concluded that vaccination and immunoglobulin replacement therapy, but not prophylactic antibiotics, could reduce infections to some degree, although no intervention reduced all-cause mortality.[9] Although retrospective, the work by Tadmor et al for the first time demonstrates that an intervention to reduce the severity of the infection by early antiviral treatment upon a positive SARS-CoV-2 test can reduce the risk of hospitalization and death for patients with CLL.

Mortality and morbidity from infections for patients with hematological malignancies in general, and for patients with CLL in particular, need ongoing attention. Clinical trials and retrospective assessment of real-world data are needed to test the efficacy of different prophylactic interventions and treatment approaches to reduce the morbidity and mortality due to immune dysfunction and infections in patients with hematological malignancies and in particular for patients with CLL.

Elderly patients with CLL and other comorbid conditions or heavily pretreated for CLL are at the highest risk of morbidity and mortality from infections. Importantly, Tadmor et al identify this patient population as those with the greatest potential benefit from early treatment with the combination of nirmatrelvir plus ritonavir upon a positive SARS-CoV-2 test. This study highlights the importance of identifying patients with CLL at highest risk of infections for inclusion in trials testing interventions to reduce the risks of infections. As next steps toward identifying such patient populations, machine learning algorithms based on data-driven pattern recognition using real-world data and clinical trial data should be explored.[10]

Most current guidelines for patients with CLL recommend vaccination against pneumococci, influenza, and COVID-19 along with passive immunization against COVID-19 and immunoglobulin replacement therapy for specific subgroups. Furthermore, prophylaxis toward pneumocystis pneumonia and herpesvirus is recommended in specific treatment situations. These guidelines may now be supplemented with the recommendation of nirmatrelvir plus ritonavir as soon as possible after testing positive for SARS-CoV-2 in CLL patients who are above 65 years of age, are heavily pretreated, have comorbidity associated with increased risk of infections, or are receiving immunoglobulin replacement therapy. Furthermore, optimization of early treatment and prevention of infections in patients with CLL should be tested as next steps toward improving outcomes for patients.

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MYELOID NEOPLASIA

Comment on Piqué-Borràs et al, page 2245

NFIA-ETO2, *TP53*, and erythroid leukemogenesis

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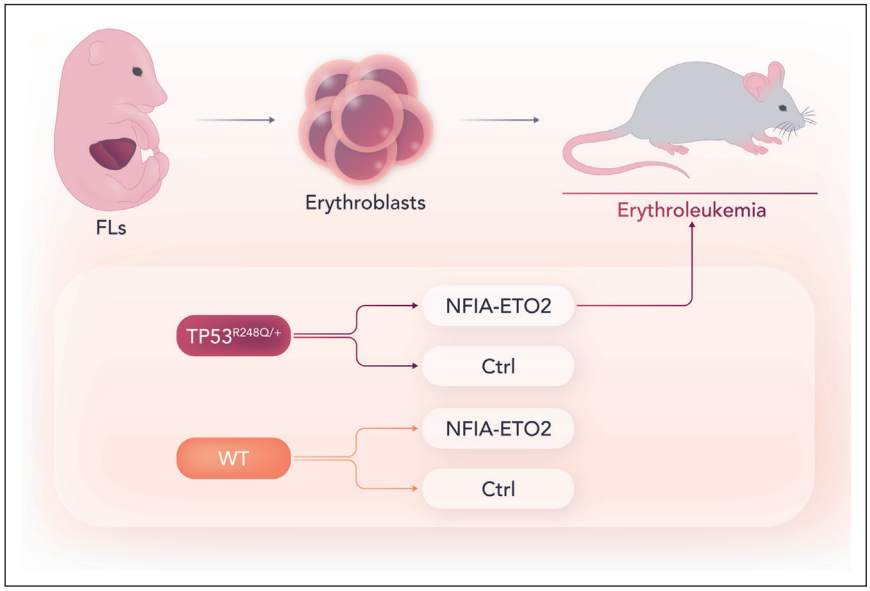
In this issue of *Blood*, Piqué-Borràs et al[1] investigate how the NFIA-ETO2 fusion perturbs erythropoiesis and show how this oncoprotein cooperates with mutant *TP53* in leukemogenesis.

Pure erythroid leukemia (PEL) is a rare and aggressive subtype of acute myeloid leukemia (AML), and driver *NFIA-ETO2* fusions have been restricted to pediatric PEL patients to date. The transcription factor NFIA is a key regulator of erythroid differentiation during early hematopoiesis.[2] Similarly, ETO2 (also known as CBFA2T3) is a transcriptional corepressor that appears to also play a role in erythroid differentiation through the coregulation of GATA1 targets.[3] In addition, the *ETO2* gene is a frequent fusion partner with *GLIS2* and *RUNX1*



Fetal liver–derived *TP53*$^{R248Q/+}$ murine erythroblasts retrovirally transduced with the *NFIA-ETO2* fusion and transplanted into recipients induces a highly penetrant, transplantable erythroleukemia. By contrast, expressing *NFIA-ETO2* in other genetic contexts (wild-type or *Tp53*$^{+/-}$ mutant cells) and transducing *TP53*$^{R248Q/+}$ mutant cells with the control vector did not cause leukemia. Ctrl, control; FL, fetal liver; WT, wild type. Professional illustration by Somersault18:24.

in megakaryoblastic and myeloblastic forms of AML, respectively. This suggests that ETO2 broadly impacts cell fate decisions in hematopoietic stem and progenitor cells (HSPCs) in a cell context–dependent manner. Although the NFIA-ETO2 oncoprotein is exclusively associated with PEL, how it functions in leukemogenesis remains poorly understood. In this study, the authors used multiple complementary model systems to show that NFIA-ETO2 impairs erythroid differentiation while simultaneously driving proliferation but is not sufficient to induce full leukemic transformation. By contrast, enforced NFIA-ETO2 expression in primary murine hematopoietic cells harboring a recurring point mutation (*Tp53*$^{R248Q/+}$) induced a highly penetrant erythroleukemia in mice.

Piqué-Borràs et al first showed that expressing NFIA-ETO2 in mouse erythroleukemia (MEL) cells and in primary murine erythroblasts impaired terminal erythroid differentiation in vitro. Mutagenesis experiments demonstrated that the DNA-binding domain of NFIA and the protein interaction NHR2-4 motifs of ETO2 are essential for inducing this differentiation block. Paired chromatin immunoprecipitation sequencing and transcriptome (RNA-seq) analyses showed NFIA-ETO aberrantly binds near key erythroid differentiation regulators such as *Tal1*, *Gfi1b*, and *Klf1* and that this is associated with decreased messenger RNA expression. By contrast, *Myc* and *Myb* expression was markedly upregulated. Mechanistically, the use of RNA interference to reduce *Myb* expression was sufficient to overcome the erythroid differentiation block. The authors went on to validate these general observations in MEL cells by performing RNA-seq in primary mouse fetal liver–derived erythroblasts engineered to express either "wild-type" NFIA-ETO2 or an inactive control fusion lacking the ETO2 NHR4 domain. Altogether, these data support a model whereby NFIA-ETO2 acts, in part, by enforcing an immature and proliferative state in cells that are partially committed to the erythroid lineage through aberrant expression of MYC, MYB, and perhaps other myeloid transcription factors.

Despite these widespread effects on cell fates and key transcriptional networks, exogenous NFIA-ETO2 expression neither enhanced colony replating potential ex vivo nor initiated leukemia in vivo in a transduction/transplantation assay. These data are consistent with an essential role for cooperating mutations in leukemic transformation, which is a biologic property of other AML-associated transcription factor fusions. An intriguing aspect of *NFIA-ETO2*–driven PEL is that it has only been reported in pediatric patients. This suggests that fetal/neonatal HSPCs are particularly (and perhaps uniquely) vulnerable to transformation by *NFIA-ETO2* as has been observed for some *KMT2A* (also known as *MLL*) fusions in infant AML and for mutant Ras pathway genes in juvenile myelomonocytic leukemia. Future studies might address the effects of expressing NFIA-ETO2 in HPSCs isolated from human umbilical cord blood vs adult bone marrow.

TP53 is frequently altered in PEL, most often owing to point mutations with or without concurrent *TP53* deletions (often manifesting as chromosome 17 partial deletions).[4] A key question for addressing the effects of mutant *TP53* in mouse models of hematologic malignancies involves the relative merits of using a "first-generation" *Tp53* null allele or subsequent knockin models encoding highly prevalent point mutations found in human cancers. These respective strains spontaneously develop a different spectrum of primary malignancies.[5,6] Although there remains some debate about the precise mechanisms underlying *TP53*-mediated oncogenesis in different tissues contexts, these in vivo data and studies in AML cell lines support dominant-negative biologic activity of mutant *TP53*.[7] In this context, it is notable that Piqué-Borràs et al compared the transforming activity of expressing NFIA-ETO2 in bone marrow cells from both heterozygous *Tp53* knockout (*Tp53*$^{+/-}$) and *TP53*$^{R248Q/+}$ knockin mice. Remarkably, recipients of *NFIA-ETO2; TP53*$^{R248Q/+}$ "double mutant" cells developed a fully penetrant transplantable erythroleukemia, whereas mice transplanted with *NFIA-ETO2; Tp53*$^{+/-}$ cells did not. These data have implications for characterizing potential dominant-negative and gain-of-function activities of mutant *TP53* in leukemogenesis and for future studies of how it cooperates with other leukemia-associated transcription factor oncoproteins.

Mechanistically, the authors' studies support a model whereby NFIA-ETO2 promotes aberrant proliferation of early erythroid cells and impairs their differentiation with mutant *TP53* cooperating by promoting self-renewal (see figure). These data are broadly similar to previous studies that investigated the cooperating and competing effects of oncogenic *Nras/Kras* alleles and *Tp53* inactivation in leukemogenesis.[8,9] Although somatic

TP53 mutations are highly prevalent in other subtypes of PEL, it should be noted that they have not been detected in patients with *NFIA-ETO2* fusions. This raises potential questions about using the new transplantable model reported here for future biologic and preclinical studies. With this said, few model systems for pure erythroleukemia exist, and the authors' findings that transcriptional rewiring induced by NFIA-ETO2 expression in primary bone marrow cells cooperate with mutant *TP53* to induce erythroleukemia represent a novel contribution to the field.

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PLATELETS AND THROMBOPOIESIS

Comment on Ver Donck et al, page 2261

SLFN14 ribosomopathy and platelet dysfunction

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In this issue of *Blood*, Ver Donck et al,[1] investigated a dominantly inherited platelet defect with a mild-to-moderate bleeding diathesis in a family due to a mutation in SLFN14,[1,2] which is involved in RNA degradation, especially ribosomal RNA (rRNA) but also transfer RNA (tRNA) and messenger RNA (mRNA).[2,3] The authors demonstrate a loss of rRNA in platelets in affected family members and studied the other consequences of this mutation.[1] These findings offer insights into how a potential endoribonuclease SLFN14 may affect megakaryocyte development, and have pleiotropic effects on both megakaryocytes and platelets. The study also suggests a potential therapeutic target for these patients, with implications for understanding the differentiation of large, polyploidy megakaryocytes. Overall, the molecular bases of inherited platelet disorders continue to provide valuable insights into megakaryocyte and platelet biology.

The authors' studies used patient platelets and megakaryocytes and CRISPR/Cas9–engineered cells from an immortalized megakaryocyte progenitor cell line (imMKCL) to define the consequences of the K219N amino acid substitution in SLFN14.[1] imMKCL is an immortalized induced pluripotent stem cell (iPSC) intermediate line that, upon withdrawal of doxycycline, completes its differentiation into megakaryocyte-like cells.[4,5] Overall, studies using the primary cells and the imMKCL were consistent in terms of morphologic changes in the megakaryocytes.[4,5] Further studies of the imMKCL demonstrated multiple, apparently downstream, pleiotropic effects of the $SLFN14^{K219N}$ mutation on differentially expressed genes in the mutated imMKCL.[1] Of particular importance was the enhancement of genes involved in rRNA processing, which may explain why the levels of 28S and 18S rRNAs in the mutant imMKCL megakaryocytes were normal yet associated with enhanced levels of degraded rRNA bands. Furthermore, mitochondrial translation and protein expression pathways were enhanced in the mutant imMKCL, consistent with the mitochondrial morphologic abnormalities observed via electron microscopy and the marked reduction in mitochondrial oxygen consumption rate by mutated imMKCL-derived megakaryocytes.[1]

The authors point out that the changes in both ribosomal and mitochondrial biogenesis suggest that the $SLFN14^{K219N}$ mutation enhances the mammalian target of rapamycin complex 1 (mTORC1) pathway. To test this, the authors treated the differentiating imMKCL megakaryocytes with rapamycin to inhibit mTORC1 pathway activity and saw decreased megakaryocyte differentiation concomitant with decreased levels of 28S rRNA and markedly enhanced levels of rRNA degradation fragments, supporting the model proposed in the figure.

Thus, the authors have shown that the K219N mutation in the endoribonuclease SLFN14 appears to involve rRNA degradation and alterations in the mTORC1 pathway (see figure), although other pathways may be affected in the developing megakaryocytes because there were robust changes in many important intracellular pathways. Why the degradation of rRNAs leads to such widespread effects, whether the endoribonuclease activity of SLFN14 targeting rRNAs accounts for all the pathology because SLFN14 may also target tRNAs and mRNAs, and whether these, as yet unidentified tRNAs or mRNAs, may