings remain promising, they should be interpreted with caution, especially in patients with β-thalassemia intermedia. The confidence intervals in studies reporting the highest response rates are expected to be wide considering the small sample sizes. Moreover, interpretation of the observed benefits may be challenging, as the indications for transfusion therapy with respect to target total hemoglobin levels vary considerably between studies and centers. The documented final total hemoglobin level achieved upon transfusion independence ranged between 60 and 100 g/L. Finally, recent evidence supports a benefit of transfusion therapy for the prevention or management of several morbidities in patients with β-thalassemia intermedia,57,86 and the role of hydroxyurea as an alternate therapy should be evaluated against similar end points, particularly given that analogous transitions in patients with SCD have not consistently met with success.59 However, it is noteworthy that improvements in transfusion requirements in most β-thalassemia patients were also associated with a favorable reduction in iron overload and hemolytic indices.

In studies including transfusion-independent patients with β-thalassemia, the primary hematological outcome was improvement in total hemoglobin level. Mean increases within studies ranged approximately between 5 and 25 g/L, with an average around 15 g/L (Table 1), which is comparable to findings in patients with SCD.43,53 However, a high variance is noted in total hemoglobin response in most studies, indicating that although some patients achieve considerable elevations, others have minimal or no change. The proportion of patients having total hemoglobin increases >10 g/L ranged between 40% and 70%.50,65,76,77,85 Improvement in anemia was usually associated with better exercise tolerance, appetite, and sense of well-being. Elevations in mean corpuscular volume and hemoglobin were consistently noted along with improvements in total hemoglobin level.

Predictors of response

Responses in β-thalassemia patients were observed at hydroxyurea doses ranging between 10 and 20 mg/kg per day, with most investigators opting to use a fixed low dose (10 mg/kg per day), while others escalated the dose according to toxicity (maximal tolerated dose) up to a maximum of 20 mg/kg per day (Table 1). These doses remain lower than those used in patients with SCD, which are often in excess of 20 mg/kg per day.51,53 Whether dose increments above 20 mg/kg per day could lead to more favorable responses warrants further study; however, a 2009 report suggests that a dose increase to 30 mg/kg per day in a small group of nonresponsive patients did not provide any additional benefit.86

Experience from patients with SCD established the long-term efficacy of hydroxyurea.53,55 In β-thalassemia, most studies evaluated outcomes after 6, 12, or 24 months of therapy, although results from longer follow-up were also reported (Table 1). Response to hydroxyurea therapy was commonly noted in the first 3 to 6 months of therapy, with further improvements noted up to 12 months, and sustained responses observed over long-term follow-up.65,69,73,78-80,82,85 However, some studies noted a decline in hematological response beyond 12 months.50,76 Because of these observations, it has been theorized that long-term treatment with hydroxyurea may result in an impairment in the ability of certain hematopoietic stem cells to give rise effectively to erythroid lineage cells. Basic science studies suggest that peripheral blood hematopoietic stem and progenitor cells from β-thalassemia patients under long-term treatment with hydroxyurea lose the ability to undergo erythroid differentiation, which may lend some support to such theories.50
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Age (y)*</th>
<th>HU dose (mg/kg per d)*</th>
<th>Duration (mo)</th>
<th>Total Hb (g/L)*</th>
<th>Fetal Hb*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karimi et al&lt;sup&gt;65&lt;/sup&gt;</td>
<td>126 TM</td>
<td>22.3</td>
<td>10</td>
<td>96-156</td>
<td>Baseline: 85-100 Final: ≥90 (n = 51 [40%]) Final: ≥80 to &lt;90 (n = 44 [35%]) Final: ≥70 to &lt;80 (n = 16 [13%]) Final: &lt;70 (n = 15 [12%]) Δ: +≥20 (n = 12 [11%]) Δ: +10 to &lt;20 (n = 44 [42%]) Δ: +&lt;10 (n = 47 [44%]) Δ: no change (n = 3 [3%])</td>
<td>—</td>
<td>Ti: NTD or TD [intermittent] TM: 86 [68.3%] TD → NTD, 25 [19.8%] TD → 1 or 2 transfusions</td>
</tr>
<tr>
<td></td>
<td>106 TI</td>
<td>18.1</td>
<td>10</td>
<td>96-156</td>
<td>Baseline: 96-156 Final: $90 (n = 15 [40%]) TM: 86 [68.3%] TD: 25 [19.8%]</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Amoozgar et al&lt;sup&gt;66&lt;/sup&gt;</td>
<td>33 TI</td>
<td>20</td>
<td>10</td>
<td>12</td>
<td>Baseline: 98.4 Baseline: 79.3 g/L</td>
<td>Δ: +13.4†  Δ: +8.6 g/L‡</td>
<td>Ti: NTD or TD [intermittent]</td>
</tr>
<tr>
<td></td>
<td>51 TI</td>
<td>17</td>
<td>None</td>
<td>12</td>
<td>Baseline: 92.8</td>
<td>Δ: +4.7‡</td>
<td>—</td>
</tr>
<tr>
<td>Ansari et al&lt;sup&gt;67&lt;/sup&gt;</td>
<td>119 TM</td>
<td>2.6-25</td>
<td>16</td>
<td>24</td>
<td>Baseline: 75 Δ: +13† (responders, n = 33 [27.7%]) Baseline: 82 Δ: -141 partial responders, n = 57 (47.9%) Baseline: 79 Δ: -131 (nonresponders, n = 29 [24.4%])</td>
<td>Baseline: 11.2% Baseline: 88.4% (responders)</td>
<td>Ti: NTD Responders: TD → NTD Partial responders: &gt;50% ↓ in transfusion need Nonresponders: &lt;50% ↓ in transfusion need</td>
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<tr>
<td></td>
<td>27 TI</td>
<td>3-13</td>
<td>16</td>
<td>24</td>
<td>Baseline: 67 Δ: +11†</td>
<td>Baseline: 98.9% Δ: +1.4%</td>
<td></td>
</tr>
<tr>
<td>Italia et al&lt;sup&gt;68&lt;/sup&gt;</td>
<td>13 Hb E/β-thalassemia</td>
<td>8-34</td>
<td>15-20</td>
<td>17-23 (median 20)</td>
<td>Baseline: 61 Δ: +11† (responders, n = 6 [46.2%]) Baseline: 63 Δ: 0† (partial responders, n = 4 [30.8%]) Baseline: 76 Δ: -12† (nonresponders, n = 3 [23.1%])</td>
<td>-Baseline: 22.3% Δ: +16† (responders) F cells: +25.8†</td>
<td>Ti: NTD Responders: TD → NTD Partial responders: ↓ in transfusion need (30% to 50%) Nonresponders: no change in transfusion need</td>
</tr>
<tr>
<td>Karimi et al&lt;sup&gt;69&lt;/sup&gt;</td>
<td>143 TI</td>
<td>21</td>
<td>10.7</td>
<td>18-120 (median 68.4)</td>
<td>Baseline: 74 Δ: +23†</td>
<td>—</td>
<td>Ti: NTD or TD</td>
</tr>
<tr>
<td>Rigano et al&lt;sup&gt;50&lt;/sup&gt;</td>
<td>24 TI</td>
<td>37</td>
<td>14.6</td>
<td>12</td>
<td>Baseline: 78 Δ: +15†</td>
<td>Baseline: 46.5% Δ: +10.7%†</td>
<td>Ti: NTD 17 patients with ΔHb &gt; 10 g/L followed for &gt;12 mo (mean: 68 mo); 9 maintained response, while 8 had reduction</td>
</tr>
<tr>
<td>Ehsani et al&lt;sup&gt;70&lt;/sup&gt;</td>
<td>16 TI</td>
<td>10.7</td>
<td>20</td>
<td>6</td>
<td>Δ: +16Δ: +17 g/L</td>
<td>Ti: NTD</td>
<td></td>
</tr>
</tbody>
</table>

Hb, hemoglobin; HU, hydroxyurea; NTD, nontransfusion dependent; TD, transfusion dependent; TI, β-thalassemia intermedia; TM, β-thalassemia major.

*All values represent the central tendency (mean or median) unless otherwise indicated. For HU dose, the fixed or actual mean/median maximal tolerated dose is indicated when reported; otherwise, the dosing scheme is described.
†Statistically significant (P < .05).
‡Statistically insignificant (P > .05).
Table 1. (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Age (y)*</th>
<th>HU dose (mg/kg per d)*</th>
<th>Duration (mo)</th>
<th>Total Hb (g/L)*</th>
<th>Fetal Hb*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italia et al54</td>
<td>41 TM</td>
<td>12-28</td>
<td>15-20</td>
<td>20-24</td>
<td>-Baseline: 81</td>
<td>-Baseline: 5.4%</td>
<td>TI: TD (intermittent) or TD Responders: TD → NTD</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Δ: -51 (partial responders, n = 13 [31.7%])</td>
<td>Δ: +32%† (partial responders)</td>
<td>Partial responders: &gt;50% ↓ in transfusion need</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>F: +90.9%†</td>
<td>F: +13.8%†</td>
<td>Dose increases to 30 mg/kg per day in nonresponders had no benefit</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: -3%</td>
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<td></td>
<td></td>
<td></td>
<td>Δ: +8.2%‡ (nonresponders)</td>
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<td></td>
<td></td>
<td></td>
<td>F: +13.8%†</td>
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<tr>
<td></td>
<td>38 TI</td>
<td>5-40</td>
<td>15-20</td>
<td>20-24</td>
<td>-Baseline: 76</td>
<td>-Baseline: 51.8%</td>
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<td></td>
<td></td>
<td>Δ: +15† (responders, n = 22 [57.9%])</td>
<td>Δ: +35.4%† (responders)</td>
<td>F: +27.4%†</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>-Baseline: 79</td>
<td>F: +33.3%†</td>
<td>F: +13.8%†</td>
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<td></td>
<td></td>
<td>Δ: -24 (partial responders, n = 6 [15.8%])</td>
<td>Δ: +31.1%‡ (partial responders)</td>
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<td></td>
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<td></td>
<td>-Baseline: 63</td>
<td>F: +25.9%</td>
<td>F: +13.8%†</td>
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<td></td>
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<td></td>
<td></td>
<td>Δ: -2‡ (nonresponders, n = 10 [26.3%])</td>
<td></td>
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</tr>
<tr>
<td>Zamani et al71</td>
<td>49 TM</td>
<td>18.4</td>
<td>10</td>
<td>12</td>
<td>Baseline: 85.2</td>
<td>—</td>
<td>Mean number of transfused units ↓ from 22.8 → 6.0†</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: -0.7†</td>
<td></td>
<td>12 [24.5%] became NTD</td>
</tr>
<tr>
<td>Koren et al72</td>
<td>11 TM</td>
<td>18</td>
<td>10.9</td>
<td>5-60 (median 24)</td>
<td>Responders, n = 9 [81.8%]</td>
<td>Baseline: 83%</td>
<td>Ti: NTD or TD (intermittent) Responders: TD → NTD (maintaining an Hb of 70 g/L in TM and 60 g/L in TI)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Δ: -1% (1 y) †, -5% (2 y) †</td>
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</tr>
<tr>
<td></td>
<td>7 TI</td>
<td>18</td>
<td>10.9</td>
<td>36-96 (median 60)</td>
<td>-Baseline: 67</td>
<td>Baseline: 15%</td>
<td></td>
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<td></td>
<td></td>
<td>Δ: +2 (5 TD, responders, n = 5 [100%])</td>
<td>Δ: +13%‡ (1 y), +8% (2 y) †</td>
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<td></td>
<td></td>
<td>-Baseline: 66</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: +18 (2 NTD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mtvarelidze et al73</td>
<td>6 TM</td>
<td>9.8</td>
<td>15</td>
<td>60</td>
<td>Baseline: 94</td>
<td>Baseline: 23%</td>
<td>3 [50%] patients: TD → NTD 2 [33.3%] patients: ↓ in transfusion need after 1 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: +1 (12 mo), +3 (60 mo)</td>
<td>Δ: +8.3% (12 mo), +20.4 (60 mo)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Baseline: 23%</td>
<td>Baseline: 23%</td>
<td></td>
</tr>
<tr>
<td>Ansari et al74</td>
<td>23 TM</td>
<td>8.7</td>
<td>16</td>
<td>24</td>
<td>Baseline: 86</td>
<td>—</td>
<td>42.8% ↓ in blood volume transfused</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: -14</td>
<td></td>
<td>68.7% ↓ in transfusion interval</td>
</tr>
<tr>
<td>Bradai et al75</td>
<td>45 TM</td>
<td>10</td>
<td>17.4</td>
<td>12</td>
<td>Δ: +15 (good responders, n = 20 [44.4%])</td>
<td>—</td>
<td>Ti: TD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: +7 (partial responders, n = 9 [20%])</td>
<td></td>
<td>Good responders: &gt;70% ↓ in transfusion need</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Δ: +2 (nonresponders, n = 16 [35.6%])</td>
<td></td>
<td>Partial responders: 40% to 70% ↓ in transfusion need</td>
</tr>
<tr>
<td></td>
<td>9 TI</td>
<td>12.5</td>
<td>17</td>
<td>12</td>
<td>Δ: +37 (8 good responders [88.9%], 1 nonresponder [11.1%])</td>
<td>—</td>
<td>Nonresponders: &lt;40% ↓ in transfusion need</td>
</tr>
</tbody>
</table>

Hb, hemoglobin; HU, hydroxyurea; NTD, nontransfusion dependent; TD, transfusion dependent; TI, β-thalassemia intermedia; TM, β-thalassemia major.

*All values represent the central tendency (mean or median) unless otherwise indicated. For HU dose, the fixed or actual mean/median maximal tolerated dose is indicated when reported; otherwise, the dosing scheme is described.
†Statistically significant (P < .05).
‡Statistically insignificant (P > .05).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Age (y)*</th>
<th>HU dose (mg/kg per d)*</th>
<th>Duration (mo)</th>
<th>Total Hb (g/L)*</th>
<th>Fetal Hb*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mancuso et al&lt;sup&gt;76&lt;/sup&gt;</td>
<td>18 Ti</td>
<td>37</td>
<td>14.6</td>
<td>12</td>
<td>Δ: +15†</td>
<td>—</td>
<td>Ti: NTD 11 patients with ΔHb &gt; 10 g/L were followed for &gt;12 mo (mean: 66 mo); 7 maintained response, while 4 had reduction</td>
</tr>
<tr>
<td>Taher and Sheikh-Taha&lt;sup&gt;84&lt;/sup&gt;</td>
<td>7 Ti</td>
<td>24</td>
<td>10-20 (median 12)</td>
<td>Baseline: 70</td>
<td>Δ: +5</td>
<td>—</td>
<td>Ti: NTD</td>
</tr>
<tr>
<td>Singer et al&lt;sup&gt;85&lt;/sup&gt;</td>
<td>45 Hb E/β-thalassemia</td>
<td>13</td>
<td>18-20</td>
<td>24</td>
<td>Baseline: 68</td>
<td>Δ: +6</td>
<td>Baseline: 29.8% Patients weaned off transfusions before study</td>
</tr>
<tr>
<td>Dixit et al&lt;sup&gt;77&lt;/sup&gt;</td>
<td>37 TM + Ti</td>
<td>10</td>
<td>10-&gt;20</td>
<td>6</td>
<td>-Baseline: 65</td>
<td>-Baseline: 67%</td>
<td>Δ: +16† (major responders, n = 17 [45.9%]) Δ: +9%† (major responders) Δ: +8.5%† (minor responders) Δ: +1%† (nonresponders) Minor responders: TD → NTD (Hb &gt; 80 g/L), or Hb ↑ &gt; 20 g/L ↓ in transfusion need, or Hb ↑ 10 to 20 g/L Nonresponders: &lt;50% ↓ in transfusion need, or Hb ↑ &lt;10 g/L</td>
</tr>
<tr>
<td>Karimi et al&lt;sup&gt;78&lt;/sup&gt;</td>
<td>163 Ti</td>
<td>13.5</td>
<td>10</td>
<td>72</td>
<td>Baseline: 86.8</td>
<td>Δ: +9.6† (group 2 + 3, 12 mo)</td>
<td>Nonsignificant changes</td>
</tr>
<tr>
<td>Aleybouyeh et al&lt;sup&gt;79&lt;/sup&gt;</td>
<td>36 TM</td>
<td>16.3</td>
<td>20</td>
<td>&gt;6</td>
<td>Baseline: 100</td>
<td>Δ: +7</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>9 Ti</td>
<td>14.7</td>
<td>20</td>
<td>34-58 (median 40)</td>
<td>Baseline: 93</td>
<td>Δ: +11</td>
<td>—</td>
</tr>
<tr>
<td>Yavarian et al&lt;sup&gt;80&lt;/sup&gt;</td>
<td>133 TM</td>
<td>17.1</td>
<td>10-15 (median 42)</td>
<td>24-60</td>
<td>Good responders: n = 81 [60.9%] Moderate responders: n = 31 [23.3%]</td>
<td>—</td>
<td>Good responders: TD → NTD (Hb &gt; 95 g/L) Moderate responders: transfusion interval &gt;6 mo (Hb 75 to 96 g/L) Nonresponders: TD after 12 mo</td>
</tr>
<tr>
<td>Bradai et al&lt;sup&gt;81&lt;/sup&gt;</td>
<td>7 TM + Ti</td>
<td>12</td>
<td>18.4</td>
<td>13-21 (median 19)</td>
<td>-Baseline: 45</td>
<td>Baseline: 90.9%</td>
<td>Δ: +6.8%</td>
</tr>
</tbody>
</table>

Hb, hemoglobin; HU, hydroxyurea; NTD, nontransfusion dependent; TD, transfusion dependent; Ti, β-thalassemia intermedia; TM, β-thalassemia major.
*All values represent the central tendency (mean or median) unless otherwise indicated. For HU dose, the fixed or actual mean/median maximal tolerated dose is indicated when reported; otherwise, the dosing scheme is described.
†Statistically significant (P < .05).
‡Statistically insignificant (P > .05).
Alongside dose and duration of therapy, several other factors were assessed for their association with hematological response in patients with β-thalassemia. Findings regarding the roles of age and HbF level at the start of treatment are conflicting. Moreover, although some studies found certain β-globin genotypes to be predictors of a favorable response, others failed to establish such an association. Similar discrepancies are noted for β-globin haplotypes. Patients with Lepore or δβ-thalassaemia genotypes usually showed a better response.

Of note, several reports confirm that the effects of hydroxyurea in patients with hemoglobin S/β-thalassemia may be better than those even reported for homozygous hemoglobin S disease, because the synthesized γ-chains not only inhibit the sickling process, but they also neutralize the noxious effects of the excess α-chains and cut down on ineffective erythropoiesis. Co-inheritance of α-thalassemia was described as a predictor of good response in some studies, but it was found to have no effect in others. Homozygosity for the Xmn1 polymorphism (−158C>T) was a strong predictor of favorable responses, although the case was different in some studies, especially those including patients with hemoglobin E/β-thalassemia. A 2012 study also showed that the rs766432 polymorphism at intron 2 of the BCL11A gene correlates strongly with a response to hydroxyurea therapy; further studies in this direction are encouraged. However, it is important to bear in mind that when genetic markers are studied, the markers themselves may not be causally linked to such effects, but rather variants in linkage disequilibrium with these markers may be the mediators of such effects.

There is some basic evidence that response to hydroxyurea may be affected by the degree of iron overload. However, clinical studies evaluating such an association are lacking.

### Other clinical outcomes

Hydroxyurea therapy was also found to decrease the frequency of certain morbidities in patients with β-thalassemia. A beneficial role in patients with pulmonary hypertension was suggested, especially upon combination with the antioxidant t-carnitine, although these findings primarily relied on echocardiographic findings. In smaller studies, hydroxyurea therapy was also associated with improvements in endocrine function, leg ulcers, and extra- medullary hemtopoietic tumors. These findings are further confirmed through a 2010 cross-sectional study of 584 β-thalassemia intermedia patients from the Middle East and Italy, where hydroxyurea therapy was associated with reduced adjusted odds of extra- medullary hemtopoietic tumors (0.52, 95% CI, 0.30-0.91), pulmonary hypertension (0.42, 95% CI, 0.20-0.90), leg ulcers (0.10, 95% CI, 0.02-0.43), hypothyroidism (0.05, 95% CI, 0.01-0.45), and osteoporosis (0.02, 95% CI, 0.01-0.09). These effects were independent of total hemoglobin level or transfusion status, which further suggests that the benefit from hydroxyurea could extend beyond HbF induction and subsequent improvement of anemia. It should be noted that hydroxyurea’s effects, especially on phosphatidylserine exposure and hypercoagulability, are most notable in splenectomized β-thalassemia patients, which represent the subgroup of patients with a considerably high risk for morbidity from thrombotic events.

### Safety of hydroxyurea in β-thalassemia patients

Hydroxyurea therapy was generally well tolerated at the doses used in β-thalassemia studies, with some studies reporting no adverse events at all, even with long-term therapy. The rate of...
myelotoxicity ranged between 2% and 30%.\textsuperscript{\textit{44,49,71,74,75,77,82,85} while some studies did not report any hematological toxicities.\textsuperscript{\textit{65,69,70,72}} Myelotoxicity was usually dose dependent, especially when doses >20 mg/kg per day were used, and it could be reversed upon dose reduction. The bone marrow of β-thalassemia patients may be more sensitive to myelosuppression by hydroxyurea than occurs in other disorders, possibly due to mediulary inflammation.\textsuperscript{\textit{86,103}} There is only 1 report of leukemic transformation in a β-thalassemia intermedia patient following 3 years of hydroxyurea therapy at 19 mg/kg per day.\textsuperscript{\textit{75}} The rate of gastrointestinal adverse events ranged between 1% and 30%.\textsuperscript{\textit{20,65,67,69,71,74,77,81}} Some studies also reported dermatological (hyperpigmentation, alopecia, maculopapular rash, or facial erythema)\textsuperscript{\textit{65,69}} and neurological (headache or dizziness)\textsuperscript{\textit{65,69}} adverse events on long-term therapy, although others did not observe such symptoms or attributed them to other disease-related risk factors.\textsuperscript{\textit{67,74,104}} No renal or hepatic side effects were reported with hydroxyurea therapy.\textsuperscript{\textit{67,69,71,74,75,82,85}} Although some reports suggested that hydroxyurea may adversely affect gonadal function,\textsuperscript{\textit{87}} others failed to document such an association even on long-term therapy.\textsuperscript{\textit{105}} Interestingly, 2 patients became pregnant while on hydroxyurea and delivered normally, without any congenital malformations in the infants.\textsuperscript{\textit{72}}

**Short-chain fatty acid induction of fetal hemoglobin**

In the middle of the 1980s, as the initial clinical trials of 5-azacytidine and hydroxyurea were being reported, Perrine et al.\textsuperscript{\textit{106}} as well as Bard et al.\textsuperscript{\textit{107}} reported that the infants of diabetic mothers have a delayed fetal-to-adult hemoglobin switch. While the exact underlying mechanisms were not clear, hypotheses were put forth about potential mechanisms. Because it was known that hydroxyurea is elevated in mothers with diabetes, Perrine and colleagues\textsuperscript{\textit{106}} tested the idea that butyrate or other similar short-chain fatty acids may be effective as inducers of fetal hemoglobin in sheep. An initial trial involving a 2- to 3-week infusion of arginine butyrate (at a dose of 500 mg/kg per day) in 3 SCD and 3 β-thalassemia patients (2 who were transfusion dependent) showed promise. Nearly all the patients in the trial showed an increase (2- to 6-fold) in γ-mRNA levels and in the synthesis of this globin chain, leading to improvement in the globin chain ratios.\textsuperscript{\textit{109,110}} The 2 transfusion-dependent patients showed lower levels of plasma-free hemoglobin, which is an indicator of ineffective erythropoiesis. In addition, 1 of these patients showed a steady rise in total hemoglobin level from 45 to >100 g/L over the course of 7 weeks of therapy. No other markers of disease progression were measured, and no further assessment of ineffective erythropoiesis was done. A follow-up trial extended such therapy to 9 to 13 weeks, with doses of arginine butyrate escalating from 500 to 2000 mg/kg per day for 6 days per week, and it studied the responses in 5 SCD and 5 β-thalassemia patients.\textsuperscript{\textit{111}} This trial had primary end points involving hematologic responses, as defined by an increase in total hemoglobin concentration of 20 g/L in patients with β-thalassemia. The 5 β-thalassemia patients had variable transfusion requirements, with some being transfusion independent (2 patients) and others requiring intermittent (2 patients) or regular transfusions (1 patient). Unfortunately, the primary hematologic end points were not achieved in this longer-term trial. It has been suggested that the loss of response to butyrate over long-term therapy may be a result of the antiproliferative effects on the bone marrow.\textsuperscript{\textit{112}} This hypothesis is supported by data confirming that intermittent or pulse-butyrate therapy is associated with well-tolerated, marked, and sustained response in patients with SCD.\textsuperscript{\textit{112}} A separate cohort study of oral sodium phenylbutyrate therapy at a dose of 20 g per day over the course of 41 to 460 days showed that 4 of 11 β-thalassemia patients had increased levels of total hemoglobin >10 g/L (mean increase: 21 g/L), along with an increased production of Hbf.\textsuperscript{\textit{113}} All these responders had higher average levels of erythropoietin at baseline (all >120 mU/mL) and were transfusion independent. Changes in the percent of Hbf, absolute Hbf levels, or α/γ-α-globin ratios did not correlate with response to treatment, nor did the β-genotype. In addition, the reduction of markers of ineffective erythropoiesis and hemolysis, including lactate dehydrogenase and indirect bilirubin, was also noted in the patients who showed responses. Two cohort studies have examined the efficacy of the oral butyrate derivative isobutyramide to induce Hbf. The first study was an open-label, phase 2 trial on a group of 12 patients with transfusion-independent β-thalassemia intermedia (mean age: 31 years) for a period of 28 days.\textsuperscript{\textit{114}} There was some increase in the percent of Hbf following treatment with 150 mg/kg per day of isobutyramide (P = .06, with wide variability among patients). No change in globin chain imbalance or in markers of ineffective erythropoiesis was noted in this study. Another study examined 8 patients with transfusion-dependent β-thalassemia and involved treatment of these patients with 350 mg/kg per day of oral isobutyramide for 126 to 384 days.\textsuperscript{\textit{115}} Hbf increased from 3% to 6%, while a drop in plasma-free hemoglobin was noted. Two of the 8 patients showed a decreased frequency of transfusion requirements in the setting of receiving this treatment, and a reduction in iron burden was also noted in some patients. Response to treatment was associated with high pretreatment Hbf (>4.5%), high parental Hbf, and increased erythropoietin levels. Treatment with short-chain fatty acids was generally tolerable in published trials, with side effects being minimal or limited to gastrointestinal disturbances. It has been suggested that the relatively poor response to butyrate therapy in patients with β-thalassemia compared with patients with SCD may be attributed to the effects of these agents on other globin genes. It was shown that butyrate exposure increases α-globin expression in progenitor-derived erythroid cells from patients with β-thalassemia, while it decreases α-globin mRNA levels in patients with SCD. Thus, the favorable effects of the butyrate-induced increase in γ-globin expression on α/β-chain imbalance in β-thalassemia may be reduced as a result of the associated increase in α-globin expression.\textsuperscript{\textit{116}}

Trials of other derivatives of these agents, such as 2,2-dimethylbutyrate, were reported in 2012 in patients with SCD, and data from patients with β-thalassemia are awaited.\textsuperscript{\textit{117}} These short-chain fatty acids are thought to work as inhibitors of HDACs, and specific inhibitors of these molecules have been produced, which may also be promising for future trials aimed at inducing Hbf.\textsuperscript{\textit{10,16}}

**Combination therapy**

The use of recombinant human erythropoietin or the newer erythropoietic-stimulating agent darbepoetin alfa in patients with β-thalassemia is associated with increases in total hemoglobin level.\textsuperscript{\textit{118-123}} A correlation between serum erythropoietin and Hbf levels also exists.\textsuperscript{\textit{124}} Earlier studies from primates and from patients with SCD suggested that erythropoietin augments the Hbf
response attributed to hydroxyurea therapy.\textsuperscript{125-127} More-prominent effects were also noted when hydroxyurea was combined with sodium phenylbutyrate.\textsuperscript{126} Consequently, several trials evaluated the value of combinations of these 3 agents for the management of \(\beta\)-thalassemia patients. Loukopoulos et al\textsuperscript{128} demonstrated that the combination of hydroxyurea and erythropoietin (50 000 U 3 times a week) is associated with higher increments in total hemoglobin level than hydroxyurea alone (17 vs 2 g/L after 6 months of therapy) in patients with \(\beta\)-thalassemia intermedia. Lower erythropoietin doses (10 000 U) did not achieve such effects. Long-term transfusion independence in a \(\beta\)-thalassemia major patient was also reported with this combination.\textsuperscript{129} In the late 1990s, Olivieri et al\textsuperscript{130} reported 2 \(\beta\)-thalassemia major siblings (homozygous for \(g\)-globin Lepore) who showed remarkable total hemoglobin responses and transfusion independence from HbF induction with the combination of sodium phenylbutyrate and hydroxyurea. However, further observations in this direction were not always promising. In 2 patients with \(\beta\)-thalassemia intermedia, the addition of sodium phenylbutyrate to hydroxyurea treatment failed to produce an increase in total hemoglobin, despite increasing HbF levels.\textsuperscript{64} Similarly, in another study on 45 patients with hemoglobin E/\(\beta\)-thalassemia treated with hydroxyurea, the addition of sodium phenylbutyrate had no benefit, while the addition of erythropoietin did benefit selected patients.\textsuperscript{65} In addition, there may exist some bias in the literature toward the reporting of positive results.

### Other approaches

Thalidomide, a drug known for its immunomodulating and anti-angiogenic properties, has been suggested to induce \(\gamma\)-globin gene expression and to increase the proliferation of erythroid cells using in vitro culture models.\textsuperscript{130} Two case reports reported that thalidomide therapy at 75 to 100 mg/kg per day caused a progressive and rapid increase in total hemoglobin and HbF levels in \(\beta\)-thalassemia major patients.\textsuperscript{131,132} Promising roles of thalidomide derivatives (pomalidomide and lenalidomide) for the induction of HbF also were reported in 2008 and 2011 from in vitro and animal studies.\textsuperscript{133,134} These findings support the evaluation of such agents as a potential new therapy that may not have the cytotoxic side effects associated with other HbF inducers.

### Concluding remarks: lessons from the past and hope for the future

For HbF induction therapy to become part of the standard management for patients with \(\beta\)-thalassemia, there needs to be a great deal of work from both basic scientists and clinical researchers. Ideally, efforts to understand the exact mechanisms through which current and future agents exert their effects should go in parallel with the establishment of large randomized clinical trials and formal clinical development programs evaluating the efficacy and safety of these agents.

Several lessons can be learned from the pitfalls and successes of the available studies (Table 2), which should help the design of future trials in \(\beta\)-thalassemia cohorts. We herein provide some recommendations.

### Study population

One of the main limitations in previous studies is the inclusion of a heterogeneous group of patients, especially with regard to transfusion dependence, which precludes the interpretation of study outcomes. Although there exists a variety of genotypes that can lead to a \(\beta\)-thalassemia syndrome, the distinction of various phenotypes is primarily attained through clinical parameters.\textsuperscript{1,135} As the hallmark of disease in these syndromes is ineffective erythropoiesis and subsequent anemia, transfusion dependence has classically been an essential factor in characterizing the various phenotypes and their severity. It should be noted, however, that commitment to transfusion therapy may be the consequence of

### Table 2. Summary of findings and limitations of fetal hemoglobin inducer studies in patients with \(\beta\)-thalassemia

<table>
<thead>
<tr>
<th>Agent</th>
<th>Main positive findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decitabine</td>
<td>Hematological responses achieved.</td>
<td>Few studies. Small sample sizes.</td>
</tr>
<tr>
<td></td>
<td>Favorable effects on red cell indices noted.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment was well tolerated.</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>Hematological responses achieved.</td>
<td>Heterogenous phenotypes studied together.</td>
</tr>
<tr>
<td></td>
<td>Favorable effects on red cell, hemolysis, and hypercoagulability indices noted.</td>
<td>Heterogeneous study end points evaluated together.</td>
</tr>
<tr>
<td></td>
<td>Favorable effects on clinical morbidities noted.</td>
<td>Ideal dose and duration of therapy still controversial.</td>
</tr>
<tr>
<td></td>
<td>Treatment was well tolerated.</td>
<td>Lack of efficacy on long-term therapy.</td>
</tr>
<tr>
<td>Short-chain fatty acids</td>
<td>Hematological responses achieved.</td>
<td>Small sample sizes.</td>
</tr>
<tr>
<td></td>
<td>Favorable effects on red cell and hemolysis indices noted.</td>
<td>Lack of efficacy on long-term therapy.</td>
</tr>
<tr>
<td></td>
<td>Treatment was well tolerated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Favorable effects on combination with hydroxyurea noted.</td>
<td>High doses required.</td>
</tr>
<tr>
<td></td>
<td>Treatment was well tolerated.</td>
<td>No additive effects with short-chain fatty acids.</td>
</tr>
<tr>
<td></td>
<td>Treatment was well tolerated.</td>
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</tbody>
</table>
physician, patient, or family preferences rather than a reflection of disease severity. For the purpose of selecting a patient cohort for inclusion in an HbF inducer trial, patients should essentially be divided into 2 distinct groups: (1) transfusion-dependent patients, and these include phenotypes where patients require regular-transfusion therapy for survival, such as β-thalassemia major or severe hemoglobin E/β-thalassemia, and (2) nontransfusion-dependent thalassemia (NTDT) patients, and these include patients not requiring regular-transfusion therapy for survival, such as β-thalassemia intermedia or mild/moderate hemoglobin E/β-thalassemia. Study end points for the 2 distinct groups may be dissimilar, as outlined below. One challenge, however, is that some patients with NTDT may still require occasional blood transfusions during infection or pregnancy or before surgery. They may also require more regular, yet temporary, transfusions in the case of poor growth or development during childhood or in the management of specific complications in adulthood where the benefit of transfusion therapy has been established. For this latter group, we recommend that patients should be off transfusions for at least 6 months before inclusion in an HbF inducer trial.

Study design

The ideal design would be that of a randomized, placebo-controlled trial, although data from well-conducted, single-arm, phase 2 studies would also be valuable. Once the benefit is established in an evidence-based manner, agents may be evaluated in comparative trials against each other or against other conventional therapies. HbF-inducing agents may also be trialed in combination, either together or with conventional therapies. Although, as outlined in “Primary study end points,” a reduction in the regular-transfusion requirement is a study end point in transfusion-dependent patients, the effect of a short-term treatment strategy that includes an HbF-inducing agent alongside transfusion therapy may still be worth evaluating for the management of certain morbidities in NTDT patients.

Primary study end points

Another challenge in the available studies is the definition of end points and response criteria, which does not allow for a systematic comparison of results. An essential question to answer is what response is expected from an HbF inducer in a β-thalassemia patient. In untreated patients with β-thalassemia, ineffective erythropoiesis and premature red cell death are the hallmarks of disease leading to chronic anemia and hypoxia, increased intestinal iron absorption, intra- and extravascular hemolysis, and a hypercoagulable state. These mechanisms collectively lead to a variety of clinical morbidities involving almost every organ system. The ineffective erythropoiesis in patients with β-thalassemia is estimated to be 10 to 20 times the normal basal erythropoietic level. Thus, the main objective of intervention would be to ensure an effective supply of normal erythrocytes and partially suppress ineffective erythropoiesis and subsequent pathophysiologic mechanisms that lead to clinical morbidity. Erythroid activity decreases to 1 to 2 times normal levels with total hemoglobin values between 100 and 110 g/L, 1 to 4 times normal levels with values between 90 and 100 g/L, and 2 to 6 times normal levels with values between 86 and 90 g/L. Achieving a total hemoglobin level >90 g/L has been associated with significant reduction in morbidity in β-thalassemia patients, although lower levels (>70 g/L) may be equally beneficial in children with hemoglobin E/β-thalassemia who have a remarkable facility for adaptation to low hemoglobin levels. Moreover, total hemoglobin elevations of 10 to 20 g/L have been associated with improvement in disease severity in some studies.

In light of these observations, in patients with NTDT, response to HbF induction therapy may be defined as follows: Excellent Response, achieving an elevation in total hemoglobin level of 10 to 20 g/L and reaching a final total hemoglobin level >90 g/L; Good Response, achieving an elevation in total hemoglobin level of 10 to 20 g/L or reaching a final total hemoglobin level >90 g/L; Poor Response, achieving an elevation in total hemoglobin level <10 g/L, and No Response, no elevation in total hemoglobin level. For transfusion-dependent patients the following definitions may be used: Excellent Response, transfusion independence and reaching a pretransfusion total hemoglobin level >90 g/L; Good Response, ≥50% reduction in pretreatment transfusion requirement and reaching a pretransfusion total hemoglobin level >90 g/L; Poor Response, <50% reduction in pretreatment transfusion requirement and reaching a pretransfusion total hemoglobin level >90 g/L; and No Response, no change in transfusion requirement to reach a pretransfusion total hemoglobin level >90 g/L.

Elevations in HbF level should not be used as a primary study end point. As described earlier in this review, several studies reported discordance between changes in HbF and total hemoglobin levels. This may be a particular problem given that HbF inducers may exert their effects through pathways other than γ-chain expression.

Secondary study end points

Alongside changes in HbF level and safety measures, it would be worthwhile evaluating alterations in indices of pathophysiologic mechanisms (eg, globin chain ratios, hemolysis, iron overload, hypercoagulability), which could shed more light on the specific mechanisms of the action of Hbf-inducing agents. Moreover, the evaluation of the effects of Hbf inducers on the incidence of clinical morbidities (eg, pulmonary hypertension, leg ulcers, extramedullary hematopoietic pseudotumors) remains an area of extreme importance.

Dose and duration of therapy

Previous studies have mainly used 2 dosing strategies. For example, in the hydroxyurea studies reviewed in this review, most used a fixed starting dose <20 mg/kg per day, while some studies escalated the dose to the maximal tolerated dose. We recommend the latter approach for the following reasons: (1) There is a lack of pharmacokinetic or dynamic studies to identify the optimal dose in β-thalassemia patients. (2) Such studies were not always helpful in dosing hydroxyurea for patients with SCD. (3) Data on the efficacy or safety of high dosing in β-thalassemia patients is limited to single case reports or small case series. The duration of therapy should rely on the evaluated drug and the anticipated outcomes (end points). Total hemoglobin levels should generally be evaluated after 6 and 12 months of therapy. In light of the few reports, reviewed herein, that showed diminished response after 12 months of therapy, it may be necessary to reevaluate long-term response through cohort studies. This is also especially relevant in patients with β-thalassemia who show worsening of anemia and disease severity as they advance in age, suggesting that alterations in the dosing or course of management may be necessary. Secondary study end points can be evaluated pre- and posttherapy and, potentially, early on for markers of hemolysis and ineffective erythropoiesis. When the incidence of clinical morbidities is a study end point, it may be difficult to evaluate outcomes in short-term studies, except in cases when the trial is designed for the management of a specific morbidity.
Predictors of response

There is great controversy from the available studies on what factors predict good response to therapy. Conducting studies with large samples will allow for association analysis, and the role of the following factors should be evaluated: age, splenectomy status, β-globin genotype, α-globin genotype, molecular determinants of increased HbF production, and baseline hematologic profile (eg, total hemoglobin level, HbF level). Although through randomization the confounding effects of such risk factors are diminished, assessment through stratification remains essential.

Conclusions

Optimal design of future studies should assist with the improved allocation of therapy to those patients who demonstrate clear evidence of benefit from treatment. While the efforts to induce HbF in β-thalassemia patients by using the available agents have been ongoing for over 3 decades, recent molecular studies suggest that there is great promise that more-effective targeted therapies to induce HbF can be developed. For clinical investigators interested in testing these potential therapies, many challenges lay ahead. We hope that the lessons from prior trials in this field can serve as a guide for these future efforts.

Acknowledgment

This work was not supported by a funding source.

Authorship

Contribution: Conception and design: K.M.M. and V.G.S. Literature review and interpretation: K.M.M., A.T.T., M.D.C., and V.G.S. Manuscript drafting: K.M.M. and V.G.S. Manuscript review for important intellectual content: K.M.M., A.T.T., M.D.C., and V.G.S. All authors gave final approval of the manuscript for submission.

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

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Clinical experience with fetal hemoglobin induction therapy in patients with β-thalassemia

Khaled M. Musallam, Ali T. Taher, Maria Domenica Cappellini and Vijay G. Sankaran