

## The Role of JAK Pathway Dysregulation in the Pathogenesis and Treatment of Acute Myeloid Leukemia

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### Abstract

The discovery of the Janus kinase 2 (*JAK2*) V617F mutation has improved our understanding of the pathophysiology of myeloproliferative neoplasms such as polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Before discovery of the *JAK2* V617F mutation, there were no specific targeted therapies for patients with myeloproliferative neoplasms. More recently, several small-molecule inhibitors have been developed that have shown therapeutic potential in the clinical setting. There is evidence that the *JAK2* pathway is dysregulated in some acute myeloid leukemias and may also represent a novel therapeutic target in this disease. In this review, we describe the preclinical, clinical, and pathophysiologic evidence for using JAK inhibitors in the treatment of acute myeloid leukemias. *Clin Cancer Res*; 19(2); 327–35. ©2012 AACR.

### Introduction

#### Acute myeloid leukemia: current landscape

Acute myeloid leukemia (AML) is a hematopoietic clonal stem cell disorder characterized by abnormal differentiation and proliferation of immature blast cells in the bone marrow. AML is defined as 20% or more blasts in the bone marrow or less than 20% blasts with recurrent cytogenetic abnormalities, according to the 2008 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia (1, 2). AML accounts for approximately 80% of all adult leukemia cases, and incidence increases with age, with a median age at diagnosis of 67 years (3).

AML may arise *de novo* by transformation of the hematopoietic stem cell or progenitor cells of the myeloid lineage (4). Secondary AML can develop as a consequence of chemoradiotherapy or evolution of preexisting myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN). Both forms of secondary AML are associated with unfavorable chromosomal abnormalities and worse prognosis as compared with *de novo* AMLs (5). The primary goal of AML therapy is to achieve complete remission (CR) with induction chemotherapy, defined as bone marrow blasts less than 5%, neutrophil count more than  $1.0 \times 10^9/L$ , and platelet count more than  $100 \times 10^9/L$  independent of transfusion (6). Recent improvements in chemotherapeutic agents and supportive care have resulted in CR rates as high

as 85% in patients with AML. However, relapse occurs frequently as approximately 50% to 70% of patients with AML who achieve CR as a result of frontline therapy will experience relapse within 3 years (7). As a result, consolidation chemotherapy or allogeneic stem cell transplantation is often used to prevent relapse, with varying degrees of success.

In spite of advances in therapeutic options, overall patient survival rate has not markedly improved, and AML therapy remains an area of unmet medical need. A large proportion of patients with AML display mutations that lead to signaling pathway dysregulation. The most well-known of these mutations is the FMS-like tyrosine kinase-3 (FLT3) mutation, which occurs in approximately 30% of patients with AML. The FLT3 mutation is generated by either internal tandem duplication of the juxtamembrane domain or point mutations within the tyrosine kinase domain, both resulting in constitutive activation of tyrosine kinase (8, 9).

#### The JAK/STAT Pathway

The JAK/STAT pathway has increasingly been implicated in the pathogenesis of AML. The Janus kinases (JAK) and downstream components of the pathway are involved in cellular proliferation and differentiation and immunologic regulation. In humans, the JAK family of nonreceptor tyrosine kinases consists of 4 known members: JAK1, JAK2, JAK3, and TYK2 (10). JAK1, JAK2, and TYK2 are ubiquitously expressed, whereas JAK3 is expressed exclusively in hematopoietic, vascular smooth muscle, and endothelial cells (8). The JAK/STAT pathway is initiated via the extracellular binding of cytokines, including interleukins, IFNs, neurotrophic factors, and hormones, to their respective transmembrane receptors. Cytokine binding induces receptor dimerization, thereby bringing JAK proteins, which are constitutively bound to the intracellular region

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### Translational Relevance

Therapy for acute myeloid leukemia (AML) has not changed significantly since the "War on Cancer" was declared 40 years ago. Although we have made advances in understanding the genetics and pathophysiology of AML, patient outcomes have not improved substantially. Recognition of the heterogeneity of the disease has been accentuated by genomic data suggesting that the driving mutations may be different in individual patients with AML. Here, we show that the dysregulated Janus kinase (JAK) pathway may represent a novel therapeutic target for a subset of patients with AML. Given the recent clinical successes of JAK inhibitors in the treatment of myeloproliferative neoplasms, we review preclinical and clinical data supporting the use of JAK inhibitors in AML and summarize relevant evidence that may revolutionize the current paradigm of AML treatment.

of the receptors, into close proximity, resulting in transautophosphorylation of JAK molecules. Receptor-associated phosphorylated JAK (p-JAK) subsequently phosphorylates several sites on their respective receptors, thereby exposing an SH2 docking site for activation of the signal transducer and activator of transcription (STAT) transcription factors (11).

Activated STATs homodimerize or heterodimerize and translocate into the nucleus to induce transcription of various downstream targets (Fig. 1; ref. 10). Transcriptional targets of the JAK-activated STAT family, specifically STAT3 and STAT5, include genes involved in the regulation of cell survival, proliferation, and differentiation, including *MYC*, *cyclin D1*, *survivin*, and *BCL2* (9, 12, 13). Thus, abnormal activation of the JAK/STAT pathway is thought to play an important role in the pathogenesis of some malignancies, and it is becoming increasingly clear that the STATs also play roles in both the intrinsic and extrinsic cancer-associated inflammatory microenvironment (14, 15).

### JAK Dysregulation in Myeloproliferative Neoplasms

Aberrant JAK/STAT hyperactivation and its role in cancer biology have been best defined with respect to hematopoietic malignancies, including MPNs such as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In 2005, the JAK2 V617F gain-of-function mutation was discovered and observed in more than 90% of patients with PV and more than 50% of patients with PMF and ET (16–19). Additional JAK2-activating mutations have been observed in the clinic. Furthermore, MPNs have shown the abnormal elevation of several cytokines including serum interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), and TNF- $\alpha$ , which result in the activation of the JAK2 signaling pathway (20, 21). JAK/STAT

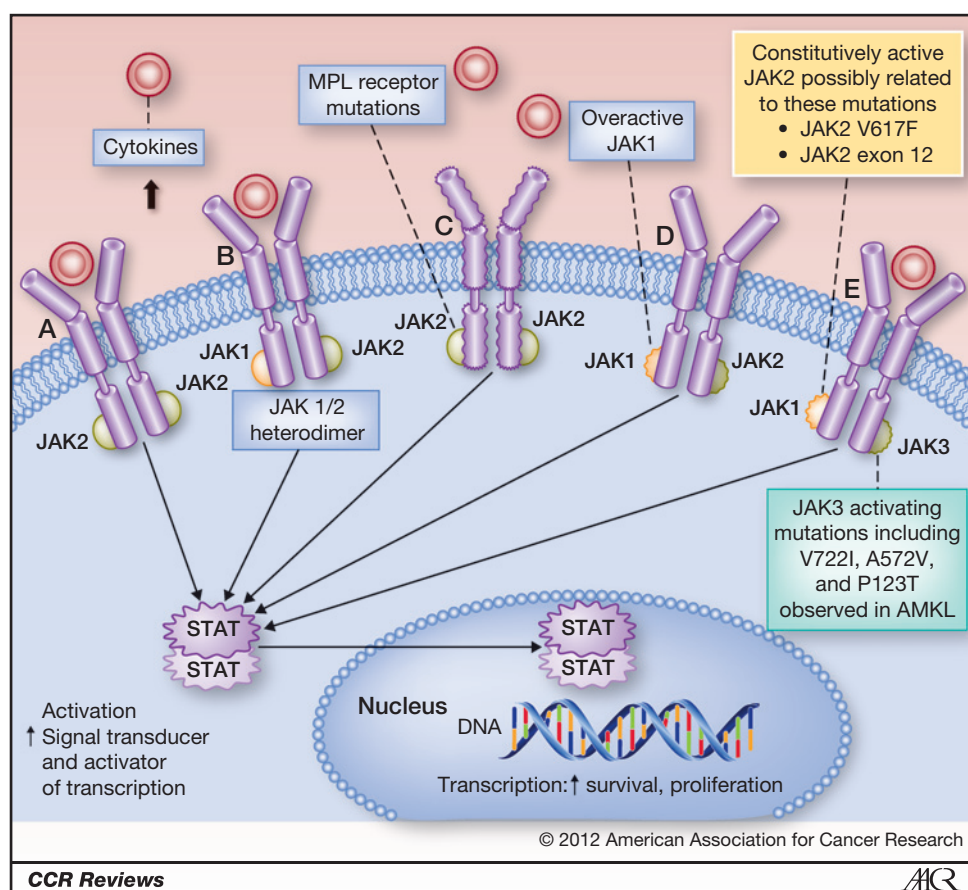
dysregulation through JAK2-activating mutations, the release of JAK2-activating cytokines from stromal cells, or the autocrine loop of JAK2-mediated cytokine production has been postulated as a possible mechanism by which MPNs sustain their proliferative and cytoprotective advantage (22, 23).

A number of JAK inhibitors have been developed. Of note, ruxolitinib, the only JAK inhibitor to complete phase III trials, was approved by the U.S. Food and Drug Administration for the treatment of intermediate- or high-risk MF and more recently by Health Canada and the European Commission for the treatment of MF-related splenomegaly or symptoms on the basis of results from the phase III COMFORT-I and -II studies, in which ruxolitinib therapy resulted in pronounced reductions in splenomegaly as well as improvements in disease-related symptoms compared with both placebo and best available therapy (24, 25). A follow-up in both the phase I/II 251 study and the COMFORT-I study reported an overall survival advantage in the ruxolitinib arm (24, 26). In the COMFORT-I study, an unplanned survival analysis during a planned safety update revealed that ruxolitinib significantly increased overall survival compared with placebo [13 (8.4%) deaths in ruxolitinib group and 24 (15.7%) deaths in placebo group]. Additional JAK inhibitors that are currently in clinical trials for treatment of MPNs include lestaurtinib (CEP-701), SAR302503 (TG101348), CYT387, AZD1480, and SB1518. Similar to ruxolitinib, a number of JAK inhibitors were effective in reduction of splenomegaly and constitutional symptoms (11).

### Myeloproliferative Neoplasm Progression to AML

It has been established that patients with an MPN are at an elevated risk for leukemic transformation. Progression to AML occurs in a subset of patients with an MPN, but progression to acute lymphoblastic leukemia is uncommon (27). Leukemic transformation may be secondary to the use of cytoreductive therapy, such as hydroxyurea; however, this hypothesis remains controversial (28). Another hypothesis is that progression to acute leukemia occurs through additional acquisition of key genetic mutations, which provide a survival or proliferative advantage similar to that observed in the progression of chronic myeloid leukemia from chronic phase to blast crisis (29, 30).

Much of our understanding of the pathophysiologic progression of MPN to AML comes from studies using mouse models. For example, deficient interferon consensus sequence binding protein (ICSBP)-mediated expression of the DNA-repair protein Fanconi F (FANCF) results in transformation to AML in myeloproliferative disease mouse models (31). Furthermore, activating mutations in the tyrosine phosphatase *SHP2* gene in combination with ICSBP haploinsufficiency accentuate the progression to AML in an MPN mouse model (32). Although incomplete, the preclinical data suggest a complex mechanism of MPN to AML progression involving multiple cooperative pathways.

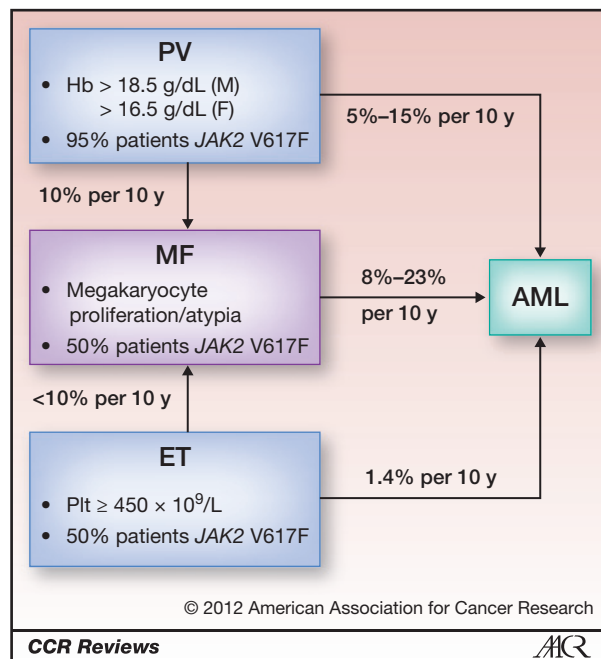


**Figure 1.** JAK receptor signaling and activation of STAT proteins. A and B, normal JAK/STAT signaling after specific binding of cytokine receptors by ligand. Cytokine binding to JAK2/JAK2 homodimers (A) or JAK1/JAK2 heterodimers (B) results in increased activation of STAT proteins, which leads to subsequent transcriptional changes that induce cellular survival and proliferation. C, MPL receptor mutations can result in ligand-independent activation of the JAK/STAT pathway. D, mutations in JAK1 or JAK2, specifically *JAK2* V617F, which is often observed in myelofibrosis, PV, or ET, can lead to constitutively active JAK function independent of ligand binding. E, mutations in JAK3, which have been observed in patients with acute megakaryoblastic leukemia (AMKL) can lead to elevated STAT activity.

The clinical data about AML transformation from a pre-existing MPN is less well understood. It is evident that a prolonged history of PV/ET results in an increased risk of bone marrow fibrosis and, correspondingly, it has been shown that increased bone marrow reticulin content and hypercellularity in patients with PV/ET increase risk for myelofibrosis or AML transformation (33). Moreover, investigators have identified increased levels of growth factors transforming growth factor (TGF)- $\beta$ , basic fibroblast growth factor (bFGF), and VEGF secreted by megakaryocytes, monocytes, or both in bone marrow specimens from patients with a history of PV/ET. Several genes are frequently found to be mutated in the MPN blast phase, such as *TET2*, *IDH1*, *IDH2*, *IKZF1*, and *RUNX1* (34–37). Recently, it was shown that mutations in the serine/arginine-rich splicing factor 2 (*SRSF2*) gene are more prevalent in patients with AML that has progressed from a preexisting MPN than in patients with *de novo* AML (18.9% and 5.6%, respectively; ref. 38).

In the clinic, the overall survival rates of patients whose AML progresses from MPN are inferior to those of patients with *de novo* AML (29). Leukemic transformation occurs in

approximately 15% of patients with PMF, 10% of those with PV, and 4% of those with ET (Fig. 2; refs. 39–43). Not surprisingly, the *JAK2* V617F mutation is found in a significant percentage of patients with AML arising from a pre-existing MPN (44, 45). Conversely, less than 10% of patients with *de novo* AML harbor the mutation (46). However, there is evidence that *JAK2* V617F-positive MPNs can progress to *JAK2* V617F-negative AML at disease transformation (47). Work by Theodorides and colleagues suggested that *JAK2* V617F-positive MPN progression to *JAK2* V617F-negative AML occurs through clonal evolution of a common *JAK2* V617F-negative progenitor (47). Interestingly, patients with V617F-negative AML who previously had a V617F-positive MPN had a significantly shorter interval between MPN diagnosis and AML transformation than those who developed V617F-positive AML. Furthermore, patients whose V617F-positive MPN progressed to V617F-positive AML had a more than 50% V617F positive-to-negative allelic ratio in blasts, indicating that high allelic burden may be an important factor in the clonal evolution to *JAK2* V617F-positive AML.



**Figure 2.** MPNs associated with the JAK2 V617F mutation. The JAK2 V617F mutation occurs in 95% of patients with PV and 50% of patients with ET or PMF. The approximate transformation rates are indicated. Plt, platelet; Hb, hemoglobin; M, male; F, female; y, years.

The prognostic implication of JAK2 V617F in this setting remains unclear. One study reported a significantly inferior survival in JAK-mutated AML, whereas other studies have not detected any prognostic or predictive implications (48–50). Currently, no well-defined treatment is available for AML arising from a preexisting MPN, and such patients would likely receive standard *de novo* AML therapies or be referred to a clinical trial.

### Rationale for Targeting JAK/STAT in AML

Given the high occurrence of JAK dysregulation in MPN, it may be suggested that JAK/STAT pathway dysregulation plays a role in the pathogenesis of secondary AML. Yet JAK/STAT dysregulation may not be exclusive to secondary AML, as elevated JAK and STAT levels have been observed in patients with *de novo* AML. As p-JAK levels are increased in patients with AML, and the JAK2 V617F mutation is present in only a small percentage of these patients, this specific mutation cannot be exclusively responsible for JAK dysregulation. It is possible that the classic V617F mutation, when present, may function either as a driver mutation in the initial stages of AML or as a passenger mutation in later stages by virtue of increased genomic instability and mutation load. Besides the JAK2 V617F mutation, several novel JAK2 activation mutations have been observed in AML patient samples, including PCM1-JAK2, JAK2 K607N, and JAK2 T875N (Table 1; refs. 51–56).

A recent study found elevated levels of activated p-JAK2 in bone marrow samples from a large cohort of patients

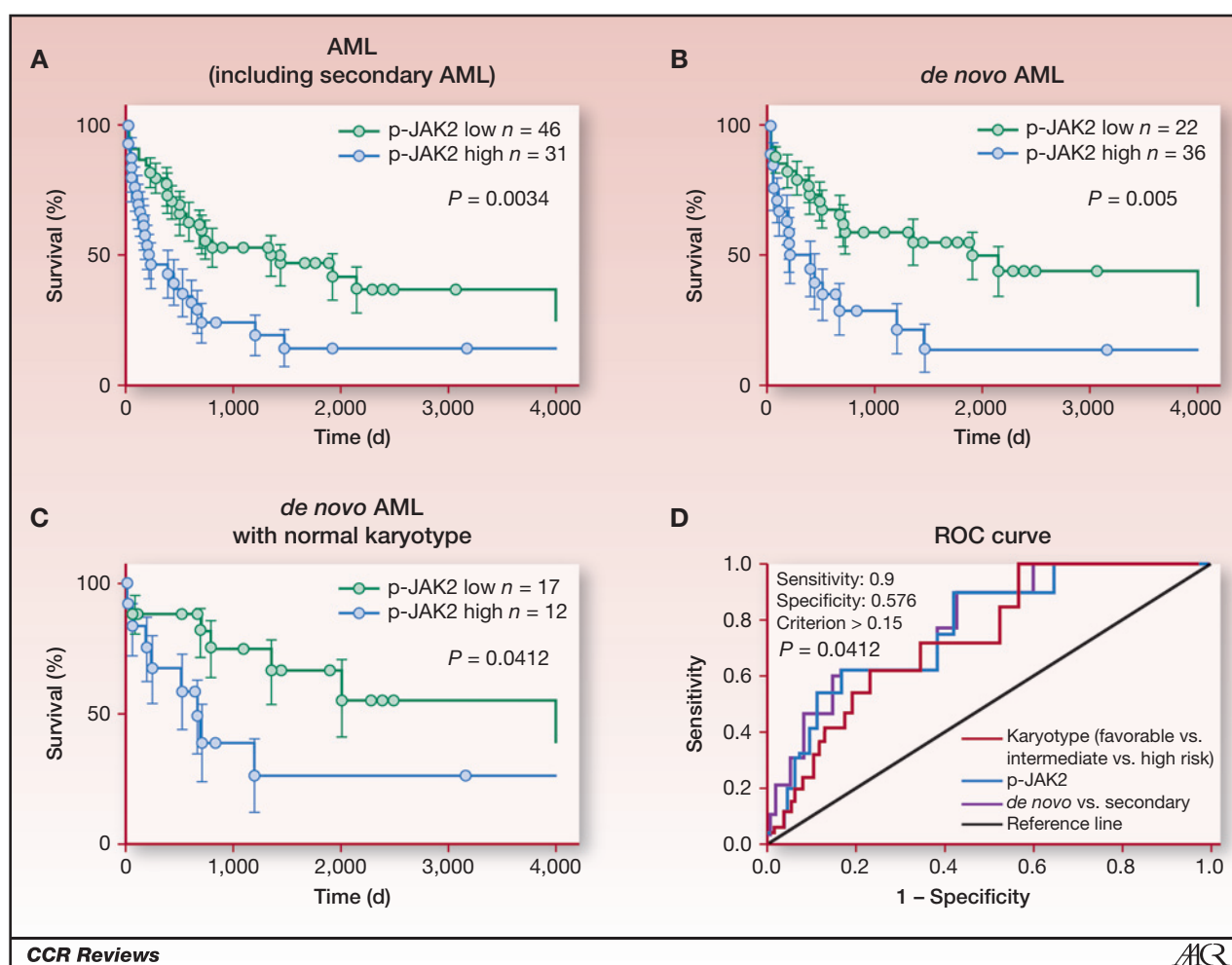
**Table 1.** Clinical observed JAK mutations in AML

JAK family member	Specific GOF mutation	Disease subtype	Reference
JAK1	T478S	AML	54
	V623A	AML	54
JAK2	V617F	AML	55
	K607N	AML	57
	T875N	AMKL	53
JAK3	PCM1-JAK2	AML	52
	V722I	AMKL	56
	A572V	AMKL	56
	P132T	AMKL	56

Abbreviations: AMKL, acute megakaryoblastic leukemia; GOF, gain-of-function

with AML ( $n = 77$ ). Elevated p-JAK2 correlated with high white blood cell count, low platelet count, and shorter survival in patients with either *de novo* or secondary AML (Fig. 3; ref. 54). Although elevated p-JAK2 levels were clearly observed, JAK2 V617F mutation was noted in only one of the 77 patients with AML, indicating that JAK/STAT dysregulation occurs through alternative mechanisms. Accordingly, treatment of AML cell lines with AZ960, a JAK2 inhibitor, reduced growth in AML cell lines, and induced apoptosis. Furthermore, inhibition of JAK/STAT by AZ960 resulted in increased apoptosis in CD34<sup>+</sup>/CD38<sup>-</sup> cells, which are proposed to contain a subpopulation of dormant leukemia stem cells (58).

The role of JAK/STAT dysregulation in AML has been further elucidated by several groups. Notably, inhibition of IL-27R, an activator of JAK/STAT signaling and a cell surface marker of AML, induced apoptosis and cell-cycle arrest in 32D myeloid cells (59). Furthermore, *in vitro* inhibition of JAK2-mediated phosphorylation of STAT3 and STAT5 in AML cell lines inhibited cell growth and induced apoptosis (60). More specifically, addressing a common cytogenetic abnormality, JAK inhibition with TG101348 inhibited monosomy 7 MDS bone marrow cell proliferation *in vitro*, whereas diploid cell numbers remained stable (61). Whether the result of genetic or epigenetic JAK/STAT pathway modifications, JAK hyperactivation and pathway dysregulation in AML has been shown, and inhibition of JAK/STAT in AML cell lines resulted in the inhibition of cell proliferation. Chou and colleagues recently published an elegant study elucidating one possible mechanism for this observation. Core-binding factor leukemia cells were shown to evade p53-dependent apoptosis by the upregulation of Bcl-xL through thrombopoietin and its receptor MPL, which interacts directly with p53 to regulate cell death. Thrombopoietin signaling, previously associated with providing a proliferative advantage through the JAK/STAT pathway, is thought to provide a "survival signal" via its interaction



**Figure 3.** Recent data about the association of elevated levels of phosphorylated JAK2 (p-JAK2) and survival in patients with *de novo* or secondary AML. A to C, high levels of p-JAK2, as determined by immunohistochemical analysis of patient samples, had a statistically significant negative effect on survival in all patients with AML (A), *de novo* AML (B), or *de novo* AML with intermediate/normal karyotype (C). D, area under curve (AUC) and receiver operating characteristic (ROC) analysis indicates a moderate predictive power of p-JAK2 level on AML outcome (54). Adapted from Ikezoe and colleagues (54) with permission of John Wiley & Sons, Inc.

with the p53 pathway. Accordingly, the authors speculate that small-molecule inhibitors could be useful in blocking this interaction (62).

The role of JAK/STAT pathway dysregulation and the potential benefit of JAK inhibition in patients with AML have been further elucidated by recent work. Clinical trials for patients with AML have shown that inhibition of the commonly mutated form of FLT3 is cytotoxic to leukemic cells in the peripheral blood, yet has limited efficacy in the bone marrow. It has been postulated that bone marrow stroma protects FLT3-mutated leukemia cells by providing a microenvironment rich with proliferative cytokines and antiapoptotic signals (63). Interestingly, combining FLT-3 inhibitors with JAK1/2 inhibitors, such as ruxolitinib, potentiates FLT3 inhibition, leading to a significant increase in cytotoxicity in *in vitro* FLT3-positive stromal models (64). Some JAK2 inhibitors (e.g., CEP-701, SB518, and TG101348) in development do

have activity against FLT3, but the consequences of dual FLT3/JAK2 inhibition have not been fully studied outside of MPNs (11). Furthermore, activating mutations in JAK1 have been described at low rates (1%–2%) in AML and therefore JAK2 inhibitors that also target JAK1, such as ruxolitinib and CYT387, may be the most useful in this context (53).

### Clinical Results: Patient Data and Therapeutic Toxicity

Early evidence regarding the efficacy of JAK inhibition in the treatment of AML shows promise. In a phase II clinical trial, 23 patients with relapsed or refractory AML, ALL, MDS, or CML (median age, 68 years) were administered ruxolitinib and were initially evaluated after one cycle of therapy or 28 days (65). Eight of the patients had *de novo* AML, and 8 had secondary AML derived from progression

of a prior MPN. Of 8 patients who exhibited the *JAK2* V617F gain-of-function mutation, 6 had secondary AML. As expected, *STAT3* levels were elevated in most patients, particularly those with the *JAK2* V617F mutation suggestive of *JAK/STAT* dysregulation. Ruxolitinib was well tolerated and efficacious. Of the 8 patients who had clinical improvement [either CR, partial remission (PR), or SD], 4 had secondary AML.

This study was expanded to include a total of 38 patients; 18 had secondary AML and 10 had *de novo* AML. The *JAK2* V617F mutation was observed in 12 patients, 3 with *de novo* AML, 7 with secondary AML, and 2 with MDS (66). Clinical benefit (CR, PR, or SD) was observed in 15 patients, although it was limited to SD in 12. Of the 3 patients achieving CR or PR, all had secondary AML, which was positive for *JAK2* V617F in 2. In these 3 patients, ruxolitinib not only produced a response but also reduced spleen size. Ruxolitinib therapy was well tolerated, with minimal grade 3 or more toxic effects in this heavily pretreated population. This may represent a potential treatment option for patients with refractory disease.

In the COMFORT trials, anemia and thrombocytopenia were the most frequently reported adverse events in the ruxolitinib arms of both studies, though they rarely led to treatment discontinuation and were generally manageable with dose modifications and/or transfusions (24, 25). Accordingly, patients with AML treated with *JAK1/2* inhibitors may develop or experience worsening of their anemia and thrombocytopenia. As the *JAK/STAT* pathway is a major regulator of erythropoiesis, it is anticipated that *JAK1/2* inhibitor therapy would lead to some myelosuppression, including anemia. However, with the possible suppression of the malignant clone, there is a possibility of a net improvement in blood counts if *JAK1/2* inhibitors suppress the mutant clone.

### Future Direction/Perspectives

The *JAK/STAT* pathway may represent a potential therapeutic target for AML. The effectiveness and therapeutic benefit obtained from *JAK* inhibitors will depend on accurate patient selection and improved understanding of AML biology. If appropriately harnessed, *JAK2* inhibitors have the potential to improve therapy and outcomes for certain subsets of AML, such as AML evolved from myelofibrosis. The benefit of *JAK1/2* inhibitor therapy may be greatest in patients with symptomatic disease with splenomegaly, cachexia, and pruritus. Furthermore, current *JAK2* inhibitors target both mutant and wild-type *JAK2*, and, therefore, patients with elevated *JAK2* or *STAT3* activity, not just those patients who are *JAK2*-mutation positive, would be appropriate.

Targeted molecular therapeutic approaches have yielded successful outcomes in other branches of oncology. A recent example of such targeted therapeutics led to the approval of a novel small-molecule inhibitor, crizotinib, for lung cancer. Crizotinib targets the *EML4-ALK* fusion, which is observed in approximately 4% of lung cancers. In the

clinical trial of crizotinib in lung cancer, approximately 1,500 patients had to be screened to identify 82 patients with this fusion protein. However, in this highly selective population, crizotinib yielded a 90% clinical benefit, 57% responses and 32% SD (67). Given the heterogeneity and complex molecular genetics of AML, it is unlikely that any one single agent will emerge as a curative option. Combination therapies will likely remain superior to single-agent therapy, and personalized treatment based on careful patient selection and accurate molecular stratification is the ultimate goal. Thus, further clinical trials are warranted to define the precise role of *JAK* inhibitors in AML and other non-MPN malignancies.

Aberrant *JAK/STAT* signaling is thought to occur during leukemic clonal evolution. Sustained proliferative signaling is a proven pathway facilitating leukemogenesis. However, other abilities, such as evasion of growth suppression [e.g., suppressor of cytokine signaling (SOCS)], resistance to cell death, and acquisition of genomic instability, work together with the tumor-promoting inflammation/microenvironment to promote leukemic transformation (68). Combination therapy with agents that target different aspects of cancer biology may be useful. For example, hypermethylation of key tumor suppressor genes *p15<sup>INK4b</sup>* and *p16<sup>INK4a</sup>* has been reported in leukemic transformation of agnogenic myeloid metaplasia (69). The same group has reported the use of azacitidine, a hypomethylating agent, in the treatment of Philadelphia chromosome-negative MPN that has progressed to MDS/AML. The overall response rate was 52% without significant side effects in this high-risk population (70). To this end, a hypomethylating agent and a *JAK2* inhibitor may represent a logical combination for patients with secondary MDS/AML who are not candidates for intensive chemotherapy. Furthermore, combining a *JAK* inhibitor with an immunomodulator such as lenalidomide or IL-6 antibodies that change the leukemic "niche" and/or interact with immunomodulatory cells, such as regulatory T cells, may represent a promising alternative therapeutic strategy (71, 72).

The ultimate goal of such strategies is "personalized medicine," wherein clinicians will be able to optimize AML therapy based on each individual's genomic data. Under such an approach, identification of tyrosine kinase mutations in patients with AML may suggest the use of specific small-molecule kinase inhibitors. Moreover, if dysregulation of a specific pathway was identified, treatment with an appropriate inhibitor would represent a rational approach (73, 74). Given recent advances in our understanding of genomic pathways as well as the economic feasibility of individual sequencing, it is reasonable to foresee a time when clinicians will be able to personalize the therapy offered to individual patients with AML.

### Disclosure of Potential Conflicts of Interest

F. Ravandi-Kashani has a commercial research grant from Incyte and has a honoraria from speakers' bureau from Novartis. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**Conception and design:** H.J. Lee, H.M. Kantarjian, F. Ravandi-Kashani  
**Development of methodology:** H.J. Lee, N. Daver, S. Verstovsek  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** H.J. Lee, H.M. Kantarjian  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** H.J. Lee, N. Daver, H.M. Kantarjian  
**Writing, review, and/or revision of the manuscript:** H.J. Lee, N. Daver, H.M. Kantarjian, S. Verstovsek, F. Ravandi-Kashani

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** H.J. Lee, H.M. Kantarjian

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