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Myelofibrotic transformation in essential thrombocythemia. 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 criteria[2] 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.[3] 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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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.[1,2] 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.[3,4] 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.[5] 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.[6-10] 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.[3] 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.[11] We detected the het-

erozygous *JAK2*-V617F mutation in 3 patients (6.7%) with t(8;21) AML, which was consistent with previous studies.[6-10] 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,[3] 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 *FLT3*, *KIT*, or *N-RAS* in AML patients with the *JAK2* mutation has been reported.[7-9] In the current study, a total of 23 (51%) patients had mutations in *KIT*, *FLT3* and *JAK2*, suggesting that activating mutations in the *RTK* and *JAK2* play a critical role as a secondary event leading to the development of t(8;21) AML.

We examined the clinical significance of *KIT*, *FLT3* and *JAK2* mutations as a collective group because the present study was limited to a small number of *JAK2*

mutated cases for the comparison of clinical features, and these mutations activate the same STAT signal transduction pathway and belong in the same class I mutation.[2] There was no significant relationship between the mutations and age, sex, leukocyte counts, platelet counts, CD56 expression, or additional chromosomal aberrations. However, t(8;21) AML patients with an activating mutation in *KIT*, *FLT3* and *JAK2* had significantly greater marrow blast percentages and serum lactate dehydrogenase levels than those without a mutation (*data not shown*). Considering that the *JAK2* mutation confers a proliferative and survival advantage on hematopoietic cells,[5] these clinical profiles appear to be associated with these mutations.

A total of 44 patients received intensive chemotherapy based on the Japan Adult Leukemia Study Group (JALSG) protocols in the AML87, AML89, AML92, AML95 and AML97 studies.[12] Although patients were treated with different schedules, all received regimens consisting of anthracyclines and cytarabine as induction therapy. Cytarabine plus one of the anthracyclines, high-dose cytarabine, or allogeneic hematopoietic stem cell transplantation (HSCT) was used as post-remission therapy. Patient 1 carrying both *KIT* and *JAK2* 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
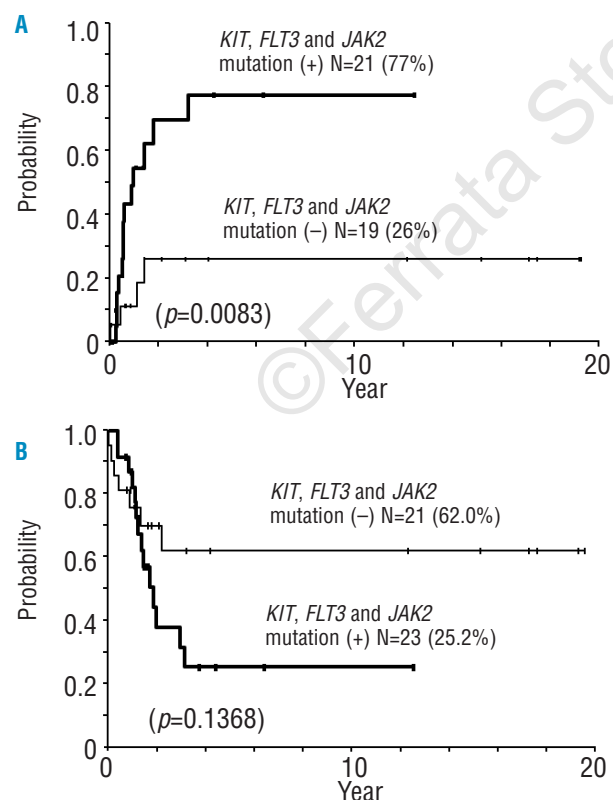
Figure 1. Cumulative incidence of relapse (A) and overall survival (B) in patients with t(8;21) acute myeloid leukemia by mutations in *KIT*, *FLT3* and *JAK2*. Estimation of survival distributions was performed using the Kaplan–Meier method and the differences were compared using the log-rank test.

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

Patient N.	1	2	3
JAK2	V617F	V617F	V617F
KIT	N822Y	-	-
K-RAS	-	G12D	-
Age (years)	31	68	26
Sex	Male	Female	Female
Hemoglobin (g/dL)	6.9	8.1	7.1
Platelet (x10⁹/L)	13	57	15
Leukocyte (x10⁹/L)	43.9	30	32
Circulating blast (%)	35	18	78
Marrow blast (%)	22.5	40	NT
WBC index[a]	9.88	12	NT
LDH (U/L)[b]	1,829	2,398	1,120
CD56 expression	+	+	NT
Additional chromosome abnormality	add(7)(q22)	-	-X
Induction	Failure	CR	CR
Relapse-free survival (years)	0	1.4	12.0+
Overall survival (years)	1.3	1.8	12.0+

[a]*Leukocyte count x marrow blast,* [b]*Normal range of lactate dehydrogenase is 112 - 213 U/L. NT: not tested, CR: complete remission.*

clinical outcome. Illmer *et al.*[8] also showed that 4 of 5 t(8;21) or inv(16) AML patients with a *JAK2* mutation had early relapses within 20 months after diagnosis. Taken together, these results suggest that mutations in the *JAK2*, *KIT* and *FLT3* genes are associated with unfavorable clinical outcome in patients with t(8;21) AML.

Our study also implies that patients with *RTK* and *JAK2* mutations may benefit from allogeneic HSCT. Three patients with mutations received allogeneic HSCT after relapse and have achieved continuous second CR. Three patients in each group also received allogeneic HSCT at the first CR. As a consequence, 6 out of 9 patients with AML harboring *KIT*, *FLT3* and *JAK2* mutations who continued CR received allogeneic HSCT. When patients who underwent HSCT were censored at the date of the HSCT, the 6-year overall survival in patients with mutations was 25% compared to 62% in those without mutations (p=0.1368) (Figure 1B). These findings are of significant clinical import as activating mutations in *KIT*, *FLT3* and *JAK2* could be potential therapeutic targets for specific tyrosine kinase inhibitors and JAK2 pathway inhibitors in patients with t(8;21) AML harboring the mutations.

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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).[1] 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.[2-4] In T-ALL, *JAK1* mutations were identified in approximately 20% of adult T-ALL cases, with a much lower frequency in childhood T-ALL.[2] 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.[2-4]

Leukemia cell lines with mutations in *FLT3*, *JAK2* and *NOTCH1* have been described as useful models for pre-clinical testing of small molecule inhibitors.[5-8] 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