these results support a model in which increased levels of the RUNX1A isoform not only actively upregulates MYC/E2F-induced proliferative program but also perturbs RUNX1C/GATA1-induced megakaryopoiesis. In line with this model, genetic or pharmacologic inhibition of MYC/MAX complexes, downstream of RUNX1A, decreases cellular growth and induces differentiation of ML-DS blasts.

Although the exact mechanism(s) governing the altered level of expression of RUNX1 transcripts in TAM/ML-DS is still unknown, this study suggests that it may be at least in part intrinsic to trisomy 21 through the increased expression of the chromosome 21 gene *U2AF1*, which encodes a splicing factor subunit often mutated in myeloid malignancies (myelodysplastic syndrome and acute myeloid leukemia [AML]). Preliminary data show that GATA1s also regulate this imbalance, suggesting that a feed forward loop may occur during the stepwise pathogenesis of TAM/ML-DS itself. Strikingly, several subtypes of AML, with or without gain of the chromosome 21, have a high RUNX1A/RUNX1C ratio and are also sensitive to *MAX* interference and pharmacologic inhibition of MYC/MAX complexes. This emphasizes the broad therapeutic potential of the study and warrants further investigation of mechanisms that can promote this disequilibrium and how they cooperate to drive leukemogenesis in non–Down syndrome cases. It would also be of great interest to assess whether non-myeloid leukemia harboring complete or partial gain of the chromosome 21 exhibits an altered RUNX1A/C ratio and whether this is pathogenic in different hematopoietic lineages.

Altogether, Gialesaki et al show for the first time that ML-DS blasts are dependent on RUNX1 and demonstrate that increased level of RUNX1A has a pivotal role in Down syndrome leukemogenesis. Notably, this feature is not unique to ML-DS pathogenesis, highlighting a potentially underestimated role for these onco-transcripts. Therefore, this work emphasizes the need to seek new alternative transcripts in hematologic malignancies and to identify their downstream effectors and functional consequences on tumor development.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Izutsu et al, page 1159

Synthetic lethality in ATL

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In this issue of *Blood*, Izutsu et al report the results of the phase 2 trial of valemetostat, a dual inhibitor of enhancer of zeste homolog 1 (EZH1) and EZH2, in relapsed or refractory adult T-cell leukemia/lymphoma (ATL).[1] Heavily pretreated patients with a median of 3 prior lines (range, 1-8) of treatments were enrolled and received 200-mg daily doses of valemetostat until progression or intolerance. The authors reported encouraging activity with an overall response rate (ORR) of 48% with no differences in ORR by disease status (relapse vs refractory) or previous exposure to mogamulizumab and lenalidomide.

Notably, 5 patients achieved complete response with the duration of response being more than 1 year. Hematologic toxicities, particularly thrombocytopenia (grade ≥3: 32%, 3 patients with grade 4) were common but valemetostat was reasonably well tolerated with minor grade ≥3 non-hematologic toxicity. Based on the trial, valemetostat is approved for relapsed or refractory ATL in Japan. Many patients with ATL are not candidates for aggressive treatment, including allogeneic transplants with curative potential. Thus, this trial provides hope for patients with a great unmet need.

Multiple groups engaged in understanding the molecular biology of ATL provided the framework needed for the development of this drug. Yamagishi et al found an abnormal accumulation of histone H3 Lys27 trimethylation (H3K27me3) in ATL cells.[2,3] This accumulation of H3K27me3 causes epigenetic silencing in various transcription factors, epigenetic modifiers, developmental genes, and loss of microRNA including miR-31 which activates the NF-κB pathway by MAP3K14 expression.[3] The accumulation is seen not only in acute or lymphoma subtype of ATL but also in smoldering/chronic ATL or

even in human T-lymphotropic virus type 1 (HTLV-1) infected T cells from patients with asymptomatic HTLV-1. This suggests that abnormal gene downregulation occurs early in disease progression. EZH1/2-containing polycomb repressive complex 2 catalyzes H3K27me3 (ie, accumulation is EZH1/2 dependent), and EZH2 is overexpressed in HTLV-1–infected ATL cells.[4] EZH1 is also highly expressed in peripheral T cells, compensates the EZH2 functions, and contributes to the accumulation of H3K27me3. Simultaneous depletion of both EZH1 and EZH2 causes synthetic lethality, significantly decreases cellular H3K27me3 level and dramatically inhibits ATL cell growth compared with single depletion.[5] These findings laid the molecular groundwork for developing the dual EZH1/2 inhibitor, valemetostat.

Although the scientific background of the trial and reported activity are exciting, we need to be careful when applying it to typical patients with relapsed or refractory ATL. The median time since the last treatment was 60 days (and up to 1400 days), the median lines of previous treatment were 3 (range, 1-8), and only 2 patients (8%) had Eastern Cooperative Oncology Group performance status (PS) of 2. The typical course or presentation of symptomatic and often the treatment of patients with refractory ATL may differ from participants in the trial who could wait for the treatment without declining PS. A similar challenge of selecting patients with ATL for clinical trials was seen in mogamulizumab development. Initial phase 2 trial in Japan of patients treated with mogamulizumab with the relapsed (not refractory) disease, the majority of whom had good PS in second-line treatment, showed encouraging ORR of 50%.[6] However, a global randomized phase 2 study of mogamulizumab vs investigator choice conducted in the United States, Europe, the Caribbean, and South America showed a lower best ORR of 28% and only 11% of patients showed persistent response for more than 8 weeks in the mogamulizumab arm likely because of enrolling more high-risk patients on the trial (40% PS = 2, 40% progressive disease to prior line). In fact, patients with refractory disease had difficulty completing the first cycle and thus the protocol was amended to exclude patients who had received >2 lines of treatment and had not achieved a response or stable disease ≥12 weeks for the immediate prior therapy. Owing to this "negative" study, mogamulizumab is not approved for the treatment of ATL outside Japan. This trial is one of many that taught us that agents require a well-thought-out trial to evaluate the appropriate target population and full potential of the activity. The global study, VALENTINEPTCL01 using valemetostat, targeting both peripheral T-cell lymphoma (PTCL) and ATL is ongoing (NCT04703192). We hope to see confirmation of activity in ATL and would be delighted to see activity in other PTCLs.

Where do we go from here? ATL remains one of the most fatal lymphoid malignancies with 5-year overall survival of less than 20%.[7] Although we now have 3 active agents for ATL, which are mogamulizumab, lenalidomide,[8] and valemetostat, much more is clearly needed. A combination treatment such as lenalidomide and valemetostat may work synergistically, and trials evaluating these combinations with well-designed correlative studies are warranted. Another epigenetic modulatory agent, romidepsin, a histone deacetylase inhibitor in combination with lenalidomide was evaluated in the phase 1 trial showing ORR of 53% in PTCL including ATL[9]; however, the treatment was relatively toxic and 63% of patients required dose reduction. The safety profile of valemetostat may help facilitate combination trials. ATL is an endemic disease and thus clinical investigations to explore new agents have been very challenging in areas where it is not endemic, such as Europe and in the United States, where most new drug developments are usually undertaken. Global collaboration will be a key to facilitating clinical investigation.

It is estimated that there are at least 10 to 20 million people worldwide with HTLV-1 infection. HTLV-1 is present throughout the world with clusters of areas of high endemicity in developing countries, such as the Caribbean, Central and South America, and tropical Africa, where patients have no way to access cutting-edge clinical research and expensive, new targeted agents. In addition to continuing clinical investigation in developed countries, it is imperative to support research, treatment, and drug access in developing countries where the majority of patients reside. Because of long latency and low lifetime risk of developing ATL (<5%), the accurate prevalence of HTLV-1 carriers is extremely challenging to capture but the incidence of ATL is rising in areas of nonendemicity, including the United States, most likely owing to migration.[10] We should continue exploring the effective prevention strategy by appropriate screening process considering the nature of extremely fatal HTLV-1–driven disease.

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GENE THERAPY

Comment on Hardouin et al, page 1169

β-Thalassemia: all about that base, no cutting

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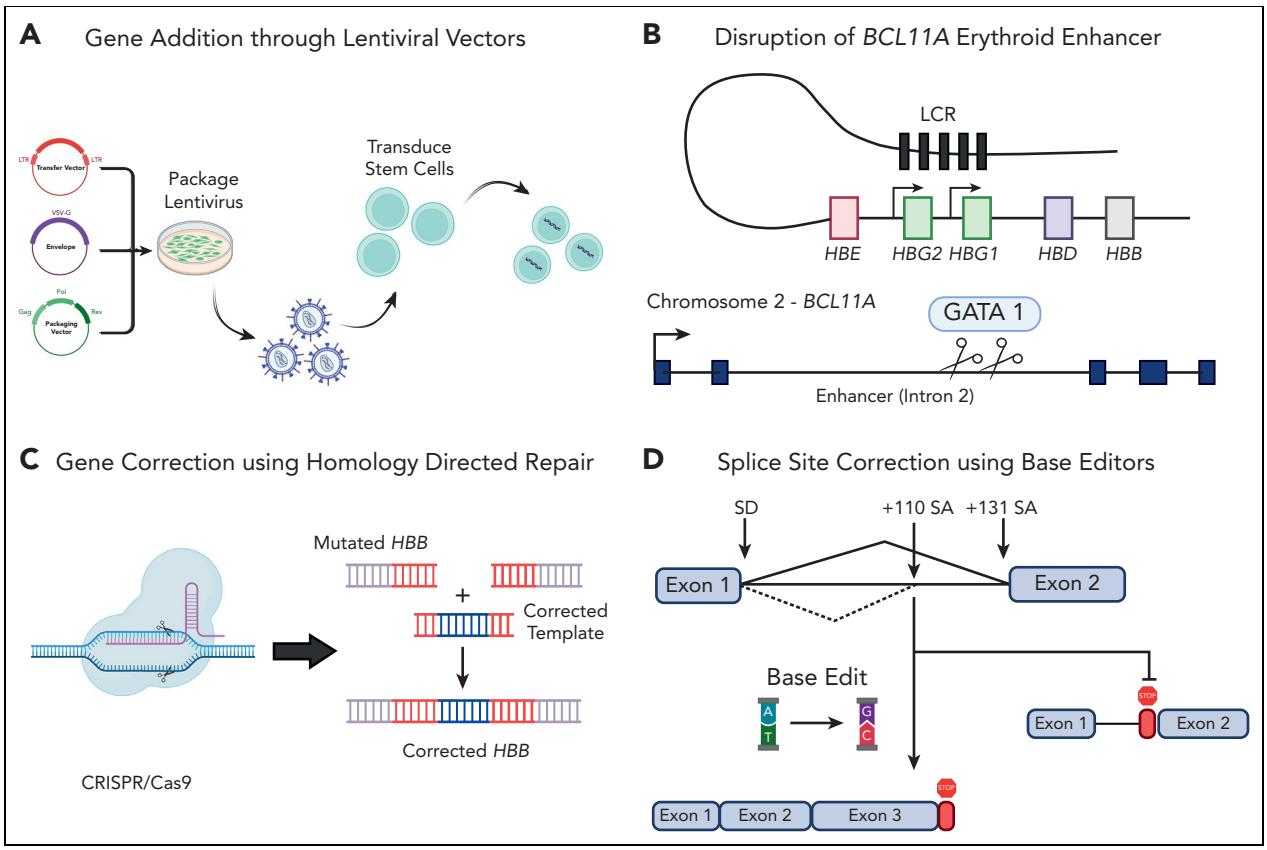
In this issue of *Blood*, Hardouin et al report on the development of an adenine base-editing approach capable of correcting the most common β-thalassemia mutation in the eastern Mediterranean, IVS1-110.[1]

β-Thalassemia, a common recessive genetic disorder characterized by reduction in β-globin production, results in mild to severe transfusion-dependent anemia. There have been several recent advances in the field of gene therapy to combat β-thalassemia through lentiviral vector and CRISPR/Cas9-based gene therapies (see figure); however, these therapies have some inherent safety concerns and a high cost of goods, a challenge Hardouin et al tackled.

In August 2021, the Food and Drug Administration (FDA) approved betibeglogene autotemcel, an ex-vivo lentiviral vector gene therapy for patients with transfusion-dependent β-thalassemia. This treatment was based on data from a phase 3 trial in which 32 of 36 patients attained transfusion independence after receiving the gene therapy. Although largely successful at ameliorating the disease phenotype, this lentiviral gene therapy (and many before it) suffers from a high cost of goods, owing to its long vector size and relatively low-titer and suboptimal gene transfer. Other efforts at targeting β-thalassemia have been attempted on the gene-editing front using CRISPR/Cas nuclease-induced double-stranded break (DSB) to repair disease-causing mutations in the gene for β-globin (*HBB*) by means of homology-directed repair.[2,3] More recently, Cromer et al have attempted to correct the imbalance of α- and β-globin present in patients with β-thalassemia by integrating a β-globin transgene downstream of the *HBA2* promoter of α-globin to not only knock down excess α-globin production but also increase the dearth of β-globin expression.[4] Although these methods are effective at producing properly regulated



Schematic of various gene therapy strategies to treat β-thalassemia. (A) The use of lentiviral vectors to incorporate a full-length *HBB* gene or shmiRs targeting *BCL11A* for the upregulation of HbF. The use of CRISPR/Cas9 machinery can either knock out *BCL11A* to upregulate HbF (B) or incorporate a corrected *HBB* cassette with HDR machinery (C). Finally, adenine base editors can be used to convert A to G nucleotides to correct single-point mutations or incorrect splice sites, such as IVS1-110 (D). Created with BioRender.com.