Fujikawa et al described the epigenetic landscape in ATL using an integrative analysis of transcriptome and epigenome.[1] They demonstrate that early after HTLV-1 infection, the viral transactivator of the X gene region, Tax, activates the transcription of key components of the polycomb-repressive complex 2, mainly the enhancer of zeste homolog 2 (EZH2), resulting in global alteration of trimethylation at histone H3Lys27 (H3K27me3) and epigenetic reprogramming involving more than half of cellular genes. These epigenetic changes could be reproduced through Tax transduction into normal T lymphocytes (see figure) whereas other viral proteins failed to do it, including the product of the HTLV-1 basic leucine zipper (HBZ) gene encoded by the antisense transcript. Importantly, the same profile is persistent in ATL even at late stages of the disease. Critically, this epigenetic-dependent global modification of host gene expression in ATL was reversible and was not due to EZH2 mutations found in other types of lymphoma, demonstrating that the ATL phenotype is Tax dependent.

It is well established that the HTLV-1 oncoprotein Tax initiates transformation[2] in ATL. In addition to its effects on the viral promoter, Tax alters many cellular pathways[3]: it activates transcription factors such as the nuclear factor-κB and the cAMP response element-binding protein (CREB), upregulates antiapoptotic proteins, represses the tumor suppressor p53, DNA polymerase β, proliferating cell nuclear antigen, and mitotic arrest deficient-1 checkpoint protein, and interferes with several cell cycle regulators and DNA repair. The current study by Fujikawa et al adds a new dimension in Tax oncogenic properties, namely the epigenetic-dependent global modification of host gene expression.[1]

Through all of these activities, Tax acts as a powerful oncogene as demonstrated in transgenic mice models, whereby expression of Tax alone can induce ATL. However, that continuous Tax expression is required to sustain the malignant phenotype remains controversial. Indeed, Tax levels are very low in most ATL patients, making them undetectable by routine techniques. Furthermore, some ATL clones bear mutations in Tax predicted to abrogate its expression. This has suggested that, although Tax may have an initiating role in ATL, it could allow the accumulation of subsequent genetic changes that are the actual drivers of transformation.[3] On the other hand, with regulatory proteins that are expressed at very low levels, sensitivity of the detection method always remains an issue. For example, in Tax transgenic mice, Tax mRNA is present at very low levels, similar to acute ATL patients, and the protein remains undetectable in the transformed T cells.[4] Furthermore, ATL-derived cells and HTLV-1–transformed cells are addicted to continuous Tax expression even when Tax protein is undetectable,[5] providing a strong rationale for targeting Tax in ATL therapy. The recent demonstration of the efficacy of the Tax peptide-pulsed dendritic cell vaccine in treating ATL patients[6] further strengthens this concept.

Despite significant progress in ATL therapy using the antiviral combination of zidovudine and interferon-α, most ATL patients continue to die rapidly from their disease, often in <12 months, stressing the need for novel effective targeted therapies.[7] The current report by Fujikawa et al unravels that Tax fingerprint is almost everywhere in ATL through a powerful epigenetic-dependent global modification of host gene expression, either through upregulation or downregulation.[1] These findings provide a strong rationale for directly targeting Tax in ATL therapy using arsenic trioxide and interferon α4 or anti-Tax vaccines[6] as well as targeting this Tax global fingerprint on cellular genes using EZH2 inhibitors. Indeed, pharmacologic inhibition of EZH2 reversed the epigenetic disruption and selectively eliminated leukemic and HTLV-1–infected cells.

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The Tax fingerprint is everywhere in ATL. Venn diagram showing overlap between regions with significant H3K27me3 gain in ATL cells and Tax-transduced T cells compared with normal CD4$^+$ T cells. See Figure 6G in the article by Fujikawa et al that begins on page 1790.

● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Bernaudin et al, page 1814

Hydroxyurea for abnormal TCDs: safe to switch?

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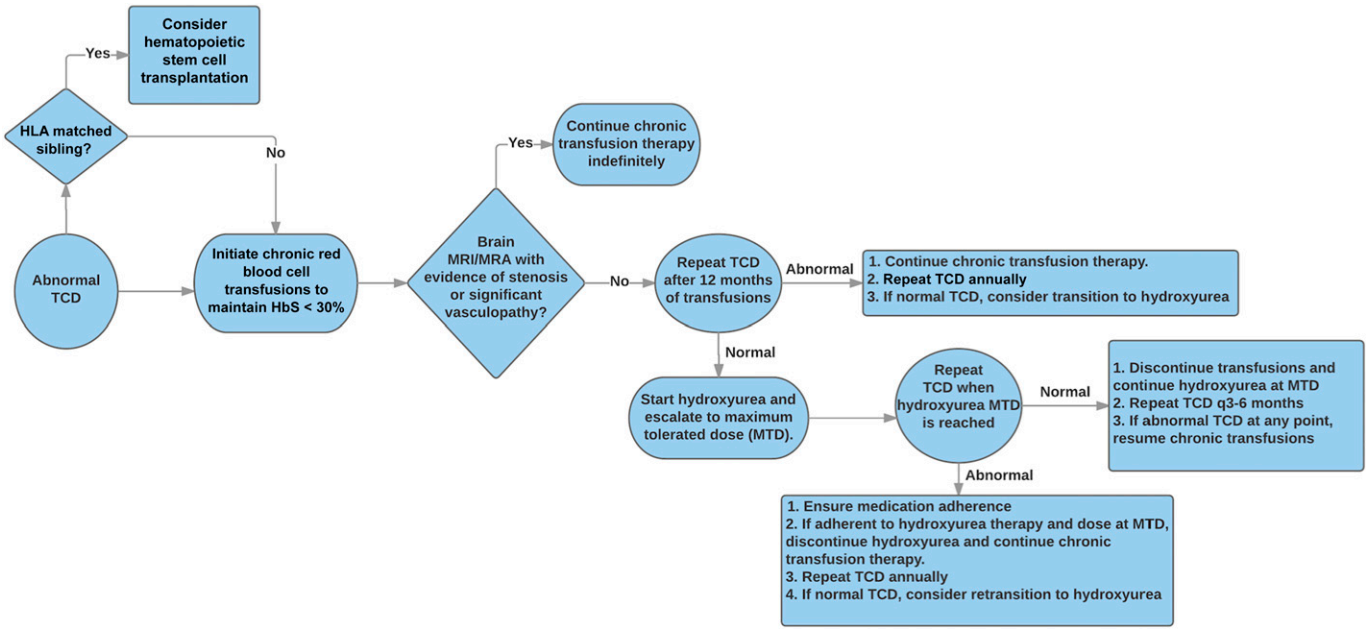
In this issue of *Blood*, Bernaudin et al report that chronic red blood cell transfusions can be safely replaced with hydroxyurea therapy or bone marrow transplantation for a cohort of children with sickle cell anemia (SCA) and

abnormal transcranial Doppler (TCD) velocities.[1] These data nicely complement the recently published results from the phase 3 multicenter TCD With Transfusions Changing to Hydroxyurea (TWiTCH) study and suggest that it may be safe to carefully transition a subset of patients from chronic transfusions to hydroxyurea therapy.[2]

Over the last 20 years, we have seen dramatic reductions in the frequency of stroke for children with SCA. Prior to active surveillance with TCD and routine use of either chronic blood transfusions or hydroxyurea therapy, the Cooperative Study of Sickle Cell Disease demonstrated that 11% of children with SCA will suffer a stroke before 20 years of age.[3] At that time, there were no screening tests to identify those at highest risk and treatment included supportive care once a stroke did occur. At that point, unfortunately, the damage had been done. In the 1990s, we learned that TCD velocities can be used to identify children at high risk for stroke, and the subsequent Stroke Prevention Trial in Sickle Cell Anemia (STOP) clearly demonstrated that chronic transfusion therapy reduces the risk of stroke for children with abnormal TCD velocities.[4,5] This work led to a paradigm shift in the standard of care to now include screening TCDs for all children with SCA and chronic blood transfusion programs as primary stroke prophylaxis for those at highest risk. This aggressive screening and preventive approach has resulted in significant reductions in the frequency of stroke.[6]

Although the benefits of TCD screening and chronic blood transfusions for primary stroke prevention are indisputable, they do not come without cost, both literally and figuratively. If a child has abnormal TCD velocities at age 2 years of age, current guidelines would suggest that this child receive monthly blood transfusions indefinitely. Chronic blood transfusion therapy requires prolonged monthly hospital visits, costly iron chelation therapy, and oftentimes surgical procedures to implant central venous catheters. In addition to the cost and inconvenience, chronic transfusion therapy is associated with serious and life-threatening hemosiderosis, most commonly in the liver, as well as the development of auto- or alloantibodies to erythrocyte antigens that can make it difficult to find compatible blood. For these reasons, in combination with the emergence of hydroxyurea therapy as a widely available and safe disease-modifying therapy, there has been great interest in identifying safe alternatives to chronic transfusion therapy with improved iron management, particularly for the prevention and treatment of the neurologic consequences of SCA.

The current report by Bernaudin et al carefully demonstrates that there are alternatives to chronic transfusion therapy for children with abnormal TCD velocities and no significant intracranial stenosis. The authors report normalization of TCD velocities in 23 of 24 patients who received bone marrow transplantation with a matched sibling donor, and all transplanted patients survived transplant without any reported long-term toxicities. Forty-six children were transitioned to hydroxyurea therapy, with the majority of patients doing well with normalization of TCD velocities and no reported serious neurologic events. These data are a timely parallel to the recently published results from TWiTCH, which was a multicenter, open-label, phase 3, noninferiority trial, in which children with abnormal TCDs were transitioned to hydroxyurea therapy after ≥1 year of chronic transfusion therapy. The study was halted early when hydroxyurea was found to be noninferior to chronic transfusion therapy as defined by the study's primary end point: 24-month TCD velocity. The patient population for TWiTCH was carefully selected, and the process of hydroxyurea dosing and monitoring was rigorous. It is therefore important to validate these results in a more practical setting and the current report does just that. Although



This proposed algorithm provides guidance for the management of children with abnormal TCD velocities, including decision points regarding the initiation or discontinuation of chronic blood transfusions and hydroxyurea therapy. HbS, sickle hemoglobin; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging.

this French cohort is particularly well cared for and carefully monitored by a very experienced clinical research team, the current report was not in the context of a rigorous randomized clinical trial and thus represents early "effectiveness" data to indicate that there are safe alternatives to lifelong chronic transfusion therapy.

While certainly encouraging, these findings are not yet a "slam dunk" to suggest that hydroxyurea is now a universal replacement for chronic transfusions to prevent neurologic complications for children with SCA. Thirteen of the 36 patients transitioned to hydroxyurea demonstrated reversion to abnormal TCD velocities, although 4 occurred within the first 6 months of hydroxyurea therapy before maximum tolerated dose had been reached. Once an abnormal TCD was identified, the patients were again placed on chronic transfusions, and, ultimately, 6 of 13 were transplanted and 4 of 13 were able to be successfully transitioned a second time back to hydroxyurea therapy with normalization of TCD velocities. These carefully documented observations provide important data to suggest that a combination of therapies with careful surveillance may be a safe and effective alternative to indefinite chronic transfusions alone. The figure provides a potential clinical algorithm for the short- and long-term management of children with abnormal TCD velocities.

With easily available TCD and MRI/MRA studies and multimodal disease-modifying therapy starting early in life, we continue to see changes with the natural history of SCA and are moving toward the goal of a stroke-free childhood for patients with SCA. The early initiation of hydroxyurea therapy for infants with SCA is likely to reduce the number of children who develop abnormal TCD velocities in the first place, and a careful treatment and follow-up strategy for children with abnormal velocities will further reduce the frequency and severity of neurologic complications for children with SCA. It will be critical to further validate these results to determine whether it truly is safe to "flip the switch" from chronic transfusions to hydroxyurea, particularly in the context of the real-world clinical challenges around medication adherence, appropriate dose escalation, and timely monitoring and follow-up.

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TRANSFUSION MEDICINE

Comment on Arthur et al, page 1823

Platelet refractoriness: it's not the B-all and end-all

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In this issue of *Blood*, Arthur et al uncover that HLA alloantibodies cannot solely account for the immune mechanism in platelet refractoriness.[1]

The explosion of blood transfusion therapy about 60 years ago was accompanied by the frequent complication of alloimmunization to the cellular components of blood, mainly toward leukocytes, red cells, or platelets. Correspondingly, leukoreduction of transfused blood products decreased the prevalence of alloantibodies in heavily transfused patients; nevertheless, sensitization remains a serious clinical problem. Platelets harbor antigens that could potentially be mismatched between donor and recipient, including HLA class I, ABO blood group antigens, and human platelet antigen 1. Alloantibodies to all three groups of platelet antigens have been demonstrated in platelet-refractory patients, and have also been associated with increased clearance and destruction of transfused platelets.[2]

Laboratory investigation of platelet refractory patients has focused on HLA matching or crossmatching of platelets, which in turn has led to increased health care costs and significant delays in treatment.

Antibodies to platelets have been described since the late 1960s with much focus centered on anti-HLA antibodies.[3] By the early 1990s, characterization of donor and recipient HLA antigens led to some clinical improvement in a small fraction of platelet-refractory patients, yet large numbers of patients remain at risk, with estimates as high as half of all patients treated for hematologic malignancies. Although anti-platelet alloantibodies have received most of the attention, several clinical observations have prompted intense investigation to uncover other causative factors. First, most patients who are highly sensitized to HLA antigens do not exhibit platelet refractoriness. Second, many cases of platelet refractoriness do not appear to have any anti-platelet antibodies detectable by standard laboratory assays. Third, interventions to alter antibody production or suppress B cells have not resulted in improved outcomes.[2]

The concomitant diseases and therapies in patients inherently confound studies of platelet refractoriness. Mouse models to study platelet refractoriness were pioneered 20 years ago, and elegantly demonstrated that T cells play a major role in the immune response toward transfused platelets.[3,4] In these initial studies, both anti–major histocompatibility complex (MHC) antibodies and CD8 T-cell–mediated cytotoxic T-lymphocyte (CTL) responses were found to be active in the disease.[5,6] Further work also implicated natural killer (NK) cells in driving an anti-platelet antibody response. Moreover,