The modern use of hydroxyurea for children with sickle cell anemia

Charles T. Quinn[1,2] and Russell E. Ware[1-3]

[1]Division of Hematology, Cincinnati Children's Hospital Medical Center; [2]University of Cincinnati College of Medicine and [3]Global Health Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Abstract

Over the past 40 years, the introduction and refinement of hydroxyurea therapy has led to remarkable progress in the care of individuals with sickle cell anemia (SCA). From initial small proof-of-principle studies to multicenter phase III controlled clinical trials and then numerous open-label studies, the consistent benefits of once-daily oral hydroxyurea have been demonstrated across the lifespan. Elevated fetal hemoglobin (HbF) serves as the most important treatment response, as HbF delays sickle hemoglobin polymerization and reduces erythrocyte sickling. Increased amounts of HbF, especially when distributed across the majority of erythrocytes, improve clinical outcomes by reducing hemolytic anemia and preventing vaso-occlusion, thereby ameliorating both acute and chronic - and overt and covert - complications. Additional benefits of hydroxyurea beyond HbF induction include lower neutrophil and platelet counts, reduced inflammation, and improved rheology. Toxicities of hydroxyurea in SCA are typically mild and predictable; modest cytopenia is expected and actually therapeutic, while occasional gastrointestinal and cutaneous manifestations are well-tolerated. Long-term risks of hydroxyurea for SCA are mainly theoretical but require ongoing surveillance. Accordingly, hydroxyurea should be initiated as part of standard of care, ideally in the first year of life. Proper dosing of hydroxyurea is critical, aiming through stepwise dose escalation to achieve modest but safe myelosuppression, with periodic adjustments for weight gain. Precision dosing using pharmacokinetics may facilitate optimal dosing without frequent dose adjustments. Although transformative and even curative therapies for SCA are emerging, hydroxyurea is the only available and accessible disease-modifying treatment that can address the global burden of disease, especially in low-resource settings within sub-Saharan Africa.

Introduction

Forty years have passed since the first publication regarding hydroxyurea treatment for sickle cell anemia (SCA).[1] In that proof-of-principle report, oral hydroxyurea given to two patients increased fetal hemoglobin (HbF), which was recognized as a therapeutic goal to reduce sickling. Hundreds of publications have followed, collectively documenting a consistent safety and efficacy profile of this small and simple compound for the treatment of such a complex and life-threatening disease.

In 2014, the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health published guidelines for the management of SCA.[2] In that consensus document, a strong recommendation was made to offer hydroxyurea to all infants and toddlers with SCA as young as 9 months of age, and to treat older persons based on various clinical settings. Even though newer therapeutic agents have become available, hydroxyurea remains the only disease-modifying treatment that is considered the standard of care for individuals with SCA. This manuscript focuses on the "modern use" of hydroxyurea for children with SCA, intending to distill many lessons learned through a long series of controlled trials and years of clinical experience with this treatment. Our goal is to summarize the published literature and provide useful suggestions for safe and effective hydroxyurea treatment, arguing that everyone with SCA should receive hydroxyurea with optimal dosing, and that treatment should begin early in life before the onset of organ damage. Herein, SCA refers to both homozygous HbSS and compound heterozygous sickle-β^0-thalassemia (HbSβ^0); these are distinct genotypes but have similar severe phenotypes and identical clinical management.

Rationale and early trials of hydroxyurea treatment

Clinical observations made several decades ago identified HbF as a key determinant of clinical severity in SCA. The initial observation was that newborns were asymptomatic because of physiologically high HbF levels, and that disease manifestations began during the physiological HbF decline in the first year of life.[3] Additional evidence came from patients with SCA and naturally elevated HbF levels,[4] including the rare but striking phenotype of HbS with pancellular (gene-deletion) hereditary persistence of fetal hemoglobin (HbS/HPFH), which is clinically benign.[5] Both the Jamaican newborn cohort[6] and the US-based Cooperative Study of Sickle Cell Disease[7] subsequently confirmed that HbF was a critical protective biological feature.

Based on these observations, HbF became a principal pharmacological target, with the aim being to delay the age-related HbF decline or ideally to reverse it. Several agents were studied that might "de-repress" the physiological HbF switch, including chemotherapeutic agents such as 5-azacytidine[8] and cytosine arabinoside.[9] Hydroxyurea was a known cytostatic agent with clinical benefits for hematologic malignancies and myeloproliferative disorders, and thus an attractive treatment option for SCA due to its oral dosing, mild side-effects, and low leukemogenic risk. The proof-of-principle experiment involved two young females with SCA, who received oral hydroxyurea at 50 mg/kg/day in three divided doses for 5 days, followed by three additional pulses. The number of circulating reticulocytes containing HbF rose quickly in both patients and led to overall increases in percentage of HbF.[1] A progression of formal trials followed (Figure 1). Among these, two important phase I/II open-label trials in adults[10] and children[11] documented the safety and benefits of once-daily oral hydroxyurea with dose escalation. Subsequently, placebo-controlled phase III trials in both adults[12] and then children[13] documented the benefits of hydroxyurea for decreasing acute vaso-occlusive painful events, transfusions, and hospital admissions. Additional trials focused on neuroprotection in high-risk children, and more recently the safety and feasibility of treatment in low-resource settings (Figure 1). Although HbF induction remains the main mechanism of action of hydroxyurea in SCA, additional treatment benefits not directly related to HbF are also recognized, including lower neutrophil counts and platelet counts, reduced inflammatory markers, increased mean corpuscular volume with better erythrocyte flexibility, improved rheology, and potential nitric oxide release for vasodilation.[14]

Treatment with hydroxyurea

Indications

The natural history of SCA is well-documented as a lifelong, severe disease characterized by chronic hemolytic anemia, recurrent acute complications, progressive multiorgan damage, and early mortality. While there is variation in disease expression, some of which can be explained by genetic modifiers (e.g., polymorphisms affecting HbF expression, α-thalassemia, and others), the great majority of patients suffer from recurrent acute clinical complications and almost all will die from consequences of their disease. Accordingly, the indication for treatment of SCA with hydroxyurea should be the *disease itself*, just as it is for other severe, life-threatening, but treatable chronic diseases.

The occurrence of disease manifestations, such as painful episodes, acute chest syndrome, or stroke, should not be a prerequisite for starting hydroxyurea therapy. Such overt complications represent only a fraction of the morbidity of SCA, and the best therapeutic opportunity is lost by wait-
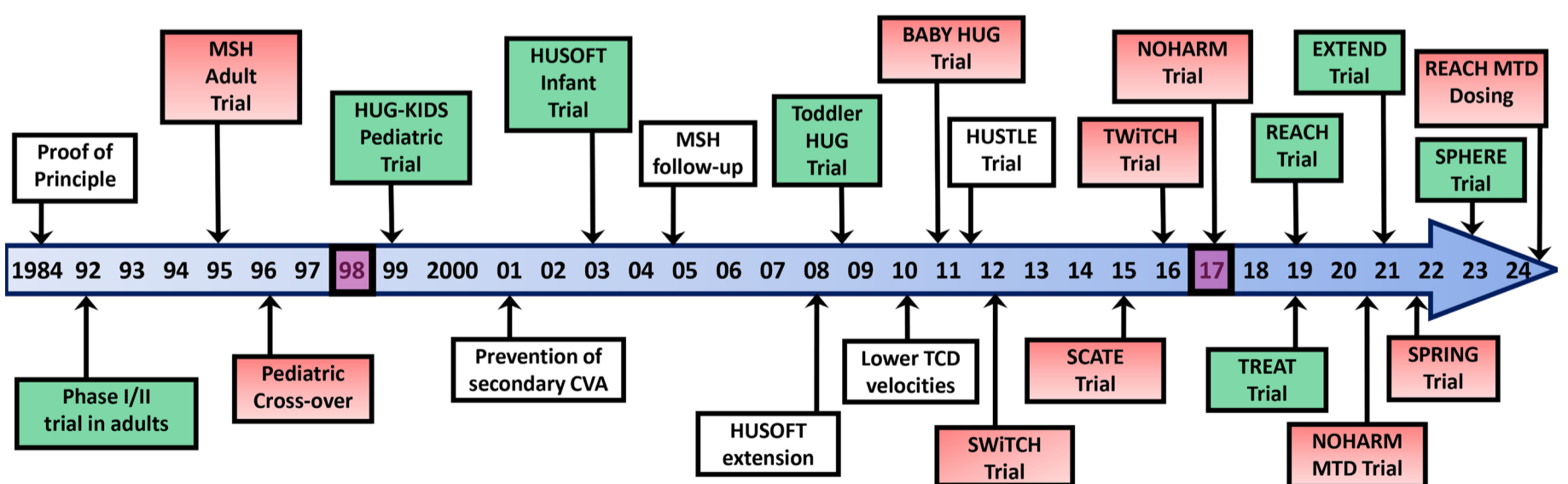

Figure 1. Timeline of hydroxyurea trials. This 40-year timeline illustrates the expanding portfolio of rigorous clinical trials that have consistently demonstrated the efficacy and effectiveness of hydroxyurea treatment for sickle cell anemia across the lifespan. Green-shaded boxes represent phase I/II trials, while red-shaded boxes represent phase III trials. Purple boxes denote the year of regulatory approval for adults (1998) and children (2017).

ing until the frequency and severity of these events prompt treatment. Chronic organ injury begins in the first few months of life and accumulates thereafter, even if a first "crisis" has not yet manifest clinically or has occurred only occasionally. Once progressive organ injury becomes clinically apparent, in the course of waiting for overt disease manifestations, crucial opportunities for prevention and protection have been lost. Treatment of SCA should not be predicated on the occurrence of an arbitrary number or type of overt clinical events. As an analogy, one does not wait to treat a patient with diabetes mellitus until the first occurrence of diabetic ketoacidosis; rather, treatment is initiated before such events, when possible, to prevent or mitigate them and the host of other manifestations of chronic organ damage associated with diabetes. We know the natural history of this disease, and people with SCA should no longer be destined to live and experience that suffering.

Age to initiate treatment

Given that having SCA is the best indication for hydroxyurea therapy, and that there is harm in waiting for overt clinical manifestations to develop, it follows that hydroxyurea should be initiated early in life. How early? Splenic ischemia and infarction begin in the first months of life, which renders the child susceptible to sepsis from encapsulated bacteria.[15] This fact supports the rationale for newborn screening programs and antibiotic prophylaxis to prevent fatal bacterial sepsis as a life-threating consequence of progressive organ (splenic) injury.[16]

As HbF falls throughout infancy, hemolytic anemia begins and becomes progressively severe. Anemia limits oxygen-carrying capacity and leads to hyperemia, which causes organ injury such as glomerular hyperfiltration and eventual renal disease. Other organs are also affected early in life. While systematic documentation of covert organ injury in infants with SCA is challenging, brain injury, including rising intercranial arterial velocities and silent cerebral infarction, can begin in the first year of life.[17] To maximize the opportunity to prevent or mitigate organ injury, hydroxyurea should be started as early as is feasible, ideally between 6-12 months of age. Not coincidentally, this is the age when endogenous HbF falls below protective levels.[18,19]

The 2014 NHLBI sickle cell treatment guidelines contain a strong recommendation to offer treatment with hydroxyurea to all infants beginning at 9 months of age, regardless of clinical severity.[2] This starting age was based on the BABY HUG trial results, reflecting the lower end of the enrollment age range.[20] However, hydroxyurea appears to be safe and effective in infants as young as 5-6 months of age,[21,22] and strong arguments have been made to start treatment at that time.[23] We use a starting age of 6-12 months as a reasonable and feasible target. This allows sufficient time, all before initiating hydroxyurea, to confirm the genetic diagnosis; integrate the patient, family, and extended caregivers into the clinical program; establish trust and provide education; and

initiate prophylactic penicillin plus appropriate vaccinations. Starting hydroxyurea early in life allows a conversation with families easily framed around a standard progression of age-based therapeutic and preventive measures: (i) the diagnosis is made at birth; (ii) immunizations begin shortly after birth; (iii) prophylactic penicillin is started by 3 months of age; (iv) hydroxyurea is initiated by 6-12 months of age; and (v) transcranial Doppler screening starts at 2 years of age. At a time when parents and family often feel guilt and fear about caring for their newborn with a serious illness, early initiation of preventive therapy empowers and engages them in the care of their child. Using this paradigm, we have achieved essentially universal uptake of hydroxyurea for our patients within the first year of life.[24]

Of note, one SCA genotype does not need hydroxyurea therapy, specifically compound heterozygous HbS/HPFH. Without family testing or genetic testing, this benign phenotype may not be clinically distinguishable from SCA in the first year of life. Therefore, we perform genetic testing on all patients at the time of the confirmatory newborn screening test to exclude HbS/HPFH.[25] This approach confirms the genotype diagnosis early in life, usually by the time of the first clinic visit, and permits early discussions and initiation of hydroxyurea at 6-12 months of age.

Dosing strategy

Our philosophy is that every child with SCA should receive hydroxyurea at the highest daily dose *without any toxicity*. This is commonly referred to as the maximum tolerated dose (MTD), although this is a misnomer and also misleading, because it implies administering the greatest amount of drug that can physically tolerated, regardless of toxicities. Our preferred term is "optimal dose" because that correctly denotes the dose that provides maximum HbF induction with only mild myelosuppression and no clinically significant cytopenias, so it is both effective and safe. However, since published literature describes dose escalation to MTD, we retain the use of this term.

Starting dose

In a phase I/II open-label trial of hydroxyurea, 49 young adults with SCA (mean age 27.6 years) started treatment with a single daily oral dose of 10-20 mg/kg.[10] Dose escalation, by 5 mg/kg/day, was permitted every 8 weeks, based on blood counts and other signs of tolerability, to the MTD of 40 mg/kg/day. Although these doses were deemed reasonable and safe, they were also somewhat arbitrary, considering that in the initial proof-of-principle study, hydroxyurea was administered at 50 mg/kg in three divided doses for 5 days.[1] The subsequent pediatric phase I/II open-label trial used a similar starting dose (15 mg/kg/day) and dose escalation up to 35 mg/kg/day.[11] In both trials, dose escalation to the MTD was safe and well-tolerated. Following these two prospective

studies, hydroxyurea starting doses in clinical practice have been relatively stable, although more children with SCA are now started at 20-25 mg/kg/day, aiming to minimize the time needed to achieve the MTD.

Dose escalation

Even with a starting dose of 20-25 mg/kg/day and dose escalations guided by experienced clinicians, it frequently takes at least 6-9 months to achieve a stable, optimal dose. This is not ideal, since over-dosing can lead to marrow toxicity and dose interruptions, while under-dosing is associated with an inferior treatment effect. The adult phase I/II trial ended with only 26 of the original 49 participants (53%) achieving MTD at an average dose of 21.3 mg/kg/day.[10] In contrast, the pediatric HUG-KIDS trial reported that 68 of 84 (81%) enrolled participants achieved MTD, at an average dose of 25.6 mg/kg/day.[11] Healthier hematopoiesis in younger patients with less cumulative marrow damage, plus faster renal clearance of hydroxyurea, help to explain why children usually tolerate higher daily doses than do adults.

Table 1 summarizes several pivotal pediatric trials conducted over the past 25 years, most of which included step-wise dose escalation to MTD. In over 1,700 treated children with SCA, the effects of hydroxyurea were remarkably consistent, achieving average hemoglobin values of ~9 g/dL without significant cytopenias, especially neutropenia. The average

HbF values were usually 20-30% but more variable than the hemoglobin response, suggesting that the daily hydroxyurea dose is not the only determinant of HbF induction. Equally important are the target levels of myelosuppression and the absolute neutrophil count (ANC) thresholds for toxicity that lead to temporary dosing interruptions. Average HbF values of 30% were regularly achieved in studies that specified lower acceptable neutrophil targets (2.0-3.0x10⁹/L) and only withheld the hydroxyurea dose when the ANC was <1.0x10⁹/L (Table 1). Lower cytopenia thresholds allow the daily dose to be given without frequent dose interruptions, which is always preferred for families and providers.

Dose maintenance

After the optimal dose has been achieved, maintaining the same mg/kg/day dose in growing children is essential to maintain the beneficial treatment effects. Too often, children are allowed to "outgrow" their dose, because the dose is not increased commensurate with weight gain. In our practice, we adjust the absolute dose every 1-3 months for infants and toddlers to maintain the optimal mg/kg/day dose, while in older children, we typically adjust every 3-6 months. Another important consideration is that these dose increases for weight gain should occur, *even when the ANC is in the target range*. Although this can seem counterintuitive, the ANC will not decrease significantly with a

Table 1. Treatment characteristics of children with sickle cell anemia in major trials of hydroxyurea treatment.

Study	ClinicalTrial.gov	Year	N	Age in years	Dose mg	Hb g/dL	MCV fL	HbF %	ANC ×10⁹/L	ANC Target ×10⁹/L	ANC Toxicity ×10⁹/L
HUG-KIDS	NA	1999	84	9.8	25.6	9.0	100	16.9	4.8	-	2.0
HUSOFT	NA	2001	28	1.3	20.0 *	8.8	90	20.3	4.2	-	1.5
HUSOFT EXT	NA	2005	21	3.4	30.0	9.1	95	23.7	-	-	1.5-2.0
DUKE	NA	2004	106	10.3	25.9	9.5	107	19.7	3.6	-	1.5-2.0
DUKE TCD	NA	2007	37	6.8	27.9	9.4	104	23.3	-	-	1.5-2.0
TODDLER HUG	NA	2008	14	2.9	28.0	9.5	99	25.9	3.0	-	1.5
BABY HUG	NCT00006400	2011	96	1.1	20.0 *	9.1	92	22.4	4.5	-	1.25
HUSTLE	NCT00305175	2011	115	9.6	25.1	9.7	107	26.9	3.2	2.0-4.0	1.0
SWiTCH	NCT00122980	2012	60	13.0	26.2	9.0	103	29.1	3.8	2.0-4.0	1.0
SCATE	NCT01531387	2015	11	5.4	25.0	8.4	96	18.8	2.7	2.0-4.0	1.0
TWiTCH	NCT01425307	2016	60	9.7	27.4	9.1	107	24.4	3.6	2.0-4.0	1.0
NOHARM	NCT01976416	2017	104	2.2	20.0 *	8.7	88	22.9	5.2	2.0-4.0	1.0
TREAT	NCT02286154	2019	50	0.9	26.7	10.1	92	33.3	3.3	2.0	1.0
REACH	NCT01966731	2020	606	5.5	22.5	8.3	91	23.4	4.2	2.0-4.0	1.0
NDEPTH	NCT02042222	2020	68	4.8	25.0	9.8	-	27.6	2.7	1.0-3.0	1.0
NOHARM-MTD	NCT03128515	2021	93	4.8	29.5	8.7	98	30.5	3.3	2.0-4.0	1.0
EXTEND	NCT02556099	2021	43	7.7	25.4	9.6	108	29.2	2.7	2.0-4.0	1.0
SPRING **	NCT02560935	2022	111	7.0	20.0 *	9.0	91	18.2	4.4	-	1.0
SPRINT **	NCT02675790	2023	50	6.6	20.0 *	8.8	96	15.8	4.1	-	1.0
SPHERE	NCT03948867	2023	45	6.3	28.7	9.3	104	27.7	3.0	2.0-3.0	1.0
SACRED	NCT02769845	2024	57	7.0	25.7	8.9	106	30.8	4.5	2.0-3.0	1.0

*Denotes fixed-dose study design; **only data from the moderate treatment dose (20 mg/kg) arm are included. MCV: mean corpuscular volume; HbF: fetal hemoglobin; ANC: absolute neutrophil count; NA: not applicable.

modest dose increase, and failure to perform such a dose increase will inevitably lead to a lower and less effective daily dose. Table 2 summarizes our pragmatic guidance for the traditional treatment strategy of stepwise escalation to MTD, emphasizing dose adjustments based primarily on ANC and absolute reticulocyte count, but not HbF. We titrate hydroxyurea to maintain the ANC between $1.0-2.0 \times 10^9$/L and do not hold the hydroxyurea dose unless the ANC is consistently below 1.0×10^9/L.

Precision dosing

Hydroxyurea is known to have variable pharmacokinetics (PK),[26,27] so we reasoned that personalized hydroxyurea dosing was possible using PK-guided dosing. After establishing a reliable PK dosing model,[28,29] we found that a single test-dose can be used to predict the optimal daily dose. The prospective TREAT trial documented the safety and benefits of this approach, with most patients needing no dose adjustments except small increases to maintain the same mg/kg/dose with growth.[30]

Current limitations of using PK-guided hydroxyurea dosing include a lack of familiarity with the process in the broad SCA community, the logistics of sample collection with quantitative serum hydroxyurea measurements, and need for a PK dosing calculator to generate the optimal daily dose. However, efforts are underway to overcome each of these hurdles, including improved and simplified measurement of hydroxyurea concentrations, and online tools to allow the calculation of the optimal hydroxyurea dose.[31] Over time, precision dosing of hydroxyurea according to individualized PK profiles should become more widely available. This strategy can reduce drug-related toxicities and frequency of laboratory monitoring, thereby saving time, effort, and costs.

Goals of treatment

The purpose of hydroxyurea treatment should be maximized therapeutic benefits, which are pleiotropic. By using a fixed, low dose of hydroxyurea or titrating the dose to a partial clinical effect (e.g., earliest detectable reduction in painful episodes or subjective improvement), the opportunity to prevent or mitigate chronic organ injury is likely lost. As previously discussed, the overt complications of SCA considered when assessing "clinical effect" represent only a fraction of the disease morbidity. A choice to focus mainly on decreasing overt manifestations of the disease is also a choice to discount the insidious and inexorable progression of organ injury, which often becomes apparent only in adulthood. As an analogy, one would not treat hypertension with the lowest dose needed to prevent hypertensive encephalopathy; rather, treatment is titrated to normalize the condition and prevent or mitigate as much disease-related morbidity as possible. Accordingly, the goal of hydroxyurea therapy should be to minimize or prevent all SCA complications, both overt and covert, and improve quality of life.

Laboratory monitoring

Standard laboratory monitoring

Key components of laboratory testing to support hydroxyurea therapy include complete blood counts, reticulocyte counts, and measurements of HbF (Table 2), in addition to any age-appropriate laboratory tests and imaging studies for individuals with SCA. The use of blood counts to adjust the hydroxyurea dose is necessary and logical. However, maximizing the hematologic benefits of hydroxyurea through robust HbF induction will prevent or mitigate most disease manifestations, so measuring these effects over time is important. In our clinical experience, optimal hydroxyurea dosing can routinely achieve >30% HbF without clinically significant myelosuppression, especially when treatment commences early in life (Figure 2).

Although the mean corpuscular volume (MCV) is not used to adjust the dose of hydroxyurea directly, an MCV of 90-110 fL usually reflects optimized therapy (in the absence of other effects on MCV). Note that individuals with α-thalassemia trait and those with HbSβ^0 will have baseline microcytosis, so their MCV will still increase on treatment but to a lower value. Both one-gene and two-gene deletional α-thalassemia trait have significant effects on the laboratory and clinical treatment responses.[32]

Table 2. Hydroxyurea dose adjustment criteria in our clinical practice. Dosing parameters will vary in specific clinical trials, especially in low-resource settings.

Laboratory parameter	Toxicity any of the following[a]	Escalation all of the following[b]	Adjustment for weight gain all of the following[c]
Absolute neutrophil count, $\times 10^9$/L	<1.0 (consistently)	>3.0	1.0-3.0
Platelet count, $\times 10^9$/L	< 100	>150	>150
Hemoglobin (Hb), g/dL	<5.5	>6.0	>6.0
Absolute reticulocyte count, $\times 10^9$/L	<50 (if Hb <7.0)	≥50 (if Hb ≥7.0)	>100 (if Hb <8.0) or >75 (if Hb ≥8.0)

[a]Hydroxyurea should be temporarily stopped, with a possible dose reduction, if any toxicity criteria are met. [b]With traditional, stepwise dose escalation, doses are increased (usually in 5 mg/kg increments) when all escalation criteria are met. [c]Once the optimal dose is reached, ongoing adjustments for weight gain are needed to maintain the same dose in mg/kg/day as long as all criteria weight gain adjustment are met.

The main goal of hydroxyurea therapy is to increase HbF levels as high as possible. Importantly, there is no HbF threshold (e.g., 20%, 30%, or even 40%) that is sufficient or clinically dangerous and prevents dose optimization - essentially, more HbF is always better than less. Measuring %HbF by electrophoresis or high-performance liquid chromatography readily reflects the treatment response, but has limitations. First, the HbF value is not suitable for hydroxyurea dose titration; instead, the degree of myelosuppression should be used (Table 2). Second, the HbF level does not indicate that all erythrocytes contain that amount of HbF. These measurements are performed on blood lysates, so the %HbF result reflects the average amount of intracellular HbF across all erythrocytes. Finally, in untreated or under-treated SCA, only a subpopulation of erythrocytes has detectable HbF (F-cells), while most have little or no detectable HbF. This HbF expression pattern is termed heterocellular, and it is a critical factor because only F-cells have any protection against sickling.

Advanced laboratory monitoring

In contrast to electrophoresis, flow cytometry can distinguish and quantify the F-cell population (Figure 3). In our clinical practice, we routinely perform F-cell analysis together with HbF quantitation by electrophoresis at least yearly. With dose optimization, hydroxyurea therapy substantially increases both HbF level and percentage of F-cells, because there is a close but non-linear relationship between these two parameters.[33] HbF inhibits intracellular sickling, so the highest achievable F-cell fraction ("protected" red blood cell fraction) is the goal, limited only by myelotoxicity. In general, once HbF increases to 20%, the F-cell fraction will be about 60-70%. While this is laudable, a substantial portion of the erythrocytes still remain unprotected. With dose-optimization in young children with SCA, one can often achieve HbF values of 30% and an F-cell fraction of 80-90% or higher, and in many cases achieve a pancellular HbF distribution (Figure 3).[34] Notably, these HbF values are similar to stated goals of current gene therapies for SCA, but do require long-term therapy at optimized doses and excellent medication adherence.

The amount of HbF within each F-cell is also important because it determines the degree of protection against sickling. An F-cell with ≥10 pg of HbF is thought to be fully protected.[35] Calculation of the mean HbF per F-cell (F/F-cell) is straightforward, but accurate single-cell measurements of F/F-cell that reflect the range of F/F-cell across the entire F-cell population would give the clearest insight into the degree of red blood cell protection (Figure 3C). Such assays are not yet widely available, but are likely to best reflect therapeutic effect. While the goal of hydroxyurea therapy is the highest possible HbF concentration, this should si-
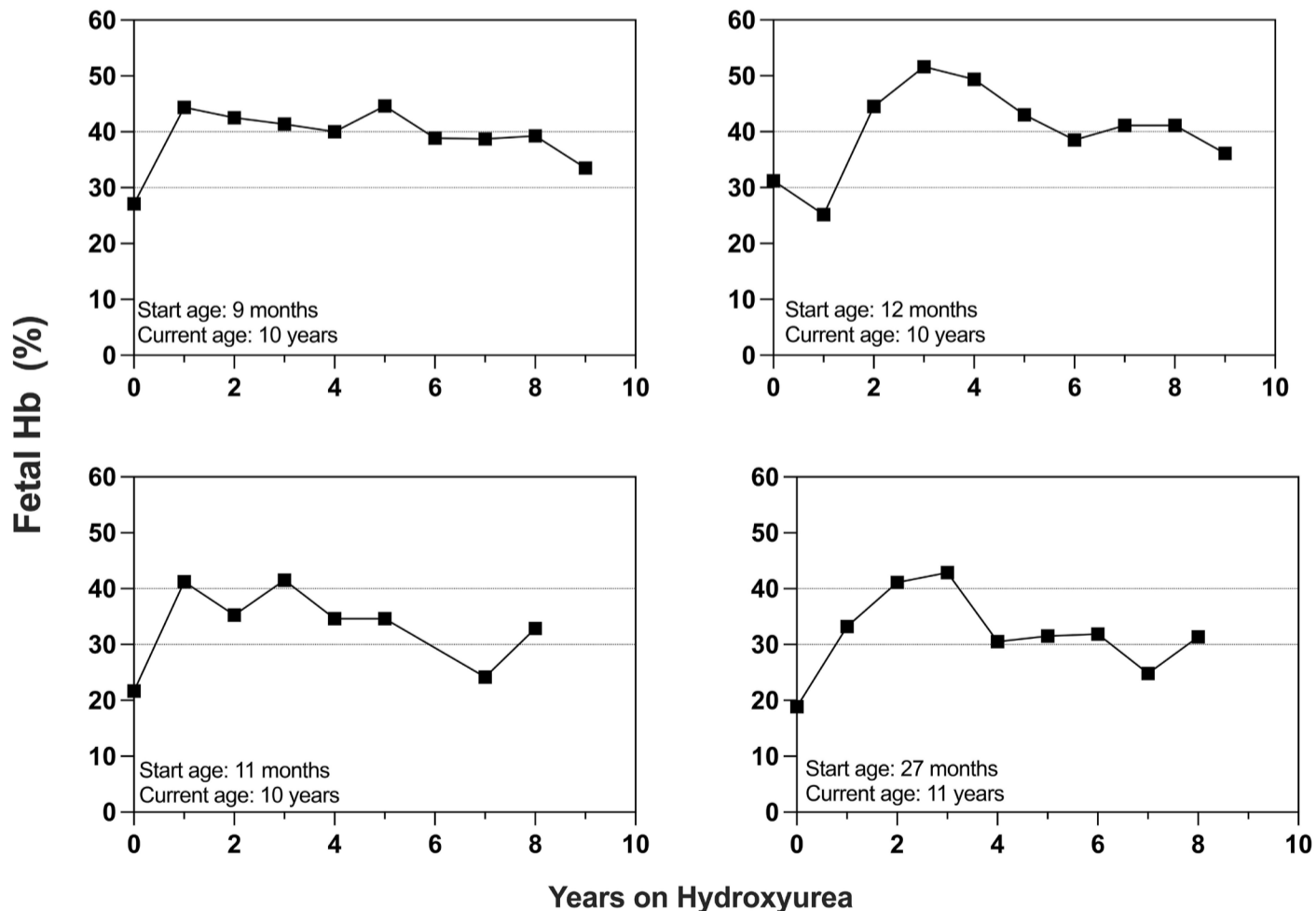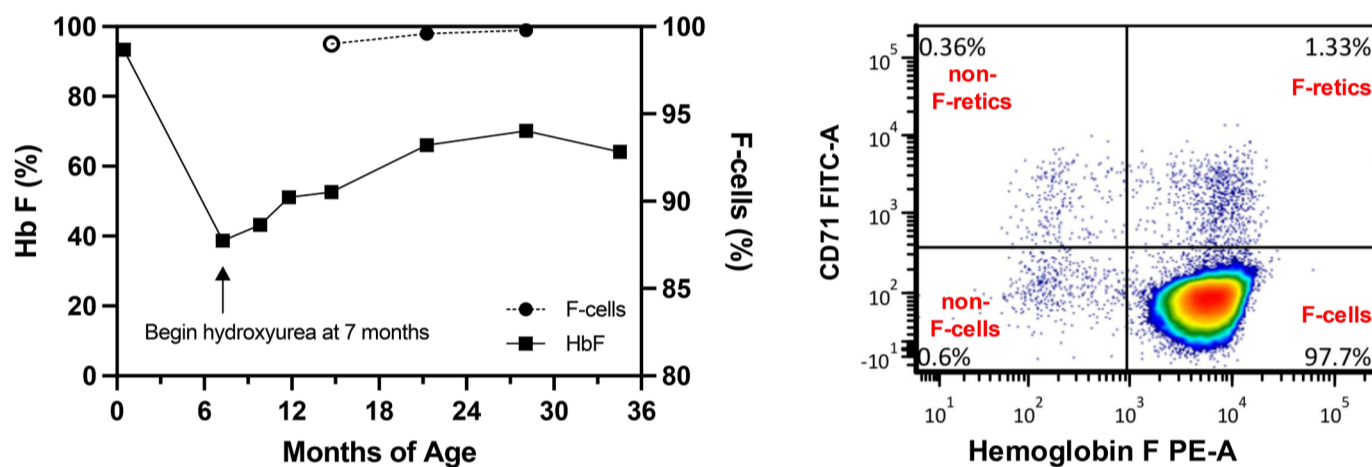

Figure 2. Sustained high values of fetal hemoglobin in children treated from infancy. Four representative examples are provided from over 40 children who initiated hydroxyurea before 3 years of age (9-27 months) who have had sustained treatment responses of fetal hemoglobin (HbF) >30% for 8-9 years. Reference lines are shown for HbF values of 30% and 40%. Note that some patients have occasional struggles with medication adherence, indicated by transient declines in HbF (e.g., the patients shown in the bottom two graphs at 7 years of therapy), which our medical and psychosocial teams can successfully address.

A Flow cytometric classification and quantitation of F-cells in sickle cell anemia.

	Patient 1	Patient 2	Patient 3	Patient 4
HbF:	<0.1%	23%	28%	42%
F-cells:	0.6%	22%	74%	99%
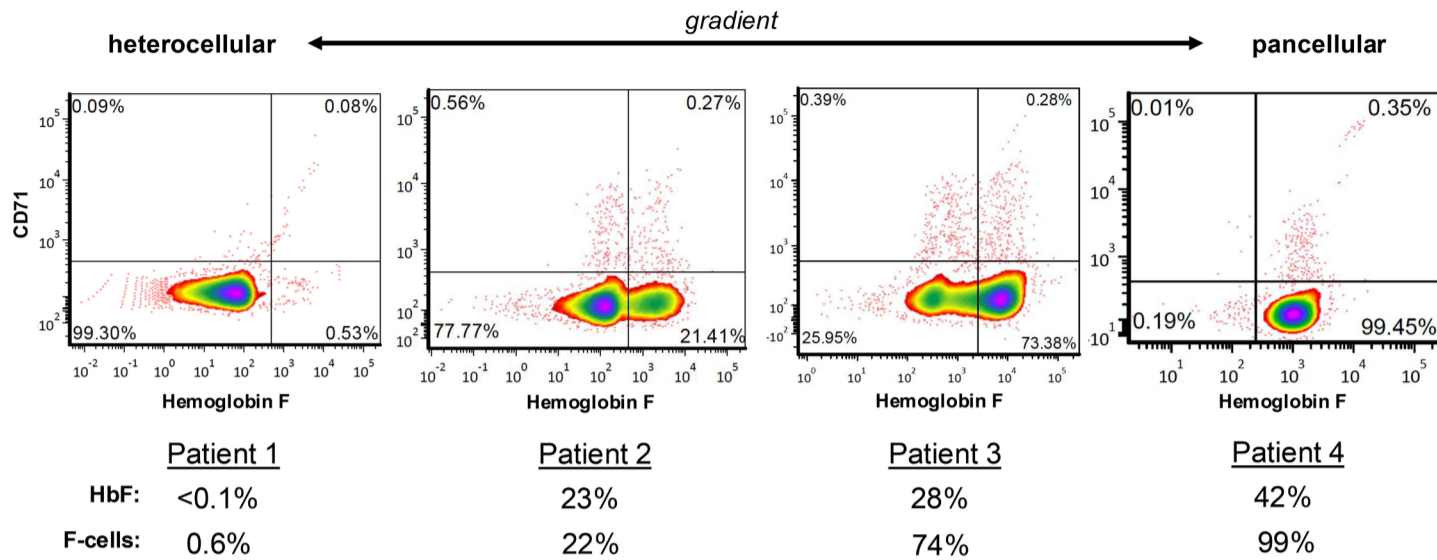
B Initiation of hydroxyurea in infancy with robust HbF induction and pancellular distribution.

C Distribution of single cell measurements of F/F-cell with hydroxyurea therapy.

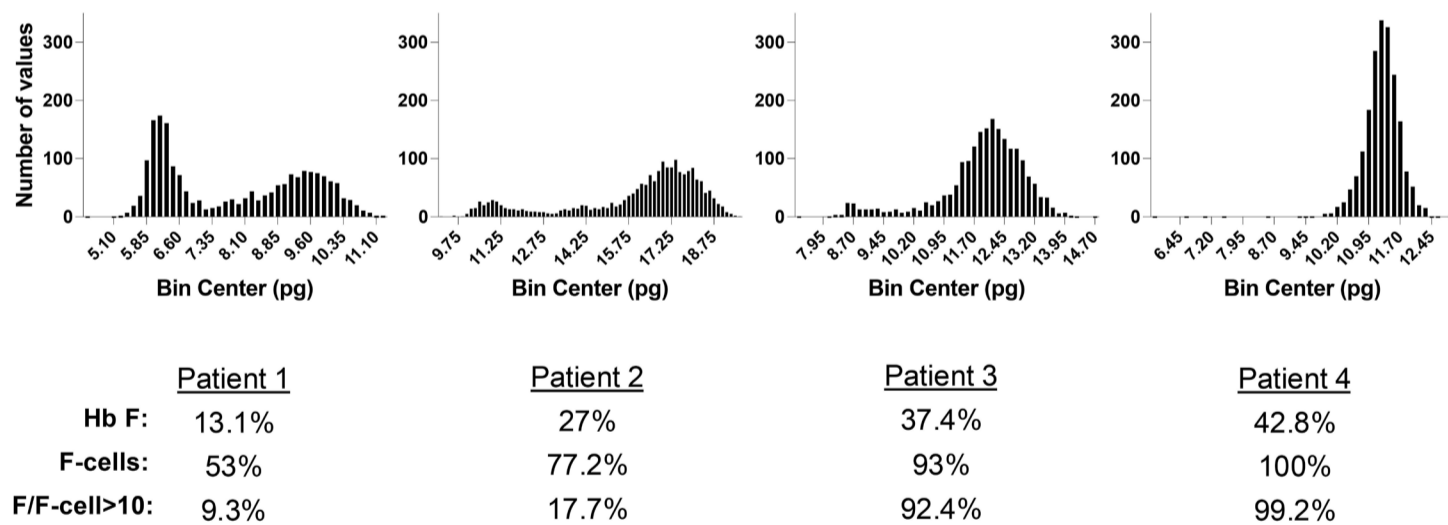|	Patient 1	Patient 2	Patient 3	Patient 4
Hb F:	13.1%	27%	37.4%	42.8%
F-cells:	53%	77.2%	93%	100%
F/F-cell>10:	9.3%	17.7%	92.4%	99.2%

Figure 3. F-cell analysis for monitoring hydroxyurea therapy. (A) The gradient of F-cell (red blood cells containing fetal hemoglobin [HbF]) expression across four individuals from heterocellular to pancellular. Patient 1 does not have sickle cell anemia (SCA); patients 2-4 have SCA and different levels of HbF induction with hydroxyurea therapy. Note the sizable difference in F-cell fraction between patients 2 and 3 even though both have similar amounts of HbF. Patient 4 is a young child who initiated phamacokinetically-optimized hydroxyurea therapy by 9 months of age. Each graph shows singlet red blood cell events classified by HbF expression (x-axis) and CD71 expression (y-axis). The two right quadrants of each graph indicate F-cells. The top two quadrants of each graph indicate immature reticulocytes. The total F-cell fraction is the sum of the right upper quadrant (F-retics: circulating reticulocytes containing HbF) and right lower quadrant (mature F-cells). (B) Data for an infant who began hydroxyurea at 7 months of age. The left graph shows HbF values (left y-axis) and F-cell fraction (right y-axis) before and during hydroxyurea therapy. The open circle (o) indicates the timing of the complete F-cell study shown in the right graph, demonstrating pancellular expression of HbF that recapitulates the HbF expression in compound heterozygosity for HbS and gene-deletion hereditary persistence of HbF (HPFH). (C) Histograms of single-cell estimates of mean HbF per F-cell (F/F-cell) (pg) in the F-cell populations of four individuals. Patients 1-3 have SCA, are treated with hydroxyurea, and have increasing amounts of HbF induction. Patient 4 has compound heterozygosity for HbS and gene-deletion HP-FH. Patients 3 and 4 have >90% of F-cells with >10 pg HbF, indicating that their red blood cells are fully protected from sickling. F-cells: red blood cells containing HbF; F-retics: circulating reticulocytes containing HbF; F/F-cell: mean HbF per F-cell.

multaneously achieve the highest F-cell fraction with the highest F/F-cell content.

The hematologic benefits of robust HbF induction include alleviation of the hemolytic anemia with decreased reticulocytosis, which are readily measurable effects. However, to better demonstrate the manifold effects of hydroxyurea, additional laboratory techniques are available, including automated quantitation of dense cells and rheological assessments including whole blood viscosity and ektacytometry, plus point-of-sickling using an oxygen gradient and measurements of splenic function (Figure 4).[36,37] These assays are not yet integrated into clinical practice but should be increasingly considered, because so many manifestations of SCA are covert (not outwardly obvious or clinically apparent, unlike painful episodes and acute chest syndrome) and not fully reflected by common laboratory tests. Table 3 summarizes foundational, basic, and expanded testing as part of hydroxyurea monitoring.

Risks and toxicities

Because the intention of hydroxyurea therapy is to achieve mild myelosuppression, the main potential risks of treatment are cytopenias. Indeed, leukocyte and platelet counts will be lower than pre-treatment values. These cells can form multicellular aggregates with adhesive sickle erythrocytes to promote vaso-occlusion, so their reduction is likely a therapeutic mechanism beyond HbF induction. There is no evidence that hydroxyurea increases the risk of infection because of the intended modest reduction in ANC. Indeed, there is evidence that the risk of infections may actually be decreased with hydroxyurea therapy at optimal dosing in low-resource settings in sub-Saharan Africa.[38]

The risk of under-treating this serious and morbid disease should be balanced against prescribers' fears, often unnecessarily grave, about mild-moderate neutropenia. Certainly, if there is severe neutropenia (ANC <0.5x10⁹/L), then hydroxyurea should be stopped temporarily, and the usual precautions and considerations about neutropenia apply. The most common and recurring scenario is that ANC falls to 0.75-1.0x10⁹/L, usually because of intercurrent infections. Rather than stopping effective treatment, it is reasonable to continue the hydroxyurea without a dose reduction with repeat blood counts in 1-2 weeks. If there is persistent moderate neutropenia, then a dose reduction may be needed (Table 2).

Initiation of hydroxyurea early in life delays splenic involution. While the preservation of splenic tissue is immunologically beneficial, it can result in chronic splenomegaly and may extend the risk period for splenic sequestration. Some children have chronically lower blood counts with splenomegaly.[39] This is due to hypersplenism and not drug toxicity. For these children, dose optimization can be challenging, and patient-specific toxicity criteria may be considered (e.g.,

continue hydroxyurea unless ANC <0.8x10⁹/L or platelets <80x10⁹/L).

Many individuals with SCA will also have the common, normal Duffy-null associated neutrophil count (DANC). DANC does not increase the risk of bacterial infection but often lowers the ANC below neutropenia thresholds derived from
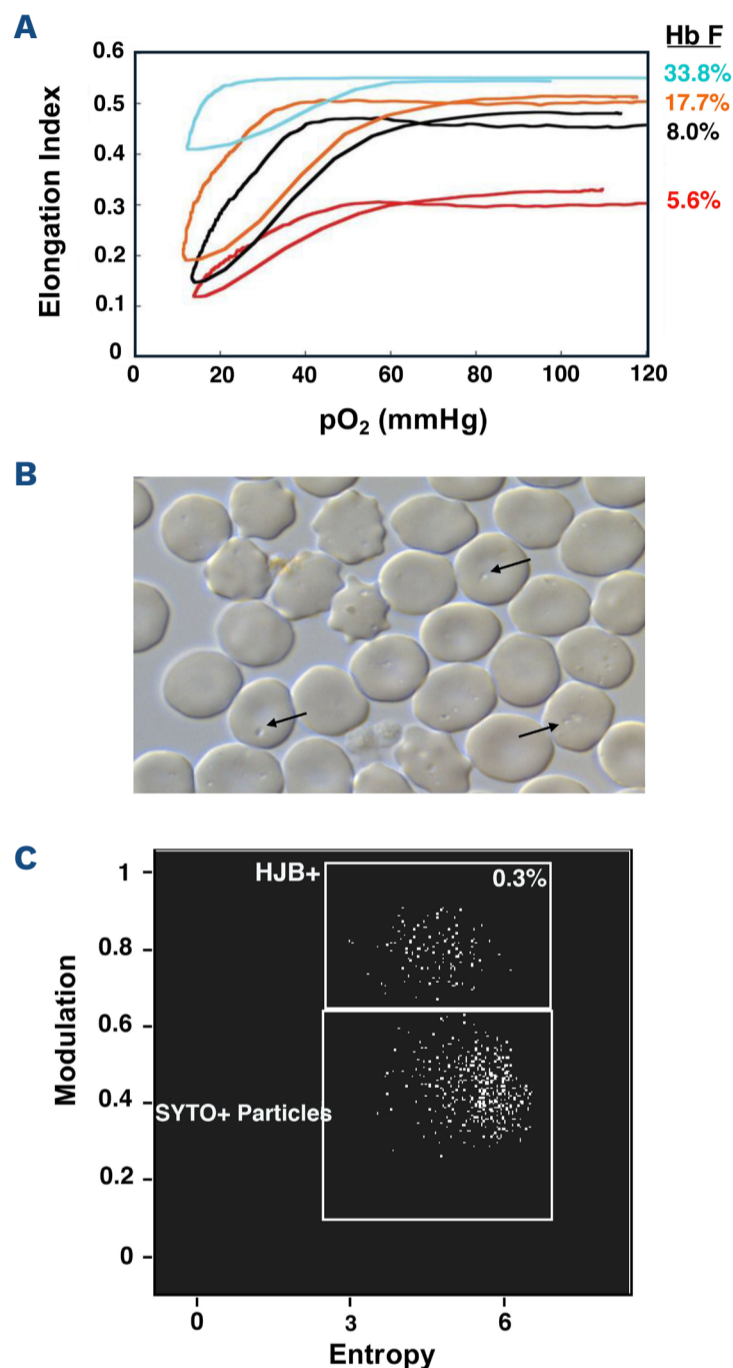

Figure 4. Advanced laboratory testing for monitoring of hydroxyurea therapy. (A) Oxygen-gradient ektacytometry depicts the relationship between red blood cell (RBC) deformability (elongation index), as an index of onset of sickling, and the partial pressure of O_2 (pO2). Patients who have higher amounts of fetal hemoglobin (HbF) expression (identified on the right of the graph) have a progressively delayed onset of sickling that occurs at successively lower pO2. (B) Phase-contrast microscopy demonstrates pocked or pitted RBC, which correspond to submembrane inclusions that are normally removed by the spleen (examples shown by black arrows). The RBC pit count is a semiquantitative measurement of splenic function. (C) A novel flow cytometric quantitative method is illustrated; it is designed to detect and enumerate Howell-Jolly bodies (HJB), which are nuclear remnants that are normally removed by the spleen and increased in number in hyposplenic states. Here, RBC are stained with double-stranded DNA-avid dye, and morphological characteristics (size, shape) of the intra-RBC DNA inclusions are used to identify HJB.

Table 3. Foundational, basic, expanded, and research laboratory and clinical testing for hydroxyurea therapy in children with sickle cell anemia.

Test	Frequency	Notes
Foundational tests		
β-globin gene (*HBB*) sequencing and copy number variation analysis of the β-globin gene cluster	Once	To ensure the correct diagnosis, especially for infants to exclude S/HPFH before starting hydroxyurea
α-globin gene (*HBA1*, *HBA2*) sequencing and copy number variation analysis of the α-globin gene cluster	Once	Concomitant α-thalassemia is a disease-modifier that also lowers the MCV, which is used to monitor hydroxyurea
ACKR1: c.-67 T/C polymorphism status (rs2814778)	Once	Diagnose Duffy null-associated neutrophil count, which may influence hydroxyurea dosing
Basic tests		
Complete blood count, Differential leukocyte count Reticulocyte count, HbF quantitation	Every 3-4 months	Minimum group of key tests to monitor hydroxyurea therapy: benefits and toxicity
Peripheral blood morphology	Every 3-4 months	If feasible, reviewing morphological improvements with patients and families promotes hydroxyurea adherence
Metabolic panel (renal, hepatic)	Every 6-8 months	Evaluation for very rare toxicities of hydroxyurea, but mainly to monitor the effects of SCA on end-organs
Expanded tests		
F-cell analysis	Every 6-12 months	Quantify the cellular distribution of HbF across RBC with the goal of approaching and maintaining a near-pancellular distribution.
F/F-cell analysis	Not standardized or research-based	To quantify the amount of HbF in F-cells with the goal of therapy to achieve a high F/F-cell
Ektacytometry (osmotic and oxygen gradient)	Not standardized or research-based	To monitor RBC deformability, RBC hydration, and point of sickling
Dense cells	Not standardized or research-based	Automated determination provided by ADVIA hematology analyzers without density gradient separation
Whole blood viscosity	Not standardized or research-based	To monitor for improvement in the hematocrit-to-viscosity ratio
RBC pit count or flow cytometric Howell-Jolly body quantitation	Not standardized or research-based	To assess preservation of splenic dysfunction
Proteinuria, albuminuria, GFR measurement	Not standardized	To assess renal function
Brain MRI/MRA	Not standardized	To assess silent infarction and vasculopathy
Cardiac MRI	Research-based	To assess myocardial fibrosis

HbS/HPFH: pancellular (gene-deletion) hereditary persistence of fetal hemoglobin; MCV: mean corpuscular volume; SCA: sickle cell anemia; HbF: fetal hemoglobin; F-cells: red blood cells containing HbF; F/F-cell: mean HbF per F-cell; GFR: glomerular filtration rate; MRI: magnetic resonance imaging; RBC: red blood cells; MRA: magnetic resonance angiography.

individuals of European ancestry. The impact of DANC on hydroxyurea therapy in SCA has not been studied prospectively, but published data for African-American children do not suggest a substantial effect.[40,41]

Long-term risks of hydroxyurea exposure are relevant because infants and toddlers will receive hydroxyurea for decades. Early concerns about mutagenicity and leukemogenicity associated with hydroxyurea have not been realized.[42] However, several cases of malignant transformation after gene therapy have been published,[43,44] with earlier onset and higher incidence than leukemia in SCA prior to hydroxyurea treatment.[45-47] In one recent study, hydroxyurea was not associated with increased clonal hematopoiesis,[48] although further research is needed. Serial analysis of genomic DNA from children and adults for acquired somatic mutations is needed, to help assure the safety of early treatment initiation and long-term exposure to hydroxyurea.

Effects of hydroxyurea on fertility are extremely important to understand but challenging to disentangle from the effects of untreated SCA on gonadal function. Adults in the phase III MSH trial had ~100 known offspring with no clear deleterious treatment effects.[49] Analysis of testicular tissue identified no adverse effects on spermatogonia in prepubertal males,[50,51] although hypospermia and even azoospermia have been reported in males with SCA, a result of testicular damage from the underlying disease, hydroxyurea exposure, or both.[52-54] Ovarian reserve may be diminished in SCA, especially when assessed by hormonal surrogate measures.[55] However, a large retrospective study showed no hydroxyurea-related reduction in ovarian reserve by direct measurement of ovarian follicle density, leading to the conclusion that fertility preservation measures were not a prerequisite for treatment initiation.[56] Finally, the risks of hydroxyurea treatment during pregnancy and lactation have not been fully defined. No evidence of hydroxyurea teratogenicity in SCA has been documented to date, and its theoretical risks during pregnancy must be balanced by the predictable deleterious effects of stopping an effective treatment. During lactation, hydroxyurea appears to

be transmitted in very small amounts through breastmilk,[57] well below World Health Organization thresholds for drug safety in human breastmilk.[58]

Other disease-modifying treatments

There have been four US Food and Drug Administration-approved disease-modifying pharmacotherapies for SCA: hydroxyurea, L-glutamine, crizanlizumab, and voxelotor. Although L-glutamine and crizanlizumab can decrease the frequency of painful events, they do not improve blood counts for relief of anemia and inflammation.[59,60] Conversely, voxelotor could improve anemia, but did not appreciably decrease the frequency of pain.[61] Voxelotor was recently withdrawn from the market due to concerns about increased mortality and vaso-occlusive complications, but few details are currently publicly available.

In contrast to these newer agents, hydroxyurea at MTD reduces almost all manifestations of SCA and has been reported to decrease mortality in both high- and low-resource settings.[62-66] Therefore, dose-optimized hydroxyurea should be the backbone of pharmacotherapy for SCA to which additional agents can be added. Although combinatorial therapies have not been properly evaluated in randomized trials, for patients who still have painful events or marked anemia despite dose-optimized hydroxyurea, we offer additive therapy based on indication (pain, anemia) and patient preference (oral, intravenous). One challenge with additive therapy is how to avoid disruptions to adherence with hydroxyurea therapy, as "too many medications" is a potential barrier to adherence.

In the modern era with increasing options for curative and transformative therapies, optimized disease-modifying pharmacotherapy remains vital - from infancy until transplantation or genetic therapy is performed. Individuals with less cumulative organ injury will likely tolerate these procedures better and have better long-term outcomes.

Some benefits and risks of hydroxyurea have not been established by the highest possible quality of clinical evidence, specifically as the primary endpoint of a randomized, placebo-controlled trial. However, given several well-substantiated benefits and the broad clinical use of hydroxyurea, many such additional trials may now be unethical to perform. Therefore, we need to incorporate other levels of clinical evidence, derived from open-label trials and epidemiological studies with appropriate insight and caution, especially with reference to the well-documented natural history of SCA to guide our current practice.

Patient support and infrastructure

The prescription of hydroxyurea at an optimized dose is a necessary first step, but it is not sufficient. There are many barriers to optimal treatment, including challenges with medication adherence, with reported rates of adherence to hydroxyurea ranging from 12% to 100% in children.[67] Like any chronic and often apparently quiescent disease, there will be barriers and interruptions to chronic medication therapy that should be expected and mitigated with dedicated infrastructure. Given that a subset of barriers are social challenges, at minimum, a dedicated social work program is needed. We also recommend dedicated psychologists to improve adherence and self-management.

Hydroxyurea increases the MCV and HbF, so displaying long-term trends in these measures is useful to understand medication effects (dosing, adherence) over time and a helpful way to reinforce medication adherence, along with monitoring pharmacy refills.[68] Reviewing morphological improvements on the blood smear (comparison of before and during hydroxyurea therapy) with patients and families can also promote adherence.

Consistency in prescribing practices across the medical team is needed, supported by institutional guidelines. All members of the team (nurses, nurse practitioners, social workers, etc.) need to know the importance of optimized disease-modifying therapy to provide regular reinforcement and support patients who have this severe chronic disease. A population-level quality improvement effort is also needed to monitor hydroxyurea prescription rate, HbF levels, and clinical outcomes.[24]

Treatment for other genotypes

Although homozygous HbSS is the most common and severe form of sickle cell disease, approximately 30-40% of affected African-Americans have another, compound heterozygous genotype, including sickle-Hb C disease (HbSC), HbSβ⁰, sickle-β⁺-thalassemia (HbSβ⁺), HbSD-Punjab, or HbSO-Arab. As noted earlier, HbSβ⁰ and HbSS have similar phenotypes and respond equivalently to hydroxyurea (although the absolute MCV value will be different). In contrast, there are scant and mostly anecdotal data on the use of hydroxyurea for other genotypes.

Individuals with HbSC or HbSβ⁺ have an overall milder phenotype than those with HbSS, but can have clinical severity that warrants hydroxyurea treatment. In our experience, the laboratory and clinical benefits are less consistent than with SCA. The largest retrospective review of HbSC and hydroxyurea included 133 children and adults who were treated with an average dose of 20 mg/kg/day.[69] There were the expected increases in HbF and MCV, and decreases in ANC and absolute reticulocyte count, but the hemoglobin concentration remained relatively stable, allaying concerns about hyperviscosity. The rate of painful events decreased after 6-12 months of hydroxyurea, especially among patients >15 years of age. Transient cytopenias occurred in 22% of cases.

A formal prospective phase I/II trial of hydroxyurea in HbSC is currently underway in Ghana. Prospective Identification of Variables as Outcomes for Treatment (PIVOT, PACTR 202108893981080) is a double-blind, placebo-controlled, randomized trial in which both children and adults are being treated. The PIVOT trial baseline data are now published,[70] and primary study results are expected in early 2025. PIVOT results should inform the design and execution of a formal phase III trial of hydroxyurea in HbSC, which is needed before widespread use can be recommended.

Use in low-resource settings

The global burden of SCA is greatest in low-resource countries and especially in sub-Saharan Africa, so it is necessary to determine the feasibility and effectiveness of hydroxyurea use in these settings. Over the past 10 years, several prospective trials in Africa have demonstrated the safety and benefits of hydroxyurea. The NOHARM trial was a double-blind, placebo-controlled, randomized trial using fixed-dose hydroxyurea (20 mg/kg/day) for very young children with SCA in Uganda. The primary endpoint was malaria, and no trend toward an increased number or severity of infections was observed.[71] Additional laboratory and clinical benefits of hydroxyurea were similar to those in US-based trials. The NOHARM MTD trial further randomized this cohort to fixed-dose hydroxyurea (20 mg/kg/day) or MTD. MTD (~30 mg/kg/day) was strikingly superior to fixed-dose hydroxyurea; a significantly higher proportion of children (86% vs. 37%) achieved the composite laboratory endpoint of HbF >20% or hemoglobin >9 g/dL, and there were also significant decreases in painful vaso-occlusive events and other sickle-related morbidity.[65]

Similarly, the REACH trial (NCT01966731) provided open-label hydroxyurea to a large cohort of children with SCA across four countries in sub-Saharan Africa: Angola, Democratic Republic of Congo, Kenya, and Uganda.[72] Hydroxyurea was safe and feasible in these settings, with tolerable rates of cytopenia allowing dose escalation to MTD. In this large cohort, the clinical benefits of hydroxyurea at MTD included reduced rates of painful events, malaria, infections, transfusions, and other key adverse outcomes with over 4,000 patient-years of follow-up.[66]

Additional clinical trials in sub-Saharan Africa aimed to reduce the risk of stroke. In children identified by transcranial Doppler screening as being at high risk, hydroxyurea has been used at differing doses to lower transcranial Doppler velocities and overt stroke rate. The SPHERE trial documented the benefits of hydroxyurea at MTD in Tanzanian children with conditional and abnormal transcranial Doppler velocities, with an average decrease of 33 cm/sec,[73] similar to the effects observed in the EXTEND trial conducted in Jamaica.[74] Trials in northern Nigeria have documented neuroprotective benefits using lower hydroxyurea doses.[75,76]

There is great potential for the implementation of PK-guided precision dosing of hydroxyurea in low-resource settings, because children might require less frequent monitoring and surveillance. Creative methods for the measurement of hydroxyurea concentrations and calculation of optimal doses are currently under investigation in three prospective clinical trials in sub-Saharan Africa (SHPERE, NCT03948867; ADAPT, NCT05662098; and REACH, NCT06171217).

Summary

In this modern era with rapid advances in the treatment of SCA, whether pharmacological or genetic, we must remain mindful of the safe and broadly effective disease-modifying therapy provided by hydroxyurea - now with the express goal of preventing chronic organ damage that can develop while awaiting genetic or curative therapy. Simultaneously, we must modernize our use of hydroxyurea and not continue to prescribe it as we did several decades ago. The greater risk, by far, is under-treatment of a severe and unforgiving disease. Hydroxyurea should be offered much more widely and at optimal doses, ideally using precision dosing. As we have opined about hydroxyurea: do not leave for tomorrow what you can do today;[77] prescribe it early and often.[78]

Notes added during proofing
After the submission of this manuscript, Pfizer withdrew voxelotor from worldwide markets on September 25, 2024. The primary results of the PIVOT trial were published on December 8, 2024 at https://pubmed.ncbi.nlm. nih.gov/39647172/

References

1. Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. J Clin Invest. 1984;74(2):652-656.

2. Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. JAMA. 2014;312(1):1033-1048.

3. Watson J. The significance of the paucity of sickle cells in newborn Negro infants. Am J Med Sci. 1948;215(4):419-423.

4. Perrine RP, Pembrey ME, John P, Perrine S, Shoup F. Natural history of sickle cell anemia in Saudi Arabs. A study of 270 subjects. Ann Intern Med. 1978;88(1):1-6.

5. Jacob GF, Raper AB. Hereditary persistence of foetal haemoglobin production, and its interaction with the sickle cell trait. Br J Haematol. 1958;4(2):138-149.

6. Bailey K, Morris JS, Thomas P, Serjeant GR. Fetal haemoglobin and early manifestations of homozygous sickle cell disease. Arch Dis Child. 1992;67(4):517-520.

7. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994;330(23):1639-1644.

8. Ley TJ, DeSimone J, Noguchi CT, et al. 5-azacytidine increases gamma-globin synthesis and reduces the proportion of dense cells in patients with sickle cell anemia. Blood. 1983;62(2):370-380.

9. Papayannopoulou T, Torrealba de Ron A, Veith R, Knitter G, Stamatoyannopoulos G. Arabinosylcytosine induces fetal hemoglobin in baboons by perturbing erythroid cell differentiation kinetics. Science. 1984;224(4649):617-619.

10. Charache S, Dover GJ, Moore RD, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. Blood. 1992;79(10):2555-2565.

11. Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Blood. 1999;94(5):1550-1554.

12. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med. 1995;332(20):1317-1322.

13. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomized, controlled trial (BABY HUG). Lancet. 2011;377(9778):1663-1672.

14. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood. 2010;115(26):5300-5311.

15. Pearson HA, Spencer RP, Cornelius EA. Functional asplenia in sickle-cell anemia. N Engl J Med. 1969;281(17):923-926.

16. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. N Engl J Med. 1986;314(25):1593-1599.

17. Wang WC, Pavlakis SG, Helton KJ, et al. MRI abnormalities of the brain in one-year-old children with sickle cell anemia. Pediatr Blood Cancer. 2008;51(5):643-646.

18. Maier-Redelsperger M, Noguchi CT, de Montalembert M, et al. Variation in fetal hemoglobin parameters and predicted hemoglobin S polymerization in sickle cell children in the first two years of life: Parisian Prospective Study on Sickle Cell Disease. Blood. 1994;84(9):3182-3188.

19. Marcus SJ, Ware RE. Physiologic decline in fetal hemoglobin parameters in infants with sickle cell disease: implications for pharmacological intervention. J Pediatr Hematol Oncol. 1999;21(5):407-411.

20. Thompson BW, Miller ST, Rogers ZR, et al. The pediatric hydroxyurea phase III clinical trial (BABY HUG): challenges of study design. Pediatric Blood Cancer. 2010;54(2):250-255.

21. Wang WC, Wynn LW, Rogers ZR, Scott JP, Lane PA, Ware RE. A two-year pilot trial of hydroxyurea in very young children with sickle-cell anemia. J Pediatr. 2001;139(6):790-796.

22. Schuchard SB, Lissick JR, Nickel A, et al. Hydroxyurea use in young infants with sickle cell disease. Pediatr Blood Cancer. 2019;66(7):e27650.

23. Elenga N, Kayemba-Kay's S, Nacher M, Archer N. A call to start hydroxyurea by 6 months of age and before the advent of sickle cell disease complications. Pediatric Blood Cancer. 2022;69(2):e29423.

24. Karkoska K, Todd K, Niss O, et al. Implementation of near-universal hydroxyurea uptake among children with sickle cell anemia: a single-center experience. Pediatr Blood Cancer. 2021;68(6):e29008.

25. Shook LM, Haygood D, Quinn CT. Clinical utility of the addition of molecular genetic testing to newborn screening for sickle cell anemia. Front Med. 2021;8:734305.

26. Yan JH, Ataga K, Kaul S, et al. The influence of renal function on hydroxyurea pharmacokinetics in adults with sickle cell disease. J Clin Pharmacol. 2005;45(4):434-445.

27. de Montalembert M, Bachir D, Hulin A, et al. Pharmacokinetics of hydroxyurea 1,000 mg coated breakable tablets and 500 mg capsules in pediatric and adult patients with sickle cell disease. Haematologica. 2006.91(12):1685-1688.

28. Ware RE, Despotovic JM, Mortier NA, et al. Pharmacokinetics, pharmacodynamics, and pharmacogenetics of hydroxyurea treatment for children with sickle cell anemia. Blood. 2011:118(18):4985-4991.

29. Dong M, McGann PT, Mizuno T, Ware RE, Vinks AA. Development of a pharmacokinetic-guided dose individualization strategy for hydroxyurea treatment in children with sickle cell anaemia. Br J Clin Pharmacol. 2016:81(4):742-752.

30. McGann PT, Niss O, Dong M, et al. Robust clinical and laboratory response to hydroxyurea using pharmacokinetic-guided dosing for young children with sickle cell anemia. Am J Hematol. 2019:94(8):871-879.

31. Power-Hays A, Dong M, Punt N, et al. Rationale, development, and validation of HdxSim, a clinical decision support tool for model-informed precision dosing of hydroxyurea for children with sickle cell anemia. Clin Pharmacol Ther. 2024;116(3):670-677.

32. Darbari DS, Nouraie M, Taylor JG, Brugnara C, Castro O, Ballas SK. Alpha-thalassaemia and response to hydroxyurea in sickle cell anaemia. Eur J Haematol. 2014;92(4):341-345.

33. Marcus SJ, Kinney TR, Schultz WH, O'Branski EE, Ware RE. Quantitative analysis of erythrocytes containing fetal hemoglobin (F cells) in children with sickle cell disease. Am J Hematol. 1997;54(1):40-46.

34. Quinn CT, Niss O, Dong M, et al. Early initiation of hydroxyurea (hydroxycarbamide) using individualised, pharmacokinetics-guided dosing can produce sustained and nearly pancellular expression of fetal haemoglobin in children with sickle cell anaemia. Br J Haematol. 2021;194(3):617-625.

35. Steinberg MH, Chui DHK, Dover GH, Sebastiani P, Alsultan A. Fetal hemoglobin in sickle cell anemia: a glass half full? Blood. 2014;123(4):481-485.

36. Sadaf A, Quinn CT, Korpik JB, et al. Rapid and automated quantitation of dense red blood cells: a robust biomarker of hydroxyurea treatment response. Blood Cells Mol Dis. 2021;90:102576.

37. Sadaf A, Seu KG, Thaman E, et al. Automated oxygen gradient ektacytometry: a novel biomarker in sickle cell anemia. Front Physiol. 2021;12:636609.

38. Namazzi R, Bond C, Conroy AL, et al. Hydroxyurea reduces infections in children with sickle cell anemia in Uganda. Blood. 2024;143(14):1425-1428.

39. Allali S, Galacteros F, Oevermann L, et al. Hydroxyurea is associated with later onset of acute splenic sequestration crisis in sickle cell disease: lessons from the European Sickle Cell Disease

Cohort-Hydroxyurea (ESCORT-HU) study. Am J Hematol. 2024;99(4):555-561.

40. Schaefer BA, Flanagan JM, Alvarez OA, et al. Genetic modifiers of white blood cell count, albuminuria and glomerular filtration rate in children with sickle cell anemia. PLoS One. 2016;11(10):e0164364.

41. Oladipupo F, Stanek J, Walden J, et al. Prevalence of Duffy null and its impact on hydroxyurea in young children with sickle cell disease in the United States. Pediatr Blood Cancer. 2024;71(6):e30945.

42. Ware RE, Dertinger SD. Absence of hydroxyurea-induced mutational effects supports higher utilisation for the treatment of sickle cell anemia. Br J Haematol. 2021;194(2):252-266.

43. Goyal S, Tisdale J, Schmidt M, et al. Acute myeloid leukemia case after gene therapy for sickle cell disease. N Engl J Med. 2022;386(2):138-147.

44. Kanter J, Thompson AA, Pierciey FJ Jr, et al. Lovo-cel gene therapy for sickle cell disease: treatment process evolution and outcomes in the initial groups of the HGB-206 study. Am J Hematol. 2023;98(1):11-22.

45. Seminog OO, Ogunlaja OI, Yeates D, Goldacre MJ. Risk of individual malignant neoplasms in patients with sickle cell disease: English national record linkage study. J R Soc Med. 2016;109(8):303-309.

46. Brunson A, Keegan THM, Bang H, Mahajan A, Paulukonis S, Wun T. Increased risk of leukemia among sickle cell disease patients in California. Blood. 2017;130(13):1597-1599.

47. Schultz WH, Ware RE. Malignancy in patients with sickle cell disease. Am J Hematol. 2003;74(4):249-253.

48. Liggett LA, Cato LD, Weinstock JS, et al. Clonal hematopoiesis in sickle cell disease. J Clin Invest. 2022;132(4):e156060.

49. Ballas SK, McCarthy WF, Guo N, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. J Natl Med Assoc. 2009;101(10):1046-1051.

50. Joseph L, Jean C, Manceau S, et al. Effect of hydroxyurea exposure before puberty on sperm parameters in males with sickle cell disease. Blood. 2021;137(6):826-829.

51. Gille AS, Pondarre C, Dalle JH. Hydroxyurea does not affect the spermatogonial pool in prepubertal patients with sickle cell disease. Blood. 2021;137(6):856-859.

52. Berthaut I, Guignedoux G, Kirsch-Noir F, et al. Influence of sickle cell disease and treatment with hydroxyurea on sperm parameters and fertility of human males. Haematologica. 2008;93(7):988-933.

53. DeBaun MR. Hydroxyurea therapy contributes to infertility in adult men with sickle cell disease. Expert Rev Hematol. 2014;7(6):767-773.

54. Sahoo LK, Kullu BK, Patel S, et al. Study of seminal fluid parameters and fertility of male sickle cell disease patients and potential impact of hydroxyurea treatment. J Assoc Physicians India. 2017;65(6):22-25.

55. Pecker LH, Hussain S, Mahesh J, Varadhan R, Christianson MS, Lanzkron S. Diminished ovarian reserve in young women with sickle cell anemia. Blood. 2022;139(7):1111-1115.

56. Diesch-Furlanetto T, Sanchez C, Atkinson A, et al. Impact of hydroxyurea on follicle density in patients with sickle cell disease. Blood Adv. 2024;8(19):5227-5235.

57. Ware RE, Marahatta A, Ware JL, McElhinney K, Dong M, Vinks AA. Hydroxyurea exposure in lactation: a pharmacokinetics study (HELPS). J Pediatr. 2020:222:236-239.

58. Anderson PO, Sauberan JB. Modeling drug passage into human milk. Clin Pharmacol Ther. 2016:100:42-52.

59. Niihara Y, Miller ST, Kanter J, et al. A phase 3 trial of l-glutamine in sickle cell disease. N Engl J Med. 2018;379(3):226-235.

60. Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. N Engl J Med.

2017;376(5):429-439.

61. Vichinsky E, Hoppe CC, Ataga KI, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381(6):509-519.

62. Steinberg MH, McCarthy WF, Castro O, et al. The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: 17.5 year follow-up. Am J Hematol. 2010;85(6):403-408.

63. Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle-cell syndromes: results of a 17-year, single center trial (LaSHS). Blood. 2010;115(12):2354-2363.

64. Lobo CL, Pinto JF, Nascimento EM, Moura PG, Cardoso GP, Hankins JS. The effect of hydroxycarbamide therapy on survival of children with sickle cell disease. Br J Haematol. 2013;161(6):852-860.

65. John CC, Opoka RO, Latham TS, et al. Hydroxyurea dose escalation for sickle cell anemia in sub-Saharan Africa. N Engl J Med. 2020;382(26):2524-2533.

66. Aygun B, Lane A, Smart LR, et al. Hydroxyurea dose optimization for children with sickle cell anaemia in sub-Saharan Africa (REACH): extended follow-up of a multicentre, open-label, phase 1/2 trial. Lancet Haematol. 2024;11(6):e425-e435.

67. Loiselle K, Lee JL, Szulczewski L, Drake S, Crosby LE, Pai AL. Systematic and meta-analytic review: medication adherence among pediatric patients with sickle cell disease. J Pediatr Psychol. 2016;41(4):406-418.

68. Thornburg CD, Calatroni A, Telen M, Kemper AR. Adherence to hydroxyurea therapy in children with sickle cell anemia. J Pediatr. 2010;156(3):415-419.

69. Luchtman-Jones L, Pressel S, Hilliard L, et al. Effects of hydroxyurea treatment for patients with hemoglobin SC disease. Am J Hematol. 2016;91(2):238-242.

70. Segbefia CI, Smart LR, Stuber SE, et al. Baseline characteristics of Ghanaian children and adults enrolled in PIVOT, a randomised clinical trial of hydroxyurea in HbSC disease in sub-Saharan Africa. Br J Haematol. 2024;205(6):2470-2480.

71. Opoka RO, Ndugwa CM, Latham TS, et al. Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM): a trial for children with sickle cell anemia. Blood. 2017;130(24):2585-2593.

72. Tshilolo L, Tomlinson G, Williams TN, et al. Hydroxyurea for children with sickle cell anemia in sub-Saharan Africa. N Engl J Med. 2019;380(2):121-131.

73. Ambrose EE, Latham TS, Songoro P, et al. Hydroxyurea with dose escalation for primary stroke risk reduction in children with sickle cell anaemia in Tanzania (SPHERE): an open-label, phase 2 trial. Lancet Haematol. 2023;10(4):e261-e271.

74. Rankine-Mullings A, Reid M, Soares D, et al. Hydroxycarbamide treatment reduces transcranial Doppler velocity in the absence of transfusion support in children with sickle cell anaemia, elevated transcranial Doppler velocity, and cerebral vasculopathy: the EXTEND trial. Br J Haematol. 2021;195(4):612-620.

75. Abdullahi SU, Jibir BW, Bello-Manga H, et al. Hydroxyurea for primary stroke prevention in children with sickle cell anaemia in Nigeria (SPRING): a double-blind, multicentre, randomized, phase 3 trial. Lancet Haematol. 2022;9(1):e26-e37.

76. Abdullahi SU, Sunusi S, Sani Abba M, et al. Hydroxyurea for secondary stroke prevention in children with sickle cell anemia in Nigeria: a randomized controlled trial. Blood. 2023;141(8):825-834.

77. Quinn CT. Do not leave for tomorrow what you can do today. Pediatr Blood Cancer 2015;62(11):1879-1880.

78. Ware RE, McGann PT, Quinn CT. Hydroxyurea for children with sickle cell anemia: prescribe it early and often. Pediatr Blood Cancer. 2019;66(8):e27778.