Myelofibrotic transformation in essential thrombocytopenia. Author reply

We thank Juergen Thiele and Hans Kvasnicka for commenting on our recently published paper including 605 patients with essential thrombocythemia (ET).1 We provided evidence that progression to myelofibrosis (post-ET MF) has a prevalence of 2.8% (10-year risk of 3.9%), and that progression to acute leukemia (AL) has a prevalence of 2.3% (10-year risk of 2.6%). The first question of Thiele and Kvasnicka concerns the evolution of ET in post-ET MF and AL. They asked that the prevalence of post-ET MF and AL in patients diagnosed according to the PVSG criteria2 and with the WHO criteria,3 be evaluated separately. The analysis suggested would be strongly biased by the fact that PVSG-classified patients have longer follow-up than WHO-classified patients. In the paper, we mentioned that the longer the follow-up, the higher the risk of transformation into myelofibrosis or leukemia. We regret that their request could not be satisfied, but a direct comparison of these two cohorts with different follow-up may give a misleading message. The second question from Thiele and Kvasnicka concerns the diagnostic differentiation between ET and prefibrotic/early fibrotic phase of primary myelofibrosis (PMF), an entity recognized on the basis of bone marrow features by the WHO classification of 2001.4 However, the recent WHO classification requires the combination of histological picture, clonal markers and clinical parameters to diagnose PMF at prefibrotic or fibrotic phase.5 In our series we excluded cases of PMF (excluded combination of leukoerythroblastosis, anemia, elevated LDH, spleen enlargement). Concerning the discussion on sequential bone marrow evaluations, we perform bone marrow biopsy at diagnosis in all the patients and during follow-up when we suspect clinical progression of the disease. We are glad to know that the prevalence of myelofibrosis reported by Thiele and Kvasnicka ranges between 2.8% and 3.5%. We find that this is a reassuringly low prevalence for patients with ET.

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References


A JAK2-V617F activating mutation in addition to KIT and FLT3 mutations is associated with clinical outcome in patients with t(8;21)(q22;q22) acute myeloid leukemia

A JAK2-V617F mutation was found in 3 of 45 (6.7%) patients with t(8;21) acute myeloid leukemia (AML), whereas only one of 137 (0.7%) patients with de novo AML other than t(8;21) had the same mutation (p=0.047). We examined the clinical significance of KIT, FLT3 and JAK2 mutations as a collective group. There was a significant difference in the cumulative incidence of relapse: 77% in the 21 patients with the mutations and 26% in 19 lacking mutations respectively (p=0.0083). Our study highlights the importance of JAK2 mutations in addition to KIT and FLT3 mutations as a prognostic factor in t(8;21) AML patients.

RUNX1(AML1)-RUNX1T1(MTG8) generated by t(8;21)(q22;q22) contributes to leukemic transformation, but additional events are required for full leukemogenesis.12 Mutations in the receptor tyrosine kinases (RTK) including the KIT and FLT3 genes are the genetic events that appear to cause acute myeloid leukemia (AML) harboring t(8;21) and are associated with unfavorable prognosis.34 The activating missense mutation in the pseudo-kinase domain of the JAK2 cytoplasmic tyrosine kinase has been identified in a significant proportion of patients with myeloproliferative disorders.1 Although the same somatic mutation has been found in a small number of AML patients, a relatively high incidence of JAK2-V617F mutation is often seen in de novo and therapy-related t(8;21) AML patients.46 Nevertheless, whether JAK2-V617F mutation is associated with other biological parameters including clinical prognosis in patients with t(8;21) AML remains to be fully determined.

To examine its biological and prognostic impact, we studied the JAK2 mutation in 45 patients with de novo t(8;21) AML. Approval for this study was obtained from the Institutional Review Board of Kumamoto University School of Medicine. The results of KIT, FLT3, N-RAS, K-RAS and PDGFRα mutations in 37 of the 45 patients have been reported previously.4 Of the 45 patients, activating mutations in KIT and internal tandem duplications in FLT3 were observed in 18 (40%) and 3 (6.7%) respectively. Mutations of JAK2-V617F were identified by allele specific RT-PCR and direct sequencing.41 We detected the het-
erzogous JAK2-V617F mutation in 3 patients (6.7%) with t(8;21) AML, which was consistent with previous studies. None of the 3 t(8;21) AML patients had a history of previous myeloproliferative disorders. No mutations other than V617F were found in the exons 12-14 of JAK2. Among 137 patients with de novo AML other than t(8;21), there was only one patient who had JAK2 mutation (p=0.047). This patient had M2 with 46,XY,add(7)(q11),del(20)(q13). Thus, the present study confirmed that the JAK2 mutation is highly associated with t(8;21) AML.

Although the occurrence of KIT and FLT3 mutations was mutually exclusive in t(8;21) AML patients, one patient harboring a JAK2 mutation also had a KIT mutation and the other patient had a K-RAS mutation (Table 1). Although we cannot exclude the possibility that two different subclones in leukemic cells had each mutation, it is also likely that the same leukemic cells carry both mutations because heterozygous JAK2 and KIT or K-RAS mutations are identified as equivocal peaks in the electropherogram of direct sequencing (data not shown). It is of note that a high prevalence of co-operating mutations of KIT and/or FLT3 and JAK2 are identified as equivocal peaks in the electropherogram of direct sequencing (data not shown). In the current study, a total of 23 (51%) patients had mutations in KIT, FLT3 and JAK2, suggesting that activating mutations in the JAK2, KIT, and FLT3 mutations did not respond to multiple induction chemotherapies including high-dose cytarabine therapy (Table 1). Patient 2 with the JAK2 and K-RAS mutations achieved a complete remission (CR) but later relapsed. Patient 3 received allogeneic HSCT during the first CR and continued in CR. Twenty-one out of 23 (91%) patients with the mutations achieved CR, while 19 out of 21 (90%) patients lacking mutations obtained CR (p=0.9240). On the other hand, there was a significant difference in the cumulative incidence of relapse: 77% in the 21 patients with the mutations and 26% in 19 lacking mutations respectively (p=0.0083) (Figure 1A). It is likely that the poor outcome cannot be attributable only to JAK2 mutation in patients with a KIT or K-RAS mutation although JAK2 mutation together with other mutations may confer additive effects on the clinical outcome.

Table 1. Clinical profiles of t(8;21) acute myeloid leukemia patients harboring the JAK2 mutation.

<table>
<thead>
<tr>
<th>Patient N.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
<td>V617F</td>
<td>V617F</td>
<td>V617F</td>
</tr>
<tr>
<td>KIT</td>
<td>N822Y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K-RAS</td>
<td>-</td>
<td>G12D</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31</td>
<td>68</td>
<td>26</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>6.9</td>
<td>8.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Platelet (x10^9/L)</td>
<td>13</td>
<td>57</td>
<td>15</td>
</tr>
<tr>
<td>Leukocyte (x10^9/L)</td>
<td>43.9</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Circulating blast (%)</td>
<td>35</td>
<td>18</td>
<td>78</td>
</tr>
<tr>
<td>Marrow blast (%)</td>
<td>22.5</td>
<td>40</td>
<td>NT</td>
</tr>
<tr>
<td>WBC index</td>
<td>9.88</td>
<td>12</td>
<td>NT</td>
</tr>
<tr>
<td>LDH (U/L)^a</td>
<td>1,829</td>
<td>2,386</td>
<td>1,120</td>
</tr>
<tr>
<td>CD56 expression</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>Additional chromosome abnormality</td>
<td>add(7)(q11),del(20)(q13)</td>
<td>-X</td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>Failure</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>Relapse-free survival (years)</td>
<td>0</td>
<td>1.4</td>
<td>12.0^a</td>
</tr>
<tr>
<td>Overall survival (years)</td>
<td>1.3</td>
<td>1.8</td>
<td>12.0^a</td>
</tr>
</tbody>
</table>

^aLeukocyte count x marrow blast, ^aNormal range of lactate dehydrogenase is 112 - 213 U/L. NT: not tested, CR: complete remission.
Letters to the Editor

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Key words: JAK2, KIT, FLT3, t(8;21), acute myeloid leukemia

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References


Haematologica 2006;91:1569-70.


JAK1 mutation analysis in T-cell acute lymphoblastic leukemia cell lines

T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of T-cell precursors that mainly occurs in children and adolescents. A variety of oncogenic events that are involved in the pathogenesis of T-ALL have been identified, including NOTCH1 and PTEN mutations, overexpression of TAL1, LYL1, and TLX1, and deletion of CDKN2A (p16). Apart from mutations in FLT3 and NRAS, and chromosomal aberrations generating the NUP214-ABL1 fusion, mutations that drive proliferation and survival of T-ALL cells are still unknown in the majority of patients. Recently, activating point mutations in the JAK1 gene were identified in patients with ALL, and rarely also in acute myeloid leukemia (AML) patients. In T-ALL, JAK1 mutations were identified in approximately 20% of adult T-ALL cases, with a much lower frequency in childhood T-ALL. These mutations are very heterogeneous in the sense that they are dispersed over several JAK1 domains, and differ in their ability to transform hematopoietic cells and to activate downstream signaling pathways such as the STAT, PI3K and MAPK cascades.

Leukemia cell lines with mutations in FLT3, JAK2 and NOTCH1 have been described as useful models for preclinical testing of small molecule inhibitors. Given the recent identification of JAK1 mutations in T-ALL, we investigated if JAK1 mutations could be detected in a panel of 18 common T-ALL cell lines. By sequencing of the JAK1 open reading frame at cDNA level in these cell lines, we identified 2 transcript variants, one non-synonymous substitution, as well as several synonymous substitutions (Table 1).

A first transcript variant was identified in the HPB-ALL cell line (Figure 1A, 1B). This transcript lacks nucleotides 2896-2967, encoding amino acids 966-989 that are located between the P-loop and the activation loop in the kinase domain. When sequencing HPB-ALL genomic DNA, we could not detect the presence of a deletion, but we detected a single nucleotide change (2897 A>T) generating a novel GT splice donor site in