Morphologic and Molecular Characteristics of De Novo AML With *JAK2* V617F Mutation

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Abstract

Background: *JAK2* V617F mutation (mut) in acute myeloid leukemia (AML) is rare. We describe the clinicopathologic findings of a single-institution series of 11 de novo AML cases with *JAK2* V617. **Methods:** We identified cases of de novo AML with *JAK2* V617F over a 10-year period. We reviewed diagnostic peripheral blood and bone marrow (BM) morphologic, cytogenetic, and molecular studies, including next-generation sequencing. The control group consisted of 12 patients with *JAK2* wild-type (wt) AML matched for age, sex, and diagnosis. **Results:** We identified 11 patients (0.5%) with *JAK2* V617F, with a median age at diagnosis of 72.5 years (range, 36–90 years). Ten neoplasms were classified as AML with myelodysplasia-related changes and 1 as AML with t(8;21)(q22;q22). All *JAK2*mut AML cases showed at least bilineage dysplasia, 7 of 11 showed fibrosis, 8 of 11 had an abnormal karyotype, and 5 had deletions or monosomy of chromosomes 5 and 7. Using the European LeukemiaNet (ELN) classification, 9 patients (82%) with *JAK2*mut AML were intermediate-2 and adverse risk. Cases of *JAK2*mut AML did not have mutations in other activating signaling pathways (*P*=.013); 7 (64%) showed additional mutations in at least one gene involving DNA methylation and/or epigenetic modification. Patients with *JAK2*mut AML had a significantly higher median BM granulocyte percentage (12% vs 3.5%; *P*=.006) and a higher frequency of ELN intermediate-2 and adverse risk cytogenetics (*P*=.04) compared with those with *JAK2*wt AML. *JAK2*mut AML showed higher circulating blasts, but this difference was not significant (17% vs 5.5%; *P*=not significant). No difference was seen in the median overall survival rate of patients with *JAK2*mut AML versus those with *JAK2*wt AML (14 vs 13.5 months, respectively). **Conclusions:** De novo *JAK2*mut AML is rare and frequently found in patients with dysplasia, BM fibrosis, and abnormal karyotype with intermediate- or high-risk features; gene mutations in DNA methylation and epigenetic-modifying pathways; and absence of gene mutations in activating signaling pathways.

Driver gene mutation status has been incorporated into the classification and prognostic assessment of patients with acute myeloid leukemia (AML) and, in some cases, these data are used for treatment decisions.[1] JAK2 is a nonreceptor tyrosine kinase that plays a fundamental role in hematopoiesis as a key signaling intermediate.[2] The *JAK2* V617F mutation is present in >95% of pa-

tients with polycythemia vera, and in 50% to 60% of patients with essential thrombocythemia and primary myelofibrosis (MF).[3] Bone marrow (BM) specimens from these patients usually show characteristic findings, including atypical megakaryocytic hyperplasia with or without reticulin fibrosis. *JAK2* V617F mutation also has been reported in chronic myelomonocytic leukemia

(CMML) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) with ring sideroblasts and thrombocytosis, with a frequency of 8% to 10% and 60%, respectively.[4,5] Therapy with *JAK2* inhibitors has improved overall survival and disease-related symptoms, and stabilized or improved BM fibrosis in patients with polycythemia vera and primary MF.[6–9]

In addition to MPN and MDS/MPN, *JAK2* V617F mutation has been reported in de novo AML at a low frequency (<5%; Table 1).[10–16] Although a limited number of cases have been studied, de novo AML with *JAK2* V617F mutation is more common in patients with erythroid or megakaryoblastic AML,[15] tends to be associated with aberrant expression of CD19 and CD56, and to have a diploid karyotype.[10,12,17] However, a detailed description of BM morphologic features in *JAK2* mutation (mut) AML is not available and the affected molecular pathways and clinical outcomes in patients with de novo AML with *JAK2* V617F have not been completely elucidated.[5]

This study describes the clinical characteristics, pathologic findings, and outcomes of patients with AML with *JAK2* V617F mutation. Using systematic mutation profiling of de novo AML cases with *JAK2* V617F mutation, we show molecular alterations that provide insights into underlying pathogenesis and offer potential therapeutic targets that might be useful to treat this patient subset.

Methods

Study Group

We searched the database of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from 2005 to 2015 for cases of de novo AML with the *JAK2* V617F mutation. The study was conducted under an Institutional Review Board–approved protocol. All patients signed a consent form before enrollment in accordance with the Declaration of Helsinki. We specifically excluded patients with AML that arose from an antecedent myeloid neoplasm, as well as patients who previously received chemotherapy or radiotherapy. We also excluded cases of acute promyelocytic leukemia and cases of AML with *BCR/ABL1* rearrangement.[18,19] We identified 12 cases of *JAK2* wild-type (wt) AML that were matched by age, sex, and WHO classification diagnosis and identified them as a control group, which consisted of 11 de novo AML cases and 1 case with a history of treated CMML. All relevant clinical data were collected from the medical database at the time of AML diagnosis. Splenomegaly was defined as a palpable spleen (≥5 cm below the left costal margin) or increased spleen size measured by MRI or CT scans. Wright-Giemsa–stained peripheral blood smears, BM aspirate smears and touch imprints, and hematoxylin-eosin–stained BM aspirate clot and biopsy specimens were reviewed. We also reviewed the results of flow cytometry immunophenotypic analysis

Characteristics	Lee et al,[10] 2006	Jelinek et al,[15] 2005	Döhner et al,[14] 2006	Steensma et al,[13] 2006	Veiga et al,[11] 2007	Swaminathan et al,[12] 2010	Balatzenko et al,[16] 2015	Eghtedar et al,[37] 2012
Pts with de novo *JAK2* mutant AML, n (%)	3 (2.7)	2 (5.1)	4 (2.4)	3 (1.8)	8 (2.3)	6 (2.3)	3 (0.8%)	10 (26.0%)
Age at diagnosis, y	32–67	51–52	ND	ND	44–82	15–65	42–73	45–88
Dysplasia	ND	1	ND	ND	ND	ND	ND	ND
FAB/WHO diagnosis	2 pts t(8;21) & 1 pt without maturation	2 pts M7	4 pts t(8;21)	1 pt M6	4 pts M2	4 pts t(8;21)	3 pts M0	ND
				1 pt M7	2 pts M1	2 pts M4		
				1 pt M2	1 pt t(8;21)			
					1 pt inv(16)			
Fibrosis	ND	ND	ND	ND	ND	ND	ND	ND
Complex karyotype	0	1	ND	ND	0	0	ND	ND
Splenomegaly	ND	1	ND	ND	ND	ND	ND	ND

Table 1. Review of Cases of De Novo AML and *JAK2* Mutation Reported in the Literature

Abbreviations: AML, acute myeloid leukemia, FAB, French-American-British; ND, no data; pts, patients.

and relevant immunohistochemical studies; MF was defined as described previously.[18]

Cytogenetic Studies

Conventional chromosomal analysis was performed on G-banded metaphases prepared from unstimulated 24- and 48-hour BM aspirate cultures using standard techniques described previously.[20] The results were reported using the 2016 edition of the International System for Human Cytogenetic Nomenclature.[21–23]

Molecular Studies

We performed targeted next-generation sequencing (NGS)–based somatic mutation analysis using a 28-gene panel.[24] We also analyzed additional genes, including splicing factors (*SF3B1*, *SRSF2*, and *U2AF1*) and *CEBPA*, using PCR followed by Sanger sequencing. Internal tandem duplication of the *FLT3* gene was assessed by PCR followed by capillary electrophoresis. All molecular tests were performed on genomic DNA extracted from whole blood.[24–26] In patients with AML diagnosed after 2012 (n=19), *JAK2*mut testing was performed at the time of diagnosis as a part of routine NGS-based multigene profiling in a 28-gene panel. In 3 patients with AML diagnosed before 2012, *JAK2*mut testing was performed after the mutation was first described. In these patients, for the purpose of this study, a 28-gene panel was performed on diagnostic BM samples. Quantification of the *JAK2*mut allele burden was obtained by NGS-based sequencing.

Statistical Analyses

Overall survival was calculated from the date of diagnosis to the date of death. Surviving patients were censored at the date on which they were last known to be alive. Frequencies and percentages were calculated for categorical variables, and means (range) were calculated for continuous variables. All statistical analyses were performed using GraphPad Prism 6. *P* values ≤.05 were considered significant.

Results

Study Group

We reviewed 2,206 patients with AML over a 10-year period and identified 132 (6%) who had a *JAK2* V617F mutation. Of these, 11 patients (0.5%) had de novo AML (which formed the study group), with a median age at diagnosis of 72.5 years (range, 36–90 years). For comparison, we identified a group of 12 patients with *JAK2*wt AML who were matched for age, sex, and diagnosis. Table 2 summarizes the characteristics of these 2 groups of patients.

Peripheral Blood and BM Findings

The CBC count in patients with *JAK2*mut AML showed median hemoglobin of 9.6 g/dL, leukocyte count of 5.1 x 10⁹/L, and platelet count of 47 x 10⁹/L; the median blast percentage was 17%. The hemoglobin level, leukocyte count, and blast count were higher in patients with *JAK2*mut AML than in the control group, but these differences were not significant.

In the BM, 10 patients with *JAK2*mut AML were classified as AML with myelodysplastic-related changes (MRC); 9 had dysplasia in >50% of cells of ≥2 lineages and 1 had complex cytogenetic changes. One patient was classified as AML with t(8;21) (q22;q22)/*RUNX1-RUNX1T1*, and showed bilineage dysplasia in the erythroid and myeloid lineages. The median BM blast count was 40% (range, 20%–70%), median BM cellularity was 77.5%, and BM megakaryocytes were 2.5 high-power fields. Of 10 patients with available data, 7 had MF (5 with MF-1, 2 with MF-2). None of the patients had MF-3, and no osteosclerotic changes were present (Figure 1).

Trilineage dysplasia was more common in *JAK2*wt AML than *JAK2*mut AML (6/12 [50%] vs 4/11 [36%]; *P*=not significant). These findings were not significantly different from the control group. However, one difference was the percentage of BM granulocytes (mainly mature): 12% in *JAK2*mut AML but 3.5% in *JAK2*wt (*P*=.005; Table 2).

Cytogenetic Data

The conventional cytogenetic data are shown in detail in the supplemental eTable 1 (available with this article at JNCCN.org). Of 11 patients with *JAK2*mut AML, 8 had an abnormal karyotype, 5 showed monosomy or deletion of chromosomes 5 or 7, and 3 had a complex karyotype (including 2 patients with monosomy 7). Using the European LeukemiaNet (ELN) classification, 2 patients had favorable and 9 had intermediate-2 or adverse risk cytogenetics.[27] In the *JAK2*wt AML group, 4 of 12 patients had an abnormal karyotype with monosomy or deletion of chromosomes 5 or 7 in 3 patients. The only significant difference was that patients with *JAK2*mut

Table 2. Characteristics of Patients With AML With *JAK2*mut AML Versus *JAK2*wt AML

Characteristics	*JAK2*mut AML (N=11)	*JAK2*wt AML (N=12)	*P* Value
Age, y	73	76	Match
Sex, male/female	4/7	5/7	Match
Diagnosis (WHO)	10 pts: AML-MRC	12 pts: AML-MRC	–
	1 pt: t(8;21)		
Diagnosis (FAB)	2 pts: RAEB-T	5 pts: RAEB-T	–
	3 pts: M0	6 pts: M1	
	2 pts: M1	1 pts: M4	
	2 pts: M2		
	1 pts: M4		
	1 pts: M7		
Hb, g/dL (range)	9.6 (7.8–17.5)	8.7 (7.8–10.6)	.10
WBC, x 10⁹/L (range)	5.1 (1.9–26.1)	3.05 (0.9–97)	.32
Platelets, x 10⁹/L (range)	47 (8–215)	62 (18–154)	.96
PB blast, % (range)	17 (0–67)	5.5 (0–54)	.34
BM cellularity, %	77.5	57.5	.17
BM metamyelocytes, % (range)	5 (0–16)	1.5 (0–14)	.22
BM granulocytes, % (range)	12 (2–41)	3.5 (0–11)	.006
PB neutrophils, % (range)	37 (3–70)	14 (1–65)	.10
BM blast, % (range)	40 (20–70)	37 (20–83)	.57
MF-0, pts	3 (30%)	1 (9%)	–
MF-1, pts	5 (50%)	9 (82%)	
MF-2, pts	2 (20%)	1 (9%)	
MF-3, pts	0	0	
Not available	1	1	
Splenomegaly, pts	1 (10%)	0 (0%)	–
ELN classification[a]			
Favorable	2 (18%)	0 (0%)	
Intermediate-1	0 (0%)	8 (67%)	
Intermediate-2/Adverse	9 (81%)	4 (33%)	.04
Complex karyotype,[a] pts	3 (27%)	2 (16.6%)	.25
Diploid karyotype, pts	3 (27%)	8 (67%)	.10

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplastic-related changes; BM, bone marrow; ELN, European LeukemiaNet; FAB, French-American-British; Hb, hemoglobin; MF, myelofibrosis; MRC, myelodysplasia-related changes; mut, mutant; PB, peripheral blood; pts, patients; RAEB-T, refractory anemia with excess of blast in transformation; wt, wild-type.

[a]Complex karyotype full description is provided in supplemental eTable 1, available with this article at JNCCN.org.

AML had a higher frequency intermediate-2 and adverse risk cytogenetics (*P*=.04).

Mutation Analysis

In *JAK2*mut AML, the median allelic frequency for *JAK2*mut was 31.8% (range, 13%–53%). The median number of mutations per sample was 2 (including *JAK2*): 3 patients had *JAK2* V617F as a sole abnormality (splicing factor genes not tested in 1 case), 3 patients had 1 additional abnormality, and 5 had multiple (≥3) additional abnormalities. In comparison, the median number of mutations in *JAK2*wt AML was 2.5: 1 patient had no mutations (splicing factor genes not tested), 2 patients had 1 mutation, 3 patients had 2 mutations, and 6 patients had multiple (≥3) mutations. One patient with *JAK2*wt AML had 6 co-mutated genes, including *FTL3*, *KRAS*, *DNMT3A*, *GATA2*, *CEBPA*, and *PTPN11*. Mutation results and allelic burden for each patient are shown in Figure 2.

Segregation of genes based on the functional classification per The Cancer Genome Atlas Research Network[28] showed that *JAK2*mut AML was not associated with activating or other mutations in other signaling pathways when compared with *JAK2*wt AML (*P*=.013; *FLT3* [0% vs 33%], *KRAS* [0% vs 16%], *NRAS* [0% vs 16%], and *PTPN11* [0% vs 8.3%]). There were no significant differences between groups in mutations of most genes implicated in DNA methylation, epigenetic modifiers, or tumor suppressor genes, such as *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *TET2*, *TP53*, and *WT1*, except that *DNMT3A* may be less frequently mutated in *JAK2*mut AML (1/11; 9%) versus *JAK2*wt (4/12; 33%) (*P*= not significant). Among myeloid transcription factor genes, *CEBPA* was mutated in 1 patient with *JAK2*mut AML and 3 patients with *JAK2*wt. *RUNX1* was mutated in 2 patients with *JAK2*mut AML (both also had an *IDH1*mut) and in 1 patient with *JAK2*wt (who also had an *IDH2*mut).

DNA was available for assessment of spliceosome gene mutations in 16 patients, including 9 with *JAK2*mut AML and 8 with *JAK2*wt. The most frequently mutated gene was *SRSF2*, found in 4 patients with *JAK2*mut AML and 3 patients with *JAK2*wt; *U2AF1*mut was found in 1 patient with *JAK2*wt AML, and no patients had *SF3B1*mut.

When stratified according to the ELN classification, patients in the adverse prognosis risk group
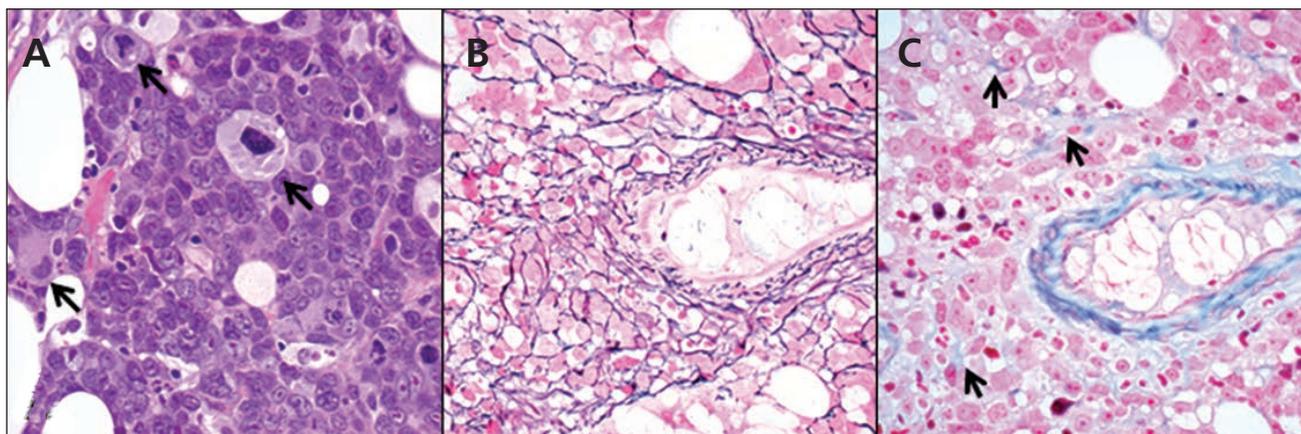
Figure 1. Morphologic features of a bone marrow (BM) sample from a patient with de novo acute myeloid leukemia with the *JAK2* V617F mutation. (A) BM infiltrate showed sheets of immature cells of medium size with irregular shaped nuclei, 1 or 2 small nucleoli and abundant cytoplasm, and small, hypolobated, dysplastic megakaryocytes indicated by the arrows (hematoxylin-eosin, original magnification x500). (B) Diffuse and dense increase in reticulin fibrosis (MF-2), with extensive intersections (reticulin, original magnification x400). (C) Arrows point to focal bundles of collagen (trichrome, original magnification x400). No osteosclerotic changes were observed (image not shown).

with *JAK2*mut AML harbored <2 additional mutations (n=4) or no other mutation (n=1). In contrast, 2 patients with an adverse prognosis in the *JAK2*wt AML group harbored multiple mutations, including *TP53*.

Survival Analysis

A total of 18 patients in this cohort (9 from each group) were treated at our institution on clinical trials; 2 patients were lost to follow-up (1 in each group). The remaining patient with *JAK2*mut AML received standard induction chemotherapy (7+3). Of the remaining 2 patients with *JAK2*wt AML, 1 refused treatment and the other received subcutaneous cytarabine. The median overall survival from disease onset was 14 months for patients with *JAK2*mut AML and 13.5 months for those with *JAK2*wt.

Discussion

We describe the clinical, morphologic, and genetic findings of 11 patients with AML associated with *JAK2* V617F mutation. All cases showed multilineage dysplasia and/or cytogenetic abnormalities diagnostic of AML with MRC. Monosomy or deletions of chromosomes 5 or 7 were present in 45% of patients, and approximately one-third of patients had a complex karyotype. Therefore, patients with *JAK2*mut AML have poorer-risk features, including multilineage dysplasia and poorer risk cytogenetic data which, when coupled with approximately

1-year survival, suggests the need for alternate treatment options. In addition, these findings support the idea that *JAK2* V617F provides the signal transduction defect in some patients with de novo AML and that *JAK2*mut is associated with monosomy of chromosomes 5 or 7.

Constitutive activation of the JAK/STAT signaling pathway plays an important role in the pathogenesis of MPN and in a subset of patients with AML with *FLT3*mut and acute lymphoblastic leukemia.[29–34] JAK/STAT signaling is important for AML stem cell growth and survival.[35] Mice with AML and treated with a STAT3 inhibitor had lower BM blast counts and significantly prolonged survival compared with control mice.[36] Patients with AML had higher levels of phosphorylated STAT3 at baseline; decreases in STAT3 levels correlated with the administration of JAK1/2 inhibitors.[37] Still, further research is needed to investigate the role of JAK/STAT pathway activation in AML.

In this cohort, *JAK2*mut was mutually exclusive with mutations in other activating signaling pathway genes. Approximately two-thirds of patients with *JAK2*mut were associated with concomitant mutations in genes involved in DNA methylation and epigenetic modification, whereas *JAK2*mut was the sole mutation in 3 patients (27%). In 2 patients at relapse, 1 had persistence of *JAK2*mut whereas mutation status was not available in the other patient. These findings support the importance of constitutive activation of the JAK/STAT pathway in

ELN Classification		Complex*	Complex	Complex	-7	del(5q)	Diploid	Diploid	+8	del(20q)	Diploid	t(8;21)	Complex	Complex	del(7q)	+8	Diploid	Diploid	Diploid	Diploid	Diploid	Diploid	Diploid	Diploid
		Adverse					Intermediate II					Favorable	Adverse		Inte. II		Intermediate I							
Activating Signaling	JAK2	26.9	32.6	53.8	N/A	26	36.6	37.7	31	13.6	15.4	39.9												
	MLL																							
	MPL																							
	KIT																							
	EGFR																							
	FLT3													3.3		N/A					N/A	0.27		
	BRAF																							
	PTPN11															9.1								
	KRAS													39				10.5						
	NRAS															26.7				13.3				
	HRAS																							
Epigenetic Modifier	ASXL1						47.5		31.8		17.3			46.2									3.6	32.2
		36.6														15.1								
DNA methylation	DNMT3A			48										31.7		50.2	62.6		31*					
	TET2							44.7		43.6										38*			34.3	
	IDH1							4.8	20.9					32.4									8.3	
	IDH2			N/A		36.6										46.8				30.8				
Myeloid transcription	RUNX1							44.7	27.6						1.7									
	GATA1																							
	GATA2															30.6								
	CEBPA										N/A			N/A		N/A				N/A				
	NPM1																							
Tumor suppressor	TP53	11*											15*											
	WT1																							
Spliceosome	SRSF2																							
	SF3B1																							
	U2AF1																							
Others	BZF2																							
	MDM2																							
	NOTCH11																							
	ABL1																							
	MYD88																							
	ATM																							

Figure 2. Molecular studies of patients with acute myeloid leukemia (AML) with *JAK2* mutated AML compared with *JAK2* wild-type AML. The first row describes cytogenetics and European LeukemiaNet (ELN) classification. Each mutated gene with its respectively allelic burden is highlighted in red; genes not mutated are shown in white. Gray selections are not tested.
Abbreviations: Int, intermediate; N/A, not available.
*Full description of complex karyotypes is provided in supplemental eTable 1, available with this article at JNCCN.org.

the genesis of AML. Further, these results highlight the role of *JAK2*mut as a driver mutation or as a second hit in cases with other gene mutations, such as *DNMT3A*, *ASXL1*, and *TET2*.[38] Based on data from Lindsley et al,[39] the presence of mutations in *SRSF2*, *ASXL1*, and *EZH2* suggests an underlying myeloid neoplasm, such as an MPN or MDS/MPN, despite a de novo AML presentation; of 11 patients with *JAK2*mut AML, 6 showed one of these mutations.

Cytogenetic findings are among the most important prognostic factors in AML.[27] In this study group, *JAK2*mut AML more frequently had a higher ELN risk classification than *JAK2*wt AML (81% vs 33%). Despite the similar outcome between the groups, these findings highlight the different pathogenesis of *JAK2*mut and *JAK2*wt AML, and these data are reinforced by differences in gene mutation frequency.

Our study was limited by a small number of samples and retrospective design. We identified only 11 patients during a 10-year period and, therefore, *JAK2*

V617F is a rare finding in de novo AML. However, the implementation of *JAK2* screening as a standard of care in patients with AML-MRC should be considered. This is feasible by including *JAK2* in the currently used, NGS-based multigene mutation panels. Nevertheless, this study offers the advantage of a single referral center with standard clinical and diagnostic care and long follow-up. Many questions remain regarding the underlying biology of BM characteristics and the role of *JAK2* in the pathogenesis of AML.

Conclusions

JAK2 V617F mutation in de novo AML is rare and associated with approximately 1 year of survival. It is more commonly seen in patients with intermediate-2 and adverse risk cytogenetics. *JAK2*mut AML is frequently found in patients with BM dysplasia and MF, an abnormal karyotype and mutations in genes involved in DNA methylation and epigene-

tic-modifying pathways, and the absence of gene mutations in activating signaling pathways other than JAK2. In patients with *JAK2*mut AML, the option of using JAK1/2 inhibitors as part of first-line therapy warrants exploration.

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