

Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias

We investigated a large number of acute myeloid leukemia (AML) samples (n=959) for the presence of the JAK2 V617F mutation. We found a low incidence of the mutation in these AML samples (1%). JAK2 V617F mutations clustered in AML samples with an aberrant karyotype ($p < 0.05$). The incidence of JAK2 V617F in patients with a core binding factor (CBF) leukemia was 3.6% ($p < 0.01$). Moreover, JAK2 V617F mutations in CBF leukemias were associated with an aggressive clinical course with 80% of the patients relapsing.

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Elegant molecular analyses showed a single mutation at amino acid position 617 of the Janus-kinase 2 gene with subsequent activation of tyrosine kinase to be at the origin of myeloproliferative disorders.^{1,2} Additionally, there is now evidence in the literature that patients with acute myeloid leukemia (AML) with an antecedent myeloproliferative disease often have JAK2 V617F mutations.³

Only recently, a few patients with AML without previous hematologic disorders were found to have the JAK2 V617F mutation.⁴ It can be assumed that mutations of JAK2 V617F lead to a more aggressive subtype of leukemia because of the activation of the JAK2-STAT5 cascade which substantially alters apoptotic response, self-renewal and proliferative capacity of myeloid cells.^{5,6} We investigated a large homogeneously treated AML population for the presence of the JAK2 V617F mutation.

Patients were included between February 1996 and February 2000 and treated within the German multi-center trial of the SHG AML96 study group. The treatment schedule of the SHG AML96 trial has been published elsewhere.⁷

JAK2 mutations were investigated by amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) method using recently published primers.⁸ The generic outer primers were labelled with 6-FAM and HEX and PCR products were analyzed on an automated sequencer, essentially as recently described.⁹ The sensitivity of the assay was determined with a threshold of 1 JAK2 V617F mutated cell in 100 investigated cells. Ten cases of JAK2-V617F mutations were found in 959 AML patients for an overall incidence of 1%. The JAK2 V617F mutations

were almost exclusively found in patients with *de novo* AML (9 out of 785). One other patient with therapy-related AML was affected (1 out of 34). No AML patient with prior myelodysplastic syndrome was found to carry the JAK2 V617F mutation (0 out of 131).

Mutations of JAK2 V617F occurred preferentially in AML patients with karyotypic aberrations (8 out of 476 AML patients with aberrations vs. 1 out of 424 patients without aberrations – $p < 0.05$, karyotype was unknown in 50 patients).

Remarkably, a high proportion of JAK2 V617F positive AML was seen in patients with core binding factor (CBF) leukemias. Whereas 5 out of 138 patients (3.5%) with either t(8;21) (n=2) or inv16 (n=3) proved to be positive for the JAK2 mutation only 5 out of 811 (0.6%) of the remaining AML patients were diagnosed with the mutation ($p < 0.01$).

In contrast to the striking association with aberrant karyotypes no correlation was seen with either age or leukocyte count. We also investigated whether the observed JAK2 mutations coincided with other important molecular alterations in AML. We observed a FLT-3-ITD in one of the patients with the JAK2 mutation whereas none of these patients showed mutations of c-KIT exon 17.

Next, we examined the impact of JAK2 V617F on the prognosis of the patients. To exclude potentially confounding factors and prove an independent impact of JAK2 mutations on survival, we conducted a multivariate analysis (Table 1). The analysis demonstrated a strong independent impact of JAK2 V617F on disease-free survival. This was mainly due to early relapses with nine out of the ten patients with JAK2 V617F relapsing within 20 months after diagnosis. In patients with CBF leukemias overall survival rates were not affected by the presence of the JAK2 V617F mutations. However, we found a remarkable high relapse rate of JAK2V617F patients with either inv(16) or t(8;21) and detected recurrent disease in less than 24 months in four out of five such patients (*data not shown*) which translated into a lower disease-free survival rate (Figure 1) ($p < 0.05$).

In conclusion, we report a low incidence of JAK2 V617F mutation in patients with *de novo* AML in whom the mutation might represent a second hit alteration in leukemogenesis in a particular subset of patients, as indicated by the significant association with cytogenetic aberrations especially with t(8;21) and inv(16). Moreover, the coincidence of the JAK2-mutation with a FLT-3-ITD in one of the patients with a normal karyotype argues for the need for an additional molecular event in the process of transformation into AML. Although the number of patients with JAK2

Table 1. Multivariate analysis for overall survival (OS) and disease-free survival (DFS) using a Cox proportional hazard model.

	OS		DFS		OS		DFS	
	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value	HR (CI)	p-value
Age	1.03 (1.024-1.036)	<0.001	1.03 (1.02-1.035)	<0.001	1.03 (1.02-1.04)	<0.001	1.02 (1.003-1.03)	<0.05
Cytogen. GR*	n.a.	n.a.	n.a.	n.a.	0.29 (0.15-0.57)	<0.001	0.2(0.08-0.5)	<0.01
Cytogen. HR [§]	n.a.	n.a.	n.a.	n.a.	1.7 (1.3-2.2)	<0.001	1.75 (1.2-2.5)	<0.01
NPM1	0.7 (0.58-0.85)	<0.001	0.78 (0.62-0.99)	0.05	0.74 (0.56-0.98)	<0.05	–	n.s.
FLT-3-ITD (HR) [§]	1.6 (1.2-2.1)	<0.01	–	n.s.	1.8 (1.2-2.7)	<0.005	–	n.s.
JAK-2/V617F	–	n.s.	2.24 (0.98-5.98)	0.05	–	n.s.	3.0 (1.3-6.8)	<0.01

*; [§]patients >60 years of age were treated without risk stratification – therefore cytogenetic risk stratification is not available; [§]FLT-3-ITD risk groups were determined building the ratio of mutated vs. non-mutated alleles as previously described. [§]NPM1: presence of nucleophosmin mutation.

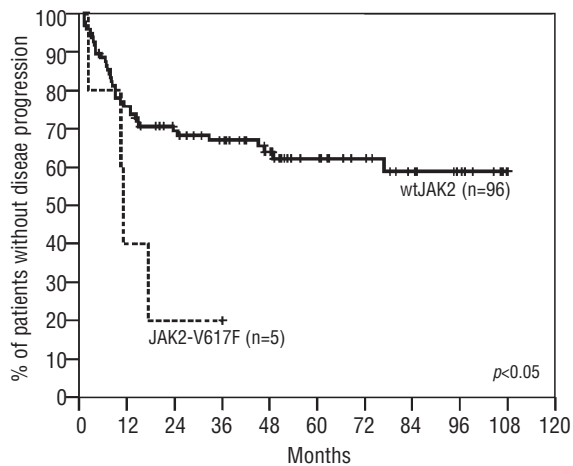


Figure 1. Disease-free survival in patients with CBF-leukemias with wild type (wt) JAK2 vs. JAK2-V617F.

mutations is small, the abnormality might represent an important additional factor in the assessment of prognosis.

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