Chronic Myeloproliferative Disorders

A longitudinal study of the *JAK2*^{V617F} mutation in myelofibrosis with myeloid metaplasia: analysis at two time points

Serial analysis for the activating *JAK2*^{V617F} mutation performed in 44 patients with myelofibrosis with myeloid metaplasia showed no interval change in 88% (22/25) of patients over a median interval of 18.6 months. The increase in *JAK2* expression observed in three patients did not correspond to disease progression or leukemic transformation.

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An activating *JAK2*^{V617F} mutation occurs in the majority of patients with polycythemia vera (PV) and approximately half of those with either essential thrombocythemia (ET) or myelofibrosis with myeloid metaplasia (MMM).[1] In MMM, both the overall and homozygous mutational frequencies are significantly higher in post-polycythemic (PPMM; 91% and 18%) than in either agnogenic (AMM; 45.3% and 2.6%) or post-thrombocythemic (PTMM; 38.9% and 11.1%) myeloid metaplasia.[2] The particular information is consistent with both *in vitro* and *in vivo* data that have demonstrated a mutant allele dose-dependent association between *JAK2*^{V617F} and a PV-weighted myeloproliferative phenotype.[3,4]

In order to determine both time-dependent changes in mutational status as well as the role of *JAK2*^{V617F} in leukemic transformation in MMM, mutation analysis was performed in two archived bone marrow samples, separated in time by a median of 18.6 months (range, 2-105), from each of 44 patients with MMM. Mutation analysis for *JAK2*^{V617F} was performed in DNA derived from cytogenetic pellets in archived specimens obtained at the time of bone marrow biopsy. Genomic DNA was extracted using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Penzberg, Germany). Genomic DNA was amplified by polymerase chain reaction (PCR), and successful amplification was confirmed by electrophoresis on an ethidium bromide-impregnated 1% agarose gel. Samples from patients who were wild-type for the mutations were then subjected to allele-specific PCR analysis. In this assay, a mutation-specific forward primer is used in a PCR reaction with a wild-type sequence reverse primer containing a fluorescent tag. Only mutated DNA will be amplified, if present, and the PCR reaction product is analyzed using capillary electrophoresis with fluorescence detection. If the *JAK2*^{V617F} mutation is present, a PCR fragment of 136 basepairs is identified. Fragment analysis was performed in an ABI3100 analyzer with data collection software 1.1, GenoMapper 3.0 following the manufacturer's instructions. The results of the *JAK2*^{V617F} analysis were then correlated with clinical parameters, disease progression and outcomes.

Among 25 AMM patients, mutation status did not change over time in 22 patients (88%), including all three patients whose disease transformed into acute myeloid leukemia (Table 1). These latter three patients were either heterozygous (n=2) or wild-type (n=1) for *JAK2*^{V617F}. On the other hand, three clinically stable patients with AMM progressed from either wild-type to heterozygous or heterozygous to homozygous mutational status after 43-83 months (Table 1). Among 19 patients with secondary myelofibrosis, the paired samples for mutational analysis were collected before and after transformation into MMM in three patients, with no change in mutational status in the interim (Table 1). Similarly, *JAK2*^{V617F} mutational status did not change in any of the remaining 16 patients with secondary myelofibrosis (eight patients with PPMM and eight with PTMM) despite leukemic transformation in six of these patients (Table 1). Among the latter, five were heterozygous for the mutant allele

Table 1. ***JAK2V*^{617F} mutational status and disease stage in 44 patients with myelofibrosis with myeloid metaplasia at two time points in their clinical course. The three patients whose mutational status changed are italicized. Patients whose disease stage progressed over the interval investigated are shown in bold.**

Disease status Time 1	*JAK2^{V617F} status Time 1*	*JAK2^{V617F} status Time 2*	*Disease status Time 2*	*Time interval (Months)*
PTMM1	**Wild-type**	**Wild-type**	**Acute leukemia**	**2.0**
AMM1	HETERO	HETERO	AMM	2.4
AMM2	HETERO	HETERO	AMM	2.9
PPMM1	HETERO	HETERO	PPMM	5.0
AMM3	Wild-type	Wild-type	AMM	5.1
AMM4	HETERO	HETERO	AMM	6.2
PPMM2	**HETERO**	**HETERO**	**Acute leukemia**	**6.2**
AMM5	Wild-type	Wild-type	AMM	7.3
PTMM2	Wild-type	Wild-type	PTMM	7.5
PTMM3	Wild-type	Wild-type	PTMM	7.8
PV1	**HOMO**	**HOMO**	**PPMM**	**8.3**
AMM6	**HETERO**	**HETERO**	**Acute leukemia**	**9.1**
PPMM3	HOMO	HOMO	PPMM	11.5
AMM7	HETERO	HETERO	AMM	12.0
AMM8	HETERO	HETERO	AMM	14.3
AMM9	Wild-type	Wild-type	AMM	14.8
AMM10	Wild-type	Wild-type	AMM	16.0
AMM11	HETERO	HETERO	AMM	16.2
PTMM4	HETERO	HETERO	PTMM	17.6
AMM12	HETERO	HETERO	AMM	17.8
AMM13	Wild-type	Wild-type	AMM	18.0
PPMM4	**HETERO**	**HETERO**	**Acute leukemia**	**18.5**
AMM14	Wild-type	Wild-type	AMM	18.6
AMM15	HETERO	HETERO	AMM	20.0
PTMM5	HETERO	HETERO	PTMM	22.0
PTMM6	**HETERO**	**HETERO**	**Acute leukemia**	**22.9**
PTMM7	Wild-type	Wild-type	PTMM	26.7
AMM16	**Wild-type**	**Wild-type**	**Acute leukemia**	**33.5**
AMM17	Wild-type	Wild-type	AMM	34.5
AMM18	Wild-type	Wild-type	AMM	39.8
PPMM5	HETERO	HETERO	PPMM	39.9
AMM19	*HETERO*	*HOMO*	*AMM*	*42.3*
ET1	**HETERO**	**HETERO**	**PTMM**	**42.8**
AMM20	Wild-type	Wild-type	AMM	44.2
PV2	**HOMO**	**HOMO**	**PPMM**	**53.2**
PTMM8	Wild-type	Wild-type	PTMM	56.9
AMM21	Wild-type	Wild-type	AMM	59.7
PPMM6	HETERO	HETERO	PPMM	60.0
AMM22	Wild-type	Wild-type	AMM	63.6
AMM23	**HETERO**	**HETERO**	**Acute leukemia**	**69.0**
AMM24	*Wild-type*	*HETERO*	*AMM*	*77.8*
AMM25	*HETERO*	*HOMO*	*AMM*	*82.3*
PPMM7	**HETERO**	**HETERO**	**Acute leukemia**	**98.8**
PPMM8	**HETERO**	**HETERO**	**Acute leukemia**	**105.5**

AMM: agnogenic myeloid metaplasia; ET: essential thrombocythemia; PV: polycythemia vera; PPMM: post-polycythemic myeloid metaplasia; PTMM: post-thrombocythemic myeloid metaplasia.

and one had the wild-type allele. Taken together, the above findings indicate relative stability of *JAK2*^{V617F} mutational status in patients with MMM and suggest that the mutation is not essential for disease progression. The role of the *JAK2*^{V617F} in leukemic transformation from MMM is difficult to discern given the limited number of patients analyzed; however, we observed no evidence that changes in mutation status were required for transformation.

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