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Emerging Science of Hydroxyurea Therapy for Pediatric Sickle Cell Disease

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Abstract

Hydroxyurea is the sole approved pharmacologic therapy for sickle cell disease (SCD). Higher fetal hemoglobin (HbF) levels diminish de-oxygenated sickle globin polymerization in vitro and clinically reduce the incidence of disease morbidities. Clinical and laboratory effects of hydroxyurea largely result from induction of HbF expression, though to a highly variable extent. Baseline and hydroxyurea-induced HbF expression are both inherited complex traits. In children with SCD, baseline HbF remains the best predictor of drug-induced levels, but accounts for only portion of the induction. A limited number of validated genetic loci are strongly associated with higher baseline HbF levels in SCD. For induced HbF levels, genetic approaches using candidate single nucleotide polymorphisms (SNP) have identified some of these same loci as also associated with induction. However, SNP associations to induced HbF are only partially independent of baseline levels. Additional approaches to understanding the impact of hydroxyurea on HbF and its other therapeutic effects on SCD include pharmaco-kinetic, gene expression and epigenetic analyses in patients and through existing murine models for SCD. Understanding the genetic and other factors underlying the variability in therapeutic effects of hydroxyurea for pediatric SCD is critical for prospectively predicting good responders and for designing other effective therapies.

INTRODUCTION

Healthy People 2020, the federal public health agenda, has set a goal of “Increase(ing) the proportion of persons with hemoglobinopathies who receive disease-modifying therapies”¹. For the vast majority of people with sickle cell disease (SCD), the Healthy People goal will be reached through increased use of hydroxyurea (HU). Critical questions surrounding its use include how this drug works to ameliorate the clinical severity of SCD and what sub-population of children with SCD benefit most from its use. This review addresses these questions from a translational science perspective.

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Sickle cell disease (SCD) affects an estimated 90,000 people in the U.S.² with over 1900 newborns detected annually through universal newborn screening². Infant screening, early preventive therapy and parental guidance have largely eliminated early child mortality from SCD³⁻⁵. Moreover, specialized care and on-going preventive services have prolonged average life expectancy⁶. Despite these successes, multi-organ damage and mortality accumulate by early adulthood, resulting in shortened lifespan⁶.

HU holds expanding promise for improved clinical outcomes. Over two decades ago, the seminal Multicenter Study of Hydroxyurea (MSH) phase III trial for adults demonstrated the striking clinical impact of HU: 40% reduction in the incidence of acute pain episodes, acute chest syndrome and hospitalization⁷. These results led to approval in 1998 of HU for use in symptomatic SCD by the United States Food Drug Administration (FDA). HU remains the only FDA-approved drug for SCD, but approval does not extend to pediatric use. The approval gap for children is partially attributed to the lack of a commercial pharmaceutical sponsor. Helping to span gap is the FDA's recent commissioning of a pediatric study of the pharmacokinetics of HU and its relative bioavailability of the liquid formulation (<http://clinicaltrials.gov/show/NCT01506544>).

Clinical efficacy of HU treatment varies between individuals, although most patients with severe phenotypes benefit from its use^{7,8}. This review describes newly identified mechanisms for the effects of HU, including genetic regulation of fetal hemoglobin (HbF) as a disease modifier and the biologic effects of HU on blood vessels and gene regulation. These recent advances improve the prospects for prospectively assessing efficacy of HU therapy, are inspiring clinical trials for additional salutatory effects of HU and may guide future drug development.

CLINICAL EFFECTS

The profound clinical effects of HU for children with SCD have been recently reviewed⁹⁻¹¹ summarized here and in Table 1. Much of the work on HU in children with SCD has come from phase III trials led by Ware and colleagues, including pivotal studies such as HUGS, HUG-KIDS¹²⁻¹⁴, HUSOFT¹⁵, BABY-HUGS¹⁶⁻¹⁸ including an early pediatric trial published in 1999¹². French investigators have also contributed insights into the impact of HU^{19,20}. Randomized pediatric trials with HU have demonstrated decreased pain episodes¹⁸, acute chest syndrome, hospitalization^{8,11,18}, transfusion and splenic auto-infarction¹⁸ and improved quality of life^{21,22}. Prolonged use sustains the laboratory effects of decreased anemia, markers of hemolysis, white blood cell and platelet counts and increased red cell mean corpuscular volume (MCV)²³. Early HU use stabilizes renal hyperfiltration²⁴ and hyposthenuria²⁵, as well as age-dependent decreased HbF¹⁸. Induction of HbF is described below.

Of note, while the laboratory effects of HU apply across the pediatric ages tested, many of the various clinical improvements noted for one age range have not necessarily been assessed for other ranges. For example, reduced dactylitis, hyposthenuria and transfusions were noted in the BABY HUG trial of children enrolled at age 9–13 months^{17,18}. Improved transcranial Doppler blood flow through large cerebral arteries has been demonstrated in

school-aged children^{26,27}. Despite positive findings, some of these trials had mixed results. For example, the primary endpoints of the BABY-HUG study were not met¹⁸. In the SWiTCH study for secondary stroke prevention, continued chronic transfusion was advantageous over HU with phlebotomy²⁸. Moreover, HU reduces but does not eliminate the symptoms and morbidity of SCD. For example, the SWiTCH trial demonstrated that chronic transfusions more effectively prevented pain episodes than HU with phlebotomy²⁹.

Two long-term studies demonstrated substantially improved lifespans from prolonged use of HU in adults, including a study based on the MSH trial^{30,31}. Prospective lifespan data for children taking HU are not yet available due to later uptake into pediatric trials. Nonetheless, a recent retrospective study from Brazil reported improved childhood mortality for those taking HU for up to six years³². Collectively, these data are increasingly persuasive about the enduring impact of HU on SCD.

The pharmacokinetics of HU appears to follow a bi-phenotypic metabolism in children³³. Multiple single base polymorphisms (SNP) are associated with two apparent pharmacokinetic profiles of HU uptake and excretion. However, these genotypes do not correlate with response by the biomarker HbF.

FETAL HEMOGLOBIN

The clinical severity of SCD is highly variable. Children experience multiple different clinical complications of differing severity and frequency. Fetal hemoglobin (HbF) is of critical importance in the major sickle sub-type of HbSS (and HbS-Beta zero thalassemia, here collectively referred to as HbSS). Lower HbF levels correlate with overall more severe disease manifestations³⁴. Unlike HbA, HbF actively inhibits the polymerization of sickle hemoglobin, the underlying patho-physiology of SCD. In solution, fetal hemoglobin concentration higher than 15% prevents sickle globin polymerization³⁵. The cut-off for defining lower risk of severe complications has been estimated at 20%³⁶.

Sharp declines of HbF during infancy occur as HbF-producing gamma globin is replaced by beta globin. This switch leads to the predominant expression of either HbA or HbS. The F-to-S switch in children affected by HbSS³⁷ occurs more gradually than the F-to-A switch in non-anemic children. HbF levels in toddlers with SCD stabilize by age 3 or 4 and are generally constant throughout childhood. Despite bearing the same beta globin sickle variant, affected populations with African ancestry exhibit wide variations in HbF levels³⁷⁻⁴⁰. In the U.S., pediatric levels vary from 3–20% of total hemoglobin, compared to only 0.5–2% for non-anemic individuals. The average HbF level in the U.S SCD pediatric population is approximately 10%³⁶.

HU USE

Mechanism of action

The physiology of the HU effect is complex, and can generally be categorized into two overlapping pathways: effects on HbF production and improved blood flow through reduced intercellular adhesion (Figure 1). HU is a short-acting cytotoxic drug that induces a state of “stress erythropoiesis.” Enhanced HbF production from intermittent mild marrow

toxicity is believed to stem from steady shifting of marrow physiology to the stressed state. The marrow responds to the repetitive pharmacologic injury of daily use by enhanced erythropoiesis and increased HbF production^{34,41}. Paradoxically, the net effect of marrow toxicity is induced HbF and stabilization of cellular hemoglobin solubility. These effects lead to decreased RBC membrane damage and hemolysis^{34,41}.

HbF induction usually occurs within the first few months after initiating HU, and is reversible upon cessation or diminution of dosing (Figure 2). Relevance of HU induction of HbF was demonstrated through a proof-of-principle murine model for SCD. Lack of expression of human HbF precluded HU induction in those mice. In that murine model, HU itself had no effect on improving anemia or protecting organs from SCD damage. In contrast, HbF gene therapy markedly improved the blood smear, microscopic and organ-level pathologic effects of SCD⁴².

HU appears to influence red blood cell-endothelial interactions. Decreased expression of RBC, WBC and endothelial integrins and other adhesion molecules probably improves micro-vascular blood flow and reduces pro-inflammatory cell-cell interactions^{43,44}. Microvascular effects of SCD and HU appeared to be replicated using an interesting microfluidic model of blood flow and endothelialized microfluidic channels⁴⁴. Whole blood samples from SCD lead to microvascular occlusion and thrombosis. Blood samples from patients with SCD had diminished velocity and greater tendency to obstruct in the microchannels. These effects nearly normalized using blood from patients on HU⁴⁴. HU may be associated with reduced generation of microparticles, suggesting a reduction in markers of inflammation and thrombosis⁴⁵.

HU may reduce cellular adhesion in general and/or adhesion provoked by infection or inflammation. Integrins and other cell surface glycoproteins regulate neutrophil migration and red blood cell flow through endothelial interactions. In a murine model for SCD and pneumococcal pneumonia and sepsis, HU provided some protection by decreasing the recruitment of neutrophils into infected lungs. Mice genetically engineered to lack E-selectin were not protected by HU⁴⁶. This finding strengthens the view that HbF-independent effects of HU include decreasing leukocyte-endothelial adhesion.

HU may also stimulate nitric oxide production as an NO donor or through stimulating intermediates (discussed below). As a potent vasodilator, NO repletion contributes to improved vascular health in SCD (Figure 1)⁴⁷. Along with decreased “sticky” interaction between of blood cells and the endothelium, enhanced nitric oxide-induced local vasodilation may also benefit blood flow (Figure 1)^{48,49}. However, questions have arisen regarding these effects from NO⁵⁰. In all, decreased pathology from damaged red blood cells and pathologic interactions between red blood cells and endothelial cells appear to synergistically reduce clinical signs symptoms and morbidities of the disease (Table 1). The ameliorative effects of HU appear to persist for as long as it is taken and the pharmacokinetics are maintained.

HbF response to HU

The individual extent of HU-induced HbF is highly variable. Standard pediatric dosing of HU adjusts for dose-dependent myelotoxicity^{14,33}. Under these conditions, HU generally induces HbF an additional 8–18% over baseline levels^{14,33,51,52}. In contrast to the biomarker HbA1c for diabetes, no absolute HbF target exists. Nonetheless, peak attained HbF levels remain fairly constant in childhood²³. No absolute limit to the therapeutic amount of HbF induction has been described. For example, Southeast Asians or Saudis with SCD have baseline HbF levels averaging 16–20%. HU induction raises their levels 1.5 to 2-fold, associated with further diminution of their already tempered clinical symptoms⁵³.

Children with SCD generally have higher baseline HbF levels than adults and more pronounced HbF response to HU^{14,54}. Factors responsible for differences may include the need for highly regenerative marrow red cell precursors and, for HU, normal renal function for prompt excretion. Adults normally experience age-dependent decreased marrow cellularity. In SCD, disease-related marrow infarcts and other age-related physiologic effects could exacerbate normal marrow regression. Age-related diminution of response to HU increases the likelihood that genetic studies using pediatric populations may reveal more precise basic biologic insights.

Genetic analysis of HU-induced HbF

Analyses of HbF regulation are crucial to understanding the spectrum of SCD severity, variability of HU response and design of novel therapies. In addition to the established observations of ethnic variability of HbF levels, several key observations drive the rationale for identifying genetic components of HU induction of HbF in U.S. populations of SCD:

1. Baseline HbF levels in SCD have high heritability^{55,56};
2. HbF induction from HU therapy is also a heritable trait⁵⁵;
3. Genome-wide SNP studies in normal non-anemic adults identified on a few major loci associated with variation of low HbF levels. These regions are both cis and trans to the beta globin gene locus^{56,57};
4. These same loci are associated with baseline HbF in people with SCD in the U.S.^{56,58–62}. Additional loci have been identified but not yet replicated;
5. A modest correlation in children exists between levels of HbF at baseline and on HU^{14,33,51}.

Taken together, these findings lead to the prediction that genetic regulation of HbF expression at baseline overlaps with the control of HU-induced HbF. The three major loci related to HbF expression in normal and SCD populations are: a SNP upstream of the gamma globin gene within the globin locus on chromosome 11, previously identified by restriction enzyme analysis as the XmnI site^{37,60,61}; BCL11A, a transcription factor now recognized as a major silencer of HbF expression^{51,58,59,61,63}; the intergenic interval between the HBS1L and MYB^{56,58,61}. Additional loci have been identified and await replication^{59,64,65}, in addition to epigenetic effects, as well as probable epigenetic effects (Figure 3)⁶⁶.

Only a few published studies report on the genetics of HbF response to HU in SCD^{33,51,54,64}. Compared to genomic studies of more common disorders, sample sizes of HU effects on SCD are inevitably modest. Using the retrospective cohort from the MSH adult trial and assessing over two dozen candidate genes, Ma et al. reported significant associations between SNPs and HbF response to HU in loci of genes involved in the metabolism of arginine to nitric oxide and in a transcription factor that induces DNA bending. This report pre-dated the identification of BCL11A as a central regulator of HbF expression. Most of the MSH patients exhibited a small HbF response to HU⁵⁴, with less than a 5% change in HbF from baseline. This blunted response is not universal in U.S. adults with SCD, and may be influenced by patient characteristics as well as adherence to HU regimen.

Whether HbF induction by HU occurs directly through via the direct influence of BCL11A, is a concept awaiting direct testing. BCL11A effects on HbF are probably mediated through its protein partners, upstream or downstream effectors, chromatin structure and/or telomerase function (recently reviewed)⁶⁷. Other reports include associations between HU response in SCD and polymorphisms in the guanosine triphosphate (GTP)-binding protein gene *sar1a*⁶⁴, underscoring the complexity of the genetic pathways regulating the HbF response to HU (Figure 3).

Two pediatric pharmacogenetics analyses using candidate single marker SNP polymorphisms suggested that just a few genes are associated with baseline HbF, including several SNPs within BCL11A (Table 2). SNP associations with induced HbF are generally not independent of baseline HbF levels^{33,51}. In contrast to the induced HbF level, the treatment-associated increment appears to be a less relevant marker. Both of these observations probably reflect the association between baseline and induced levels.

In our own, smaller multi-site analysis, baseline levels candidate were significantly associated with SNPs within the BCL11A and the beta and epsilon globin loci (HBB and HBE, respectively), with an additive attributable variance from these loci of 23% (Table 2)⁵¹. Consistent with studies by Ware and colleagues^{14,33}, we reported that baseline HbF levels explained 33% of the variance in induced levels. The variant in HBE accounted for an additional 13% of the variance in induced levels, while variants in the HBB and BCL11A loci did not contribute beyond baseline levels. Thus our data suggest that the combined effects of baseline HbF and one SNP marker contributed an estimated 46% of the variance in HbF⁵¹.

By trend analysis, children with an allele associated with higher HbF (“favorable” allele) in one of the BCL11A and/or either globin marker had significantly higher average values of baseline HbF than those who lacked a favorable allele⁵¹. Effects on baseline HbF from a SNP in each these two genes were additive, and were associated with two-fold higher HbF for patients with favorable alleles in both loci. Similarly, having at least one favorable allele in either globin locus and BL11A was associated with a higher level of induced HbF. Statistical significance did not withstand adjustment for baseline fetal hemoglobin, likely reflecting the inter-relatedness of HbF regulation under both physiologic conditions. Genetic studies examining larger pediatric populations on HU, unusual responders and the influence

of specific sequence variants are needed to the contribution of these and other genetic loci responsible for HbF response.

OTHER PHYSIOLOGIC EFFECTS OF HU

Cellular biology

The effects of HU largely depend upon its effects on nucleic acid synthesis in dividing red blood cell progenitors. HU affects the S-phase by inhibiting ribonucleotide reductase, an enzyme important for DNA synthesis. Depletion of DNA precursors by HU causes arrest of the replication fork, leading to cell death. A cell-based *ex vivo* assay for HbF induction, burst-forming unit erythroid (BFU-E) colonies grown in methylcellulose from blood of children with SCD, demonstrated that HU decreases the number of BFU-E colonies. HU and other ribonucleotide reductase inhibitors increase HbF production in that system. Interestingly, other cytotoxic agents that are not of that drug category, such as cytarabine and alkylating agents, decreased BFU-E counts but did not induce HbF^{41,68}.

HU's lethal effect of on ribonucleotide reductase and cell survival are also seen in laboratory bacteria such as *E. coli*⁶⁹. Whether the bactericidal effects influence investigation using animal models or even patients has not previously been raised. Direct bacterial effects should be addressed on the HU-dampened expression of adhesion molecules in a murine model of bacterial infection.

HbF response to temporary marrow toxicity is probably attributable to transcriptional and epigenetic effects on the progenitor developmental program^{66,70}. HU signaling appears to involve cyclic guanine monophosphate (cGMP), cAMP, p38MAPK, and others pathways. Activation of cGMP may induce HbF via enhancing production of nitric oxide (NO)^{47,68,71,72}. NO may also support HbF production⁴⁷. HU induces a small guanosine triphosphate (GTP)-binding protein, secretion-associated and RAS-related (SAR)⁷³. SAR may be involved in the activation of transcription factors and signal transduction pathways in erythroleukemia K562 cells and in human bone marrow-derived progenitor cells. HU may also function through kinase and signal transduction pathways, such as GATA-1, to enhance gamma and beta globin synthesis in erythroid cells⁴⁷.

Gene expression

Comparing whole mRNA pre- and post- initiation of therapy revealed that HU affects expression of a number of genes involved in transcription and translation, such as ribosome assembly and chromosomal organization^{66,70}. Results may vary with age, dosing or other clinical conditions. Variation in cell source would be expected to affect detection of expressed genes, whether from bone marrow or purified early reticulocytes. HU may also affect expression of genes that link HU and HbF to BLCL11A^{66,70}. Epigenetic analysis of the gamma globin promoter did not find reveal much impact from HU⁷⁴. Interestingly, HU appears to up-regulate specific miRNAs⁷⁴. These results require further investigation, but underscore the view that HU is involved with complex pathways of gene regulation.

Testing for oncogenicity

The primary effect is damaging DNA replication by inhibiting ribonucleotide reductase. This effect raises concerns about an oncogenic potential, especially after prolonged use. These fears have been amplified by its original use as chemotherapy for chronic myeloid leukemia (CML), the latent phase of acute leukemia. While links acute leukemia outside of CML have been disproven⁷⁵, concerns for the safety of long-term use in children persist. Several studies have tested DNA and cellular toxicity from pediatric HU users. No genotoxicity was detected using several different assays in vitro, including karyotype, illegitimate VDJ recombination, chromatid breaks⁹. Increased reticulocyte micronuclei were observed, but this effect was highly variable between patients and did not increase with time⁷⁶. In all, oncogenicity of HU is probably quite low or non-existent. A few cases of acute leukemia were reported in patients after many years of HU treatment, but do not appear to be more frequent than in the untreated population⁷⁵.

POTENTIAL PHARMACOLOGIC ALTERNATIVES TO HU

Other HbF inducers have been assessed over the past few decades, including nucleoside analogs such as 5-azacytidine and decitabine. However, they are often poorly tolerated potentially oncogenic and lack proof of effectiveness comparable to HU (recently reviewed)⁴¹. Additional HbF-inducing drugs are histone deacetylase inhibitors, erythropoietin (already high in SCD and shown not to induce HbF in SCD), valproate, thalidomide derivatives (e.g. pomalidomide), kit ligand. In all, a variety of cellular stresses and stimuli can promote coordinated stress responses, including activation of the gamma globin gene^{66,70}. Based on results from use of SCD mouse models, inhibitor of phosphodiesterase 9⁷¹ or inducible factor-1 α (HIF-1 α)⁷⁷, alone or in combination with HU, may be clinically useful to stimulate cyclic GMP (cGMP) and nitric oxide for HbF production and/or to enhance its anti-sickling impact⁷¹.

BARRIERS TO HU UTILIZATION

Outside of clinical trials with HU, ample documentation exists of incomplete clinical effectiveness of HU. Uneven drug adherence has been well documented^{78,79}. Provider non- and under-utilization is well documented^{75,80}. Our recent multi-site survey of parents of children with SCD revealed several family barriers to use of HU such as lack of FDA approval, near-universal safety concerns and highly varied knowledge about its benefits, including many for whom its basic property of decreasing episodes of pain was unknown⁸¹. Use of HU was positively correlated with fundamental knowledge of parents in the basic positive effects of HU on disease, independent of parental demographics such as education level, language spoken or ethnicity. Barriers in effective communication between providers and families may be exacerbated by issues arising from medical delivery systems.

The mixed uptake of HU by families may also reflect family perspectives on the long-term effects of SCD. A single site survey of parents revealed that the majority believed that the disease effects were going to diminish over time, and would not impact life goals or lifespan⁸². These poignant perceptions will need to be addressed if families are to embrace the long-term benefits of HU against its inconveniences and largely theoretical risks.

CONCLUSION

HU is a remarkably effective drug for a large proportion of children with SCD. Expanded understanding of the scientific underpinnings of its effects on SCD, the ability to predict individual response and the clinical applications for modifying disease effects are ongoing. Clinical trials will continue to test the uncertain benefits of HU (Table 1), such as primary prevention of brain infarcts (clinicaltrials.gov/show/NCT01389024). Murine models will facilitate insight into the benefits provided by induced HbF, altered expression of adhesion molecules, reduced BCL11A and other mechanisms. Genetic epidemiology will be used to identify specific variants in regulatory genes and gene pathways.

The accumulating science of HU is anticipated to lead to three direct effects for children with HbSS: 1) Use at earlier ages; 2) Wider clinical indications; and 3) Delineation of children who are less likely to enjoy substantive benefit from HU. For this last group, more aggressive consideration of chronic transfusion, hematopoietic stem cell transplantation or trial of emerging alternative agents may be warranted. To date, early clinical trials of other experimental HbF-inducing drugs have demonstrated considerable short- and long-term toxicity compared to HU. Therefore, HU is predicted to remain the mainstay of pharmacologic therapy for SCD in the foreseeable future.

Progress towards the Healthy People 2020 goals will occur through increased use of HU. Nonetheless, the entire SCD population may not benefit from HU alone. Dampened impact on clinical complications and HbF induction occurs for those with certain genotypes, many patients with HbSC, some adult patients and those with renal compromise. New, effective and safe therapies, alone or in combination with HU, are still needed to maximize pharmacologic benefit for everyone living with SCD. Constructive engagement must be made to assist families in undertaking long-term HU use to help them to balance the optimism of HU treatment and its potential toxicities with the risk of accumulating disease consequences. While several important crisis-modulators are currently under investigation, disease modifiers that prevent crises and other morbidities is arguably the primary therapeutic target.

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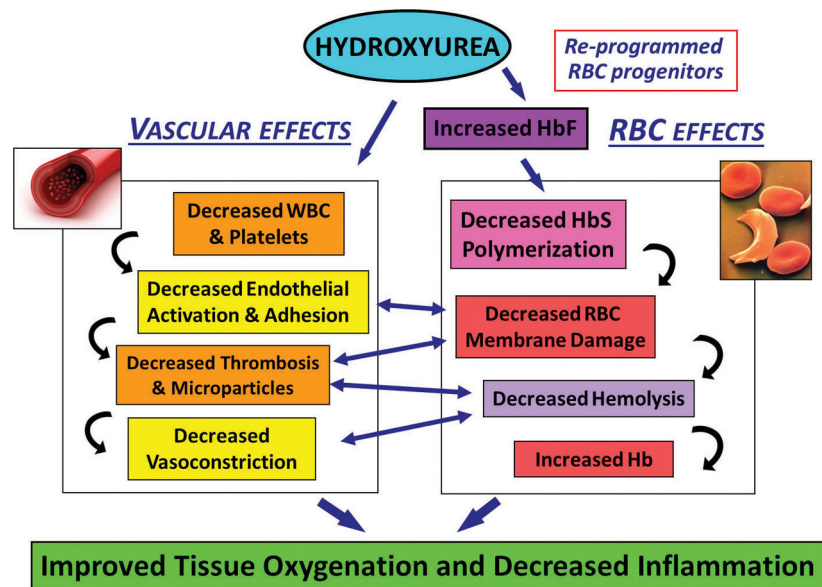


Figure 1. Physiologic Effects of Hydroxyurea on Sickle Cell Disease. Hydroxyurea has pleiotropic effects on ameliorating sickle cell disease, with complex and interacting vascular and red blood cell effects.

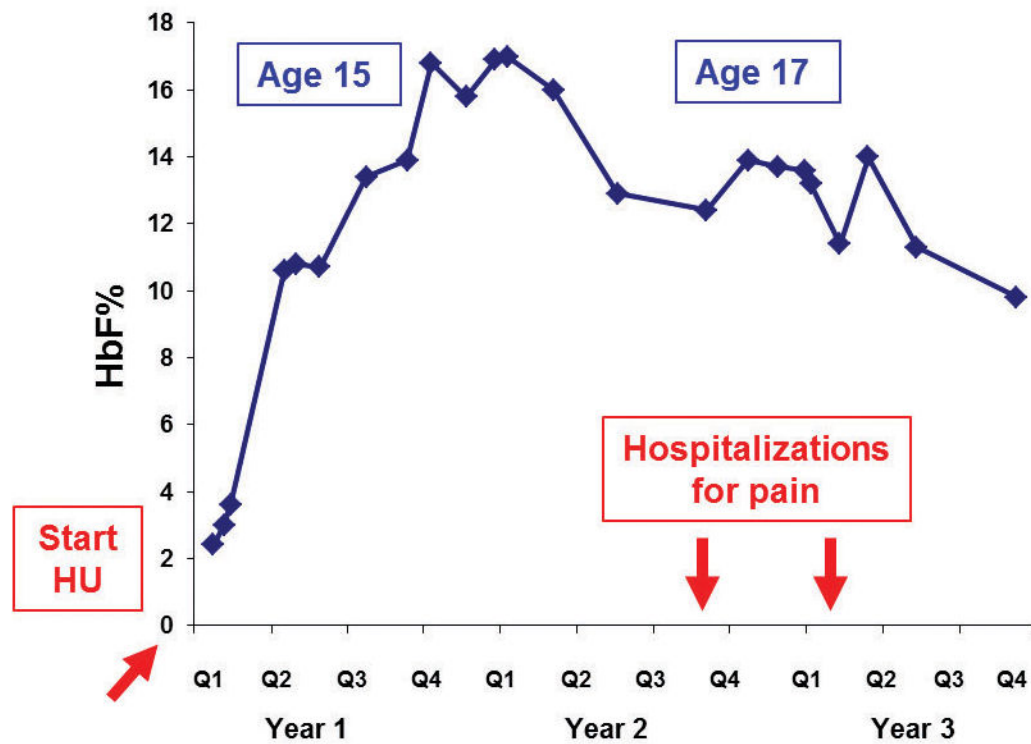


Figure 2.

HbF Levels of a Teenager with HbSS on Hydroxyurea. Prior to hydroxyurea, this teenager had 2-3 hospitalizations for pain each year. She had no admissions for 1.7 years after beginning hydroxyurea. Her HbF baseline was 2.4% and HbF maximum recorded level was 16.9%. She acknowledged intermittent adherence in years 2-3, during which time she had 2 admissions for acute pain episodes. (Blue diamonds refer to HbF data points.)

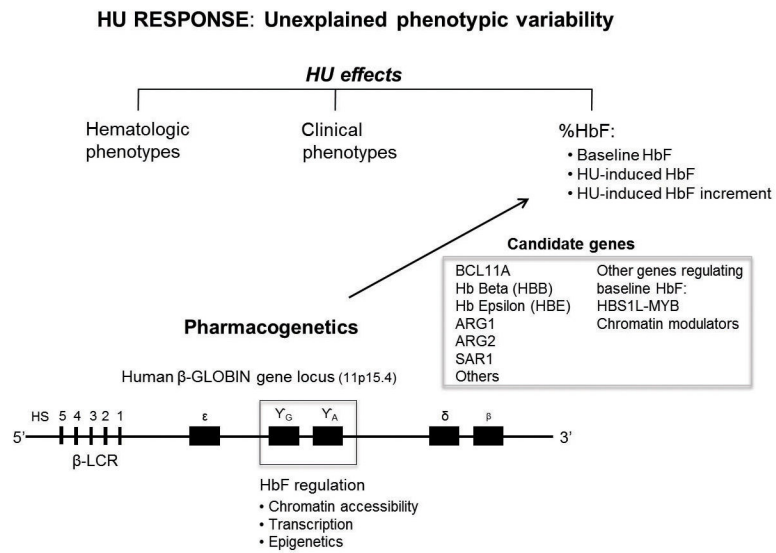


Figure 3. Phenotypic Variability in Hydroxyurea Response. A diagram synthesizes the varying clinical and genetic effects of hydroxyurea in sickle cell disease. The beta globin locus is shown below.

Table 1Clinical Effects of Hydroxurea on Children with SCD^a

Effects	Blood/Circulation	Organ/Whole body
Laboratory/Physiologic measurement	Elevate HbF levels ^{8,14,23} and stabilize high infant levels ¹⁸ Increase hemoglobin ¹³⁻¹⁵ Increase MCV ^{8,14,15,22} Decrease hemolysis ¹³⁻¹⁵ Decrease WBC and platelet counts ^{8,13-15}	Brain: Improve TCD flow velocity ^{26,27} Spleen: Preserve blood flow ^{15,18} <i>b</i> Lungs: Decrease acute chest syndrome ^{15,18} Renal: Decrease hyperfiltration ²⁴ and hyposthenuria ²⁵
Clinical/Well-being**	<i>b</i> Fewer acute pain crises ^{8,13,18} <i>b</i> Reduce dactylitis ¹⁸ <i>b</i> Fewer transfusions ¹⁸ <i>b</i> Fewer hospitalizations ^{8,13,18}	<i>b,c</i> Reduce mortality ³⁰⁻³² <i>b</i> Improve growth ^{13,15} Improve quality of life ^{21,22}
Not yet known	Stabilize HbF as adults Reduce allo-immunization (through reducing transfusion) Reduce transfusion-related iron toxicity (through reducing transfusion)	<i>b</i> Improve overall lifespan for children <i>b</i> Improve cognitive development Protect from stroke/infarct Prevent long-term renal, lung, cardiac effects Reduce cholelithiasis Reduce retinopathy <i>b</i> Normalize timing of physical maturation <i>b</i> Maintain fertility

MCV – Mean red cell volume TCD – Transcranial Doppler

^a Some of the effects have not been demonstrated across all pediatric age ranges nor by prospective randomized trials. Reports demonstrating effect by randomized trials are preferentially cited.

^b Patient-oriented outcomes

^c Established long-term outcome for adults

Table 2

SNP Polymorphisms Associated with HbF

Gene	Phenotype	Clinic	Population	Ethnicity	N	SNP	β	P	Ref. #
<i>ARG1</i>	HbF induced by HU	pediatric	USA	AA	174	rs17599586	NA	4×10^{-3}	33
<i>ARG2</i>	HbF induced by HU	pediatric	USA	AA	174	rs2295644	NA	3×10^{-3}	33
<i>ARG2</i>	HbF induced by HU	adult	USA	AA	137	rs10483801	NA	1×10^{-3}	54
<i>BCL11A</i>	HbF baseline	pediatric	USA	AA, H	108	rs4671393	2.88	5×10^{-5}	51
<i>BCL11A</i>	HbF baseline	pediatric	USA	AA	174	rs4671393	NA	3×10^{-4}	33
<i>BCL11A</i>	HbF baseline	adult	USA	AA	255	rs766432	NA	2×10^{-10}	63
<i>BCL11A</i>	HbF baseline	pediatric + adult	USA	AA	1032	rs4671393	0.60	4×10^{-37}	60
<i>BCL11A</i>	HbF baseline	pediatric + adult	USA	AA	1275	rs4671393	0.60	2×10^{-42}	61
<i>BCL11A</i>	HbF baseline	pediatric + adult	Brazil	NA	350	rs4671393	0.50	3×10^{-8}	61
<i>BCL11A</i>	HbF induced by HU	pediatric	USA	AA, H	47	rs1186868	3.37	0.019	51
<i>FTL1</i>	HbF induced by HU	adult	USA	AA	137	rs2182008	NA	0.003	54
<i>GLP2R</i>	F cells baseline	pediatric + adult	USA	AA	440	rs12103880	-1.36	3×10^{-8}	59
<i>HAO2</i>	HbF induced by HU	adult	USA	AA	137	rs10494225	NA	2×10^{-3}	54
<i>HBB</i>	HbF baseline	pediatric	USA	AA, H	108	rs7482144	3.88	2×10^{-4}	51
<i>HBB</i>	HbF baseline	pediatric + adult	USA	AA	1032	rs10128556	0.42	2×10^{-9}	60
<i>HBB</i>	HbF baseline	pediatric + adult	USA	AA	1275	rs7482144	0.41	4×10^{-7}	61
<i>HBE</i>	HbF baseline	pediatric	USA	AA, H	108	rs7130110	2.86	6×10^{-5}	51
<i>HBE</i>	HbF baseline	pediatric	USA	AA	174	rs7130110	NA	3×10^{-5}	33
<i>HBE</i>	HbF induced by HU	pediatric	USA	AA, H	38	rs7130110	6.04	0.004	51
<i>HBS1L-MYB</i>	HbF baseline	pediatric + adult	USA	AA	1032	rs9402686	0.65	2×10^{-13}	60
<i>HBS1L-MYB</i>	HbF baseline	pediatric + adult	USA	AA	1275	rs9399137	0.60	5×10^{-11}	61
<i>NOS1</i>	HbF induced by HU	adult	USA	AA	137	rs7977109	NA	0.023	54
<i>ORS1B5/B6</i>	HbF induced by HU	pediatric	USA	AA	1153	rs5006884	0.20	3×10^{-8}	65
<i>ORS1B6</i>	HbF baseline	pediatric	USA	AA, H	108	rs5024042	1.70	0.031	51
<i>SAR1A</i>	HbF induced by HU	adult	USA	AA	32	rs4282891	NA	< 0.05	64

AA: African American; H: Hispanics; AC: African Caribbean; WA: West African; N: sample size; Ali: phenotype associated allele