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The modern use of hydroxyurea for children with sickle cell anemia

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Abstract

Over the past 40 years, the introduction and refinement of hydroxyurea therapy has led to remarkable progress for the care of individuals with sickle cell anemia (SCA). From initial small proof-of-principle studies to multi-center Phase 3 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 of 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 will focus 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

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, aiming 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 like 5-azacytidine[8] and cytosine arabinoside.[9] Hydroxyurea was a known cytostatic agent with clinical benefits for hematological 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 described two young females with SCA, who received oral hydroxyurea at 50 mg/kg/day in three divided doses for five days, followed by three additional pulses. The number of circulating reticulocytes containing HbF (F-retics) rose quickly in both patients and led to overall increases in %HbF.[1]

A progression of formal trials followed (Figure 1). Among these, two important Phase 1/2 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 3 trials in both adults[12] and then children[13] documented the benefits of hydroxyurea for decreasing acute vaso-occlusive painful events, transfusions, and hospitalizations. 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 for hydroxyurea in SCA, additional treatment benefits not directly related to HbF are also recognized, including lower neutrophil count and platelet counts, reduced inflammatory markers, increased mean corpuscular volume with better erythrocyte flexibility, improved rheology, and potential nitric oxide release for vasodilation.[14]

Indications for treatment

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, alpha-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 waiting until the frequency and severity of these events prompts 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 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 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: 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 for 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: (1) the diagnosis is made at birth; (2) immunizations begin shortly after birth; (3) prophylactic penicillin is started by 3 months of age; (4) hydroxyurea is initiated by 6-12 months of age; and (5) transcranial

Doppler (TCD) 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 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), but 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 amount dose 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 its use.

Starting dose

The Phase 1/2 open-label trial of hydroxyurea for 49 young adults with SCA (mean age 27.6 years) started treatment at 10-20 mg/kg/day as a single daily oral dose.[10] Dose escalation was permitted every 8 weeks by 5 mg/kg/day, 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 the initial proof-of-principle study administered hydroxyurea in three divided doses at 50 mg/kg for 5 days.[1] The subsequent pediatric Phase 1/2 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 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 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 1/2 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 explain why children usually tolerate higher daily doses than 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 Hb values of ~9 g/dL without significant cytopenias, especially neutropenia. The average HbF values were usually 20-30% but more variable than the Hb 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 (2.0-3.0 x 10^9/L) and only held the hydroxyurea dose when the ANC was <1.0 x 10^9/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 is 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 isn't increased commensurate with weight gain. In our practice, we adjust the absolute dose every 1-2 months for infants and toddlers to maintain the optimal mg/kg/day dose, while in older children, we typically adjust every 3-4 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 modest dose increase, and failure to do so 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 ARC, but not HbF. We titrate hydroxyurea to maintain the ANC between 1.0-2.0 x 10^9/L and do not hold the hydroxyurea dose unless the ANC is consistently below 1.0 x 10^9/L.

Precision dosing

Hydroxyurea is known to have variable pharmacokinetics (PK) parameters,[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 SCD community, the logistics of sample collection with quantitative serum hydroxyurea measurements, and use of 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, as well as 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 detectible 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.

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 hematological 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 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 alpha-thalassemia trait and those with HbSβ^0 will have baseline microcytosis, so their MCV will still increase on treatment but at a lower value. Both 1-gene and 2-gene deletional alpha thalassemia trait have significant effects on the laboratory and clinical treatment responses.[32]

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 sub-population 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" RBC 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 RBC protection (Figure 3, Panel C). 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 simultaneously achieve the highest F-cell fraction with the highest F/F-cell content.

The hematological 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 the 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 <0.5 x 10^9/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 – 1.0 x 10^9/L, usually because of intercurrent infections. Rather than stopping effective treatment, continuing the hydroxyurea without a dose reduction with repeat blood counts in 1-2 weeks is reasonable. 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 < 0.8 x 10^9/L or platelets < 80 x 10^9/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 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 hydroxyurea exposure.

Hydroxyurea effects on fertility are extremely important to understand but challenging to disentangle from the effects of untreated SCA on gonadal function. Adults in the Phase 3 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 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, concluding 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 for 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 FDA-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, IV). 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 the transplant 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

This 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 rates of adherence to hydroxyurea reported from 12-100% 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 the

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), HbSβ^0, sickle-β^+-thalassemia (HbSβ^+), HbSD-Punjab, or HbSO-Arab. As noted earlier, HbSβ^0 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 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 ARC, but the Hb 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%.

A formal prospective Phase 1/2 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 treating both children and adults. 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 3 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 highest in low-resource countries and especially sub-Saharan Africa, so it is necessary to determine the feasibility and effectiveness of hydroxyurea 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 Hb >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 high-risk children identified by TCD screening, hydroxyurea has been used at differing doses to lower TCD velocities and overt stroke rate. The SPHERE trial documented the benefits of hydroxyurea at MTD in Tanzanian children with conditional and abnormal TCD velocities, with an average decrease of 33 cm/sec,[73] similar to the effects observed in the EXTEND trial conducted in Jamaica.[74] In contrast, 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 underway 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 pharmacologic 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]

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Table 1. Treatment characteristics of children with sickle cell anemia receiving hydroxyurea.

Study	ClinicalTrial.gov	Year	N	Age (years)	Dose (mg)	Hb (g/dL)	MCV (fL)	HbF (%)	ANC ($\times 10^9$/L)	ANC Target ($\times 10^9$/L)	ANC Toxicity ($\times 10^9$/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
SPHERE	NCT03948867	2023	45	6.3	28.7	9.3	104	27.7	3.0	2.0-3.0	1.0

* Denotes fixed-dose study design.

Abbreviations: ANC, absolute neutrophil count; Hb, hemoglobin; HbF, fetal hemoglobin; MCV, mean cellular volume.

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*[1]	**Escalation** *all of the following*[2]	**Adjustment for weight gain** *all of the following*[3]
Absolute neutrophil count (ANC), $x10^9$/L	< 1.0 (consistently)	> 3.0	1.0 – 3.0
Platelets, $x10^9$/L	< 100	> 150	> 150
Hemoglobin (Hb), g/dL	Hb < 5.5	Hb > 6.0	Hb > 6.0
Absolute reticulocyte count (ARC), 10^9/L	ARC < 50 (if Hb < 7.0)	ARC ≥ 50 (if Hb ≥ 7.0)	ARC > 100 (if Hb < 8.0) *or* ARC > 75 (if Hb ≥ 8.0)

[1] Hydroxyurea should be temporarily stopped, with a possible dose reduction, if any toxicity criteria are met.

[2] With traditional, stepwise dose escalation, doses are increased (usually in 5 mg/kg increments) when all escalation criteria are met.

[3] Once 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.

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

Test	Frequency	Notes
Foundational testing		
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 (DANC), which may influence hydroxyurea dosing
Basic Testing		
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 testing		

F-cell analysis	Every 6-12 months	Quantify the cellular distribution of HbF across RBCs with the goal of to approach and maintain 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

FIGURE LEGENDS

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 1/2 trials, while red-shaded boxes represent Phase 3 trials. Purple boxes denote the year of regulatory approval for adults (1998) and children (2017).

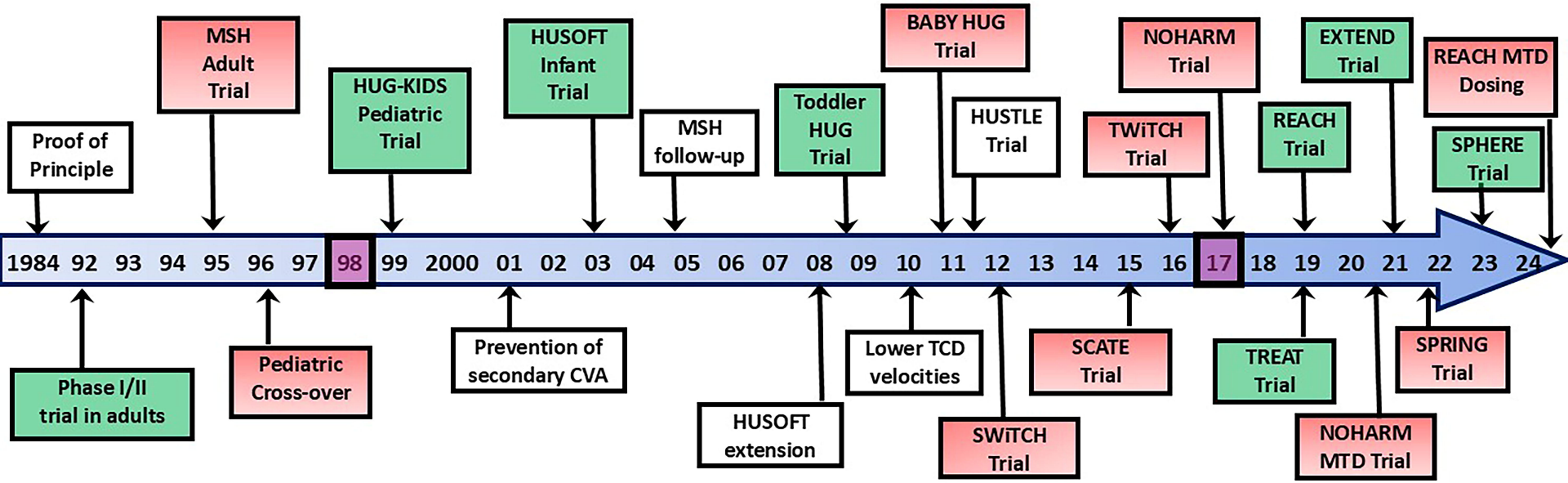
Figure 2. Sustained high HbF values in children treated from infancy. Four representative examples are provided from over 40 children who initiated hydroxyurea before 3 years of age (9-27 months) who have had sustained treatment responses of HbF >30% for 8-9 years. Reference lines are shown for HbF values 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), that our medical and psychosocial teams can successfully address.

Figure 3. F-cell analysis for monitoring hydroxyurea therapy. Panel A shows the gradient of F-cell expression across 4 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 sizeable 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 PK-optimized hydroxyurea therapy by 9 months of age. Each graph shows singlet RBC events classified by HbF expression (x-axis) and CD71 expression (y-axis). The two right quadrants of

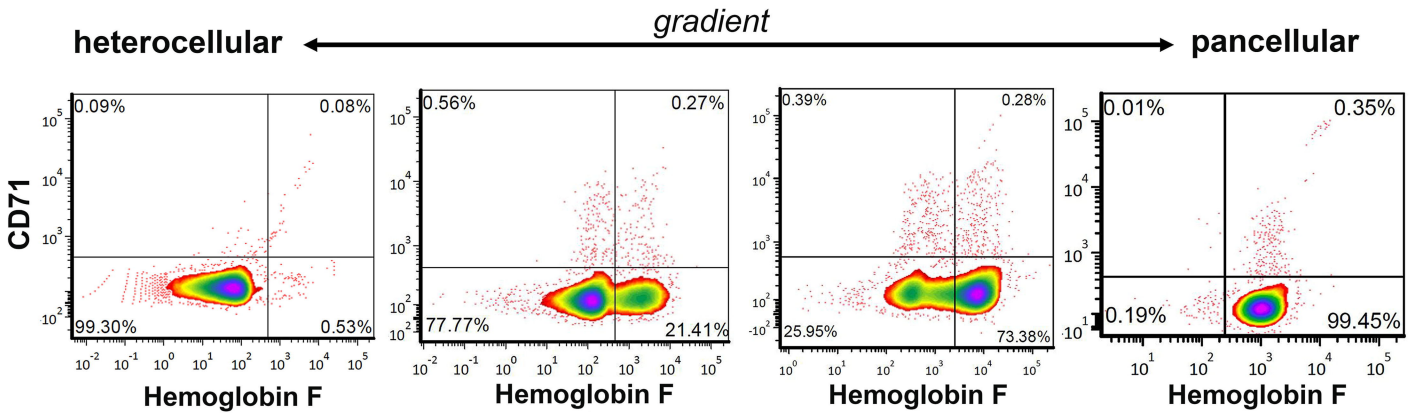
each graph indicate F-cells. The top 2 quadrants of each graph indicate immature reticulocytes. The total F-cell fraction is the sum of the right upper quadrant (F-retics) and right lower quadrant (mature F-cells). Panel B shows an infant who began hydroxyurea at 7 months of age. The graph on the left shows HbF values before and during hydroxyurea therapy (left y-axis). Also shown is the F-cell fraction measured at some of these times (right y-axis). The graph on the right demonstrates pancellular expression of Hb F measured at the indicated (*) timepoint that recapitulates the HbF expression in HbS/HPFH. Panel C shows histograms of single-cell estimates of F/F-cell (pg) in the F-cell populations of 4 individuals. Patients 1-3 have SCA, are treated with hydroxyurea, and have increasing amounts of HbF induction. Patient 4 has compound heterozygosity for Hb S and gene-deletion HFPH (S/HPFH). Patients 3 and 4 have >90% of F-cells with >10 pg HbF, indicating RBCs fully protected from sickling.

Figure 4. Advanced laboratory testing for monitoring of hydroxyurea therapy. Panel A: Oxygen-gradient ektacytometry depicts the relationship between RBC deformability (elongation index), as an index of onset of sickling, and the partial pressure of O_2. Patients who have higher amounts of HbF expression (identified on the right of the graph) have a progressively delayed onset of sickling that occurs at successively lower pO2. Panel B: Phase-contrast microscopy demonstrates pocked or pitted RBCs, 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. Panel C: A novel flow cytometric quantitative method is illustrated that 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, RBCs are stained with double-stranded DNA-avid dye, and

morphologic characteristics (size, shape) of the intra-RBC DNA inclusions are used to identify HJB.

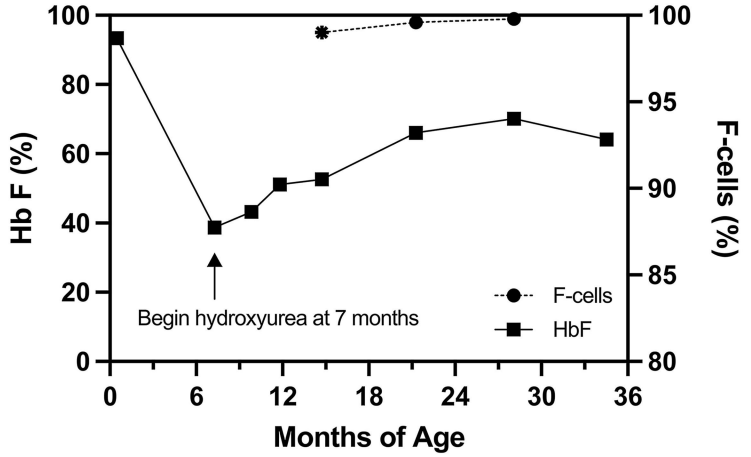

Proof of Principle
MSH Adult Trial
HUG-KIDS Pediatric Trial
HUSOFT Infant Trial
MSH follow-up
Toddler HUG Trial
BABY HUG Trial
HUSTLE Trial
TWiTCH Trial
NOHARM Trial
REACH Trial
EXTEND Trial
REACH MTD Dosing
SPHERE Trial
1984 92 93 94 95 96 97 98 99 2000 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Phase I/II trial in adults
Pediatric Cross-over
Prevention of secondary CVA
HUSOFT extension
Lower TCD velocities
SWiTCH Trial
SCATE Trial
TREAT Trial
NOHARM MTD Trial
SPRING Trial

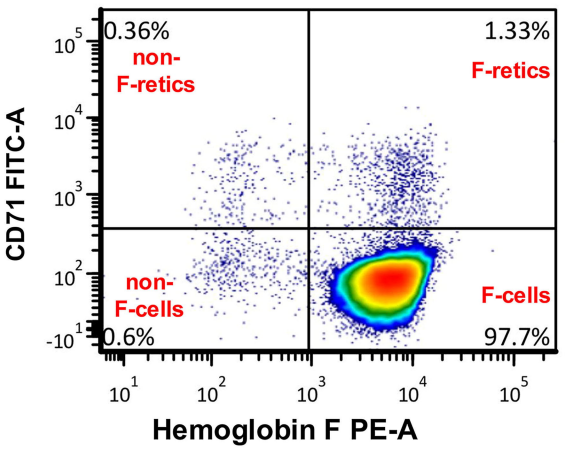
A. Flow cytometric classification and quantitation of F-cells in sickle cell anemia.



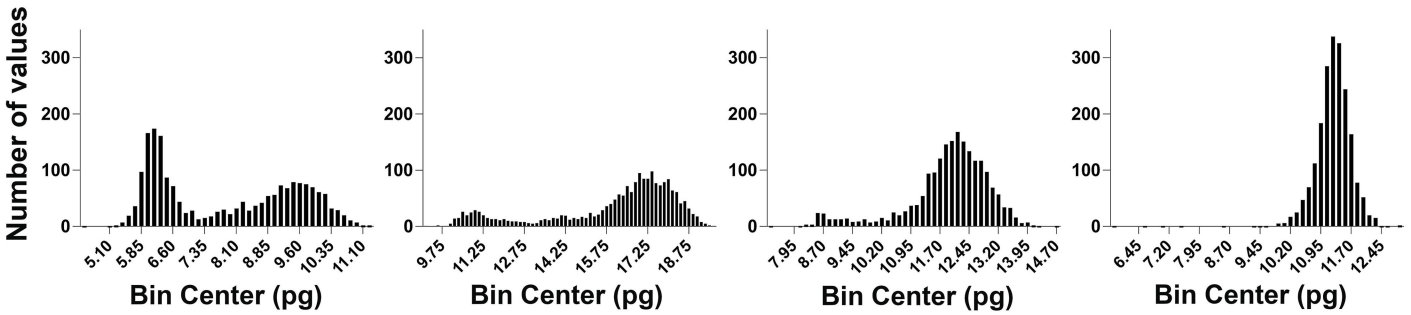
	Patient 1	Patient 2	Patient 3	Patient 4
Hb F:	<0.1%	23%	28%	42%
F-cells:	0.6%	22%	74%	99%

B. Initiation of hydroxyurea in infancy with robust HbF induction and pancellular distribution.

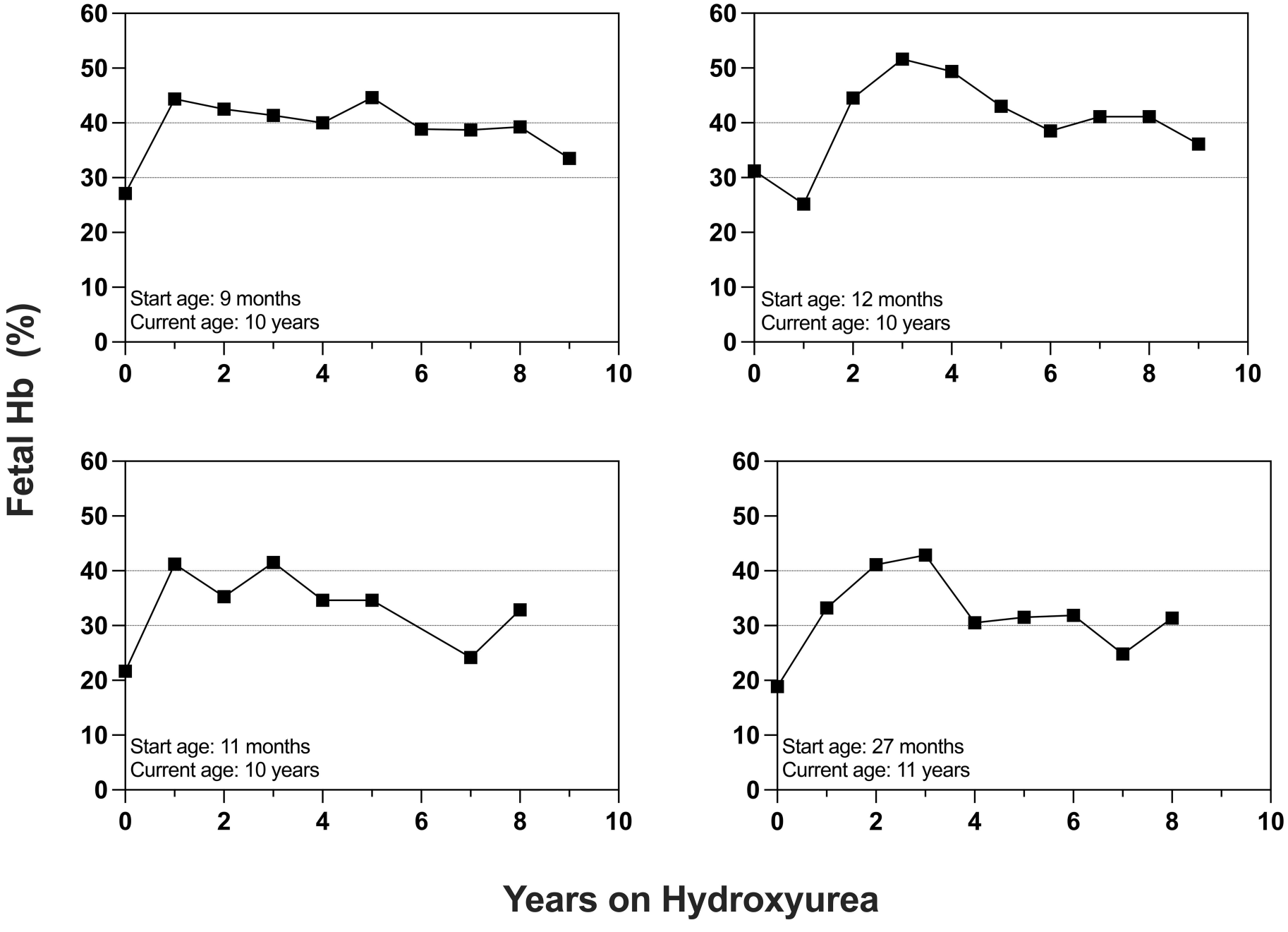


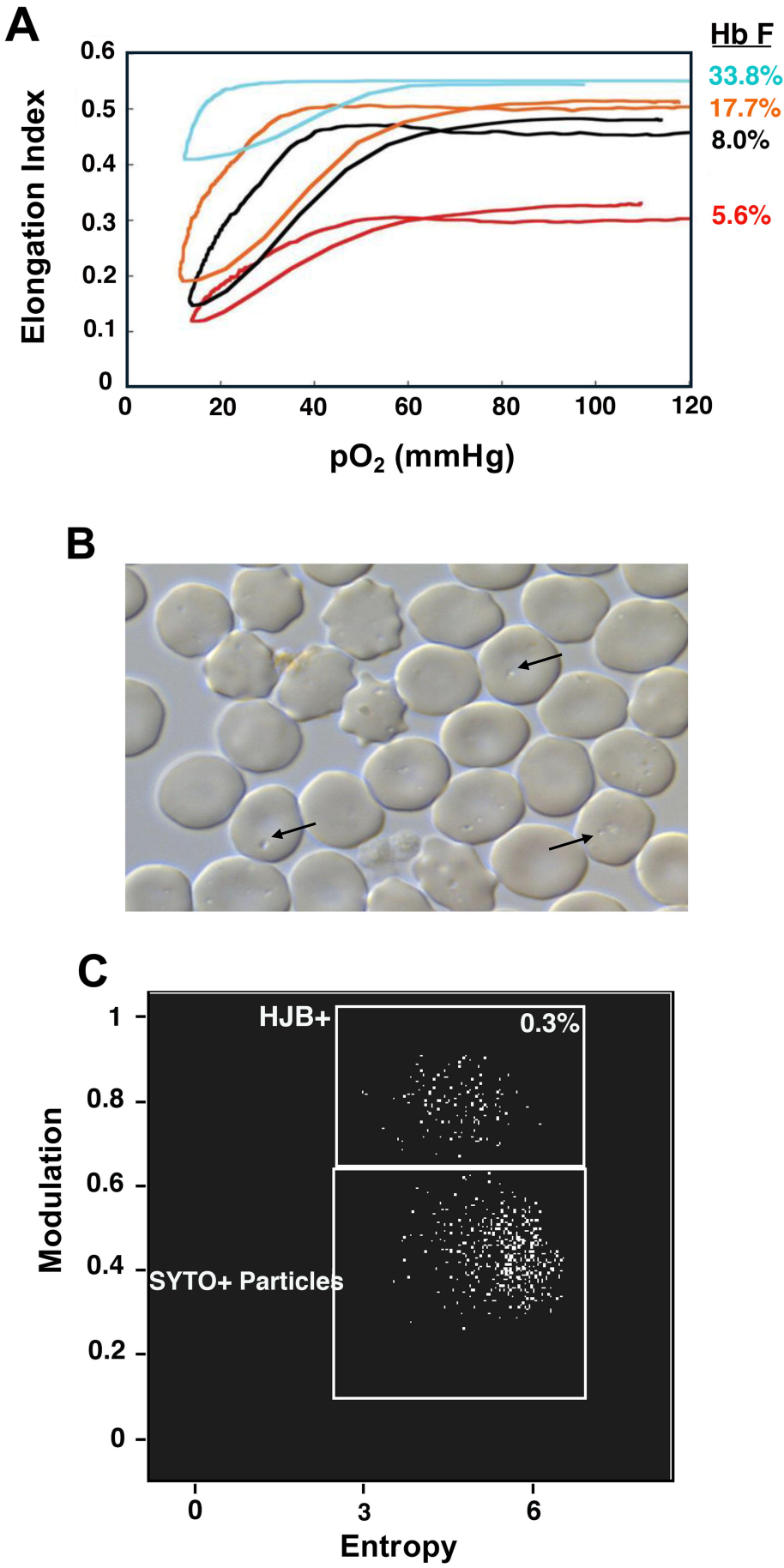


C. Distribution of single cell measurements of F/F-cell across with hydroxyurea therapy.



	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%


Fetal Hb (%)
Start age: 9 months
Current age: 10 years
Start age: 12 months
Current age: 10 years
Start age: 11 months
Current age: 10 years
Start age: 27 months
Current age: 11 years
Years on Hydroxyurea


A
Hb F
33.8%
17.7%
8.0%
5.6%
Elongation Index
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120
pO2 (mmHg)
B
C
HJB+
0.3%
SYTO+ Particles
Modulation
1
0.8
0.6
0.4
0.2
0
0
3
6
Entropy