

between inflammatory environment and somatic mutations in myeloid genes, confirming previous findings.⁴ Mutations in *TET2* and other myeloid genes appear to occur relatively more frequently than in *DNMT3A* in VEXAS syndrome compared with the clonal hematopoiesis observed in the general population. This finding, together with the analysis of clonal hierarchy, strongly suggests that these mutations occur as second hits preferentially selected in the highly inflammatory environment triggered by *UBA1* mutation. The present study also indicates that there are relevant clinical effects of additional somatic mutations in VEXAS syndrome, although a limited time to follow up and the retrospective design warrant confirmation of the clinical outcomes analysis in larger data sets and prospective studies.

Although this study confirmed the previously reported high prevalence of the evolution of VEXAS syndrome in MDS, the relationship between incidence of additional myeloid mutations, clonal patterns, and diagnosis of MDS still requires both further study and consistent definitions. Establishing a diagnosis of MDS in the context of VEXAS syndrome is extremely challenging using standard diagnostic criteria.⁵ In fact, in addition to the characteristic vacuolated myeloid and erythroid cells,⁶ *UBA1* mutation and its related inflammatory milieu are associated with changes in bone marrow cells that overlap with dysplastic features, as well as causing cytopenias, thus complicating the diagnosis of MDS. Similar challenges are usually faced when diagnosing MDS in the setting of bone marrow failure syndromes. Indeed, previous studies showed that discriminating between aplastic anemia and hypoplastic MDS relying only on conventional morphologic criteria may not be sufficiently accurate,⁷ and the same applies to making a diagnosis of MDS in the setting of a germline predisposition.⁵ In these contexts, the pattern of somatic genetic lesions, either cytogenetic abnormalities or gene mutations, has the potential to significantly improve this process. Emergence of *del(5q)*, *-7/del(7q)*, complex karyotype, multihit *TP53* mutations or *SF3B1* mutation are currently considered MDS defining, whereas additional mutation patterns have been reported associated with high positive predictive value and specificity for MDS in the context of unexplained cytopenia.⁸ Although additional studies are required

to correctly interpret acquired genetic changes in distinct contexts, moving toward a diagnosis of MDS based on specific genetic signatures will be critical to solve these diagnostic challenges and inconsistencies.

VEXAS syndrome is part of the spectrum of clonal diseases without overt malignant features, which also includes paroxysmal nocturnal hemoglobinuria and aplastic anemia with clonal hematopoiesis.⁹ The landscape of somatic mutations, recently uncovered in these conditions and ranging from drivers of typical clonal hematopoiesis to mutations enriched in myeloid malignancies,¹⁰ is making the borders between nonmalignant, premalignant, and early malignant conditions extremely subtle. Indeed, a subset of patients with VEXAS syndrome have ineffective hematopoiesis and many have dysplasia in 1 or more marrow lineages, thus meeting some of the minimum criteria for MDS. However, the relative lack of progression to increased blasts or AML appears at this stage as an unmet key criterion for a classification within the spectrum of MDS, although the severe systemic inflammation and complications related to immunosuppressive therapies may represent competing risks for early mortality, thus complicating the use of progression to AML as an endpoint. The study by Gutierrez-Rodrigues et al lays the groundwork for a better understanding of the role of mutations in *DNMT3A*, *TET2*, and other myeloid drivers in the drift of *UBA1* mutant clones toward myeloid malignancy.

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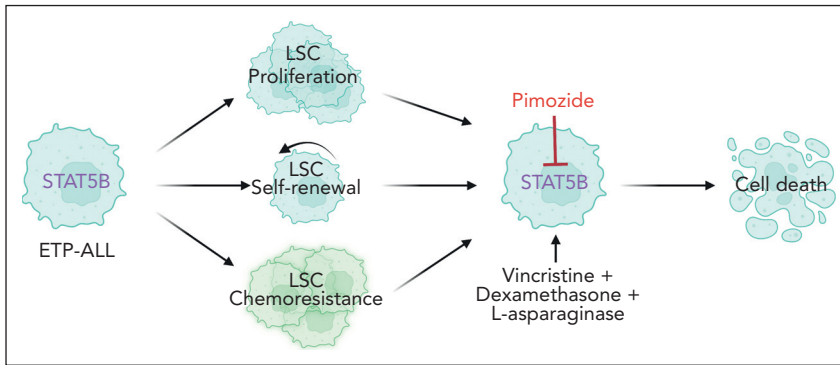
LYMPHOID NEOPLASIA

Comment on Tremblay et al, page 274

Targeting STAT5B in T-cell acute lymphoblastic leukemia

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In this issue of *Blood*, Tremblay et al demonstrate that STAT5B activation drives proliferation, self-renewal, and chemoresistance of leukemia stem cells (LSCs) in an early T-cell precursor acute lymphoblastic leukemia mouse model and explore STAT5B as a direct therapeutic target (see figure).¹



Activation of STAT5B confers proliferation, self-renewal, and chemoresistance of LSCs in early T-cell precursor acute lymphoblastic leukemia. Pimozide is a STAT5 inhibitor that sensitizes the LSCs to chemotherapy.

JAK/STAT signaling is a central pathway controlling differentiation, proliferation, and survival in the hematopoietic system and is constitutively activated in a variety of hematological malignancies.² This activation can occur through mutations in receptors, the JAK kinases, the STAT transcription factors, and/or the negative regulators of this pathway. From the different STAT proteins, STAT5 has received most attention in hematological malignancies as it is widely implicated in oncogenic signaling.^{2,3} For example, STAT5 is known to be a key downstream effector of JAK2 in cases with JAK2 fusion genes, JAK2 mutations, or receptor overexpression/mutation (such as cytokine receptor like factor 2 overexpression or erythropoietin receptor mutations). Moreover, STAT5 is involved in other pathways beyond the classic JAK/STAT pathway and is also critical downstream of many other oncogenic kinases, such as BCR::ABL1 and mutant FLT3.²⁻⁴

STAT5 is, however, a misleading name because there are 2 STAT5 genes in the human genome, STAT5A and STAT5B, which are located next to each other on chromosome 17, in the same region as STAT3. The STAT5A and STAT5B proteins are similar, and they were initially considered to have redundant functions. An elegant and detailed study from Kollmann et al recently clarified important differences between these 2 STAT proteins in the hematopoietic system.⁴ They identified STAT5B as a critical protein for the self-renewal properties of normal hematopoietic stem cells, whereas STAT5A inactivation had minimal consequences.⁴ Moreover, knockout of *Stat5b* in BCR::ABL1 LSCs prevented leukemia development in immune-deficient mice, whereas *Stat5a* knockout did not impact

leukemia development. LSCs derived from BCR::ABL1, FLT3-ITD, or JAK(V617F) leukemia models only showed strong STAT5B and not STAT5A phosphorylation in the nucleus, further indicating that STAT5B is the most important STAT5 downstream of oncogenic kinases in hematological malignancies.⁴

In T-cell acute lymphoblastic leukemia (T-ALL), STAT5B also seems to be the most important of the 2 STAT5 proteins. Several subtypes of T-ALL show a high frequency of mutations in the interleukin-7 receptor (IL-7R) signaling pathway, including mutations in the IL-7R itself, in JAK1 or JAK3 kinases, often in STAT5B, but never in STAT5A.⁵ Data from the Catalogue of Somatic Mutations in Cancer (COSMIC)⁶ reveal that the STAT5B N642H mutation is a clear hotspot mutation that is detected in various T-cell and myeloid malignancies, whereas there are no highly recurrent STAT5A mutations. Strikingly, retroviral expression of the STAT5B N642H mutant in mouse hematopoietic stem and progenitor cells is sufficient to drive their expansion *ex vivo* and to cause leukemia development *in vivo*, indicating that activation of the STAT5B target genes is critical for normal hematopoietic stem cells (HSCs) and LSCs.⁷

In the current study, Tremblay et al investigate the role of STAT5 in early T-cell precursor acute lymphoblastic leukemia (ETP-ALL). ETP-ALL cases are characterized by a high frequency of IL-7R/JAK/STAT pathway mutations and are known to be sensitive to JAK kinase inhibitors, such as ruxolitinib (which inhibits JAK2 and JAK1), even in the absence of IL-7R/JAK/STAT mutations.⁸ Using an Lmo2-driven T-ALL mouse model in which the *Il7r* gene was inactivated (*Il7r^{-/-}*), Tremblay et al

demonstrate that expression of activated STAT5B is sufficient to rescue the self-renewal capacity of the *Il7r^{-/-}* LSCs. Moreover, using a novel mouse model with inducible expression of a constitutively activated STAT5B (H298R + S716F) mutant (also known as STAT5B^{1*6}), they show that activated STAT5B enhances T-ALL development in the Lmo2 leukemia model, and confers a cell-intrinsic advantage as well as chemoresistance to the LSCs.

On the basis of the central role of STAT5B in ETP-ALL, the authors questioned if direct inhibition of STAT5 would be a viable therapeutic avenue. Pimozide is one of the older drugs known to inhibit STAT5A and STAT5B, and on the basis of recent modeling, it was suggested that this drug binds STAT5B at the Asn642 amino acid, thereby preventing its phosphorylation.⁹ Despite the fact that high concentrations of pimozide are required to reduce STAT5 phosphorylation and to block cell proliferation mediated by STAT5, these effects seem specific.¹ Indeed, cells transformed by IL-7R mutants or FLT3 mutants were sensitive to pimozide, whereas NRAS-transformed cells were not.¹ Treatment of mice transplanted with Lmo2-driven LSCs demonstrated synergy between chemotherapy and pimozide treatment, as shown by effects on leukemia burden and survival. Although pimozide alone had only mild effects on leukemia burden in patient-derived xenograft models of ETP-ALL, pimozide enhanced the response to chemotherapy.¹

This study, together with previous work on STAT5B, provides strong arguments for targeting STAT5B in T-ALL as well as other hematological malignancies.^{1,4,10} Pimozide is an interesting drug candidate, because it is already US Food and Drug Administration approved, but many questions remain. Pimozide has inhibitory effects on STAT5 but also impacts several other proteins, including receptors and ion channels. Currently, it is used as an antipsychotic drug at doses that do not affect STAT5 phosphorylation. It will be interesting to determine if pimozide or other agents that target STAT5 can be converted to more selective and more potent STAT5 targeting agents, perhaps by converting these to proteolysis-targeting chimeras. Studies to develop novel pimozide derivatives are underway,^{10,11} but the fact that STAT5 is also important in HSCs and T-cell development and that the drug would also target various ion channels might be

problematic.⁴ That should not stop us from developing and testing more potent and selective STAT5B inhibitors, as kinase inhibitors were initially also received with skepticism.

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THROMBOSIS AND HEMOSTASIS

Comment on Kaczmarek et al, page 290

Factor VIII forges its own path

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In this issue of *Blood*, Kaczmarek et al¹ elucidate distinct pathways of factor VIII (FVIII) uptake, trafficking, antigen presentation and CD4⁺ T-cell stimulation following IV administration to hemophilia A (HA) mice. FVIII avoided potential tolerizing interactions in the splenic red pulp, while innate immune and inflammatory pathways were shown to contribute to the anti-FVIII immune response.

Therapeutic options available to HA patients, whose bleeding disorder results from a lack of functional FVIII protein, have expanded considerably in the past 10 years.² In addition to the development of engineered recombinant FVIII proteins with extended half-lives (EHL), which provide the significant benefit of requiring fewer infusions, the FVIII-mimicking bispecific antibody emicizumab now enables prophylactic treatment via even less frequent subcutaneous injections. Other novel therapies targeting

anticoagulant pathways are under investigation but not currently approved by regulatory agencies, and FVIII replacement via gene therapy is also showing promise, but widespread availability, particularly for children, remains a future goal. The impact of emicizumab has been particularly striking as an effective therapy now available to the approximately 30% of patients with HA (among those with access to hemophilia care) who develop a neutralizing antibody (“inhibitor”) response to FVIII. However, despite these

impressive achievements, breakthrough bleeds still occurred in some patients who were enrolled in emicizumab clinical trials, indicating FVIII supplementation will still be required or preferred periodically.

As real-world data become available, the efficacy and safety of both EHL-FVIII and non-FVIII replacement therapies will come into better focus. Even at this early stage of gaining experience with these novel therapies, however, FVIII replacement therapy remains the standard of care to treat breakthrough bleeds and in settings of trauma and to support surgeries.³ The major impediment to effective FVIII administration remains the development of inhibitors. Therefore, further progress in understanding the molecular and cellular mechanisms contributing to FVIII immunogenicity is needed to develop targeted therapies that can prevent or reverse inhibitor development.

Therapeutic FVIII is administered IV, and therefore its initial presentation to the FVIII-naïve immune system of a patient with severe HA occurs as it is filtered by the spleen. Fifteen years ago, van Schooten et al demonstrated, by fluorescent and immunohistochemistry imaging of tissues from von Willebrand factor (VWF)-deficient mice, that IV-administered FVIII and VWF were both internalized by splenic and liver macrophages.⁴ Subsequent studies have characterized splenic trafficking and cellular interactions of FVIII in mouse HA (FVIII-knockout) models,⁵⁻⁷ as well as splenic transcriptomes,⁸ following IV FVIII infusions. Both splenectomy and marginal zone B-cell depletion attenuated the anti-FVIII antibody responses of FVIII-knockout mice.^{5,6}

The study in this issue by Kaczmarek et al sheds further light on the trafficking and immune sequelae of IV-administered FVIII. First, to follow the journey of FVIII after splenic uptake, the same dose of fluorescently labeled FVIII or ovalbumin (OVA) was administered to HA mice. Flow cytometry analysis of their splenocytes shortly afterwards showed both proteins were internalized by dendritic cells, marginal zone B cells and macrophages, and follicular and transitional B cells. Interestingly, only OVA was found in red pulp macrophages, indicating a distinct trafficking pattern of FVIII in the spleen.