

effort to combat malaria through the distribution of insecticide-treated bed nets and chemoprophylaxis has resulted in a 65% reduction in malaria-related mortality.⁵ The 2010 World Health Organization African Regional Strategy recommends that children with SCA in sub-Saharan Africa receive some form of malaria prevention.¹⁰ But bed net coverage in Africa still remains patchy, and whether or not chemoprophylaxis should occur and which specific chemoprophylaxis to use are not well established. Thus, the findings of NOHARM may not apply to children with SCA who do not receive malaria chemoprophylaxis or use insecticide-treated bed nets.

The duration of treatment with hydroxyurea was only 12 months and thus provided no information about the longer-term effects of hydroxyurea treatment in children with SCA in sub-Saharan Africa. The extended open-label phase of hydroxyurea therapy in this cohort will help generate longer-term data to guide implementation of hydroxyurea therapy in these settings. The study participants were seen for scheduled visits once per month for the first 4 months, then once every 2 months for the next 8 months, a total of 10 scheduled visits per year including the randomization visit and 2 weeks after treatment initiation. Would this frequency of clinic visits by patients and families be feasible as a standard of care in sub-Saharan Africa?

With the reassuring results of NOHARM regarding safety of hydroxyurea treatment, placebo-controlled trials in sub-Saharan Africa would no longer be justified. Rather, open-label studies with longer follow-up to explore different dosing schemes that allow for less frequent and rigorous laboratory monitoring should be conducted. Dose-limiting toxicities were low at the 20 mg/kg fixed dose of hydroxyurea in the NOHARM trial, suggesting that less frequent monitoring would be more feasible and safer with this dosing regimen compared with dose escalation to the maximum tolerated dose.

The results of the NOHARM trial help address a major barrier to using hydroxyurea treatment in children with SCA in regions where malaria is endemic, thereby moving clinicians a step closer to its wider use across sub-Saharan Africa.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Opoka RO, Nduwuga CM, Latham TS, et al. Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM): a trial for children with sickle cell anemia. *Blood*. 2017;130(24):2585-2593.
2. Piel FB, Patil AP, Howes RE, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nat Commun*. 2010; 1(8):104-111.
3. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN. Sickle cell disease in Africa: a neglected cause of early childhood mortality. *Am J Prev Med*. 2011; 41(6 suppl 4):S398-S405.
4. Quinn CT, Rogers ZR, McCavit TL, Buchanan GR. Improved survival of children and adolescents with sickle cell disease. *Blood*. 2010;115(17):3447-3452.
5. UNICEF. Malaria mortality among children under five is concentrated in sub-Saharan Africa. Updated February 2017. <http://data.unicef.org/topic/child-health/malaria/#>.
6. McAuley CF, Webb C, Makani J, et al. High mortality from *Plasmodium falciparum* malaria in children

living with sickle cell anemia on the coast of Kenya. *Blood*. 2010;116(10):1663-1668.

7. Brun M, Bourdoulous S, Couraud PO, Elion J, Krishnamoorthy R, Lapoumeroulie C. Hydroxyurea downregulates endothelin-1 gene expression and upregulates ICAM-1 gene expression in cultured human endothelial cells. *Pharmacogenomics J*. 2003;3(4):215-226.
8. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJ. Nitric oxide protection against murine cerebral malaria is associated with improved cerebral microcirculatory physiology. *J Infect Dis*. 2011;203(10):1454-1463.
9. Ramakrishnan M, Moisi JC, Klugman KP, et al. Increased risk of invasive bacterial infections in African people with sickle-cell disease: a systematic review and meta-analysis. *Lancet Infect Dis*. 2010;10(5):329-337.
10. World Health Organization Regional Office for Africa. Sickle cell disease: a strategy for the WHO African region. <http://www.who.int/iris/handle/10665/1682>.

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● ● ● LYMPHOID NEOPLASIA

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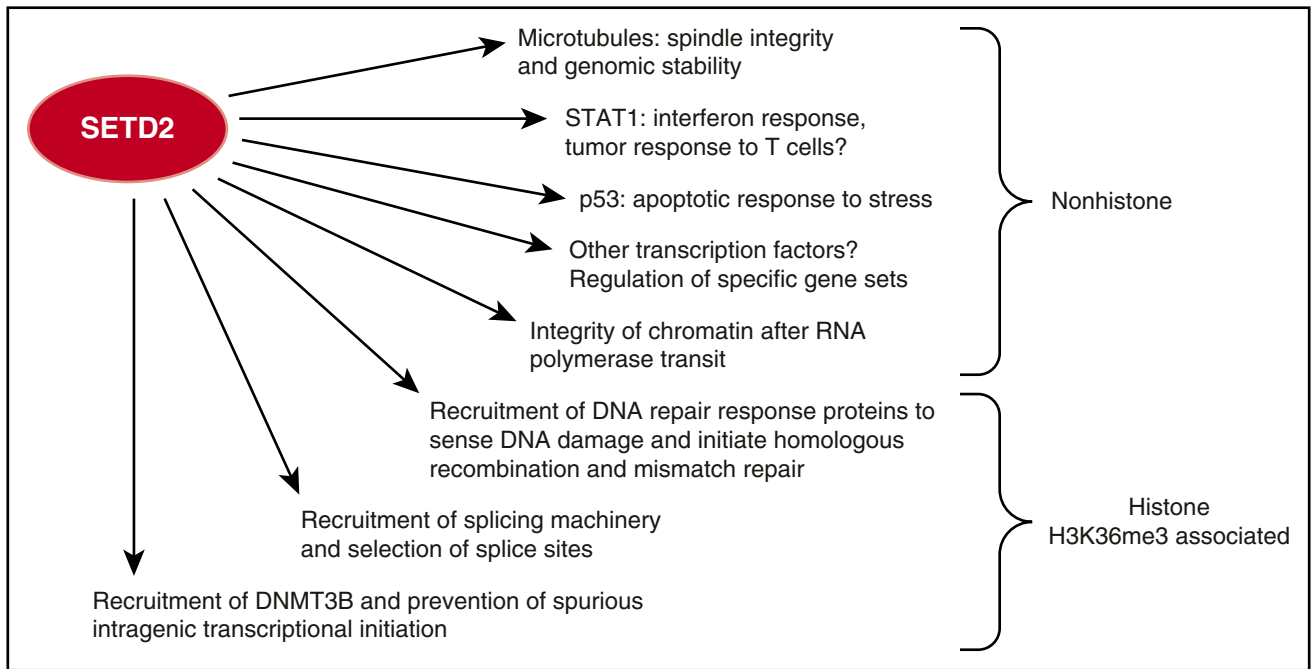
SETD2: a complex role in blood malignancy

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In this issue of *Blood*, Mar et al describe the effect of inactivating mutations of the histone methyltransferase (HMT) SETD2 in accelerating leukemia pathogenesis and conferring therapy resistance.¹ Mutations of epigenetic regulators are among the commonest lesions in malignancy. These include mutations of HMTs that modify histone tails protruding from the nucleosome. For example, inactivation of EZH2, responsible for histone H3 lysine 27 trimethylation (H3K27me3), a modification associated with gene silencing, occurs in myelodysplasia and acute myeloid leukemia (AML), whereas mutation of KMT2D, responsible for the H3K4me1 modification found at enhancers, is common in lymphoma. These enzymes change the chemical composition of chromatin at gene regulatory sites, affecting transcriptional initiation. By contrast, SETD2 has a role downstream of the transcriptional start site (TSS).

Mutations of *SETD2*, including truncating mutations, are commonest in clear cell renal carcinoma (~20%) and are found in 5% to 10% of cases of a wide variety of tumors, including melanoma, bladder, lung, and uterine (www.cbioportal.org). *SETD2* mutations were found in 12% of cases of B-cell acute lymphoblastic leukemia, 1% to 2% of cases of B-cell lymphoma, chronic lymphocytic leukemia, and AML, and occasionally cases of myeloproliferative neoplasm. SETD2 is the sole mammalian HMT that catalyzes H3K36 trimethylation (H3K36me3). SETD2

associates with elongating RNA polymerase, creating H3K36me3-modified nucleosomes 3' to the TSS that serve as docking sites for the FACT histone chaperone and assembly complex. Thus, H3K36me3 methylation by SETD2 and recruitment of tight arrays of nucleosomes prevent the spurious reinitiation of transcription within gene bodies. H3K36me3 also recruits DNMT3B, leading to the dense methylation of gene bodies, which reinforces intragenic silencing.² In addition, H3K36me3 helps direct the splicing machinery to intron/exon boundaries.



SETD2, the sole known HMT to create the histone H3 lysine 36 trimethyl (H3K36me3) modification through this histone change, mediates multiple molecular processes of gene regulation and DNA damage response. SETD2 also has nonhistone substrates with important roles in cellular homeostasis. Loss of SETD2 activity therefore may interrupt a host of critical processes that contribute to malignancy.

SETD2 has an important role in DNA repair. The MSH6 protein recognizes H3K36me3, and SETD2 activity is required for base-mismatch repair.³ SETD2 and H3K36me3 are also required for recruitment of RAD51 and LEDGF to double-stranded DNA breaks, promoting repair of active genes by homologous recombination.⁴ Furthermore, SETD2 depletion/mutation leads to decreased recruitment of DNA replication machinery and replication fork instability. SETD2 also functions outside of the nucleus, methylating tubulin, and loss of SETD2 leads to mitotic abnormalities.⁵ SETD2 also methylates STAT1 and is important for interferon-mediated antiviral⁶ and potentially immune surveillance response. In addition, SETD2 can bind and alter p53 activity.

The many functions of SETD2 suggest how its inactivation might contribute to malignancy (see figure). In renal cancer, loss of SETD2 led to depletion of nucleosomes, loss of DNA methylation, aberrant splicing, and abnormal intragenic RNAs. SETD2 mutant renal cancer cell lines are also deficient in DNA repair.⁷ Previous studies in blood malignancy showed that knockdown of *Setd2* in an MLL-fusion AML led to increased tumor cell growth. This was associated with increased expression of mTOR and STAT pathways.⁸ SETD2 mutations were found in 25% of the rare hepatosplenic $\gamma\delta$ T-cell lymphoma (HSTL), and knockdown of SETD2

in HSTL cell lines increased cell growth and altered expression of cell-cycle genes. SETD2 mutation is also found in enteropathy-associated T-cell lymphoma. The predilection of the mutation for these tumors may be explained by the observation from knockout models that SETD2 controls the ratio of $\gamma\delta$ vs $\alpha\beta$ T cells.⁹ However, the mechanism by which the depletion of H3K36me3 alters a specific gene and developmental program remains unexplained.

The work of Mar et al further implicates SETD2 as a tumor suppressor. Using CRISPR/CAS9 to generate AML cells with disruption of SETD2, they also observed that DNA damage response and repair were compromised. In a murine model in which bone marrow was transformed with MLL-AF9, disruption of both *Setd2* alleles inhibited leukemogenesis, potentially because of excessive DNA damage and impaired replication. This effect may be cell type specific because renal cancer often suffers complete loss of SETD2, whereas mutations in leukemia are heterozygous. Loss of one allele of *Setd2* led to decreased leukemia latency and resistance to chemotherapy. How loss of a single allele accelerates disease remains to be determined but may be due to deregulation of subsets of growth-activating genes or increased DNA damage and accumulation of secondary mutations.

The epigenetic lesions in malignancy are vexing because targeted therapy cannot

be applied to the common loss-of-function mutations. In the case of the HMTs, rebalancing chromatin patterns by targeting opposing enzymes can be considered. For example, inactivation of the H3K27me3 histone demethylase KDM6A in bladder cancer or myeloma can be approached by inhibition of EZH2. Consequently, Mar et al applied an inhibitor of the H3K36 demethylase KDM4A to SETD2 mutant cells and observed a rise in H3K36me3 levels. How this occurred in a cell with compound heterozygous mutation of SETD2 is unclear and may be a consequence of residual activity of SETD2 or compensation by an as yet unidentified HMT. The elevated H3K36me3 levels tracked with an increase in sensitivity to chemotherapy in vitro, and the combination of demethylase inhibitor and chemotherapy could acutely kill more leukemia cells in vivo. However, this was not sufficient to extend animal life span, perhaps because of insufficient pharmacodynamics or pharmacokinetics, the ability of the inhibitor to affect other demethylases, or other aspects of AML biology affected by *Setd2* loss.

SETD2 mutations are associated with poor prognosis in malignancy, and although rebalancing chromatin function is a rational approach, it may not be the answer. In SETD2 mutant renal cancer, ribonucleotide reductase 2 (RRM2) levels were low and further decreased by

a Wee1 inhibitor, representing a synthetic lethal approach.¹⁰ However, in the AML model, RRM2 levels were unchanged, again pointing to potentially indirect and cell type-specific effects of SETD2 loss on gene expression conceivably through nonchromatin mechanisms, such as modification of transcription factor activity. With a genetically faithful model of SETD2-associated malignancy in hand, further screens for vulnerabilities using gene editing are warranted. Thorough analysis of these mice for changes in gene expression and chromatin modifications, p53 target expression, and interferon pathways may lead to further clues to the aggressive biology generated by SETD2 loss.

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REFERENCES

1. Mar BG, Chu SH, Kahn JD, et al. SETD2 alterations impair DNA damage recognition and lead to resistance to chemotherapy in leukemia. *Blood*. 2017;130(24):2631-2641.
2. Neri F, Rapelli S, Krepelova A, et al. Intragenic DNA methylation prevents spurious transcription initiation. *Nature*. 2017;543(7643):72-77.

3. Li F, Mao G, Tong D, et al. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutS α . *Cell*. 2013;153(3):590-600.
4. Aymard F, Bugler B, Schmidt CK, et al. Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat Struct Mol Biol*. 2014;21(4):366-374.
5. Park IY, Chowdhury P, Tripathi DN, et al. Methylated α -tubulin antibodies recognize a new microtubule modification on mitotic microtubules. *MAbs*. 2016;8(8):1590-1597.
6. Chen K, Liu J, Liu S, et al. Methyltransferase SETD2-mediated methylation of STAT1 is critical for interferon antiviral activity. *Cell*. 2017;170(3):492-506.e14.
7. Carvalho S, Vitor AC, Sridhara SC, et al. SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint. *eLife*. 2014;3:e02482.
8. Zhu X, He F, Zeng H, et al. Identification of functional cooperative mutations of SETD2 in human acute leukemia. *Nat Genet*. 2014;46(3):287-293.
9. Moffitt AB, Ondrejka SL, McKinney M, et al. Enteropathy-associated T cell lymphoma subtypes are characterized by loss of function of SETD2. *J Exp Med*. 2017;214(5):1371-1386.
10. Pfister SX, Markkanen E, Jiang Y, et al. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. *Cancer Cell*. 2015;28(5):557-568.

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Carden et al, page 2654

Of pools, oceans, and the Dead Sea

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In a comprehensive study in this issue of *Blood*, Carden and colleagues describe the importance of the tonicity of IV fluids used in the treatment of patients with sickle cell disease (SCD) during vaso-occlusive crises (VOCs). Hypertonic fluids decreased sickle red blood cell (sRBC) deformability, increased occlusion, and increased sRBC adhesion in microfluidic human microvasculature models. Hypotonic fluids decreased sRBC adhesion but prolonged sRBC transit time. Fluids with intermediate tonicities resulted in optimal changes that reduced the risk of vaso-occlusion.¹

Osmosis is the movement of a neutral solvent across a semipermeable (red blood cell [RBC]) membrane from a less concentrated solution into a more concentrated one. This movement equalizes the concentration of solutes on each side of the membrane. Tonicity, on the other hand, is the ability of a solution to make water move into or out of a cell by osmosis. It depends on the concentration of all solutes in the solution (ie, its osmolarity). A solution with low osmolarity is hypotonic and vice versa. Solutions having the same osmolarity are isotonic.^{2,3}

Water in pools and rivers is hypotonic compared with sea water, but water from the

Dead Sea is hypertonic. Swimming in the ocean or sea is relatively easier than swimming in a pool or river due to the buoyancy of seawater, which contains ~3.5% salt. Floating, rather than swimming, is the rule in the Dead Sea due to its hypertonicity with ~34.2% salt. Metaphorically, the RBC maintains its normal shape, shrinks, or balloons depending on the tonicity of the extracellular solution in which it is suspended.

It is generally believed that aggressive treatment of the VOC at its onset would shorten its duration with fewer complications.⁴ The rational approach to abort a VOC is to treat it as early as possible, when tissue ischemia and

inflammation are in their early stages. The standard of care of anti-VOC therapy to abort a crisis includes a trial of hydration, anti-inflammatory, analgesics, and adjuvants.⁴ Hydration often requires the administration of IV fluids. Fluids that are usually used for hydration are shown in figure panel A. The choice of a certain fluid varies greatly among institutions and providers. The recent guidelines of the National Heart, Lung, and Blood Institute to treat the complications of SCD did not adequately address this issue.^{5,6} In addition, a recent Cochrane review of fluid replacement therapy for acute episodes of pain in people with SCD found no randomized controlled trials that have assessed the safety and efficacy of different routes, types, or quantities of fluid.⁷

In this issue of *Blood*, Carden et al describe elegant studies to determine the tonicity of the desirable IV fluid to hydrate patients with VOC. The scheme is illustrated in figure panel B. sRBC exposed to admixtures with the highest sodium level of 141 mEq/L (hypertonic solution) impacted sRBC biomechanics due to dehydration (xerocytosis), with increased mean corpuscular hemoglobin concentration (MCHC) and decreased mean corpuscular volume associated with increased cytoplasmic viscosity and increased membrane rigidity leading to decreased deformability and increased tendency for occlusion under normoxic and hypoxic conditions in the microfluidic human microvascular models used by the authors. This is similar to the xerocytosis described in hemoglobin SCD.⁸ On the other hand, the exposure of sRBC admixtures to hypotonic solution with a sodium level of 103 mEq/L caused water gain with a decreased surface area-to-volume ratio, transforming sRBCs to swollen spherocytes with significantly decreased deformability and increased risk of occlusion in the normoxic microfluidic models used due to the fact that overswollen spherocytes prolonged the transit times of sRBCs in capillary-sized microchannels. Admixtures with sodium concentrations of 111 to 122 mEq/L appeared to optimize changes in sRBC deformability, in part due to ideal changes in the MCHC, the most sensitive predictor of hemoglobin S polymerization and microvascular occlusion, and in part due to the optimization of the surface area-to-volume ratio necessary for sRBCs to traverse capillary microchannels.

In addition to the salutary effects of isotonic IV fluids to treat VOCs by preventing or minimizing