Myeloproliferative Disorders

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.

Haematologica 2006; 91:1281-1282
(*http://www.haematologica.org/journal/2006/09/1281.html*)

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 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.[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' CAGCAAGTATGATGAGCAAGCTTT-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 (*Goerttler 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, 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%).

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

Diagnosis	*JAK2*$^+$/ *PRV-1*$^+$	*JAK2*$^+$/ *PRV-1*$^-$	*JAK2*$^-$/ *PRV-1*$^+$	*JAK2*$^-$/ *PRV-1*$^-$
PV	10	1	2	1
ET	3	1	2	5

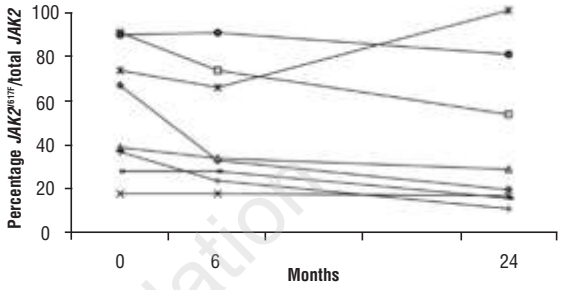

Figure 1. *JAK2* mutational status in eight patients before therapy, and after 6 and 24 months of PEG-IFN therapy. No differences with regards to clinical features, white blood cell or platelet counts prior to or after therapy were noted between patients in whom *JAK2* was downregulated and those in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

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 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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Acknowledgments: this study was initiated and performed by the Nordic study group for myeloproliferative disorders supported by an unrestricted research grant from Schering-Plough Nordic Biotech. We are indebted to Snezana Temerinac, Department of Experimental Anesthesiology University Hospital Freiburg, for technical assistance with PRV-1 analyses. We thank Olle Linder, Department of Medicine University Hospital Örebro, and Claes Malm, Department of Hematology University Hospital Linkoping for allowing us to study patients under their care. Data management was performed by Hans Garmo and Gunilla Juhlin at the regional Oncologic Center in Uppsala, Sweden. The study was supported by a grant from the Else Kröner-Fresenius Stiftung to HLP.

Key words: polycythemia vera, essential thrombocythemia, α-interferon, JAK2.

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References

1. Samuelsson J, Hasselbalch H, Bruserud O, Temerinac S, Brandberg Y, Merup M, et al. A phase II trial of pegylated interferon α-2b therapy in polycythemia vera and essential thrombocythemia. Feasibility, clinical and biological effects and impact on quality of life. Cancer 2006;106:2397-405.
2. Gugliotta L, Bulgarelli S, Tieghi A, Asioli S, Gardini G, Sabattini E, et al. PVSG and WHO bone marrow evaluation in 90 essential thrombocythemia patients treated with PEG interferon α-2-b. Preliminary results. Haematologica 2005;90Suppl 2:264a[abstract].
3. Langer C, Lengfelder E, Thiele J, Kvasnicka HM, Pahl HL, Beneke H, et al. Pegylated interferon for the treatment of high risk essential thrombocythemia: results of a phase II study. Haematologica 2005;90:1333-8.
4. Messora C, Bensi L, Vechi A, Giacobbi F, Temperani P, Berini M, et al. Cytogenetic conversion in a case of polycythaemia vera treated with interferon-α. Br J Haematol 1994;86:402-4.
5. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemias and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-e response to erythropoietin. Blood 2003;101:3294-301.
6. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. Nature 2005; 434:1144-8.
7. Klippel S, Strunck E, Temerinac S, Bench AJ, Meinhardt G, Mohr U, et al. Quantification of PRV-1 mRNA distinguishes polycythemia vera from secondary erythrocytosis. Blood 2003;102:3569-74.
8. Goerttler PS, Steimle C. Marz F, Johansson PL, Andreasson B, Griesshammer M, et al. The JAK2V617F mutation, PRV-1 overexpression, and EEC formation define a similar cohort of MPD patients. Blood 2005;106:2862-4.
9. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon α. Blood 2006;107:3339-41.
10. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, et al. High molecular response rate of polycythemia vera patients treated with interferon α-2a. Blood 2006;[E-pub ahead of print].