

Limited effects on *JAK2* mutational status after pegylated interferon α -2b therapy in polycythemia vera and essential thrombocythemia

Twenty-five patients with myeloproliferative diseases were treated with pegylated interferon α -2b. Prior to therapy, 15/25 patients had a *JAK2*^{V617F} mutation. Eight *JAK2*-positive patients were on therapy in hematological complete remission at 24 months. Five of eight patients demonstrated a 1.2-3.6 fold reduction in the percentage of *JAK2*^{V617F} cells.

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We have published clinical results of a 24-month phase II study of pegylated interferon α -2b (PegIntron®, Schering-Plough, PEG-IFN) therapy in polycythemia vera (PV) and essential thrombocythemia (ET). Briefly, patients were treated with PEG-IFN 0.5–1.0 μ g/kg/week. Twenty-nine of 42 patients (69%) achieved a complete platelet response, i.e. a platelet count $< 400 \times 10^9/L$ (symptomatic patients) or $< 600 \times 10^9/L$ (asymptomatic patients). Nineteen patients (45%) completed the 2-year treatment in complete remission.¹ Similar results have been reported by others.^{2,3} We detected normalization of initially elevated polycythemia rubra vera-1 (*PRV-1*) expression in a subset of patients.¹ Case reports have suggested that interferon can reverse chromosome abnormalities,⁴ restore polyclonal hematopoiesis and suppress erythropoietin-independent erythroid colony growth.⁵ We therefore investigated the potential of PEG-IFN to suppress the malignant clone using the *JAK2*^{V617F} mutation as a disease marker.⁶

For this investigation, frozen samples were available from 25 patients, 14 with PV and 11 with ET. Sixteen were male and nine were female; their median age was 52 years (range 29-77), and the median duration of disease was 0.5 years (range 0.01-23.2). Prior cytoreductive treatment included anagrelide (n=4), hydroxyurea (n=2), busulfan (n=1) and radioactive phosphorus (n=1). Expression of *PRV-1* mRNA was quantified in neutrophils as previously described.⁷ The allele ratio of mutant *JAK2*^{V617F} to total *JAK2* was determined by a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay of purified granulocyte RNA. Total *JAK2* mRNA was determined with a forward primer 5' CAGCAAG-TATGATGAGCAAGCTTT-3', a reverse primer 5'-TGAACCAGAATATTCTCGTCTCCAC-3' and the MGB-Probe 5'-FAM-TCACAAGCATTTGGTTTT-MGB-3'. *JAK2*^{V617F} was quantified using the same forward primer and probe but a reverse primer comprising the mutation and an additional mismatch at position 4 5'-CCAGAATATTCTCGTCTCCACTGAA-3'. The allele copy numbers were determined from a plasmid standard curve and the allele ratio was calculated. The level of *JAK2*-positivity is expressed as the percentage of mutant *JAK2* compared to total *JAK2*. A percentage of $< 1\%$ *JAK2*-positive cells was defined as *JAK2*-negative. This cut off was determined in a panel of 50 healthy controls (Goertler and Pahl, unpublished observation).

Table 1 shows *JAK2*^{V617F} and *PRV-1* status prior to therapy. A good correlation between presence of the *JAK2*^{V617F} mutation and *PRV-1* overexpression was found in PV, as previously described.⁸ In the 15 *JAK2*-positive patients,

Table 1. *PRV-1* and *JAK2* status prior to therapy according to diagnosis.

Diagnosis	<i>JAK2</i> ^{V617F} / <i>PRV-1</i> ⁺	<i>JAK2</i> ^{V617F} / <i>PRV-1</i> ⁻	<i>JAK2</i> ⁻ / <i>PRV-1</i> ⁺	<i>JAK2</i> ⁻ / <i>PRV-1</i> ⁻
PV	10	1	2	1
ET	3	1	2	5

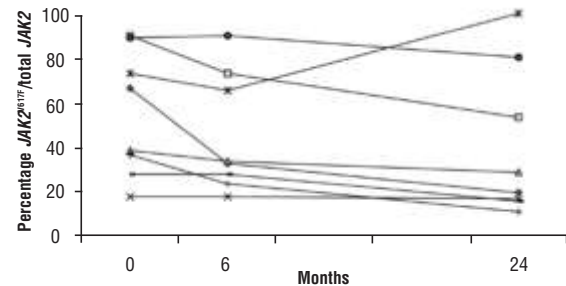


Figure 1. *JAK2* mutational status in eight patients before therapy, and after 6 and 24 months of PEG-IFN therapy. No differences with regards to clinical features, white blood cell or platelet counts prior to or after therapy were noted between patients in whom *JAK2* was downregulated and those in whom it was not (data not shown).

the percentage of mutant *JAK2*^{V617F} ranged from 18-90% (mean 44%). The mean value in PV (49%) was somewhat higher than that in ET (32%).

Thirteen of 15 *JAK2*-positive and 8/10 *JAK2*-negative patients achieved complete remission with PEG-IFN. Although the numbers are small, it does not seem that *JAK2* status is related to response to PEG-IFN. Follow-up samples for molecular studies were only available in patients still on therapy, therefore clinical response data in this highly selected subset of patients are not presented in detail. After 6 months 12 *JAK2*-positive patients were on therapy in complete remission. Four of these patients had a reduction of *JAK2*-positivity by at least 10% in five patients *JAK2*-positivity remained unchanged, and three had an increase. The further evolution of *JAK2* mutational status during therapy is shown in Figure 1. After 24 months, eight *JAK2*-positive patients (6 PV, 2 ET) were on therapy in hematologic remission: platelet count $< 400 \times 10^9/L$ and in PV also a stable hematocrit < 45 without phlebotomies. Five (3 PV, 2 ET) of eight patients had a 1.2-3.6-fold reduction of the percentage of *JAK2*^{V617F}, the percentages were unchanged in two and one had an increase.

Jones *et al.* reported that the median percentage of mutated *JAK2* alleles was not different between PV patients treated with phlebotomy alone, hydroxyurea, anagrelide or imatinib.⁹ They found a significantly lower level of *JAK2*^{V617F} in seven IFN-treated patients than in the other patient groups, and hypothesized that IFN therapy had reduced *JAK2*^{V617F} levels. Our study clearly demonstrates that, in selected patients, PEG-IFN can reduce, to a limited extent, the level of *JAK2*-positivity. In this context, it should be stressed that the goal of our clinical trial was not to suppress *JAK2*^{V617F} levels, but rather to maintain complete remission with the lowest PEG-IFN dose possible. This may have led to smaller effects on *JAK2*

due to a low mean dose, 0.3 µg/kg/week, at 24 months. In conclusion, PEG-IFN therapy can lower the percentage of circulating *JAK2*^{V617F} mutant cells, but the effect is modest. Even in sustained hematologic remission under PEG-IFN treatment, the malignant myeloproliferative clone remained present. However, Kiladjian *et al.* very recently reported a molecular response to pegylated interferon α-2a in 24/27 PV patients with levels of the mutant cells decreasing from 49 to 27% (mean); furthermore in one patient mutant *JAK2* was no longer detectable at 1 year.¹⁰

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