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Fetal Hemoglobin Induction by Epigenetic Drugs

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Abstract

Fetal hemoglobin (HbF) inhibits the root cause of sickle pathophysiology, sickle hemoglobin polymerization. Individuals who naturally express high levels of HbF beyond infancy thus receive some protection from sickle complications. To mimic this natural genetic experiment using drugs, one guiding observation was that HbF is increased during recovery of bone marrow from extreme stress. This led to evaluation and approval of the cytotoxic (cell killing) drug hydroxyurea to treat sickle cell disease (SCD). Cytotoxic approaches are limited in potency and sustainability, however, since they require hematopoietic reserves sufficient to repeatedly mount recoveries from stress that destroys their counterparts, and such reserves are finite. HbF induction even by stress ultimately involves chromatin remodeling of the gene for HbF (*HBG*), therefore, a logical alternative approach is to directly inhibit epigenetic enzymes that repress *HBG*—implicated enzymes include DNA methyltransferase 1 (DNMT1), histone deacetylases (HDAC), lysine demethylase 1 (KDM1A/LSD1), protein arginine methyltransferase 5 (PRMT5), euchromatic histone lysine methyltransferase 2 (EHMT2) and chromodomain helicase DNA binding protein 4 (CHD4). Clinical proof-of-principle that this alternative, non-cytotoxic approach can generate substantial HbF and total hemoglobin increases has already been generated. Thus, with continued careful attention to fundamental biological and pharmacologic considerations (reviewed herein), there is potential that rational, molecular-targeted, safe and highly potent disease-modifying therapy can be realized for SCD patients, with the accessibility and cost-effective properties needed for world-wide impact.

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Keywords

sickle cell disease; fetal hemoglobin; epigenetic; therapy

Introduction

The global scope of the sickle cell disease (SCD) problem, with its especially high prevalence in pediatric populations in low income countries, demands a practical, accessible oral medication solution. There are natural models for drug development to emulate – some SCD patients express high levels of fetal hemoglobin (HbF) beyond infancy, because of genetic traits separate from, and co-inherited with, the sickle mutation in the β -globin gene (*HBB^S*), and consequently have, in the best cases, asymptomatic and normal life-spans¹⁻³. Analyses of these HbF-augmenting genetic traits, coupled with biochemical studies of the β -globin locus, have identified specific molecular targets for drug development efforts that aim to recapitulate these natural exemplars of powerful disease modification.

HbF interdicts sickle pathophysiology at its inception

Red blood cells (RBCs) at the fetal stage of life contain HbF ($\alpha_2\gamma_2$). HbF intercalates with but does not polymerize with sickle hemoglobin (HbS)(normal adult hemoglobin [HbA, $\alpha_2\beta_2$] can)⁴⁻⁶. HbF thus interrupts SCD pathophysiology at its inception^{4,5}(Figure 1), and higher HbF correlates continuously with fewer vaso-occlusive pain crises, less renal damage, less pulmonary hypertension, fewer strokes and longer survival⁷⁻¹⁴(reviewed in⁶). Those few SCD patients who inherit HbF at 20-30% of total hemoglobin (hereditary persistence of fetal hemoglobin, HPFH) have essentially normal life-spans¹⁻³.

The first attempts at pharmacologic induction of HbF were based on creating bone marrow stress

Thus, several decades of translational research have been directed towards pharmacologic recapitulation of this naturally selected protective state of persistent high HbF expression (reviewed in⁶). The earliest efforts built on the observation that HbF is enriched during recovery of the bone marrow from severe insults or stress¹⁵⁻²⁰. One way of creating such stress is to administer cytotoxic (cell killing) drugs, leading to clinical evaluation in SCD of the oral ribonucleotide reductase inhibitor hydroxyurea¹⁹⁻²¹. In the pivotal trial, hydroxyurea (15-35 mg/kg) increased HbF for 2 years in ~50% of the adult SCD patients treated^{21,22}. As predicted, HbF increases with hydroxyurea correlated strongly with increased RBC half-life^{23,24}, fewer pain crises²², and better quality of life²⁵. Trial patients with HbF levels >0.5 g/dL survived longer¹⁰ (a caveat being that these analyses did not determine whether the higher HbF levels were intrinsic to the patients or a result of hydroxyurea therapy). Of significance, however, average HbF increases at 2 years were modest (3.6%)^{19-22,26}. Moreover, HbF increases were particularly unlikely in patients with the lowest baseline HbF levels and thus at highest risk of morbidity and mortality^{22,24,27,28}, and HbF increases diminished over time, even in patients with excellent initial HbF inductions^{22,29}. A reason for these properties of hydroxyurea therapy was suggested by the correlation between lower

and less durable HbF increases and fewer reticulocytes ($<300,000 \times 10^9/L$) and neutrophils ($<7.5 \times 10^9/L$) at baseline: this correlation underscored that HbF induction by cytotoxicity requires reserves of hematopoietic precursors sufficient to mount repeated recoveries from stress that destroys their counterparts^{20,22}. Such reserves are circumscribed, and moreover subject to attrition via vaso-occlusion in the marrow and to the kidneys, and from the aging process^{22,24,27,28}. This is a problem even separate from considerations of sustainable HbF-induction via cytotoxicity: SCD patients require erythropoiesis at >10 -fold the normal rate to barely sustain hemoglobin levels compatible with life, and dwindling compensatory capacity is a major cause of early death^{10,22,30,31}. Therefore, alternative, non-cytotoxic, durable, and more potent methods of inducing HbF are needed.

Directly targeting the enzymes that silence the γ -globin gene instead

DNA in nuclei is packaged together with RNA and structural proteins – histones – to form chromatin. These structures regulate accessibility of genes to the massive multi-protein machinery (~150 proteins) that executes gene transcription. Reorganization or remodeling of chromatin, to facilitate or hinder gene transcription, is signaled via post-translational modifications to histones – methylation and acetylation of lysine residues, phosphorylation of threonines and serines – and by modifications to DNA, mainly, methylation of cytosines that precede guanines (CpG). These signals motivate shifting of histones in relation to gene transcription start sites, repositioning these substantial physical barriers to either welcome or obstruct the gene transcribing basal transcription factor machinery.

Thus, whether a stimulus for *HBB* activation is natural or from without, HbF induction requires chromatin remodeling of the γ -globin gene locus (*HBB*)³². Specifically, there is decreased operation at the locus of the epigenetic enzymes that create epigenetic ‘off’ marks, or that reposition histones toward the transcription start site, and increased function of the enzymes that create epigenetic ‘on’ marks, and that reposition histones away from the transcription start site. Cytotoxic methods of inducing HbF generate these necessary epigenetic changes crudely and indirectly, via bone marrow stress^{20,32,33} (Figure 2). So why not identify repressing epigenetic enzymes and inhibit them directly? This notion has been supported by extensive pre-clinical work and even clinical proof-of-principle^{34,35}, reviewed here.

Fundamentals worth keeping in mind

The wealth of pre-clinical and clinical experience to date has underscored biological and clinical considerations fundamental to the success or failure of attempts at epigenetic induction of HbF. Here are five such fundamentals:

(i) Epigenetic enzymes by themselves do not dictate activation of genes

This is illustrated with a thought experiment: imagine rendering all the DNA accessible (opening all the chromatin) in a cell devoid of DNA-binding transcription factors – no change in gene expression would be expected – gene activation requires a cellular context primed to express the gene, with localization of a cooperating complement of sequence-specific and basal DNA binding transcription factors, and their requisite, massive, multi-

protein coregulator apparatus, exceeding more than 150 proteins. That is, sequence specific DNA-binding factors direct the activation or repression of genes, but the actual biochemical work needed for activation or repression is executed by a massive coregulator apparatus. The coregulator complexes that remodel chromatin for gene activation are termed coactivators³⁶⁻³⁸. Coactivators are opposed by corepressors that remodel chromatin to repress gene transcription instead. Coactivators and corepressors are in dynamic opposition: they can compete for the same binding sites on transcription factors³⁹, and transcriptional activity is sensitive to cellular stoichiometry of coactivators and corepressors³⁹⁻⁵¹. Moreover, coregulator complex stability can be undermined by the loss of a single sub-unit, a characteristic enabling potent pharmacologic interdiction of their function⁵².

(ii) Epigenetic enzymes by themselves do not dictate cell fates

A corollary of the above ‘fundamental’ is that the cell fate consequences of inhibiting or depleting epigenetic enzymes depends on cellular context. Specifically, the baseline expression pattern of transcription factors is a key determinant of the cell fate response to chromatin-relaxation⁵³. Moreover, not all transcription factors are equal: lineage-conversion of mature cells of one lineage into mature cells of distant lineages, or into embryonic stem cells, by forced expression of as few as two specific transcription factors, strikingly illustrates this⁵⁴. In other words, the cell fate consequences of inhibiting epigenetic enzymes depends on the baseline master transcription factor context of cells. Thus, inhibiting silencing epigenetic enzymes can produce cell fate shifts, but these are not drastic, and relate to what the cells were to begin with. This is of course critical clinically, since a candidate epigenetic therapeutic for SCD will be distributed systemically. One concern will be the potential for teratogenicity – this should be assumed for individual agents, unless shown otherwise by formal toxicological study. Another concern with systemic consequences is activation of oncogenes and predisposition or acceleration of cancer: fortunately, cancer cells, including self-replicating cancer cells (cancer or leukemia ‘stem’ cells), contain high amounts of the master transcription factors that normally activate terminal lineage-fates, and demonstrate a dependency on specific corepressors in order to avoid these terminal fates that are intended by their master transcription factor content⁵⁵. Stated simply, several corepressor components (repressing epigenetic enzymes), e.g., DNMT1, HDACs, KDM1A, have been validated as molecular targets for the treatment and prevention of cancer (reviewed in⁵⁵). The same chromatin-‘relaxing’ treatments that trigger terminal lineage-fates of cancer/leukemia stem cells preserve self-renewal of uncommitted tissue stem cells, since these cells express stem cell master transcription factors, not high levels of lineage-specifying transcription factors^{53,56}. This seems almost too good to be true, but is a highly replicated result (reviewed in^{55,57})!

(iii) Exploiting the maturational switch for HbF induction

Given fundamentals #1 and #2 above, that emphasize that the consequences of inhibiting an epigenetic ‘off’ enzyme are essentially dictated by a cells baseline state, there is a fortunate feature of *HBG* repression in adult hematopoiesis. Several groups have found that the developmental switch from *HBG* to adult β -globin gene (*HBB*) transcription is recapitulated during adult erythropoiesis. That is, the developmental switch is played out, albeit very rapidly, during maturation of erythroid progenitors (the ‘maturational switch’)⁵⁸⁻⁶². These

and other studies have indicated that the maturational switch involves removal of activating and acquisition of repressive epigenetic marks at *HBG*^{64,65}, with physical migration of the shared enhancer, the locus control region, from the *HBG* to the *HBB* locus⁶³, creating an opportunity for pharmacologic/biochemical intervention to prevent this migration, to stall the enhancer and the massive gene activating machinery at *HBG*.

That is, an epigenetic approach to HbF induction is not predicated so much on returning the enhancer from *HBB* back to *HBG*, but on preventing a switch from *HBG* to *HBB* in the first place, thereby preserving a chromatin configuration that favored *HBG* activation to begin with. Accordingly, HbF induction by an inhibitor of the silencing epigenetic enzyme EHMT2 (UNC0638) depended on the timing of its addition to cultures of synchronously maturing erythroid progenitors⁶⁴.

(iv) Baseline HbF levels dictate final HbF levels

There is a wide variation in baseline HbF levels in patients with SCD and even the general population, reflecting the influence of various genetic polymorphisms on regulation of this locus. Even if the potency of a molecular targeted therapy were to ensure similar rates of increase in HbF% (the percentage of total hemoglobin that is HbF) in all patients, the final HbF% would be dictated by where HbF% began. Moreover, in clinical trials we have conducted with the DNA methyltransferase 1 (DNMT1)-depleting drug decitabine, we have noticed a slightly lower slope to the rate of increase in HbF% in SCD patients with lower HbF% at baseline. Fortunately, HbF induced by this epigenetic strategy is well-distributed in the RBC population, and despite the lower gradient in rates of HbF% increase, the rates of increase of HbF enriched RBCs (F-cell%) was higher in patients with low F-cell% at baseline^{34,66-68}.

At some time-point after starting therapy, F-cells entering the circulation will be matched by a similar number of F-cells leaving the circulation, producing plateaus in HbF% and F-cell %.

(v) Not all repressing epigenetic enzymes (corepressor protein complexes) are logical molecular targets for therapy

Cells contain tens of epigenetic enzymes mediating gene activation and repression. For example, there are >50 lysine methyltransferases, and >30 lysine demethylases in humans. Logically, the epigenetic enzymes to target for HbF induction are those that have been directly implicated in silencing of the *HBG* locus. Multi-protein corepressor complexes directed to the *HBG* locus by the sequence-specific DNA-binding factors DRED and BCL11A have been characterized in great detail⁶⁹⁻⁷³. These corepressor complexes contain shared components: druggable epigenetic silencing enzymes DNMT1, various histone deacetylases (HDACs), lysine demethylase 1 (KDM1A, LSD1), and chromodomain helicase DNA binding protein 4 (CHD4)^{63,72-74}(Table 1). Epigenetic enzymes implicated in silencing *HBG* in other biochemical studies include euchromatic histone lysine methyltransferase 2 (EHMT2, G9a)⁷⁵, and protein arginine methyltransferase 5 (PRMT5)^{76,77}. Another approach to identification of candidate *HBG* silencing enzyme targets has been chemical screens using probes (inhibitors) known to inhibit specific epigenetic targets. This approach

has identified the histone methyltransferases EHMT1 and EHMT2 as candidates for inhibition to induce HbF^{64,78}. Notably absent, given that there are clinically available inhibitors for these targets, are epigenetic enzymes in polycomb repressor complex 2 (e.g., EZH2).

(vi) Cytotoxicity should be avoided

Many epigenetic drugs in development are intended to treat cancer, where cytotoxicity is an intended (at least per the conventional treatment paradigm). For the reasons discussed earlier, however, cytotoxicity is undesirable for long-term, durable HbF induction. Thus, a propensity of a drug to off-target anti-metabolite/cytotoxic effects, that may be acceptable in treating cancers, would be an undesirable side-effect for sustainable HbF induction. Some epigenetic targets, e.g., HDACs, have pleiotropic functions in cells - thus, even on-target HDAC inhibition can induce cytotoxicity, potentially limiting the role of such epigenetic targets and their cognate drugs in HbF induction⁸⁰⁻⁸⁴.

Candidate molecular targets for epigenetic induction of HbF are discussed in turn:

HDACs—HDACs were amongst the first candidate epigenetic targets identified for HbF induction⁷⁹. Moreover, a number of HDAC inhibitors are FDA approved to treat peripheral T-cell malignancies (Table 1). Unfortunately, despite excellent pre-clinical data, clinical application of marketed HDAC inhibitors for HbF induction is limited by pleiotropic roles of HDACs outside of chromatin, rendering it difficult to separate anti-metabolite/cytotoxicity from epigenetic effects⁸⁰⁻⁸⁴. That is, clinical side-effects, arising from HDAC participation in multiple cellular and physiological functions, limit the achievement of epigenetic pharmacodynamic effect, and thus of HbF induction in vivo⁸⁵. There are efforts to develop HDAC inhibitors that are more selective to specific HDACs than the broad HDAC inhibiting activity of current marketed drugs (Table 1), and perhaps these more selective agents will have a more suitable profile for HbF induction.

DNMT1—The chromatin modifying enzyme DNMT1 maintains methylation marks on DNA through cell division. In addition, DNMT1 is a corepressor, one that is recruited by sequence-specific DNA-binding factors DRED (TR2/TR4) and BCL11A that direct epigenetic silencing of *HBG*^{34,66,73,86-95}. The deoxycytidine analog decitabine and its pro-drug 5-azacytidine, approved in the United States to treat the myeloid malignancy myelodysplastic syndrome, can deplete DNMT1: a nitrogen substituted for a carbon in the decitabine pyrimidine ring covalently binds to DNMT1 and causes its degradation⁹⁶. The deoxyribose moiety of decitabine is unmodified, thus, it can incorporate into the elongating DNA strand during S-phase without terminating chain extension or causing cytotoxicity, contrasting with most nucleoside analogs used in the clinic to treat cancer^{97,98}. High concentrations of decitabine, however, do produce off-target anti-metabolite effects and cytotoxicity, in significant part via its uridine moiety degradation products that can misincorporate into DNA or inhibit thymidylate synthase^{99,100}.

We applied decitabine by dose, schedule and route-of-administration that exploited its capacity for non-cytotoxic, molecularly-targeted therapy of non-malignant and malignant diseases^{34,53,56,101,102}. This approach increased HbF by >10% in SCD patients who had no

HbF response (~0.3%) to hydroxyurea in the pivotal clinical trial^{34,66,67}. By depleting DNMT1 protein, decitabine disrupts its scaffolding functions for other epigenetic enzymes such as KDM1A^{120,121}. That is, decitabine does not just inhibit the enzyme function of DNMT1, but produces an even broader corepressor disrupting effect. This potent epigenetic pharmacodynamic effect explains why administration of a very small, non-cytotoxic dose of ~0.2 mg/kg 2X/week is sufficient to produce large HbF and total hemoglobin increases, even in patients in whom hydroxyurea, at >1000-fold the molar amount (decitabine ~0.2 mg/kg 2X/week vs. hydroxyurea ~20 mg/kg daily), did not induce HbF (Figure 2)^{34,35}.

Hindering wide clinical application of currently marketed decitabine in this way, however, is a very brief plasma half-life of only minutes^{103,104} and negligible solid tissue distribution and oral bioavailability. These are related pharmacologic limitations with a common cause - rapid deamination/inactivation to uridine degradation products by the pyrimidine metabolism enzyme cytidine deaminase (CDA)¹⁰⁵⁻¹⁰⁹. We therefore combined a CDA inhibitor oral tetrahydrouridine (THU) with oral decitabine^{93,106-108,110-116}; this combination was well-tolerated and safe in a Phase 1 study - the target dose of 0.16 mg/kg produced a wide decitabine concentration-time profile (low C_{max} , long T_{max}) ideal for non-cytotoxic DNMT1-depletion^{96,117-119} and decreased DNMT1 protein in peripheral blood mononuclear cells by >75% and repetitive element CpG methylation by ~10%. This increased HbF by 4-9%, doubling HbF-enriched RBC (F-cells) up to ~80% of total RBCs. Total hemoglobin increased by 1.2-1.9 g/dL ($p=0.01$) as reticulocytes simultaneously decreased; that is, better quality and efficiency of HbF-enriched erythropoiesis elevated hemoglobin using fewer reticulocytes. Also indicating better RBC quality, biomarkers of hemolysis, thrombophilia and inflammation (LDH, bilirubin, D-dimer, CRP) improved. The side-effects were a concurrent increase in platelets and decrease in neutrophils, expected with non-cytotoxic DNMT1-depletion. In the relatively short treatment duration of 8 weeks, these blood count shifts did not cross thresholds requiring treatment holds or modification, that is, neutrophil counts and platelets remained in ranges observed in SCD patients receiving standard of care therapies.

Adding substantially to the overall safety information around decitabine and related 5-azacytidine is decades of pre-clinical and clinical evaluation for HbF induction in patients with SCD and β -thalassemia, as well as >8 years of post-marketing follow-up since approval to treat myeloid malignancies. Newly developed oral THU-decitabine, designed for accessible, long-term, non-cytotoxic DNMT1-depletion, facilitates ongoing development of this approach for modification of SCD and β -thalassemia.

KDM1A (LSD1)—There are two classes of lysine demethylases: the first, containing KDM1A and KDM1B, are flavine adenine dinucleotide (FAD)-dependent monoamine oxidases, while the second, containing over 30 members are iron and α -ketoglutarate dependent enzymes. Consistent with KDM1A recruitment by DRED and BCL11A, KDM1A inhibition with either of two specific inhibitors induced HbF *in vitro*, in sickle mice and in non-human primates^{65,122-124}. Several KDM1A inhibitors are in clinical trials for cancer indications, and one is in clinical trials for SCD (Table 1). At least two of the compounds in trials (ORY-1001, GSK2879552) are built around a tranlylpropamine warhead that inhibits monoamine oxidases that metabolize catecholamine neurotransmitters in the brain. Although

cancer clinical trials are ongoing and unpublished (EudraCT number: 2013-002447-29; [ClinicalTrials.gov](#) identifiers: NCT02177812, NCT02034123), there is concern regarding side-effects related to the potential for inhibition of monoamine oxidases other than KDM1A. Thus, there are ongoing efforts to develop and evaluate KDM1A inhibitors with other scaffolds (e.g., [ClinicalTrials.gov](#) identifier: NCT01344707). At this time, there is one registered Phase 1 clinical trial evaluating a KDM1A inhibitor for HbF induction in SCD ([ClinicalTrials.gov](#) identifier: NCT03132324).

PRMT5—Methylation of histone H4 arginine 3 by PRMT5 has been implicated as a signal that recruits additional chromatin modifying enzymes and represses *HBB*⁷⁶. There is a PRMT5 inhibitor in clinical trials (GSK3326595) for cancer indications, and trials of this molecule for HbF induction in SCD are anticipated, although not registered at this time.

EHMT2 (G9a)—EHMT2 has been shown to be recruited to the β -globin locus by the sequence-specific DNA binding factor NFE2, and the EHMT2 inhibitor UNC0638 has been shown to induce HbF *in vitro*^{64,78}. As of this time, there are no registered clinical trials evaluating EHMT2 inhibition to induce HbF.

CHD4 and SMARCA5—The culmination of chromatin remodeling for gene repression or activation is nucleosome (histone octamer) repositioning around the transcription start site. This is energetically expensive work executed by SWI/SNF or ISWI family proteins containing the HELICc-DExx ATP-ase domain³⁶⁻³⁸. Since such nucleosome repositioning is the crux of chromatin remodeling, inhibition of this action should in principle offer corresponding potency. CHD4 and SMARCA5 are HELICc-DExx containing corepressors that are recruited by BCL11A and DRED to repress *HBB*⁶³. We have identified a first-in-class drug-like compound series that inhibits the HELICc-DExx domains of SMARCA5 and CHD4, and we are actively investigating the potential of this series to induce HbF (unpublished data).

Combinatorial approaches

In oncology, combinations of drugs are almost mandatory, because the target cell population is evolving, and will select to evade drug-effect. Target cells in SCD are not evolving. Combination therapy is therefore not mandatory per se. Nevertheless, it is conceivable that combinations of drugs, each used at doses low enough to avoid side-effects from off-target pharmacodynamic actions, and with non-overlapping side-effects from on-target actions, might produce greater increases in HbF than likely with single agents.

Conclusion

HbF is the most powerful known natural modulator of the root cause of SCD pathophysiology⁶. Clinical proof-of-principle that substantial HbF and total hemoglobin increases can be produced by inhibiting epigenetic enzymes that mediate HbF silencing, and by rationally avoiding cytotoxicity, has already been generated. There is thus sound basis for optimism that ongoing efforts can culminate in practical, accessible, cost-effective, safe and potent disease-modifying therapy for SCD patients, hopefully world-wide.

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References

1. Conley CL, Weatherall DJ, Richardson SN, Shepard MK, Charache S. Hereditary persistence of fetal hemoglobin: a study of 79 affected persons in 15 Negro families in Baltimore. *Blood*. 1963; 21:261–281. [PubMed: 14022587]
2. 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. [PubMed: 619731]
3. Ngo DA, Aygun B, Akinsheye I, et al. Fetal haemoglobin levels and haematological characteristics of compound heterozygotes for haemoglobin S and deletional hereditary persistence of fetal haemoglobin. *Br J Haematol*. 2012; 156(2):259–264. [PubMed: 22017641]
4. Goldberg MA, Husson MA, Bunn HF. Participation of hemoglobins A and F in polymerization of sickle hemoglobin. *J Biol Chem*. 1977; 252(10):3414–3421. [PubMed: 16902]
5. Nagel RL, Bookchin RM, Johnson J, et al. Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S. *Proc Natl Acad Sci USA*. 1979; 76(2): 670–672. [PubMed: 284392]
6. Akinsheye I, Alsultan A, Solovieff N, et al. Fetal hemoglobin in sickle cell anemia. *Blood*. 2011; 118(1):19–27. [PubMed: 21490337]
7. Platt OS, Thorington BD, Brambilla DJ, et al. Pain in sickle cell disease. Rates and risk factors. *N Engl J Med*. 1991; 325(1):11–16. [PubMed: 1710777]
8. 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. [PubMed: 7993409]
9. Powars DR, Weiss JN, Chan LS, Schroeder WA. Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? *Blood*. 1984; 63(4):921–926. [PubMed: 6200161]
10. Steinberg MH, Barton F, Castro O, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. *JAMA*. 2003; 289(13):1645–1651. [PubMed: 12672732]
11. Atweh GF, Schechter AN. Pharmacologic induction of fetal hemoglobin: raising the therapeutic bar in sickle cell disease. *Curr Opin Hematol*. 2001; 8(2):123–130. [PubMed: 11224687]
12. Powars DR, Elliott-Mills DD, Chan L, et al. Chronic renal failure in sickle cell disease: risk factors, clinical course, and mortality. *Ann Intern Med*. 1991; 115(8):614–620. [PubMed: 1892333]
13. Lebensburger J, Johnson SM, Askenazi DJ, Rozario NL, Howard TH, Hilliard LM. Protective role of hemoglobin and fetal hemoglobin in early kidney disease for children with sickle cell anemia. *Am J Hematol*. 2011; 86(5):430–432. [PubMed: 21523807]
14. Aban I, Baddam S, Hilliard LM, Howard TH, Feig DI, Lebensburger JD. Severe anemia early in life as a risk factor for sickle-cell kidney disease. *Blood*. 2017; 129(3):385–387. [PubMed: 27919909]
15. DeSimone J, Biel SI, Heller P. Stimulation of fetal hemoglobin synthesis in baboons by hemolysis and hypoxia. *Proc Natl Acad Sci U S A*. 1978; 75(6):2937–2940. [PubMed: 96444]
16. DeSimone J, Heller P, Adams JG. Hemopoietic stress and fetal hemoglobin synthesis: comparative studies in vivo and in vitro. *Blood*. 1979; 54(5):1176–1181. [PubMed: 115514]
17. DeSimone J, Heller P, Amsel J, Usman M. Magnitude of the fetal hemoglobin response to acute hemolytic anemia in baboons is controlled by genetic factors. *J Clin Invest*. 1980; 65(1):224–226. [PubMed: 6765958]
18. DeSimone J, Heller P, Biel M, Zwiers D. Genetic relationship between fetal Hb levels in normal and erythropoietically stressed baboons. *Br J Haematol*. 1981; 49(2):175–183. [PubMed: 6170305]
19. DeSimone J, Biel M, Heller P. Maintenance of fetal hemoglobin (HbF) elevations in the baboon by prolonged erythropoietic stress. *Blood*. 1982; 60(2):519–523. [PubMed: 6178456]

20. Lavelle D, DeSimone J, Heller P, Zwiers D, Hall L. On the mechanism of Hb F elevations in the baboon by erythropoietic stress and pharmacologic manipulation. *Blood*. 1986; 67(4):1083–1089. [PubMed: 2420395]
21. 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. [PubMed: 7715639]
22. Steinberg MH, Lu ZH, Barton FB, Terrin ML, Charache S, Dover GJ. Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea. Multicenter Study of Hydroxyurea. *Blood*. 1997; 89(3):1078–1088. [PubMed: 9028341]
23. Ballas SK, Marcolina MJ, Dover GJ, Barton FB. Erythropoietic activity in patients with sickle cell anaemia before and after treatment with hydroxyurea. *Br J Haematol*. 1999; 105(2):491–496. [PubMed: 10233426]
24. 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. [PubMed: 1375104]
25. Ballas SK, Barton FB, Waclawiw MA, et al. Hydroxyurea and sickle cell anemia: effect on quality of life. *Health Qual Life Outcomes*. 2006; 4:59. [PubMed: 16942629]
26. Segal JB, Strouse JJ, Beach MC, et al. Hydroxyurea for the treatment of sickle cell disease. *Evid Rep Technol Assess (Full Rep)*. 2008; 165:1–95.
27. Machado RF, Anthi A, Steinberg MH, et al. N-terminal pro-brain natriuretic peptide levels and risk of death in sickle cell disease. *JAMA*. 2006; 296(3):310–318. [PubMed: 16849664]
28. Ware RE, Eggleston B, Redding-Lallinger R, et al. Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy. *Blood*. 2002; 99(1):10–14. [PubMed: 11756146]
29. Green NS, Manwani D, Qureshi M, Ireland K, Sinha A, Smaldone AM. Decreased fetal hemoglobin over time among youth with sickle cell disease on hydroxyurea is associated with higher urgent hospital use. *Pediatr Blood Cancer*. 2016; 63(12):2146–2153. [PubMed: 27573582]
30. West MS, Wethers D, Smith J, Steinberg M. Laboratory profile of sickle cell disease: a cross-sectional analysis. The Cooperative Study of Sickle Cell Disease. *J Clin Epidemiol*. 1992; 45(8): 893–909. [PubMed: 1624972]
31. Saraf S, Farooqui M, Infusino G, et al. Standard clinical practice underestimates the role and significance of erythropoietin deficiency in sickle cell disease. *Br J Haematol*. 2011; 153(3):386–392. [PubMed: 21418176]
32. Goren A, Simchen G, Fibach E, et al. Fine tuning of globin gene expression by DNA methylation. *PLoS ONE*. 2006; 1:e46. [PubMed: 17183675]
33. 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. [PubMed: 6205021]
34. Sauntharajah Y, Hillery CA, Lavelle D, et al. Effects of 5-aza-2'-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. *Blood*. 2003; 102(12):3865–3870. [PubMed: 12907443]
35. Sauntharajah Y, Lavelle D, DeSimone J. DNA hypo-methylating agents and sickle cell disease. *British Journal of Haematology*. 2004; 126(5):629–636. [PubMed: 15327513]
36. Hartley PD, Madhani HD. Mechanisms that specify promoter nucleosome location and identity. *Cell*. 2009; 137(3):445–458. [PubMed: 19410542]
37. Parnell TJ, Huff JT, Cairns BR. RSC regulates nucleosome positioning at Pol II genes and density at Pol III genes. *EMBO J*. 2008; 27(1):100–110. [PubMed: 18059476]
38. Lemon B, Inouye C, King DS, Tjian R. Selectivity of chromatin-remodelling cofactors for ligand-activated transcription. *Nature*. 2001; 414(6866):924–928. [PubMed: 11780067]
39. Hu Z, Gu X, Baraoidan K, et al. RUNX1 regulates corepressor interactions of PU.1. *Blood*. 2011; 117(24):6498–6508. [PubMed: 21518930]
40. Perissi V, Jepsen K, Glass CK, Rosenfeld MG. Deconstructing repression: evolving models of co-repressor action. *Nat Rev Genet*. 2010; 11(2):109–123. [PubMed: 20084085]
41. Wang Z, Zang C, Cui K, et al. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell*. 2009; 138(5):1019–1031. [PubMed: 19698979]

42. Ram O, Goren A, Amit I, et al. Combinatorial patterning of chromatin regulators uncovered by genome-wide location analysis in human cells. *Cell*. 2011; 147(7):1628–1639. [PubMed: 22196736]
43. Rosenfeld MG, Lunyak VV, Glass CK. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev*. 2006; 20(11):1405–1428. [PubMed: 16751179]
44. Dannenberg JH, David G, Zhong S, van der Torre J, Wong WH, Depinho RA. mSin3A corepressor regulates diverse transcriptional networks governing normal and neoplastic growth and survival. *Genes Dev*. 2005; 19(13):1581–1595. [PubMed: 15998811]
45. Enane FO, Shuen WH, Gu X, et al. GATA4 loss of function in liver cancer impedes precursor to hepatocyte transition. *J Clin Invest*. 2017
46. Gu X, Hu Z, Ebrahim Q, et al. Runx1 regulation of Pu.1 corepressor/coactivator exchange identifies specific molecular targets for leukemia differentiation therapy. *J Biol Chem*. 2014; 289(21):14881–14895. [PubMed: 24695740]
47. Zamir I, Zhang J, Lazar MA. Stoichiometric and steric principles governing repression by nuclear hormone receptors. *Genes Dev*. 1997; 11(7):835–846. [PubMed: 9106656]
48. Soderstrom M, Vo A, Heinzl T, et al. Differential effects of nuclear receptor corepressor (N-CoR) expression levels on retinoic acid receptor-mediated repression support the existence of dynamically regulated corepressor complexes. *Mol Endocrinol*. 1997; 11(6):682–692. [PubMed: 9171232]
49. Chronis C, Fiziev P, Papp B, et al. Cooperative Binding of Transcription Factors Orchestrates Reprogramming. *Cell*. 2017; 168(3):442–459 e420. [PubMed: 28111071]
50. Sen P, Luo J, Hada A, et al. Loss of Snf5 Induces Formation of an Aberrant SWI/SNF Complex. *Cell Rep*. 2017; 18(9):2135–2147. [PubMed: 28249160]
51. Linderson Y, Eberhard D, Malin S, Johansson A, Busslinger M, Pettersson S. Corecruitment of the Grg4 repressor by PU.1 is critical for Pax5-mediated repression of B-cell-specific genes. *EMBO Rep*. 2004; 5(3):291–296. [PubMed: 14993928]
52. Marr MT 2nd, Isogai Y, Wright KJ, Tjian R. Coactivator cross-talk specifies transcriptional output. *Genes Dev*. 2006; 20(11):1458–1469. [PubMed: 16751183]
53. Hu Z, Negrotto S, Gu X, et al. Decitabine maintains hematopoietic precursor self-renewal by preventing repression of stem cell genes by a differentiation-inducing stimulus. *Mol Cancer Ther*. 2010; 9(6):1536–1543. [PubMed: 20501800]
54. Vierbuchen T, Wernig M. Molecular roadblocks for cellular reprogramming. *Mol Cell*. 2012; 47(6):827–838. [PubMed: 23020854]
55. Velcheti V, Radivoyevitch T, Sauntharajah Y. Higher-Level Pathway Objectives of Epigenetic Therapy: A Solution to the p53 Problem in Cancer. *Am Soc Clin Oncol Educ Book*. 2017; 37:812–824. [PubMed: 28561650]
56. Milhem M, Mahmud N, Lavelle D, et al. Modification of hematopoietic stem cell fate by 5aza 2' deoxycytidine and trichostatin A. *Blood*. 2004; 103(11):4102–4110. [PubMed: 14976039]
57. Sauntharajah Y, Triozzi P, Rini B, et al. p53-Independent, normal stem cell sparing epigenetic differentiation therapy for myeloid and other malignancies. *Semin Oncol*. 2012; 39(1):97–108. [PubMed: 22289496]
58. Kingsley PD, Malik J, Emerson RL, et al. “Maturation” globin switching in primary primitive erythroid cells. *Blood*. 2006; 107(4):1665–1672. [PubMed: 16263786]
59. Mabaera R, Richardson CA, Johnson K, Hsu M, Fiering S, Lowrey CH. Developmental- and differentiation-specific patterns of human gamma- and beta-globin promoter DNA methylation. *Blood*. 2007; 110(4):1343–1352. [PubMed: 17456718]
60. McGrath K, Palis J. Ontogeny of erythropoiesis in the mammalian embryo. *Curr Top Dev Biol*. 2008; 82:1–22. [PubMed: 18282515]
61. Ni H, Yang XD, Stoeckert CJ Jr. Maturation and developmental stage-related changes in fetal globin gene expression are reproduced in transiently transfected primary adult human erythroblasts. *Exp Hematol*. 1999; 27(1):46–53. [PubMed: 9923443]

62. Papayannopoulou TH, Brice M, Stamatoyannopoulos G. Stimulation of fetal hemoglobin synthesis in bone marrow cultures from adult individuals. *Proc Natl Acad Sci U S A*. 1976; 73(6):2033–2037. [PubMed: 1064874]
63. Liu X, Zhang Y, Chen Y, et al. In Situ Capture of Chromatin Interactions by Biotinylated dCas9. *Cell*. 2017; 170(5):1028–1043 e1019. [PubMed: 28841410]
64. Krivega I, Byrnes C, de Vasconcellos JF, et al. Inhibition of G9a methyltransferase stimulates fetal hemoglobin production by facilitating LCR/gamma-globin looping. *Blood*. 2015; 126(5):665–672. [PubMed: 25979948]
65. Rivers A, Vaitkus K, Ibanez V, et al. The LSD1 inhibitor RN-1 recapitulates the fetal pattern of hemoglobin synthesis in baboons (*P. anubis*). *Haematologica*. 2016; 101(6):688–697. [PubMed: 26858356]
66. Koshy M, DeSimone J, Molokie R, Dorn L, van der Galien T, Bressler L. Augmentation of fetal hemoglobin (HbF) levels by low-dose short-duration 5'-aza-2-deoxycytidine (decitabine) administration in sickle cell anemia patients who had no HbF elevation following hydroxyurea therapy. *Blood*. 1998; 92(10):30b–30b.
67. DeSimone J, Koshy M, Dorn L, et al. Maintenance of elevated fetal hemoglobin levels by decitabine during dose interval treatment of sickle cell anemia. *Blood*. 2002; 99(11):3905–3908. [PubMed: 12010787]
68. Molokie R, Lavelle D, Gowhari M, et al. Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulation in sickle cell disease: A randomized phase 1 study. *PLoS Med*. 2017; 14(9):e1002382. [PubMed: 28880867]
69. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*. 2008; 322(5909):1839–1842. [PubMed: 19056937]
70. Tanabe O, Katsuoka F, Campbell AD, et al. An embryonic/fetal beta-type globin gene repressor contains a nuclear receptor TR2/TR4 heterodimer. *EMBO J*. 2002; 21(13):3434–3442. [PubMed: 12093744]
71. Menzel S, Garner C, Gut I, et al. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. *Nat Genet*. 2007; 39(10):1197–1199. [PubMed: 17767159]
72. Cui S, Kolodziej KE, Obara N, et al. Nuclear receptors TR2 and TR4 recruit multiple epigenetic transcriptional corepressors that associate specifically with the embryonic β -type globin promoters in differentiated adult erythroid cells. *Mol Cell Biol*. 2011; 31(16):3298–3311. [PubMed: 21670149]
73. Xu J, Bauer DE, Kerenyi MA, et al. Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. *Proc Natl Acad Sci U S A*. 2013; 110(16):6518–6523. [PubMed: 23576758]
74. Constantoulakis P, Knitter G, Stamatoyannopoulos G. On the induction of fetal hemoglobin by butyrates: in vivo and in vitro studies with sodium butyrate and comparison of combination treatments with 5-AzaC and AraC. *Blood*. 1989; 74(6):1963–1971. [PubMed: 2478217]
75. Chaturvedi CP, Hosey AM, Palii C, et al. Dual role for the methyltransferase G9a in the maintenance of beta-globin gene transcription in adult erythroid cells. *Proc Natl Acad Sci USA*. 2009; 106(43):18303–18308. [PubMed: 19822740]
76. Rank G, Cerruti L, Simpson RJ, Moritz RL, Jane SM, Zhao Q. Identification of a PRMT5-dependent repressor complex linked to silencing of human fetal globin gene expression. *Blood*. 2010; 116(9):1585–1592. [PubMed: 20495075]
77. Xu Z, He Y, Ju J, et al. The role of WDR5 in silencing human fetal globin gene expression. *Haematologica*. 2012; 97(11):1632–1640. [PubMed: 22689669]
78. Renneville A, Van Galen P, Canver MC, et al. EHMT1 and EHMT2 inhibition induces fetal hemoglobin expression. *Blood*. 2015; 126(16):1930–1939. [PubMed: 26320100]
79. Ginder GD, Whitters MJ, Pohlman JK, Burns LJ. Transcriptional activation of an embryonic globin gene in adult erythroid cells in vivo. *Prog Clin Biol Res*. 1985; 191:463–474. [PubMed: 2413482]

80. Scuto A, Kirschbaum M, Kowolik C, et al. The novel histone deacetylase inhibitor, LBH589, induces expression of DNA damage response genes and apoptosis in Ph- acute lymphoblastic leukemia cells. *Blood*. 2008; 111(10):5093–5100. [PubMed: 18349321]
81. Lee JH, Choy ML, Ngo L, Foster SS, Marks PA. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. *Proc Natl Acad Sci USA*. 2010; 107(33):14639–14644. [PubMed: 20679231]
82. Conti C, Leo E, Eichler GS, et al. Inhibition of histone deacetylase in cancer cells slows down replication forks, activates dormant origins, and induces DNA damage. *Cancer Res*. 2010; 70(11):4470–4480. [PubMed: 20460513]
83. Gaymes TJ, Padua RA, Pla M, et al. Histone deacetylase inhibitors (HDI) cause DNA damage in leukemia cells: a mechanism for leukemia-specific HDI-dependent apoptosis? *Mol Cancer Res*. 2006; 4(8):563–573. [PubMed: 16877702]
84. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer*. 2006; 6(1):38–51. [PubMed: 16397526]
85. Paththamalai P, Fuchareon S, Chaneiam N, et al. A phase 2 trial of HQK-1001 in HbE-beta thalassemia demonstrates HbF induction and reduced anemia. *Blood*. 2014; 123(12):1956–1957. [PubMed: 24652964]
86. van der Ploeg LH, Flavell RA. DNA methylation in the human gamma delta beta-globin locus in erythroid and nonerythroid tissues. *Cell*. 1980; 19(4):947–958. [PubMed: 6247075]
87. Mavilio F, Giampaolo A, Care A, et al. Molecular mechanisms of human hemoglobin switching: selective undermethylation and expression of globin genes in embryonic, fetal, and adult erythroblasts. *Proc Natl Acad Sci USA*. 1983; 80(22):6907–6911. [PubMed: 6316333]
88. Tagle DA, Koop BF, Goodman M, Slightom JL, Hess DL, Jones RT. Embryonic epsilon and gamma globin genes of a prosimian primate (*Galago crassicaudatus*). Nucleotide and amino acid sequences, developmental regulation and phylogenetic footprints. *J Mol Biol*. 1988; 203(2):439–455. [PubMed: 3199442]
89. DeSimone J, Heller P, Hall L, Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. *Proc Natl Acad Sci USA*. 1982; 79(14):4428–4431. [PubMed: 6181507]
90. Ley TJ, DeSimone J, Anagnou NP, et al. 5-azacytidine selectively increases gamma-globin synthesis in a patient with beta+ thalassemia. *N Engl J Med*. 1982; 307(24):1469–1475. [PubMed: 6183586]
91. 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. [PubMed: 6191799]
92. Charache S, Dover G, Smith K, Talbot CC Jr, Moyer M, Boyer S. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the gamma-delta-beta-globin gene complex. *Proc Natl Acad Sci USA*. 1983; 80(15):4842–4846. [PubMed: 6192443]
93. Dover GJ, Charache S, Boyer SH, Vogelsang G, Moyer M. 5-Azacytidine increases HbF production and reduces anemia in sickle cell disease: dose-response analysis of subcutaneous and oral dosage regimens. *Blood*. 1985; 66(3):527–532. [PubMed: 2411310]
94. Lowrey CH, Nienhuis AW. Brief report: treatment with azacitidine of patients with end-stage beta-thalassemia. *N Engl J Med*. 1993; 329(12):845–848. [PubMed: 7689171]
95. Cui S, Kolodziej KE, Obara N, et al. Nuclear receptors TR2 and TR4 recruit multiple epigenetic transcriptional corepressors that associate specifically with the embryonic beta-type globin promoters in differentiated adult erythroid cells. *Mol Cell Biol*. 2011; 31(16):3298–3311. [PubMed: 21670149]
96. Santi DV, Garrett CE, Barr PJ. On the mechanism of inhibition of DNA-cytosine methyltransferases by cytosine analogs. *Cell*. 1983; 33(1):9–10. [PubMed: 6205762]
97. Covey JM, D’Incalci M, Tilchen EJ, Zaharko DS, Kohn KW. Differences in DNA damage produced by incorporation of 5-aza-2'-deoxycytidine or 5,6-dihydro-5-azacytidine into DNA of mammalian cells. *Cancer Res*. 1986; 46(11):5511–5517. [PubMed: 2428479]

98. Schermelleh L, Haemmer A, Spada F, et al. Dynamics of Dnmt1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation. *Nucleic Acids Res.* 2007; 35(13):4301–4312. [PubMed: 17576694]
99. Zauri M, Berridge G, Thezenas ML, et al. CDA directs metabolism of epigenetic nucleosides revealing a therapeutic window in cancer. *Nature.* 2015; 524(7563):114–118. [PubMed: 26200337]
100. Almqvist H, Axelsson H, Jafari R, et al. CETSA screening identifies known and novel thymidylate synthase inhibitors and slow intracellular activation of 5-fluorouracil. *Nat Commun.* 2016; 7:11040. [PubMed: 27010513]
101. Olivieri NF, Saunthararajah Y, Thayalasuthan V, et al. A pilot study of subcutaneous decitabine in beta-thalassemia intermedia. *Blood.* 2011; 118(10):2708–2711. [PubMed: 21700776]
102. Saunthararajah Y, Sekeres M, Advani A, et al. Evaluation of noncytotoxic DNMT1-depleting therapy in patients with myelodysplastic syndromes. *J Clin Invest.* 2015; 125(3):1043–1055. [PubMed: 25621498]
103. Liu Z, Marcucci G, Byrd JC, Grever M, Xiao J, Chan KK. Characterization of decomposition products and preclinical and low dose clinical pharmacokinetics of decitabine (5-aza-2'-deoxycytidine) by a new liquid chromatography/tandem mass spectrometry quantification method. *Rapid Commun Mass Spectrom.* 2006; 20(7):1117–1126.
104. Liu Z, Liu S, Xie Z, et al. Characterization of in vitro and in vivo hypomethylating effects of decitabine in acute myeloid leukemia by a rapid, specific and sensitive LC-MS/MS method. *Nucleic Acids Res.* 2007; 35(5):e31. [PubMed: 17264127]
105. Camiener GW, Smith CG. Studies of the enzymatic deamination of cytosine arabinoside. I. Enzyme distribution and species specificity. *Biochem Pharmacol.* 1965; 14(10):1405–1416. [PubMed: 4956026]
106. Neil GL, Moxley TE, Kuentzel SL, Manak RC, Hanka LJ. Enhancement by tetrahydrouridine (NSC-112907) of the oral activity of 5-azacytidine (NSC-102816) in L1210 leukemic mice. *Cancer Chemother Rep.* 1975; 59(3):459–465. [PubMed: 54211]
107. DeSimone J, Heller P, Molokie RE, Hall L, Zwiers D. Tetrahydrouridine, cytidine analogues, and hemoglobin F. *Am J Hematol.* 1985; 18(3):283–288. [PubMed: 2579548]
108. Beumer JH, Eiseman JL, Parise RA, Joseph E, Covey JM, Egorin MJ. Modulation of gemcitabine (2',2'-difluoro-2'-deoxycytidine) pharmacokinetics, metabolism, and bioavailability in mice by 3,4,5,6-tetrahydrouridine. *Clin Cancer Res.* 2008; 14(11):3529–3535. [PubMed: 18519786]
109. Ebrahim Q, Mahfouz R, Ng KP, Saunthararajah Y. High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. *Oncotarget.* 2012; 3(10):1137–1145. [PubMed: 23087155]
110. Riccardi R, Chabner B, Glaubiger DL, Wood J, Poplack DG. Influence of tetrahydrouridine on the pharmacokinetics of intrathecally administered 1-beta-D-arabinofuranosylcytosine. *Cancer Res.* 1982; 42(5):1736–1739. [PubMed: 6896013]
111. Kreis W, Budman DR, Chan K, et al. Therapy of refractory/relapsed acute leukemia with cytosine arabinoside plus tetrahydrouridine (an inhibitor of cytidine deaminase)—a pilot study. *Leukemia.* 1991; 5(11):991–998. [PubMed: 1961042]
112. Wong PP, Currie VE, Mackey RW, et al. Phase I evaluation of tetrahydrouridine combined with cytosine arabinoside. *Cancer Treat Rep.* 1979; 63(8):1245–1249. [PubMed: 383291]
113. Ho DH, Bodey GP, Hall SW, et al. Clinical pharmacology of tetrahydrouridine. *J Clin Pharmacol.* 1978; 18(5-6):259–265. [PubMed: 641214]
114. Kreis W, Woodcock TM, Gordon CS, Krakoff IH. Tetrahydrouridine: Physiologic disposition and effect upon deamination of cytosine arabinoside in man. *Cancer Treat Rep.* 1977; 61(7):1347–1353. [PubMed: 589600]
115. Marsh JH, Kreis W, Barile B, et al. Therapy of refractory/relapsed acute myeloid leukemia and blast crisis of chronic myeloid leukemia with the combination of cytosine arabinoside, tetrahydrouridine, and carboplatin. *Cancer Chemother Pharmacol.* 1993; 31(6):481–484. [PubMed: 8453688]
116. Goldenthal EI, Cookson KM, Geil RG, Wazeter FX. Preclinical toxicologic evaluation of tetrahydrouridine (NSC-112907) in beagle dogs and rhesus monkeys. *Cancer Chemother Rep.* 1974; 5(1):15–16. [PubMed: 4213302]

117. Santi DV, Norment A, Garrett CE. Covalent bond formation between a DNA-cytosine methyltransferase and DNA containing 5-azacytosine. *Proc Natl Acad Sci USA*. 1984; 81(22): 6993–6997. [PubMed: 6209710]
118. Patel K, Dickson J, Din S, Macleod K, Jodrell D, Ramsahoye B. Targeting of 5-aza-2'-deoxycytidine residues by chromatin-associated DNMT1 induces proteasomal degradation of the free enzyme. *Nucleic Acids Res*. 2010; 38(13):4313–4324. [PubMed: 20348135]
119. Creusot F, Acs G, Christman JK. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'-deoxycytidine. *J Biol Chem*. 1982; 257(4):2041–2048. [PubMed: 6173384]
120. Clements EG, Mohammad HP, Leadem BR, et al. DNMT1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. *Nucleic Acids Res*. 2012; 40(10):4334–4346. [PubMed: 22278882]
121. Brenner C, Luciani J, Bizet M, et al. The interplay between the lysine demethylase KDM1A and DNA methyltransferases in cancer cells is cell cycle dependent. *Oncotarget*. 2016; 7(37):58939–58952. [PubMed: 27449289]
122. Shi L, Cui S, Engel JD, Tanabe O. Lysine-specific demethylase 1 is a therapeutic target for fetal hemoglobin induction. *Nat Med*. 2013; 19(3):291–294. [PubMed: 23416702]
123. Cui S, Lim KC, Shi L, et al. The LSD1 inhibitor RN-1 induces fetal hemoglobin synthesis and reduces disease pathology in sickle cell mice. *Blood*. 2015; 126(3):386–396. [PubMed: 26031919]
124. Jagadeeswaran R, Vazquez BA, Thiruppathi M, et al. Pharmacological inhibition of LSD1 and mTOR reduces mitochondrial retention and associated ROS levels in the red blood cells of sickle cell disease. *Exp Hematol*. 2017; 50:46–52. [PubMed: 28238805]

Figure 1. Polymerization of deoxy sickle hemoglobin (HbS) drives the multi-organ cascade of SCD pathophysiology

Fetal hemoglobin (HbF), by blocking this polymerization, can interdict essentially all manifestations of disease, illustrated by the natural genetic experiment of hereditary persistence of fetal hemoglobin expression, and a model for drug development efforts to emulate. By contrast to HbF, normal adult hemoglobin (HbA, β -chains) can participate in polymerization. We published a variation of this figure in Molokie et al, PLoS Medicine 2017.

Figure 2. HbF induction requires chromatin remodeling, including DNA hypomethylation, of the HbF gene locus (*HBG*)

Bone marrow stress, e.g., from cytotoxic drugs such as hydroxyurea, can create such remodeling during the recovery phase by surviving erythroid precursors. An alternative approach is to remodel the HbF locus directly, e.g., by directly inhibiting repressing epigenetic enzymes. The relative efficiencies of these approaches are illustrated by the greater HbF increases produced in the same non-human primates or patients by decitabine ~0.2 mg/kg 2X/week, versus hydroxyurea ~20 mg/kg daily. That is, the molar amount of decitabine administered per week is <1/1000th the amount of hydroxyurea administered per week.

Table 1

Scientifically validated molecular targets for HbF induction and candidate drugs

Target	Drugs	Stage
HDACs [*]	<ul style="list-style-type: none"> - Depsipeptide (HDAC1,2,4,6) - Belinostat (broad HDAC inhibitor) - Panobinostat (broad) - Vorinostat (broad) 	<ul style="list-style-type: none"> - Marketed for peripheral T-cell lymphomas - Phase 1 in sickle cell disease (panobinostat) (clinicaltrials.gov identifier: NCT01245179) - Phase 2 in SCD and β-thalassemia (HQB-1001) (clinicaltrials.gov identifiers: NCT01642758, NCT01601340)
DNMT1	<ul style="list-style-type: none"> - Decitabine - 5-azacytidine (decitabine pro-drug) 	<ul style="list-style-type: none"> - Marketed for myelodysplastic syndromes - Oral forms, including in combination with inhibitors of degradation, are in Phase 1/2 for liquid/solid malignancies, and SCD (clinicaltrials.gov identifier: NCT01685515)
KDM1A [#]	<ul style="list-style-type: none"> - ORY-1001 (related to RN-1) - GSK2879552 - 4SC-202 - INCB059872 	<ul style="list-style-type: none"> - Phase 1/2 in liquid/solid malignancies - Phase 1 in SCD (INCB059872) (clinicaltrials.gov identifier: NCT03132324)
PRMT5	<ul style="list-style-type: none"> - GSK3326595 	<ul style="list-style-type: none"> - Phase 1 in liquid/solid malignancies
EHMT2	<ul style="list-style-type: none"> - UNC0638 	Pre-clinical in vitro
ISWI (CHD4, SMARCA5)	<ul style="list-style-type: none"> - not officially designated, patent issued 	Pre-clinical in vitro

^{*} only HDAC inhibitors approved in the USA are listed, several other compounds are in clinical trials

[#] only KDM1A inhibitors registered in clinical trials in the USA are listed, several other compounds are in development