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

Twenty-five patients with myeloproliferative diseases were treated with pegylated interferon α-2b (PegIntron®, Schering-Plough, PEG-IFN) therapy in polycythemia vera (PV) and essential thrombocytemia (ET). Briefly, patients were treated with PEG-IFN 0.5–1.0 μg/kg/week. Twenty-nine of 42 patients (69%) achieved a complete platelet response, i.e. a platelet count <400×10^9/L (symptomatic patients) or <600×10^9/L (asymptomatic patients). Nineteen patients (45%) completed the 2-year treatment in complete remission. Similar results have been reported by others. We detected normalization of initially elevated polycythemia rubra vera-1 (PRV-1) expression in a subset of patients. Case reports have suggested that interferon can reverse chromosome abnormalities, restore polyclonal hematopoiesis and suppress erythropoietin-independent erythroid colony growth. We therefore investigated the potential of PEG-IFN to suppress the malignant clone using the JAK2V617F mutation as a disease marker.

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. The allele ratio of mutant JAK2V617F 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’-CAGCAAGTATGATGACCAAAGCTTT-3’, a reverse primer 5’-TGAACCGAATATTGCCGTCCTCCAC-3’ and the MGB-Probe 5’-FAM-TCACAAACATTTTGGTTTT-MGB-3’. JAK2V617F was quantified using the same forward primer and probe but a reverse primer comprising the mutation and an additional mismatch at position 4 5’-CCAGAATTTCTGGCTCCACTGAA-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 This cut off was determined in a panel of 50 healthy controls (Goerttler and Pahl, unpublished observation).

Table 1 shows JAK2V617F and PRV-1 status prior to therapy. A good correlation between the presence of the JAK2V617F mutation and PRV-1 overexpression was found in PV, as previously described. In the 15 JAK2-positive patients, the percentage of mutant JAK2V617F 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 <400×10^9/L and in PV also a stable hemocrit <45 without phlebotomies. Five (3 PV, 2 ET) of eight patients had a 1.2-3.6 fold reduction of the percentage of JAK2V617F, 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. They found a significantly lower level of JAK2V617F in seven IFN-treated patients than in the other patient groups, and hypothesized that IFN therapy had reduced JAK2V617F 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 JAK2V617F 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 JAK2V617F 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. 


