Novel therapeutic agents for HbF induction: a new era for treatment of β thalassemia?

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Abstract

Fetal globin is endogenous, normally integrated in hematopoietic stem cells in all humans, and available for reactivation. Inducing expression of fetal globin (γ-globin) gene expression to 60-70% of α globin synthesis produces β-thalassemia trait globin synthetic ratios, and has been shown to reduce anemia to mild levels which do not require regular blood transfusion. Several classes of therapeutics have induced γ-globin expression in β thalassemia patients, raised total hemoglobin levels, and even eliminated transfusion requirements in formerly transfusion-dependent patients, demonstrating proof-of-concept of the approach. However, prior generations of therapeutics were not readily feasible for widespread use. Currently, several recently discovered oral therapeutic candidates are more potent and/ or patient-friendly, requiring low oral doses, have distinct molecular mechanisms of action, and can be used in combination regimens. Tailoring therapeutic regimens to patient subsets stratified for solely β^+ or a β^0 globin mutation, and for quantitative trait loci (QTL) which modulate HbF and clinical severity, can guide more effective and informative clinical trials. These advancements provide methods for a rational approach to applying fetal globin gene induction in therapeutic regimens suitable for use in diverse thalassemia patient populations world-wide.

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Introduction

β-thalassemia syndromes are common monogenic disorders world-wide, characterized by molecular mutations of the β-globin chain of adult hemoglobin (HbA; $\alpha_2\beta_2$), which cause deficiency of β-globin chains and an excess of unmatched α-globin chains.[1-8] The excess α-globin damages the red blood cell membrane and causes apoptosis of developing erythroblasts and intramedullary hemolysis.[1-8] Clinical observations, and previous clinical trials of fetal globin inducers, have clearly shown that patients with β-thalassemia benefit from natural persistence of, or pharmacologic induction of another type of globin which is normally suppressed in infancy, fetal or γ-globin.[1-20] Many patients with higher γ-globin levels than their counterparts with the same mutations often do not require regular transfusions on a regular basis, or as early in life as patients, with lower levels of γ-globin; inheritance of a single modifying trait which increases HbF, such as BCL-11A, without any other genetic difference, can produce a higher total hemoglobin of as much as 1 gram/dl.[51] Inducing γ-globin expression by even small increments is therefore recognized as a powerful therapeutic avenue that should be most amenable to applying world-wide, as the γ globin genes are universally present and normally integrated in hematopoietic stem cells.[1-2] While only a chemotherapeutic drug, hydroxyurea, has been commercially available and has had variable effects in the thalassemias, several important principles for application have been defined in trials of prior generations of therapeutic candidates, and the recent discovery of new therapeutic candidates now offers a renaissance for this approach.

Lessons from prior trials

Proof-of-principle was demonstrated in several previous clinical trials in which pharmacologic reactivation of γ-globin expression reduced anemia and even eliminated transfusion requirements in patients with β thalassemia. Fetal globin re-induction has been accomplished with chemotherapeutic agents, particularly 5-azacytidine, and 5-aza-2-deoxy- cytidine (decitabine),[14-20] and with short-chain fatty acids (SCFAs), such as arginine butyrate (AB) and sodium phenylbutyrate.[2, 9-10, 12-13, 37] A therapeutic which is not cytotoxic is preferable for a long-term therapy in β thalassemia, as with cumulative dosing with hydroxyurea, total hemoglobin (Hgb) levels increase, usually < 1 gm/dl, but also tend to decline over time.[16-17]

The first generation SCFAs had limitations of rapid metabolism and high dose requirements; arginine butyrate and phenylbutyrate are also global HDAC inhibitors, which inhibit erythropoiesis.[2] Erythropoiesis stimulating agents have been somewhat beneficial, but require parenteral administration and are too costly for life-long therapy.[19, 21-24] Nevertheless, these 3 classes of therapeutics reduced anemia and rendered some thalassemia patients transfusion- independent.

Therapeutics which require lower doses and oral administration, would allow broader application, particularly where thalassemia is common globally and transfusions carry particularly high risks of infections.[2]

Several observations in the earlier trials were highly informative regarding magnitude of responses, and patterns of response, in patients with differing β thalassemia mutations. Several γ-globin inducers, 5-azacytidine, phenylbutyrate, arginine butyrate, and EPO preparations, produced significant hematologic responses with rises in total hemoglobin of 2-5 g/dL or more above baseline, and although the clinical trials have been small, patients with diverse thalassemia syndromes had significant responses, including transfusion- independence.[2, 10-19] Collins and colleagues found that sodium phenylbutyrate could increase total Hgb by 2 grams/dl above baseline, and that responses occurred more frequently in patients with EPO levels > 160 mU/mL.[9] Increases in total Hgb levels of 1-5 g/dL above baseline were achieved when these agents were administered for at least 3-6 months.[9-10, 38] This is remarkable, as thalassemic cells survive for only a few days,[4-8] compared to the normal red cell survival of 120 days.

Of the chemotherapeutic agents, hydroxyurea (HU) treatment has increased total Hgb by 0.6-1.0 g/dL in HbE/β-thalassemia patients, and although not as great an effect in magnitude, is still significant in reducing hemolysis.[2, 16-17] Hajjar and Pearson reported that γ-globin increased rapidly with HU treatment, with a 6-week treatment time-frame required for a peak response, but was followed by a decline in total Hgb, suggesting cellular growth inhibition.[15] 5-azacytidine has increased total Hgb levels by an average of 2.5 g/dL (range 1-4 g/dL), even in end-stage patients with life-threatening severe anemia.[1-2, 18-19] Of the SCFAs and histone deacetylase (HDAC) inhibitors, arginine butyrate (AB), administered first frequently, 4-5 days/week, and then intermittently, twice per month, increased total Hgb levels by 1-5 g/dL (mean 2.9 g/dL) when administered for three to six months.[10] AB treatment rendered patients transfusion-independent for several years with home therapy, given 4 nights every other week avoid the anti-proliferative effects common to HDAC inhibitors. AB has been safe in long-term use, with no butyrate-related adverse events in more than 16 patient- years of home administration provided by parents. A representative profile of rises in total hemoglobin levels in a formerly transfusion-dependent patient is shown below. Isobutyramide also increased fetal globin within 28 days of treatment and reduced transfusion requirements.[12-13]

EPO preparations increased Hgb levels by 1-3 g/dL above baseline in thalassemia intermedia patients, and decreased transfusion requirements in thalassemia major.[45] However, HbF did not increase with EPO or darbopoietin, so that only thalassemic red blood cell production increased, rather than red cells *corrected* for globin chain imbalance as occurs with the HbF inducers. These trials all demonstrated proof-of-principle of the utility of therapeutic induction of γ-globin +/- enhancement of erythropoiesis in β-thalassemia patients.

Novel HbF inducers with differing mechanisms of action

Combinations of therapeutic agents are often necessary for controlling most medical conditions. γ-globin inducers with higher potency than the original therapies have been discovered recently in screening programs employing high throughput screening (HTS) or molecular modeling, and drug candidates with complimentary mechanisms of action can now be applied.[40-41, 54] Cytotoxic agents are not suitable for *simultaneously* dosed combinations, as this would likely result in greater degrees of erythroid cell apoptosis, but such agents can be used sequentially. A precedent for combination therapy with butyrate and 5-azacytidine was first noted in laboratory models by Stamatoyannopoulos; the two drugs produced a synergistic, 3- fold increase in γ-globin expression, above the significant levels induced by each drug alone.[2]

We have found higher responses, with both additive and synergistic effects, with combinations of therapeutics in erythroid cell culture studies and in animal models with hydroxyurea, decitabine, or an oral HDAC inhibitor, MS-275, plus a SCFAD which is *not* a global HDAC inhibitor, sodium 2,2 dimethylbutyrate (SDMB).[40, 45] In clinical trials of AB and EPO, β^+- thalassemia patients tended to have relatively lower HbF levels (<30%) and lower baseline EPO levels (<130 mU/mL) than patients with at least one β^0 thalassemia mutation.[45] The β^+ thalassemia group responded to a combination of Butyrate + EPO with higher rises in total hemoglobin than with either agent administered alone, whereas patients with a single β^0 thalassemia mutation (and higher baseline EPO levels >130 mU/mL) responded well to Butyrate, increasing total Hgb levels by 2-4 g/dL, while added EPO conferred no additional benefit in this group.[2, 45] In contrast, patients with baseline EPO levels <80 mU/mL required the combination of AB and EPO to elicit an equally high hematologic response (with a rise in total hemoglobin of 3 g/dL above baseline); neither agent alone was as effective as the two agents together.[45] These clinical and laboratory findings indicate that combinations of therapeutics with complimentary, yet distinct, molecular mechanisms of actions can produce significantly higher responses than single agents.[45]

Dual-action fetal globin inducers

The beneficial therapeutic effects of sodium phenylbutyrate, arginine butyrate, and Isobutyramide all suggest that an oral SCFAD which requires lower doses could offer benefit for long-term treatment in β thalassemia. An oral butyrate derivative, sodium 2,2- dimethylbutyrate (SDMB), was found to stimulate γ-globin production in erythroid cell cultures, anemic baboons, and transgenic mice.[34] SDMB also enhances thalassemic erythroid cell survival through Bcl-family pro-survival proteins,[35] has had excellent safety profile in long-term animal studies in two species, tested negative in mutagenicity testing, and has favorable pharmacokinetics in normal volunteers, with a half-life of 9-11 hours at low doses from 2 to 20 mg/kg/dose, allowing once per day dosing.[39] SDMB is therefore a good candidate for single- use therapy in some and for combined modality therapy, as it can be used with cytotoxic therapeutics which suppress erythropoiesis, such as the global HDAC inhibitors, decitabine, or hydroxyurea. SDMB has undergone undergoing initial safety evaluation in short-term studies in β-thalassemia intermedia patients with dose-escalation from 10 to 40 mg/kg/dose for 2 months, and was observed to increase HbF by a mean of 9% over baseline particularly at 20 mg/kg.[39]

With beneficial dual actions on fetal globin induction and prolongation of erythroid cell survival, SDMB might provide a maintenance therapeutic,[33-35] to which the cytotoxic agents might be intermittently added or *pulsed*. Small, but longer, 6-month trials will begin in the near future, as hematologic effects of hydroxyurea require at least 6 months of treatment.[16-17] Other agents with dual actions include Butyrate, (which has epigenetic HDAC inhibitory actions, targeted promoter actions, and suppresses BCL-11A), and DLT, which induces the γ globin promoter and suppresses BCL-11A.

The influence of quantitative trait loci on HbF

HbF and proportions of F-cells can vary by 10-fold in different normal subjects and in patients with the same molecular mutations, making it

difficult to predict whether thalassemia intermedia or major will result from the same globin mutations within individual patients. The influence of specific genetic modifiers, including single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTL), which alter basal HbF levels in both normal and hemoglobinopathy subjects and ameliorate clinical severity is now recognized to be influenced primarily by the presence of three genetic modifiers.[46-52] These include the T-allele present at promoter nucleotide (nt) –158 5" upstream of the HbG ($^{G}\gamma$-globin gene) on chromosome 11p15 (rs7482144), which is associated with elevated HbF levels during stress erythropoiesis, (as in sickle cell disease and β thalassemia).[25,29] This SNP and two other QTLs account for nearly 50% of the variation in basal HbF/ F-cell levels.[25-31, 47-48, 51-52] Genome-wide SNP association studies by Thein and colleagues found that *BCL11A* is a major QTL for HbF and F-cell production in normal individuals and in patients with β-thalassemia, and subsequently confirmed in sickle cell disease.[44, 47-48, 51-52] *BCL11A* has profound effects in preventing the fetal to adult globin switch when absent.[25-31] Thein and colleagues also showed that the *HBS1L-MYB* intergenic polymorphism (*HMIP*) on chromosome 6q23 exerts significant negative effects.[25, 27-28, 47-48] Other groups have confirmed that *BCL11A* on chromosome 2p16 is a major HbF QTL in populations with or without β-hemoglobinopathies. Furthermore, Chui and colleagues showed that BCL11A is a transcriptional repressor of HbG ($^{G}\gamma$-globin) proximal promoter activity, which is abolished by Butyrate; Uda, Galenello et al showed that a SNP in this γ globin repressor is associated with higher HbF and an increase in total Hb in thalassemia patients.[30, 44, 51] Our group previously identified alterations in protein binding in nucleated erythroid cells of patients who responded to butyrate therapy, including disappearance of a repressor protein from this region.[37] More recent findings demonstrated that treatment of erythroid progenitor cells with butyrate or the higher potency agent, RB7, cause displacement of a repressor complex containing HDAC3 from the γ globin promoter, leading to histone acetylation, new binding of EKLF and a remodeling complex Brg-Brm, followed by γ globin transcription.[32-38] HbF inducers which suppress BCL-11A, such as MS-275 and resveratrol, have been identified in high-throughput screening of diverse chemical libraries, including an already FDA-approved library.[40,54]

Differences in QTLs occur commonly, and differing QTL profiles may well have contributed to the variability in patient responses to differing therapeutic candidates, making definitive correction of globin imbalance in thalassemia appear unpredictable. In recent analyses of sickle cell and thalassemia patients enrolled in clinical trials of SDMB, >50% of the randomly enrolled subjects were found to have at least one high-basal HbF genotype at the *BCL11A* or *Xmn-1* locus, and 70% of Thai HbE-β thalassemia patients had a high HbF QTL genotype, (Fuchareon, 2010). As in the Sardinian population, 3 QTLs in the Thai thalassemia population which alter F genotypes were found to profoundly affect the severity of thalassemia, with polymorphisms in BCL-11A, (which should functionally diminish the repression of fetal globin), producing an average 1 gm/dl higher total hemoglobin levels in patients with the same thalassemia globin mutations otherwise.[26] It is likely that the presence or absence of polymorphisms in these influential genetic modifiers (QTL) will affect the ability of different therapeutics to induce HbF in diverse patients. Evaluating the QTL profiles of thalassemia patients in Phase 2 trials should provide a rational, targeted guide for successful Phase 3 trials of newer HbF inducers in the patient subsets who are most likely to respond well. In addition to QTL profiles, administering a therapeutic combination consisting of an epigenetic modifier, (eg an HDAC inhibitor such as Butyrate or MS-275, or a demethylating agent such as decitabine),[53] with a promoter *targeted* agent which is *not* a global HDAC inhibitor, such as RB7 or SDMB,[37, 40, 45] would provide complimentary therapeutic effects. Phase 3 clinical trials and ultimately definitive therapy could then be tailored to patients with a higher likelihood of success.

Summary

Proof-of-concept of fetal globin induction as an approach to partially or fully correct globin chain balance, and thus reduce the anemia in β-thalassemia, has been established with 3 different classes of therapeutics.[45] Patients with β+ thalassemia mutations, without a β^0 thalassemia mutation, typically have lower baseline EPO levels and require two different therapeutic agents for optimal hematologic responses in prior trials, whereas the presence of a single β^0 globin mutation was associated with rapid and higher responses to Butyrate alone. Chemotherapeutic agents and global HDAC inhibitors, which inhibit cellular proliferation, should be used intermittently in β thalassemia, as the cellular growth arrest they produce can aggravate erythroid cell apoptosis. New oral HbF inducers, such as SDMB, DLT, or RB7, which are not cytotoxic, can be used in combination regimens with epigenetic modifiers and cytotoxic agents. An oral formulation of decitabine is in late-stage development, and higher potency oral inducers (eg, RB7, MS-275, DLT) have been identified, some of which are FDA- approved for other medical conditions. Clinical trials can now target specific therapies to patients characterized for low vs high HbF QTL profiles. Combining therapeutics with epigenetic and promoter-targeted mechanisms of action should benefit many thalassemia patients, particularly those who chronically live with low hemoglobin levels (<7 g/dL) and without transfusions.

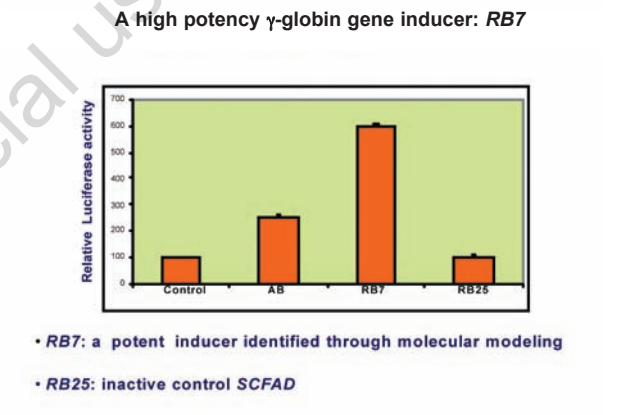

Figure 1. Comparative activity of a novel inducer, RB7, and Arginine Butyrate (AB), which has had clinical activity. RB7 has several-fold higher induction and acts at significantly lower (1/100) concentrations than does AB.

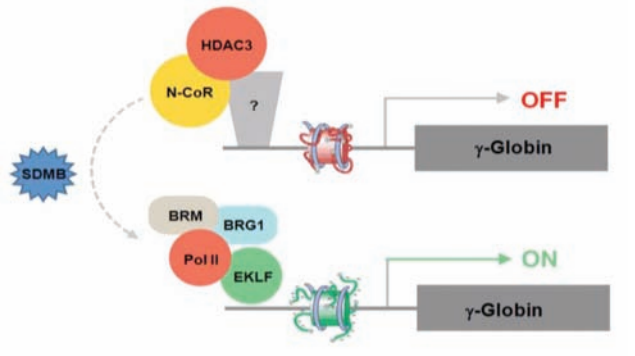

Figure 2. Example of a targeted mechanism of γ-globin promoter activation in erythroid cells treated with RB7: displacement of a repressor complex, recruitment of EKLF, the remodeling complex Brg-Brm, followed by γ globin induction.[36-37]

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