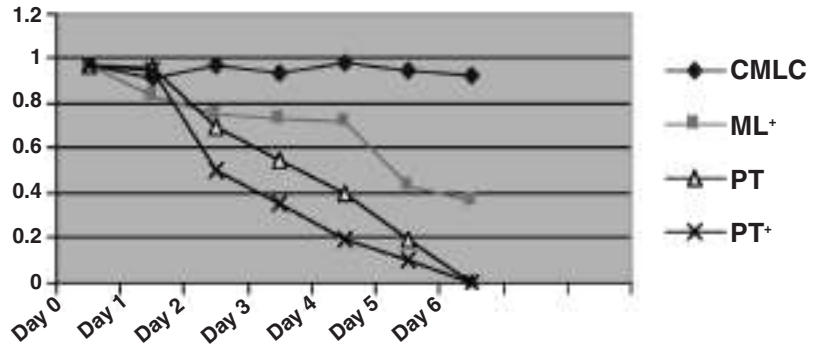

Figure 2. Viability of mononuclear cells from the patient (PT) and from 5 CML other patients exposed or not to 1 µM of Imatinib (STI).

kinase.[8] One patient with a t (5;10) was treated at diagnosis with imatinib and achieved clinical and cytogenetic responses after 3 weeks.[7] In contrast our patient did not respond to imatinib. It must be noted that in our case treatment with imatinib was begun 29 months after diagnosis and 5 years after the first signs of the disease. Moreover several clinical and biological signs including anemia, thrombocytopenia, marked reticulin fibrosis, prominent splenomegaly at diagnosis, and additional chromosome abnormalities at the time of treatment with imatinib suggested that the patient had an accelerated phase of disease. Imatinib was given at the dose of 400 mg/day, which is lower than the dose recommended for accelerated phase CML. Some CML patients have imatinib-resistant disease as a consequence of acquired mutations in the ATP binding pocket of *BCR-ABL*.[9] Direct sequencing of the *H4-PDGFRB* kinase domain (corresponding to amino acids 478-886 of *PDGFRB*) was performed. The sequence of the kinase domain showed no coding changes. We hypothesize that other mechanisms of resistance were responsible for the lack of response observed in our patient. Drugs with activity against the imatinib-resistant variants are currently being developed and will be therapeutically important.[10]

Jean-Noël Bastie, Isabel Garcia,° Christine Terré,° Nicholas C. P. Cross,# Francois-Xavier Mahon,@ Sylvie Castaigne**

**Department of Hematology and Oncology, °Department of Biochemistry, Haematology and Cytogenetics, #Wessex Regional Genetics Laboratory, Salisbury District Hospital, UK; @Unité CNRS 2617, Université Bordeaux 2, France*

Acknowledgments: the authors are grateful to Pierre Fenaux for fruitful advice.

Key words: atypical chronic myeloproliferative disorder, PDGFRB, t(5;10), imatinib mesylate.

Correspondence: Sylvie Castaigne, Department of Hematology and Oncology, Centre Hospitalier de Versailles, Le Chesnay, 78157, France. E-mail: scastaigne@ch-versailles.fr

References

1. Cross NCP, Melo JV, Feng L, Goldman JM. An optimized multiplex polymerase chain reaction (PCR) for detection of BCR-ABL fusion mRNAs in hematological disorders. Leukemia 1994; 8:186-9.
2. Apperley JF, Gardembas M, Melo JV,Russel-Jones R, Bain BJ, Baxter EJ, e al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor β. N Engl J Med 2002; 347:481-7.
3. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor β to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 1994;77:307-16.
4. Sterr EJ, Cross NCP. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor β. Acta Haematol 2002;107:113-22.
5. Siena S, Sammarelli G, Grimoldi MG, Schiavo R, Nozza A, Roncalli M, et al. New reciprocal translocation t(5;10)(q33;q22) associated with atypical chronic myeloid leukemia. Haematologica 1999;84:369-72.
6. Kulkarni S, Heath C, Parker S, Chase A, Iqbal S, Pocock CF, et al. Fusion of H4/D10S170 to the platelet-derived growth factor receptor β in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). Cancer Res 2000;60:3592-8.
7. Garcia JL, Font de Mora J, Hernandez Jesus M, Queizan JA, Gutierrez NC, Hernandez Jose M, et al. Imatinib mesylate elicits positive clinical response in atypical chronic myeloid leukemia involving the platelet-derived growth factor receptor β. Blood 2003;102:2699-700.
8. Schwaller J, Anastasiadou E, Cain D, Kutok J, Wojiski S, Williams IR, et al. H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived growth factor receptor β gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). Blood 2001;97:3910-8.
9. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003;102:276-83.
10. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 2002;2:117-25.

Chronic Myeloproliferative Disorders

The effects of hydroxyurea on *PRV-1* expression in patients with essential thrombocythemia and polycythemia vera

The study was designed to investigate the influence of hydroxyurea (HU) treatment on *PRV-1* expression. Eighteen newly diagnosed patients with essential thrombocythemia (ET) or polycythemia vera (PV) were included. HU significantly increased *PRV-1* gene expression in the early stage of treatment.

haematologica 2004; 89:1264-1266
(*http://www.haematologica.org/2004/10/1264*)

Hopes of finding a pathognomonic molecular marker for the diagnosis of polycythemia vera (PV) were raised when the polycythemia rubra vera-1 (*PRV-1*) gene was

Table 1. Clinical characteristics of the 18 patients with PV or ET.

	PV	*ET*
Number of patients	9	9
Male/Female	2/7	3/6
Age, years*	74 (66-84)	68 (51-80)
Platelet count ($\times 10^9$/L) at start of hydroxyurea*	720 (207-910)	934 (412-1170)
Platelet count ($\times 10^9$/L) after one month*	423 (209-745)	588 (264-941)
Platelet count ($\times 10^9$/L) after four months*	341 (91-742)	357 (265-502)

**mean (range).*

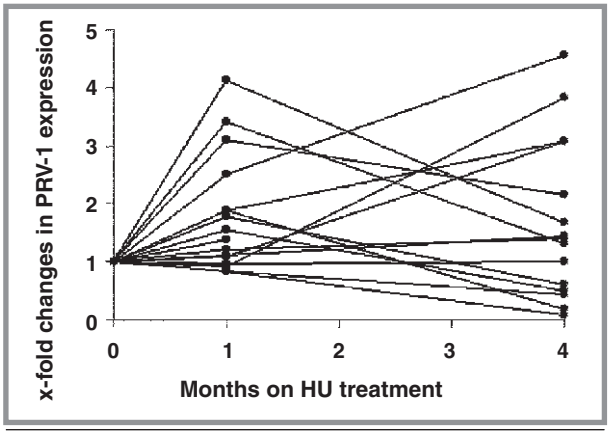

Figure 1. Changes in PRV-1 expression in 16 patients treated with hydroxyurea (HU). One patient interrupted his HU therapy after one month due to adverse events. Two patients are not out-lined because of 204 and 32-fold changes in *PRV-1* expression at one month, and 19 and 0.83-fold changes at four months.

described.[1] It was found that mRNA from the *PRV-1* gene was overexpressed in granulocytes from all patients with polycythemia vera (PV) and occasionally in patients with essential thrombocythemia (ET) and idiopathic myelofibrosis. Several groups, including ours, have recently published similar results.[2-3] However, in most studies treated and untreated patients were mixed and there were great variations in disease duration, which might have influenced the *PRV-1* gene expression. The effect of hydroxyurea (HU) on *PRV-1* expression in individual patients during treatment is not yet known.

HU is widely used as a first line myelosuppressive therapy in ET and in PV to control thrombocytosis.[4-5] The aim of our study was to investigate the effect of myelosuppressive treatment, i.e., HU, on *PRV-1* mRNA expression in granulocytes, in newly diagnosed patients with PV or ET.

Nine patients with ET and nine patients with PV, with newly diagnosed disease, were studied (Table 1). The reasons for initiating myelosuppressive treatment were: history of prior thrombosis, platelet count over 600×10^9/L in patients aged over 60 years or platelet count above 800×10^9/L in patients with PV and over 1000×10^9/L in ET patients. The goal was a platelet count below 400×10^9/L.

Ten milliliters of EDTA-anticoagulated venous blood were collected from the patients before treatment as well as one month and four months after the start of HU. A real-time reverse transcriptase polymerase chain reaction (RT PCR), recently reported by us,[2] was used to quantify *PRV-1* expression in buffy coat granulocytes. The results were expressed using the Ct method.[6]

The results for the individual fold change of *PRV-1* expression are presented in Figure 1. In 13 of the 18 patients a significant increase (p=0.0312) of *PRV-1* mRNA expression was detected after one month of HU treatment. In one patient the GAPDH value indicated poor RNA quality at one month of treatment and this sample is not included in the comparison. After 4 months of treatment 11 out of 18 patients had increased *PRV-1* expression, although this was not statistically significantly (p=0.557) different from the expression level before treatment initiation. One patient interrupted HU therapy after one month due to adverse events. There were no significant changes with regard to lymphocyte counts or GAPDH levels during treatment. Fourteen of the eighteen patients achieved a platelet count below 400×10^9/L, which was the target level. Reversion of clonal to polyclonal haematopoiesis was reported by Liu *et al.*[3] in two patients with PV treated with interferon (IFN). Fruehauf *et al.*[7] recently reported a significant decrease in *PRV-1* expression in the peripheral granulocytes of four patients when IFN therapy was initiated. *In vitro* colony assays showed similar patterns, with lower granulocyte and erythroid growth[8-9] when HU and IFN therapy were started. HU has been shown to exert a bi-modal dose-dependent effect on erythropoiesis.[10]

Our hypothesis was that *PRV-1* gene expression would decrease after initiation of HU therapy. Interestingly, however, *PRV-1* gene expression was significantly increased in 13 of 18 patients (p=0.0312) one month after HU treatment had started. Four months after HU therapy initiation there were no significant changes. One possible explanation for the differences in *PRV-1* expression between HU and IFN-treated patients is that the two treatment modalities affect myelopoiesis at different levels. It could be that the effect of HU on *PRV-1* gene expression develops more slowly than that of IFN treatment. If this is the case a longer follow-up will be needed to detect changes. Nine of our patients have been treated with HU for 12 months and six of them still have increased *PRV-1* gene expression, although this is not statistically significant (p=0.515). We conclude that HU increases *PRV-1* gene expression, at least in the early phase of treatment and therefore myelosuppressive therapies must be taken into consideration when presenting studies on *PRV-1* gene expression.

Peter Johansson,# Anne Ricksten,° Carina Wasslavik,° Berit Johansson,# Björn Andréasson*#*

**Haematology and Coagulation Section, Department of Medicine; °Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, University of Göteborg, Göteborg; #Department of Medicine, Uddevalla, Sweden*

Key words: hydroxyurea, PRV-1, polycythemia rubra vera.

Correspondence: Dr. Peter Johansson, Section of haematology and coagulation, Sahlgrenska University Hospital, 413 45 Göteborg, Sweden. Phone: international +46.52293212. Fax: international +46.52293232. E-mail: peter.l.johansson@vgregion.se

References

1. Temerinac S, Klippel S, Strunck E, Roder S, Lubbert M, Lange W, et al. Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. Blood 2000; 95:2569-76.
2. Palmqvist L, Goerttler P, Wasslavik C, Johansson P, Andréasson B, Safai-Kutti S, et al. Comparison of methods for polycythemia rubra vera-1 mRNA quantification in whole-blood leukocytes and purified granulocytes. Clin Chem 2004;50:644-7.
3. 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.
4. Cortelazzo S, Finazzi G, Ruggeri M, Vestri O, Galli M, Rodeghiero F, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. N Engl J Med 1995;332:1132-6.
5. Finazzi G, Barbui T. Efficacy and safety of hydroxyurea in patients with essential thrombocythemia. Pathol Biol (Paris) 2001;49:167-9.
6. Livak K, Schmittgen T. Analysis of relative gene expression data using realtime quantitative PCR and the 2-ΔΔC(T) method. Methods 2001; 25:402-8.
7. Fruehauf S, Topaly J, Villalobos M, Veldwijk MR, Laufs S, Ho AD. Quantitative real-time polymerase chain reaction shows that treatment with interferon reduces the initially upregulated PRV-1 expression in polycythemia vera patients. Haematologica 2003;88:349-51.
8. Castello G, Lerza R, Cerruti A, Cavallini D, Bogliolo G, Pannacciulli I. Circulating hematopoietic progenitor cells in polycythemia vera: the in vivo effect of hydroxyurea. Ann Hematol 1995;71:119-21
9. Castello G, Lerza R, Cerruti A, Cavallini D, Bogliolo G, Pannacciulli I. The in vitro and in vivo effect of recombinant interferon α-2a on circulating haemopoietic progenitors in polycythaemia vera. Br J Haematol 1994;87:621-3.
10. Wang M, Tang DC, Liu W, Chin K, Zhu JG, Fibach E, et al. Hydroxyurea exerts bi-modal dose-dependent effects on erythropoiesis in human cultured erythroid cells via distinct pathways. Br J Haematol 2002; 119:1098-105.

Acute Promyelocytic Leukemia

Molecular remission with arsenic trioxide in patients with newly diagnosed acute promyelocytic leukemia

Thirty six APML patients achieving hematologic remission with As_2O_3 were serially monitored using RT-PCR. Though only 5.5% achieved molecular remission at induction remission, 94.5% became negative during consolidation. At 20 months follow-up, 85% remain in remission but longer follow up studies are needed to monitor late relapses.

haematologica 2003; 89:1266-1267
(*http://www.haematologica.org/2004/10/1266)*

Arsenic trioxide (As_2O_3) achieves induction remission in 70-90% of patients with newly diagnosed acute promyelocytic leukemia (APL) with 65-70% long term remission rates[1,2] but there is limited data on molecular remission in these patients. We evaluated this aspect in 40 patients and describe our findings here. The study population was formed of 40 patients with t(15;17) APL treated with As_2O_3 between January 2000 and February 2004. As_2O_3 was administered in teh context of an institutional study protocol after obtaining ethical clearance in the first 5 patients, but since 2001, As_2O_3 has become standard therapy for patients who cannot afford treatment with ATRA. Intravenous As_2O_3, prepared in the hospital pharmacy at the cost of $0.5 per vial, was administered at a daily dose of 10 mg (adults) and 0.15 mg/kg/day (children) as per protocol (Figure 1). Full blood counts, coagulation parameters, as well as renal and hepatic function were closely monitored. Electrocardiograms were done if patient was symptomatic. Platelet and fresh frozen plasma transfusions were given to maintain platelet counts >20,000/mm^3 or if the patients had a coagulopathy. Bone marrow examination was done to assess remission on normalization of blood counts. The molecular monitoring was carried out by reverse transciption polymerase chain reaction (RT-PCR) to detect PML-RARα transcripts, as described by van Dongen *et al.*,[3] and was done at diagnosis, at hematologic remission, prior to consolidation therapy, twice during maintenance therapy (3 months apart) and subsequently every 6 months. This method, with nested amplification, has a sensitivity of 10^{-3} to 10^{-4}. There were 23 males and 17 females, including 31 adults and 9 children (mean age 27.8 years; range: 6-60) with hypergranular APL. The median white cell count at diagnosis was 2.5×10^9/L (range: 0.6 to 58.9). Thirty-six patients (90%) achieved hematologic remission (HCR) at a median time of 42.6 days (range: 26-60) with 4 early deaths due to intracranial hemorrhage. Molecular remission was achieved in all at a median time of 83.9 days (range: 51-136). Though only 2 (5.5%) patients became PML-RARα transcript negative at HCR, another 25 (69.5%) had became negative at the start of consolidation without further treatment. Seven patients (19.5%) became negative at the end of consolidation while 2 (5.5%) became negative during maintenance therapy. Thirty-four patients (94.5%) were in molecular remission by the end of consolidation. As far as concerns toxicity, 20 patients (50%) had leukocytosis requiring addition of hydroxyurea with temporary discontinuation of As_2O_3 in 5 patients and prolonged neutropenia in 1 patient. Asymptomatic elevation of liver enzymes was noted in 7 (17.5%) patients. There were no cases of clinical cardiac toxicity. Isoform analysis showed that 29 patients (72.5%) were bcr-1-positive, 2 (5%) were bcr-2-positive and 9 (22.5%) were bcr-3 positive. The rate

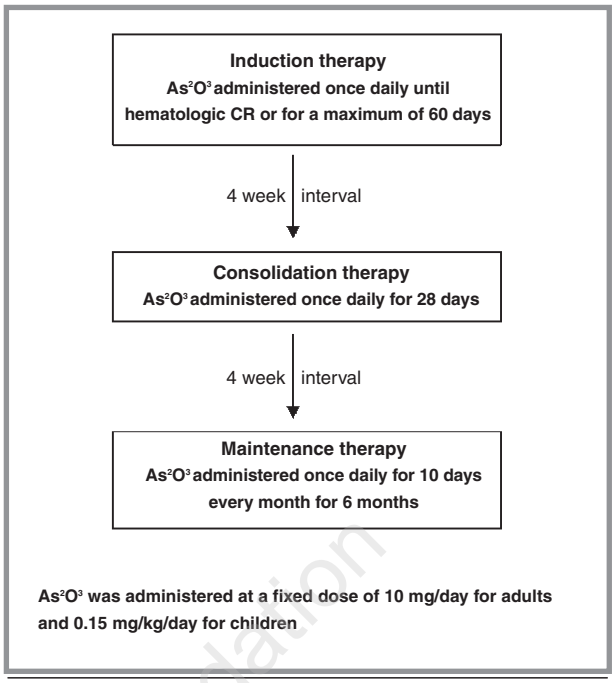

Figure 1. Protocol for tretament of patients with APML with As_2O_3.