

ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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DNA Hypomethylation Therapies And Hemoglobin Disorders

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H&O What is the standard of care for hemoglobin disorders such as sickle cell disease (SCD) and β -thalassemia?

HF Patients with hemoglobin disorders are mainly treated for the complications that result from these diseases. In SCD, the standard treatments may include medications for chronic pain, blood transfusions for vaso-occlusive crises, antibiotics to prevent infections, and folic acid to help prevent severe anemia. Hydroxyurea is the first and only member of a class of pharmacological inducers of fetal hemoglobin (HbF) that is approved by the US Food and Drug Administration for the treatment of patients with moderate or severe SCD. Although hydroxyurea may help reduce the frequency of pain crises, acute chest syndrome, and blood transfusion, its long-term effects are unknown. In addition, about one-third of patients with SCD do not respond to it.

Blood transfusion is the main treatment for patients with moderate to severe β -thalassemia. However, blood transfusions carry a risk of transmitting infections and can lead to a buildup of iron in the blood—an iron overload that damages the liver, heart, and other organs. To prevent these damages, iron chelation therapy (ie, deferoxamine and deferasirox), which removes excess iron from the body, is needed. However, this therapy can be mildly painful with side effects.¹ In addition, patients with thalassemia are given folic acid supplements.

While supportive therapies minimize long-term sequelae, currently, the only cure for these disorders is allogeneic hematopoietic cell transplantation. It has been successfully applied to patients with sickle cell- and thalassemia-producing overall long-term disease-free survival

rates.² Despite these promising results, hematopoietic stem cell transplantation is likely to be limited to a small proportion of patients, due to a lack of suitable donors.

H&O What is the biological explanation for the efficacy of DNA hypomethylation therapies available today?

HF The 2 most commonly used DNA hypomethylating agents are the nucleoside analogs 5-azacytidine and decitabine (5-aza-2'-deoxycytidine). These drugs are FDA-approved for the treatment of myelodysplastic syndrome.³ 5-azacytidine is the first prototype of an agent that induces HbF by targeting epigenetic gene silencing. It was first used in the United States during the 1970s as a cytotoxic cancer chemotherapy agent, and as a HbF inducer in patients with hemoglobin disorders in the 1980s. More recently, decitabine has been studied as a pharmacological inducer of HbF for SCD.^{4,5}

The mechanism of action of catalytic inhibitors of DNA methyltransferases (DNMTs) is somewhat unique. After phosphorylation, decitabine is incorporated preferentially into DNA, whereas 5-azacytidine can be incorporated into both DNA and RNA. Thus, in addition to its effect on transcription via toxicity associated with incorporation into DNA, 5-azacytidine alters protein synthesis. Cells that replicate in the presence of these drugs show a dramatic decrease in the overall level of DNA methylation.⁶ This hypomethylated state results from the formation of covalent complexes between DNMT and cytidine analogs that are incorporated into DNA, resulting in the depletion of functional enzyme.^{7,8} Another potential theory of 5-azacytidine and decitabine was suggested by a recent study that showed a rapid and selective degradation of DNMT by the proteasomal protein-degradation pathway upon exposure to these agents.⁹ Because both 5-azacytidine and decitabine need to be incorporated into DNA to trap DNMT, they may have additional non-specific toxicities that are a result of this or perhaps the trapping of other DNA binding proteins as well.

Reactivation of HbF expression is an important therapeutic option in patients with hemoglobin disorders. In SCD, an increase in HbF would interfere with the polymerization of sickle hemoglobin, whereas in

β -thalassemia, an increase in γ -globin chain synthesis would decrease the globin chain imbalance. During several decades, the mechanisms of regulation of globin gene expression have been the subject of intense investigation. It became clear that epigenetic factors such as DNA methylation and histone modifications played important roles in the developmental regulation of globin gene expression. Thus, it was proposed that pharmacological agents that alter the epigenetic configuration of the fetal γ -globin genes may provide a viable therapeutic approach to the induction of HbF. Several pharmacologic agents including DNA methylation inhibitors like 5-azacytidine and decitabine and histone deacetylase (HDAC) inhibitors like butyrate were shown to induce HbF in vivo in patients with hemoglobin disorders.¹⁰⁻¹³

The molecular mechanisms of HbF induction by DNA hypomethylating agents like 5-azacytidine and decitabine are not fully understood. These agents were first introduced based on their ability to inhibit methylation of newly synthesized DNA within the promoters of the fetal γ -globin genes.¹⁴⁻¹⁸ DNA methylation within the promoters favors the binding of a repressor, which may be responsible in part for fetal γ -globin repression in adult erythroid cells. In contrast, DNA hypomethylation at these promoters can enhance the binding of an activator of fetal γ -globin gene expression. However, in spite of this data, the causal role of DNA hypomethylation in the induction of HbF expression by these analogs remains controversial.

It is well known that a partial switch from adult hemoglobin to HbF production takes place during accelerated erythropoiesis, as seen following acute blood loss. This results in augmented F-reticulocytosis and an increase in HbF levels. Thus, some investigators proposed that the treatment with a cytotoxic chemotherapy agent like 5-azacytidine may result in injury to bone marrow progenitors, which may lead to accelerated erythropoiesis and an increase in HbF levels. Moreover, molecular examination of DNA methylation in bone marrow cells from a patient who failed to respond to 5-azacytidine with increased γ -globin synthesis revealed hypomethylation of the γ -globin promoters.¹⁵ Therefore, the exact mechanism of induction of HbF by hypomethylating agents remains shrouded in controversy.

Although the use of these agents may have clinical benefits, there are several problems that must be considered. The toxicity and instability under physiological conditions may complicate the use of 5-azacytidine and decitabine in the clinical setting. Furthermore, the lack of specificity of the demethylating agent, which may result in the activation of normally silenced genes and may contribute to tumorigenesis, poses as a major obstacle to this therapy for SCD and thalassemia. Also, there is ample epidemiologic and clinical evidence that the higher the HbF levels, the better the amelioration of the clinical

disorders.¹⁹ Thus, more effective and safer agents that can induce higher levels of HbF are clearly needed.

H&O What is known about the association of DNA hypomethylation therapies and tumor incidences?

HF In spite of the promising results, clinical trials in patients with hemoglobin disorders with 5-azacytidine were not continued because of concerns over potential carcinogenic effects in the long-term. An increase in the incidence of tumors was shown in an animal model.²⁰ However, relatively short term studies in patients with leukemia treated with decitabine showed no increased incidence of secondary tumors at 2–5 years after initiation of therapy.²¹ More recent studies have suggested that treatment of mice with a genetic disposition for colon or lung cancer with decitabine results in a marked reduction in tumor formation.^{22,23} It is speculated that this reduction in tumorigenesis may be a reflection of the prevention of methylation of tumor suppressor genes, a process that is believed to be important in the pathogenesis of some cancers. These studies suggested that decitabine may have some potential in the chemoprevention of cancer. However, larger and longer term studies are clearly needed to confirm the safety and efficacy of decitabine in patients with hemoglobin disorders.

H&O What do we know about DNA hypomethylation and its effect on histone acetylation?

HF The 2 components of the epigenome—DNA methylation and histone modifications—are tightly correlated. More than 3 decades ago, it was shown that inactive chromatin is enriched with hypermethylated DNA and hypoacetylated histones, whereas active chromatin is associated with hypomethylated DNA and hyperacetylated histones.⁴ These correlations were confirmed by detailed analyses of specific genes, as well as genome-wide ChIP-on-chip analyses. The interrelation between histone modifications and DNA methylation suggests that there is a crosstalk between drugs targeting histones and those targeting DNA methylation. Indeed, recent experiments in baboons showed that DNA hypomethylation induced by treatment with decitabine is associated with histone hyperacetylation at the γ -globin promoters.²⁴

Similarly, our studies have shown that induction of γ -globin gene expression by butyrate, a HDAC inhibitor, in erythroid progenitor cells is associated with an increase in histone acetylation and a decrease in DNA methylation at the γ -globin gene promoters.¹² These data suggest that pharmacological agents that directly modulate one type of epigenetic modifications may have indirect effects on other types. Increasing our knowledge about the epigenetic network that controls

gene expression should help us derive novel treatment concepts for the treatment of patients with SCD and thalassemia.

H&O What studies have supported the efficacy of this therapy? Are there studies that have investigated combination therapies (eg, with histone deacetylase inhibitors)?

HF The ability of 5-azacytidine to stimulate HbF production was first demonstrated in the anemic baboon where the HbF levels could be increased up to 70–80% of total hemoglobin.^{25,26} These observations provided strong support for the hypothesis that γ -globin gene expression could be pharmacologically induced in vivo by DNA hypomethylation at the promoters of the γ -globin genes, resulting in increased levels of HbF. These encouraging data led to clinical trials of 5-azacytidine in a small number of patients with SCD and β -thalassemia. This treatment resulted in significant increases in the levels of HbF, F cells, and total hemoglobin. The therapeutic effects of this drug were associated with a decrease in the percentage of dense cells in patients with SCD and of the chain imbalance and transfusion requirements in patients with β -thalassemia.^{14-17,27}

In phase I/II studies, decitabine was administered to patients with SCD and produced clinically significant increases in total hemoglobin and HbF levels and improved several important parameters in the pathophysiology of vaso-occlusion. The only toxicity that was observed in these studies was transient neutropenia.^{11,13,18}

These clinical studies with the single agent demonstrate that the use of epigenetic modifiers in patients with hemoglobin disorders can reactivate γ -globin gene expression in adult life. Or the complexity of the mechanisms of regulation of globin gene expression suggests that the combination of a DNMT inhibitor like decitabine with an HDAC inhibitor like butyrate might result in more potent activation of γ -globin expression. Alternatively, the combination of DNMT and/or HDAC inhibitors with agents like erythropoietin or hydroxyurea, which increase HbF by different mechanisms, may also be more effective than treatment with a single agent. Finally, the combination of agents that increase HbF levels with agents that target other aspects of the pathophysiology of hemoglobin disorders, such as cell adhesion and/or cell dehydration, may also provide more effective therapeutic strategies.

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