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Research Article

Increasing Red Cell Mass and JAK2 V617F Allele Burden in a Patient with Polycythemia Vera and Normal Bone Marrow Five Years after Discontinuation of Long-term Interferon-Alpha2b

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Abstract

Several studies have shown that treatment of polycythemia vera (PV) and essential thrombocythemia (ET) with interferon-alpha2 (IFN-alpha2) is associated with a decrease in the JAK2 V617F mutational load together with complete hematological responses and in a subset of patients even with normalisation of the bone marrow. We report a PV patient, who – after having achieved a major molecular remission with normalisation of the bone marrow and off IFN-alpha2b for about 5 years – had a rapid rise in the hematocrit value and the JAK2 V617F allele burden within months but still with a normal bone marrow and a slightly enlarged spleen. This report adds novel information on the dynamics of the JAK2 V617F allele burden from the very early PV-stage with normal peripheral blood counts and low burden JAK2 V617F to the stage, when rising JAK2 V617F allele load is associated with a definite increase in the hematocrit value.

INTRODUCTION

Polycythemia vera (PV) belongs to the Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) characterized by clonal proliferation and increased production of mature hematopoietic cells due to an acquired genetic defect in the pluripotent stem cell. The JAK2 V617F mutation is detected in more than 98% of PV patients and has been proposed as a marker to monitor disease progression and treatment response [1]. Untreated, PV is associated with a high risk of thrombohemorrhagic complications and in a subset of patients a risk of evolution into myelofibrosis or progression to acute myeloid leukemia. Current treatment strategies in PV are phlebotomies and aspirin together with cytoreduction in high-risk PV-patients [2,3]. Several studies have shown that the JAK2 V617F mutational load may decrease during long-term treatment with IFN-alpha2 to borderline detectable or undetectable levels in concert with complete hematological responses and normalisation of the bone marrow histomorphology in a subset of patients. These responses may be sustained after discontinuation of treatment [4-12]. Some investigators argue for treatment with IFN-alpha2 – earlier rather than later [3,13-16]. We report a patient with PV described in 2007, who achieved complete hematological remission (CHR), major molecular response (MMR) (JAK2 V617F < 1%), and normalization of bone marrow morphology after long-term treatment with IFN-alpha2b [5]. The responses were maintained after discontinuation of IFN-alpha2b for 47 (a rising hematocrit value), 3 (a rising JAK2 V617F allele burden > 1%), and 62 months, respectively. After 62 months the

JAK2 V617F allele burden rapidly increased with subsequent increase in the hematocrit value, necessitating phlebotomy, but still without evidence of PV features in the bone marrow.

SUBJECT AND METHODS

Case story

The patient was a 59-year-old male, when he was diagnosed with PV in 1996. The diagnosis of PV in 1996 was also obtained when using the criteria of WHO (2007 and 2008) [17-19]. At the time of diagnosis the hemoglobin concentration was 19,7 g/dL and the hematocrit value was 0,60. The plasma erythropoietin and erythropoietin-independent endogenous colonies (EECs) were not assessed. The JAK2 V617F allele burden analyzed from bone marrow samples was 54% in 1996 and 73% in 1999 prior to treatment. The bone marrow biopsy showed classical features of PV with hypercellularity, erythroid predominance and clusters of morphologically abnormal megakaryocytes, and the patient had a slightly enlarged spleen. The platelet count and the white blood cell count were within the normal range. The patient had suffered from a headache since the age of 25 years old, which disappeared after phlebotomy and never returned.

Therapeutic intervention

The patient initiated treatment with aspirin and phlebotomies in 1996. Treatment with non-pegylated interferon-alpha2b (Introna) 3-7 MIE three times per week was instituted in March 1999 after a transitory ischemic attack. In March 2002, Introna was reduced to 4 MIE once weekly. In September 2003, the treatment was changed to pegylated interferon-alpha2b (PegIntron) 30 μg once every second week, and from February 2004 30 μg once weekly. A switch from non-pegylated interferon-alpha2b to pegylated interferon-alpha2b was performed, since it was considered more convenient with fewer administrations. In March 2008 the treatment was discontinued, since treatment had been given for nine years, and the bone marrow was normal with a very low JAK2 V617F allele burden (0,7%). In August 2013 – after 65 months off-therapy – the patient resumed treatment with IFN-alpha2 as pegylated interferon-alpha2a (Pegasys) 90 μg once weekly because of concern in regard to the potential thromboembolic risk associated with the increasing JAK2 V617F mutational load [20-22] – a brisk rise to about 38% within a few months – in concert with a rising hematocrit value (56%) necessitating phlebotomies.

Methods

A highly sensitive real-time qPCR was used for quantification of the proportion of JAK2 V617F mutated alleles using bone marrow biopsies (the two first samples in 1996 and 1999) and whole-blood [23,24].

Erythropoietin-independent Endogenous Colony (EEC) – growth was analyzed from peripheral blood using MethoCult Agar-LCM according to manufacturer's instructions (Stemcell technologies, Grenoble, France).

RESULTS

Hematological response

Initially, the patient achieved CHR after 12 months on treatment with non-pegylated IFN-alpha2b (Introna) [8]. CHR was sustained for 47 months off-therapy, when the need of phlebotomy recurred. At that time the hemoglobin concentration was 15,1 g/dL and the hematocrit value 0,47. The hematocrit value increased to 0,56 and the hemoglobin concentration to 18,5 g/dL after 62 months off-therapy. The platelet count and the white blood cell count were normal.

Molecular response

Since "undetectable" JAK2 V617F mutational load ("complete molecular response (CMR)" has never been reported in Danish IFN-treated MPN-patients [4-6], when using the internationally recommended highly sensitive Danish JAK2 V617F assay (sensitivity 0,1%) [23-25], the currently used molecular response criteria were modified [8,9] excluding CMR and including major molecular response (MMR) instead defined by a JAK2 V617F mutational load < 1%.

A JAK2 V617F mutational load of 0,1% was achieved after 66 months of IFN-alpha2b treatment. After 9 years IFN-alpha2b was discontinued at a time, when the patient was still in MMR with a JAK2 V617F mutational load of 0,7%. A MMR was sustained for 3 months off-therapy, when the JAK2 V617F mutational load increased to 1,1%. In the succeeding 22 months the JAK2 V617F mutation was in the range between 1-6% and thereafter steadily rising during the following 28 months from about 8-10% to about 15-20%. After 62 months off-therapy the JAK2 V617F allele burden abrubtly increased within 3 months from 21,8% to 38% 65 months after discontinuation of IFN-alpha2b in concert with a rapid rise in the hemoglobin concentration (Figure 1.1 and 1.2).

Histomorphological response

A complete normalisation of the bone marrow was achieved after treatment with IFN-alpha2b for a total 108 months, when the JAK2 V617F allele burden was 1,1% 3 months after discontinuation of IFN-alpha2b.

Bone marrow biopsies were still without features of PV 51 months and 62 months after discontinuation of IFN-alpha2b – except for an expanded erythropoiesis at 62 months.

Red cell mass (RCM) estimation

At 52 and 58 months off IFN-alpha2b therapy Red Blood Cell (RBC) volume estimations were normal. At 63 months RCM estimation was definitely increased at a time, when the hematocrit value had increased to 56%.

Abdominal ultrasound (UL) investigations

Several abdominal UL investigations were performed after discontinuation of treatment with IFN-alpha2b and confirmed normalisation of spleen size – also when being performed after 41 months off IFN-alpha2b therapy. An UL 58 months off IFN-alpha2b therapy displayed a slightly enlarged spleen (spleen length 12,5 cm), and 7 months later (about 5 years after discontinuing IFN-alpha2b) the spleen was increased in size to 13,5 cm.

DISCUSSION

Published data on IFN-alpha2 have convincingly confirmed its efficacy in reducing the JAK2 V617F allele burden – in

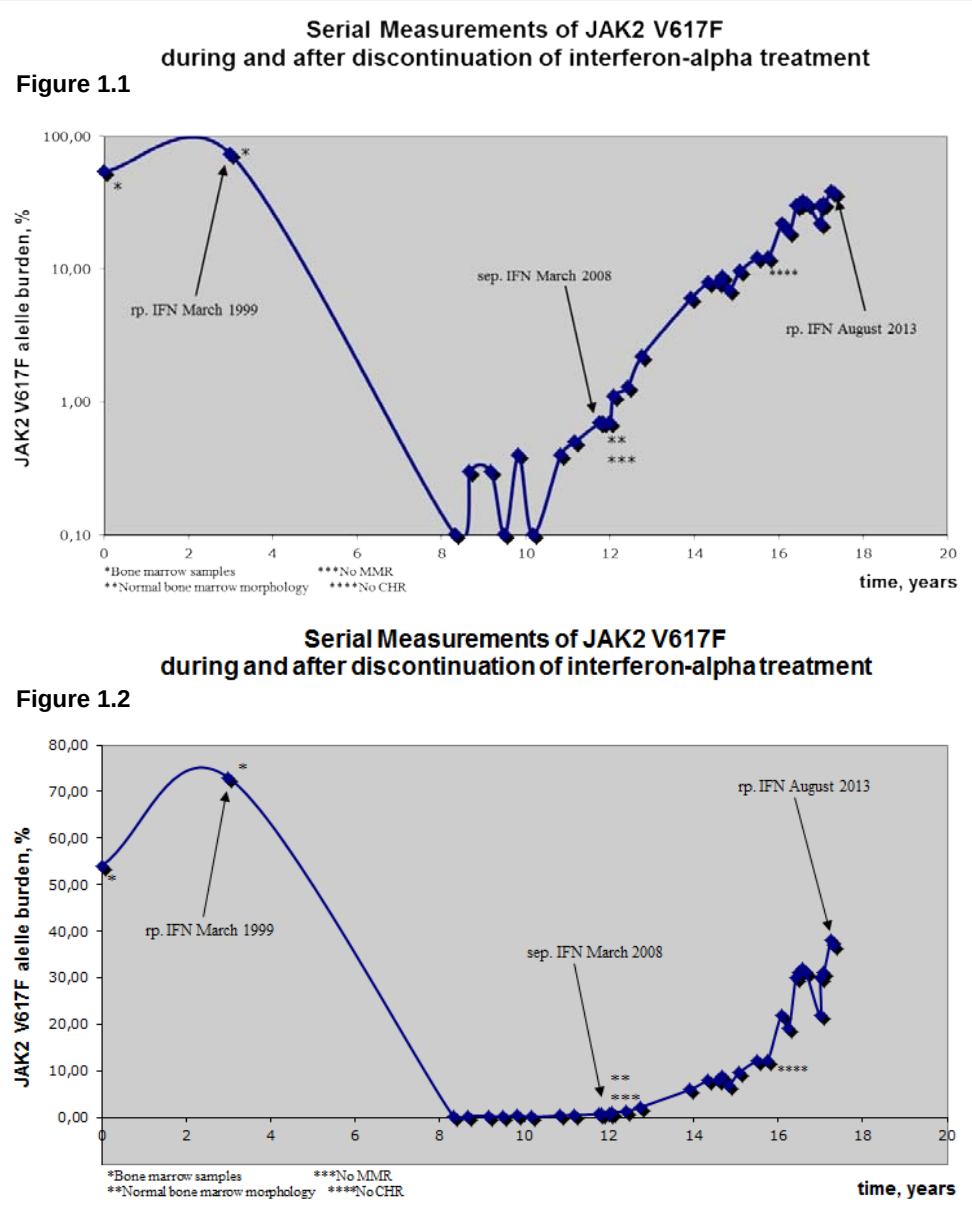

Figure 1 X,Y-scatter plot depicting serial measurements of the JAK2 V617F allele burden during and after long-term IFN-alpha2b. (figure 1.1 with logarithmic scale, figure 1.2 with linear scale).
The leukocyte and platelet counts were normal during the whole period including the 65 months from March 2008 to August 2013, when the patient was off-therapy.

some patients even being sustained with "undetectable" levels compatible with "CMR" [7-10]. CMR in one PV-patient 12 years after discontinuation of IFN-alpha has been reported [11]. A significant molecular response has also been reported in Danish studies with IFN-alpha2a or -b treatment demonstrating sustained JAK2 V617F ≤ 2% up to five years after discontinuation of long-term treatment together with normalization of the bone marrow morphology in a subset of patients [4-6].

On the other hand, preliminary data on IFN-alpha2 treatment have also shown minimal molecular response with only a slight reduction in JAK2 V617F allele burden [26,27] and hematological responses not accompanied by molecular responses [28] – the latter with a starting and maintenance dose of IFN-alpha2 1.5 to 2 times lower than the dose used in other studies. Furthermore, rapid increase in the JAK2 V617F mutational load together with recurrence of clonal hematopoiesis after discontinuation of IFN-alpha have been reported in a single patient – though with a very short duration of treatment and follow-up [29]. Thus, significant responses on IFN-alpha2 seem to require long-term treatment [6,10].

Our patient demonstrates a unique PV-course, which for several reasons is highly interesting. First, the patient achieved a long-lasting molecular and hematological remission – the latter was sustained for about 4 years after discontinuation of treatment. Second, a normal bone marrow morphology was obtained at a time, when the patient achieved "minimal residual

disease" with low-burden JAK2 V617F (1,1% mutated alleles) in peripheral blood. Third, a bone marrow biopsy obtained after about 5 years off IFN-alpha2 therapy was still normal despite rapidly rising JAK2 V617F levels to 38% within a few months in concert with a brisk increase in the hematocrit value necessitating phlebotomies. Fourth, the absence of leukocytosis and thrombocytosis at the time of diagnosis – despite typical PV-features with e.g. an increased number of megakaryocytes – illustrates that our patient likely was in the early PV stage with elevated hematocrit only, which later during evolution of the disease might have been associated with leukocytosis and thrombocytosis as well. In this context the present case report also shows that early treatment with IFN-alpha2b is able to prohibit clonal evolution with accompanying leukocytosis and thrombocytosis. Fifth, for the first time this case report depicts one of several patterns for evolution of the JAK2 V617F allele burden, implying a rapid increase in the JAK2 V617F in concert with increasing hematocrits within a few months after several years off IFN-therapy. In conclusion, the off IFN-therapy period with normal peripheral blood counts, a normal bone marrow biopsy and low-burden JAK2 V617F for several years may likely describe "the natural course of PV" with an initial phase of JAK2 V617F driven increase in erythropoiesis only as reflected by a rapid increase in the JAK2 V617F allele burden without concomitant elevation of leukocyte and platelet counts and still with a normal bone marrow but a spleen, which was slightly increased.

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