blood® commentary

MYELOID NEOPLASIA

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ERGonomics for EVI1 acute myeloid leukemia

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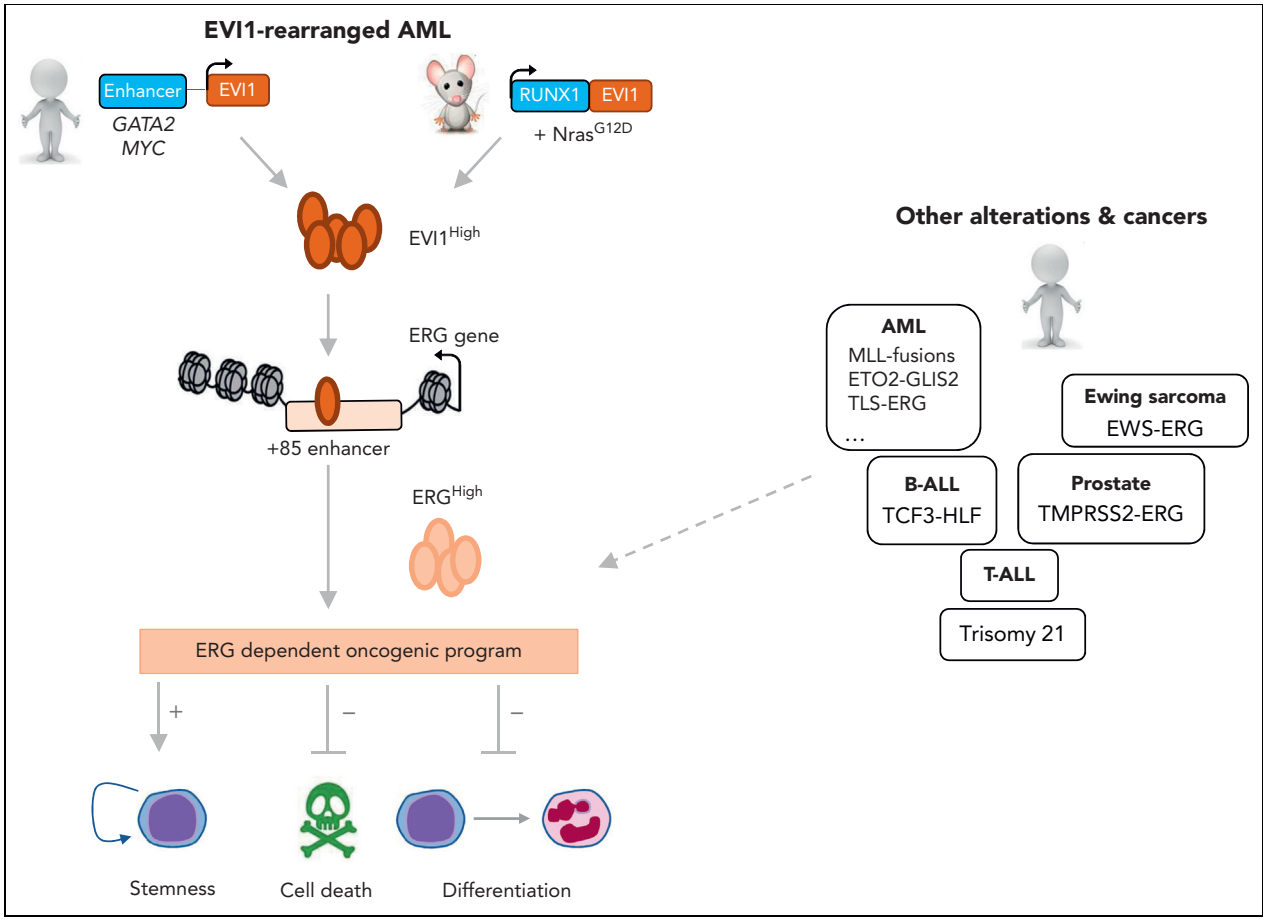
In this issue of *Blood*, Schmoellerl et al leveraged both human and murine models of ecotropic virus integration site-1 (EVI1)-driven acute myeloid leukemia (AML), on which they performed functional genome-wide genetic screens to identify genes that are essential to maintain the leukemic cells of this aggressive subtype of AML cells.[1] They identified ERG as a uniquely conserved direct transcriptional target of EVI1, which strongly contributes to enhanced cell survival and differentiation blockage in these AML cells.

AML is an aggressive malignancy driven by genetic alterations in hematopoietic stem and progenitor cells that frequently tlead to aberrant expression and/or activation of transcription factors or co-regulators. Although chemotherapy for AML has progressed over the past 40 years, several molecular subgroups, including leukemia overexpressing the EVI1 zinc finger transcription factor, remain associated with poor clinical outcome. EVI1 is encoded at the *MECOM* locus at 3q26.2, which is rearranged by several types of chromosomal alterations leading to *EVI1* overexpression (EVI1^{High}). This overexpression can result from the relocation of enhancers, including GATA binding protein 2 (*GATA2*) enhancer in inv(3)/t(3;3)(q21q26)[2] and *MYC* super-enhancer in t(3;8)(q26;q24) close to the *EVI1* gene, or from expression of fusion oncoproteins [eg, runt-related transcription factor 1 (RUNX1)-EVI1 in t(3;21) (q26;q22), ETV6-EVI1 in t(3;12)(q26;p13). Overall, the consequences of chromosomal rearrangements on *EVI1* transcription have been well characterized in recent years, and only a few unexplained cases remain. However, the gene regulatory program downstream of EVI1 is less well understood.

Schmoellerl et al created a novel murine transgenic model of RUNX1-EVI1 expression combined with a Nras^{G12D} mutation recurrently observed in EVI1^{High} AML patients (see figure). To identify genes that are transcriptionally controlled by EVI1, expression analyses were performed in this murine model and in the human HNT34 cell line carrying t(3;3)(q21;q26) after EVI1 knockdown. In these 2 contexts, a common core set of genes were positively controlled by EVI1, including transcriptional factors/coregulators (eg, *BCL11A*, *CBX6*, *ERG*, *HHEX*, *LYL1*), which were also enriched in EVI1-rearranged AML patients. At the loci of these gene targets in the RUNX1-EVI1 murine model, EVI1 enforced higher chromatin accessibility. To establish functional EVI1-specific dependencies, the authors performed genome-wide gene inactivation screens in human and murine EVI1-dependent models using a CRISPR (for clustered regularly inter-spaced short palindromic repeats)/Cas9 (for CRISPR-associated) approach and compared their hits with those obtained in other published screens. A strength of the study is that it integrated data from expression analyses and functional screens, resulting in the identification of ERG as the sole candidate that is both directly transcriptionally regulated by EVI1 and essential for the survival of EVI1-driven AML in human and murine models. Validation of these data through specific ERG knockdown showed that ERG contributes to myeloid differentiation blockage, the anti-apoptotic cell survival effect, and leukemia maintenance in both of their models. An elegant rescue experiment in the RUNX1-EVI1 murine model revealed that ERG ectopic expression was sufficient to overcome the effect of EVI1 knockdown on cell survival and restore expression of 34% of EVI1 target genes (eg, *MYC*, *KIT*), indicating strongly that ERG mediates a significant part of the EVI1 gene regulatory network involved in leukemic cell survival, proliferation, and stemness maintenance.

By demonstrating that EVI1^{High} AML cells depend on ERG, these data extend the central oncogenic role of this E-twenty six (ETS)-family transcription factor in human acute leukemia associated with poor prognosis. Indeed, EVI1^{High} ERG^{High} expression is a distinctive feature of an aggressive subset of mixed lineage leukemia (MLL)-rearranged AML.[3] An independent study on MLL-eleven nineteen leukemia (ENL)$^{+}$ cells found that EVI1^{High} cells were more dependent on ERG than EVI1^{Low} cells.[4] ERG, encoded on chromosome 21, also has functional relevance for ETO2-GLIS2^{+} AML[5] as well as acute lymphoblastic leukemia (ALL), including T-cell ALL[6] and TCF3-HLF^{+} B-cell ALL,[7] but whether EVI1 is also involved in these types of leukemia remains to be studied.

Mechanistically, EVI1 was shown here to bind and regulate the open chromatin state of the ERG locus at a +85 enhancer, a regulatory element previously shown to be controlled by a transcriptional complex referred to as the heptad complex,[8] including several factors that



Ecotropic virus integration site-1 (EVI1)-rearranged acute myeloid leukemia (AML) joins the club of ERG-dependent alterations and cancers. In this issue of *Blood*, Schmoellerl et al have studied 2 EVI1-driven human and murine leukemia models (EVI1^{High}). They showed that EVI1 binds and regulates the open chromatin state at the *ERG* gene, leading to high expression of this E-twenty six (ETS) transcription factor. ERG enforces an oncogenic program, including self-renewal and survival properties associated with hematopoietic differentiation blockade. Some of the other genetic alterations and human cancers associated with high ERG expression or dependency are indicated on the right side, including different molecular subgroups of AML, B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), Ewing sarcoma, and prostate cancer. MLL, mixed lineage leukemia; RUNX1, runt-related transcription factor 1.

are important for normal hematopoietic stem cell biology and are altered in EVI1- and ERG-dependent AML (eg, RUNX1, GATA2, ETO2). In addition, although EVI1 is often referred to as a repressor interacting with C-terminal binding protein (CtBP), it also positively controls a gene expression program including stemness genes. Therefore, the precise combinatorial interplay between EVI1 and heptad transcription factors, as well as the relative contribution of other cofactors controlling the locus-specific transcriptional activation of this stemness program is an interesting area for future investigation.

These data and other observations also indicate that an interplay occurs between the genetic-context or the cell-context and EVI1 activity. Here, Schmoellerl et al found that not all of the EVI1-rearranged AML cell lines are equally sensitive to EVI1 knockdown. Specifically, HNT34 was highly sensitive, whereas MOLM1 and Kasumi3 were less affected. Interestingly, only HNT34 cells also presented an additional SF3B1 mutation that controls the expression of an EVI1 isoform with a 6–amino-acid insertion close to the zinc fingers and an enhanced self-renewal stimulation property.[9] Whether this isoform is responsible for enhanced *ERG* expression is unknown. Regarding the importance of the cellular context, EVI1 and ERG both show decreasing expression upon differentiation of normal hematopoietic stem cells. Still unknown is whether the cell of origin in which the EVI1 rearrangement occurs determines the level of ERG expression in the resulting AML, and whether EVI1 and ERG expression levels vary during leukemic evolution. Indeed, ERG could progressively substitute for EVI1 oncogenic function through differentiation or epigenetic drift toward EVI1-independent leukemia in which ERG could enforce leukemia cell survival and maintenance.

Finally, these data provide new perspectives on future development of therapeutics. Indeed, although direct targeting of the driver EVI1 oncogene remains desirable, achieving specific inhibition of ERG activity will most likely be of wider therapeutic interest for several subtypes of aggressive AML, including EVI1^{High} AML. With this goal, ERG pharmacologic inhibitors are being developed, and ERG protein structure–based strategies may also allow for the development of inhibitory peptidomimetic approaches.[10]

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CLINICAL TRIALS AND OBSERVATIONS

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Progress in risk-adapted acute GVHD therapy

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In this issue of *Blood*, Etra et al report that itacitinib, a selective JAK1 inhibitor, has activity as the primary treatment of acute graft-versus-host disease (GVHD). The use of itacitinib spared steroid exposure and was associated with decreased infectious complications compared with a matched control population.[1]

Advances are needed to improve the safety and efficacy of acute GVHD treatment. Systemic corticosteroids (commonly, prednisone at 1 to 2 mg/kg/day starting dose) are the long-standing standard initial therapy for acute GVHD. Limitations of this practice include incomplete efficacy with subsequent steroid-refractory GVHD and associated mortality, morbidity, and complications of prolonged steroid therapy. As important, the universal application of high-intensity treatment does not respect individual disease risk. Important prior research has defined clinically based and biomarker-based tools to provide risk stratification of acute GVHD,[2,3] and the field has begun to test novel interventions in acute GVHD risk subgroups. These new strategies may hold promise to personalize GVHD therapy, in which the right intensity of therapy is delivered to the right patient.

In this current report, the investigators describe notable results of a multicenter phase 2 trial testing steroid-free initial therapy with itacitinib among patients with clinical and biomarker-defined low-risk acute GVHD. Although ruxolitinib has been studied in steroid-refractory acute GVHD (and is now approved for this indication),[4] the investigators chose to test itacitinib in this setting for potential advantage in hematologic toxicities. As well, itacitinib has been tested in combination with corticosteroids for acute GVHD treatment.[5] A total of 70 patients with low-risk (Minnesota standard risk, biomarker AA score 1) acute GVHD were treated with itacitinib and compared with a matched control population (140 patients from a prospectively assembled multicenter consortium) meeting the same eligibility criteria and treated with at least 0.5 mg/kg/day prednisone therapy. The results support that this steroid-free therapy achieved a high response rate (overall response rate [ORR] of 89% at day 28). There was no signal of inferior outcomes compared with the steroid control group considering day 28 ORR, response according to subgroups of GVHD organ involvement and severity, time to initial response, durability of response, or risk for treatment failure or GVHD flare. Importantly, the itacitinib-treated participants had significant reduction in cumulative prednisone exposure through days 28 and 56 of therapy compared with control participants and significant reduction in risk of infectious complications. Itacitinib discontinuation occurred for lack of efficacy (10% of participants), treatment-emergent (most commonly hematologic) adverse events (AEs) (29%), or relapse of malignancy (3%). A total of 30% of itacitinib-treated participants had other (nonhematologic, noninfectious) treatment-emergent ≥ grade 3 AEs, with the highest frequency events being alanine aminotransferase increase and hypertension. There was no evidence of worsened long-term outcomes, including chronic GVHD, relapse, and nonrelapse mortality. Potential limitations (nonrandomized control group, not standardized infectious prophylaxis, limitation to AA biomarker score 1 participants, possibility that topical therapy alone could be sufficient) of this work were well described.

Additional progress in the field will require selection of priority interventions and allied trial designs for risk-adapted therapy. A national Blood and Marrow Transplant Clinical Trials Network randomized trial is currently underway in high-risk acute GVHD (NCT04167514). For lower-risk acute GVHD (the majority of acute GVHD cases), at least 3 (lower-dose prednisone,[6] sirolimus,[7] itacitinib) steroid-minimizing or steroid-free primary