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 V617F. 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 JAK2mut 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 JAK2mut AML were intermediate-2 and adverse risk. Cases of JAK2mut AML did not have mutations in other activating signaling pathways (P=0.013); 7 (64%) showed additional mutations in at least one gene involving DNA methylation and/or epigenetic modification. Patients with JAK2mut AML had a significantly higher median BM nucleocyte percentage (12% vs 3.5%; P=0.006) and a higher frequency of ELN intermediate-2 and adverse risk cytogenetics (P=0.04) compared with those with JAK2wt AML. JAK2mut 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 JAK2mut AML versus those with JAK2wt AML (14 vs 13.5 months, respectively). Conclusions: De novo JAK2mut 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 patients with polycythemia vera, and in 50% to 60% of patients with essential thrombocythemia and primary myelofibrosis (MF).1 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 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.

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De Novo AML With JAK2 Mutation

(CMML) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) with ring sideroblasts and thrombocytosis, with a frequency of 8% to 10% and 60%, respectively.6,7 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

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

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Pts with de novo JAK2 mutant AML, n (%)</td>
<td>3 (2.7)</td>
<td>2 (5.1)</td>
<td>4 (2.4)</td>
<td>3 (1.8)</td>
<td>8 (2.3)</td>
<td>6 (2.3)</td>
<td>3 (0.8%)</td>
<td>10 (26.0%)</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FAB/WHO diagnosis</td>
<td>2 pts t(8;21) &amp; 1 pt without maturation</td>
<td>4 pts t(8;21)</td>
<td>1 pt M6</td>
<td>4 pts M2</td>
<td>4 pts t(8.21)</td>
<td>4 pts M0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Complex karyotype</td>
<td>0</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia, FAB, French-American-British; ND, no data; pts, patients.
and relevant immunohistochemical studies; MF was defined as described previously.\textsuperscript{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.\textsuperscript{20} The results were reported using the 2016 edition of the International System for Human Cytogenetic Nomenclature.\textsuperscript{21–23}

**Molecular Studies**
We performed targeted next-generation sequencing (NGS)–based somatic mutation analysis using a 28-gene panel.\textsuperscript{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.\textsuperscript{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 JAK2mut 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 JAK2wt 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 JAK2mut AML showed median hemoglobin of 9.6 g/dL, leukocyte count of 5.1 x 10^9/L, and platelet count of 47 x 10^9/L; the median blast percentage was 17%. The hemoglobin level, leukocyte count, and blast count were higher in patients with JAK2mut AML than in the control group, but these differences were not significant.

In the BM, 10 patients with JAK2mut 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 JAK2wt AML than JAK2mut 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 JAK2mut AML but 3.5% in JAK2wt (P=.005; Table 2).

**Cytogenetic Data**
The conventional cytogenetic data are shown in detail in the supplemental eTable 1 (available at JNCCN.org). Of 11 patients with JAK2mut 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.\textsuperscript{27} In the JAK2wt 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 JAK2mut
De Novo AML With JAK2 Mutation

Table 2. Characteristics of Patients With AML With JAK2mut AML Versus JAK2wt AML

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>JAK2mut AML (N=11)</th>
<th>JAK2wt AML (N=12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73</td>
<td>76</td>
<td>Match</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>4/7</td>
<td>5/7</td>
<td>Match</td>
</tr>
<tr>
<td>Diagnosis (WHO)</td>
<td>10 pts: AML-MRC</td>
<td>12 pts: AML-MRC</td>
<td>–</td>
</tr>
<tr>
<td>Diagnosis (FAB)</td>
<td>2 pts: RAEB-T</td>
<td>5 pts: RAEB-T</td>
<td>–</td>
</tr>
<tr>
<td>Hb, g/dL (range)</td>
<td>9.6 (7.8–17.5)</td>
<td>8.7 (7.8–10.6)</td>
<td>.10</td>
</tr>
<tr>
<td>WBC, x 10^9/L</td>
<td>5.1 (1.9–26.1)</td>
<td>3.05 (0.9–97)</td>
<td>.32</td>
</tr>
<tr>
<td>Platelets, x 10^9/L (range)</td>
<td>47 (8–215)</td>
<td>62 (18–154)</td>
<td>.96</td>
</tr>
<tr>
<td>PB blast, % (range)</td>
<td>17 (0–67)</td>
<td>5.5 (0–54)</td>
<td>.34</td>
</tr>
<tr>
<td>BM, %</td>
<td>77.5</td>
<td>57.5</td>
<td>.17</td>
</tr>
<tr>
<td>BM, %</td>
<td>5 (0–16)</td>
<td>1.5 (0–14)</td>
<td>.22</td>
</tr>
<tr>
<td>BM, %</td>
<td>12 (2–41)</td>
<td>3.5 (0–11)</td>
<td>.006</td>
</tr>
<tr>
<td>PB blast, % (range)</td>
<td>37 (3–70)</td>
<td>14 (1–65)</td>
<td>.10</td>
</tr>
<tr>
<td>MF-0, pts</td>
<td>3 (30%)</td>
<td>0 (0%)</td>
<td>–</td>
</tr>
<tr>
<td>MF-1, pts</td>
<td>5 (50%)</td>
<td>9 (82%)</td>
<td>–</td>
</tr>
<tr>
<td>MF-2, pts</td>
<td>2 (20%)</td>
<td>1 (9%)</td>
<td>–</td>
</tr>
<tr>
<td>MF-3, pts</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Not available</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Splenomegaly, pts</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>–</td>
</tr>
<tr>
<td>ELN classification</td>
<td>Favorable</td>
<td>2 (18%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>0 (0%)</td>
<td>8 (67%)</td>
<td>–</td>
</tr>
<tr>
<td>Intermediate-2/Adverse</td>
<td>9 (81%)</td>
<td>4 (33%)</td>
<td>.04</td>
</tr>
<tr>
<td>Complex karyotype, pts</td>
<td>3 (27%)</td>
<td>2 (16.5%)</td>
<td>.25</td>
</tr>
<tr>
<td>Diploid karyotype, pts</td>
<td>3 (27%)</td>
<td>8 (67%)</td>
<td>.10</td>
</tr>
</tbody>
</table>

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.

*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 JAK2mut AML, the median allelic frequency for JAK2mut 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 JAK2wt 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 JAK2wt AML had 6 co-mutated genes, including FLT3, 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 showed that JAK2mut AML was not associated with activating or other mutations in other signaling pathways when compared with JAK2wt 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 JAK2mut AML (1/11; 9%) versus JAK2wt (4/12; 33%) (P= not significant). Among myeloid transcription factor genes, CEBPA was mutated in 1 patient with JAK2mut AML and 3 patients with JAK2wt. RUNX1 was mutated in 2 patients with JAK2mut AML (both also had an IDH1mut) and in 1 patient with JAK2wt (who also had an IDH2mut).

DNA was available for assessment of spliceosome gene mutations in 16 patients, including 9 with JAK2mut AML and 8 with JAK2wt. The most frequently mutated gene was SRSF2, found in 4 patients with JAK2mut AML and 3 patients with JAK2wt; U2AF1mut was found in 1 patient with JAK2wt AML, and no patients had SF3B1mut.

When stratified according to the ELN classification, patients in the adverse prognosis risk group
with JAK2mut AML harbored <2 additional mutations (n=4) or no other mutation (n=1). In contrast, 2 patients with an adverse prognosis in the JAK2wt 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 JAK2mut AML received standard induction chemotherapy (7+3). Of the remaining 2 patients with JAK2wt AML, 1 refused treatment and the other received subcutaneous cytarabine. The median overall survival from disease onset was 14 months for patients with JAK2mut AML and 13.5 months for those with JAK2wt.

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 JAK2mut 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 JAK2mut 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 FLT3mut and acute lymphoblastic leukemia. JAK/STAT signaling is important for AML stem cell growth and survival. Mice with AML and treated with a STAT3 inhibitor had lower BM blast counts and significantly prolonged survival compared with control mice. Patients with AML had higher levels of phosphorylated STAT3 at baseline; decreases in STAT3 levels correlated with the administration of JAK1/2 inhibitors. Still, further research is needed to investigate the role of JAK/STAT pathway activation in AML.

In this cohort, JAK2mut was mutually exclusive with mutations in other activating signaling pathway genes. Approximately two-thirds of patients with JAK2mut were associated with concomitant mutations in genes involved in DNA methylation and epigenetic modification, whereas JAK2mut was the sole mutation in 3 patients (27%). In 2 patients at relapse, 1 had persistence of JAK2mut whereas mutation status was not available in the other patient. These findings support the importance of constitutive activation of the JAK/STAT pathway in
the genesis of AML. Further, these results highlight the role of JAK2mut as a driver mutation or as a second hit in cases with other gene mutations, such as DNMT3A, ASXL1, and TET2. Based on data from Lindsley et al, 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 JAK2mut AML, 6 showed one of these mutations.

Cytogenetic findings are among the most important prognostic factors in AML. In this study group, JAK2mut AML more frequently had a higher ELN risk classification than JAK2wt AML (81% vs 33%). Despite the similar outcome between the groups, these findings highlight the different pathogenesis of JAK2mut and JAK2wt 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. JAK2mut 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 JAK2mut AML, the option of using JAK1/2 inhibitors as part of first-line therapy warrants exploration.

Acknowledgments

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References

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