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JAK2 allele burden in the myeloproliferative neoplasms: effects on phenotype, prognosis and change with treatment

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***Abstract*:** The field of Philadelphia-chromosome-negative chronic myeloproliferative neoplasms (MPNs) has recently witnessed tremendous advances in the basic knowledge of disease pathophysiology that followed the identification of mutations in *JAK2* and *MPL*. These discoveries led to a revision of the criteria employed for diagnosis by the World Health Organization. The prognostic role of the *JAK2*V617F mutation and of its allelic burden has been the objective of intensive research using a variety of cellular and animal models as well as in large series of patients. While a definitive position cannot yet been taken on all of the issues, there is a consensus that the presence of higher V617F allele burden, that is on the basis of a stronger activation of intracellular signalling pathways, is associated with the clinical phenotype of polycythemia vera and with defined haematological and clinical markers indicative of a more aggressive phenotype. On the other hand, a low allele burden in myelofibrosis is associated with reduced survival. Finally, a significant reduction of *JAK2*V617F allele burden has been demonstrated in patients treated with interferon, while the effects of novel JAK1 and JAK2 inhibitors have not yet been fully ascertained.

***Keywords*:** myeloproliferative neoplasms, *JAK2*, mutations, prognosis, therapy

Introduction

The Philadelphia-chromosome-negative chronic myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [Vannucchi *et al.* 2009b]. PV and ET are relatively indolent disorders which result in a modest reduction in survival, usually after the first decade far from diagnosis; in contrast, PMF has a more severe course with a median survival of about 5 years, although some patients experience survival longer than 10 years. The course of PV and ET is marked by an excess of arterial and venous thromboses, which represent the principal cause of morbidity and mortality; major haemorrhages, evolving into postpolycythemic or postthrombocythemic myelofibrosis (PPV/PET-MF; collectively known as 'MPN-associated myelofibrosis' (MPN-MF) and including all of the MPNs with bone marrow fibrosis) [Mesa *et al.* 2010], and transformation to acute myeloid leukaemia (AML) also contribute to reduced survival.

The molecular lesion(s) that form the basis of MPNs remained unknown until 2005, when a point mutation in exon 14 of Janus kinase 2 gene (*JAK2* V617F) was discovered in patients with all three classic MPNs [Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005a; Levine *et al.* 2005]. Further studies demonstrated that the V617F allele is harboured by more than 95% of patients with PV and about 60% of those with ET or PMF [Guglielmelli *et al.* 2009b; Tefferi *et al.* 2008, 2006, 2005; Barosi *et al.* 2007; Vannucchi *et al.* 2007b; Antonioli *et al.* 2005; Wolanskyj *et al.* 2005]. Additional mutations in exon 12 of *JAK2* were described in a few patients with PV lacking the V617F allele [Scott *et al.* 2007], while mutations at codon 515 of *MPL* [Pikman *et al.* 2006] have been described in 3–8% of patients with ET and PMF [Vannucchi *et al.* 2008a; Beer *et al.* 2008; Guglielmelli *et al.* 2007; Pardanani *et al.* 2006]. These mutant alleles all result in a gain of function due to constitutive activation of tyrosine kinase-dependent cellular signalling

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pathways, particularly the JAK–STAT pathway [Levine *et al.* 2007]. However, evidence of activation of the JAK–STAT pathway is also found in patients who lack mutations in *JAK2* or *MPL*, pointing to still unknown mutations in other genes linked to this pathway. One of these is *LNK*, which encodes for a member of a family of adaptor proteins involved in the negative regulation of JAK/STAT signalling. Mutations in *LNK* have recently been reported in patients with JAK2-negative MPNs [Oh *et al.* 2010] including subjects with erythrocytosis [Lasho *et al.* 2010].

As a whole, this information has significantly advanced our understanding of the mechanisms that form the basis of MPNs, and has almost immediately prompted a modification of the diagnostic criteria redacted from the World Health Organization (WHO) [Swerdlow *et al.* 2008; Tefferi *et al.* 2007]. Furthermore, by pointing out mutated kinases as a common mechanism in MPNs, these molecular discoveries have formed the basis for the development and clinical exploitation of small inhibitors of JAK2, with the hope of reproducing the successful history of the tyrosine kinase inhibitor imatinib in chronic myelogenous leukaemia [Goldman *et al.* 2009].

The puzzle of 'one-mutation-different diseases'

One major issue that emerged from the very first studies is how a single mutation in *JAK2* could form the basis of at least three major clinical phenotypes, i.e. PV, ET and PMF. To explain this puzzle of 'one-mutation-different diseases', different hypotheses have been advocated, alone or in combination: (i) a different stem cell as the target of the mutation; (ii) variable levels of JAK2 kinase activity as a reflection of the relative proportion of mutated and wild-type protein in the cell ('mutational load theory'); (iii) the specific genetic background of the host; (iv) a pre-JAK2 molecular event; (v) a contributing nonmutational factor, such as epigenetic mechanisms [Vannucchi *et al.* 2009a], miRNA expression abnormalities [Guglielmelli, 2008], and possibly others.

Indeed, it was soon recognized that the *JAK2* V617F mutation, although integral to the myeloproliferative process in murine models (as described in the following), may not be the sole molecular event in the human diseases. The main factor supporting this theory initially came from the discovery of mutations in *TET2*, a putative tumour suppressor gene located at 4q24, by the group headed by William Vainchencker in Paris [Delhommeau *et al.* 2009]. It was found that *TET2* mutations were contained in a pre-JAK2 mutated cell through studies at the single precursor cell level. However, subsequent studies demonstrated a very complex mutational pattern in most patients with MPNs, leading to an appreciation of the mutational genetic complexity of these disorders [Kralovics, 2008]. Other mutations beyond *TET2* that can accompany, precede, or follow *JAK2* V617F mutation [Tefferi, 2010] have been reported in *ASXL1* [Carbuccia *et al.* 2009], *CBL* [Grand *et al.* 2009], *IDH1*/*IDH2* [Green and Beer, 2010; Tefferi *et al.* 2010], *EZH2* [Ernst *et al.* 2010] and *IKZF1*/*Ikaros* [Jager *et al.* 2010]. These mutations can be variably present during the chronic or blastic phase of MPNs [Abdel-Wahab *et al.* 2010; Tefferi, 2010].

The fact that almost 40% of ET or PMF patients lack a molecular marker is a strong support to the theory that mutations other than *JAK2* V617F are involved in the pathogenesis, and possibly affect the phenotype, of MPNs. In females with *JAK2* V617F-positive ET, a clone of haematopoietic cells larger than that harbouring the *JAK2* V617F mutation can exist, as shown by results of analysis of X-chromosome inactivation pattern (X-CIP) [Levine *et al.* 2006; Antonioli *et al.* 2005]. In addition, in several *JAK2* V617F-mutated patients with a del(20q), the size of del(20)q and *JAK2* V617F-mutated clones was different, suggesting that the V617F allele had been acquired on the background of a pre-existing del(20q) clone [Kralovics *et al.* 2006]. It is also of interest that the occurrence of independently acquired biallelic *JAK2* mutations has been described [Olcaydu *et al.* 2009]; however, a recent reappraisal in ET patients, using different techniques simultaneously, indicated that in contrast to previous reports [Lambert *et al.* 2009], the phenomenon is quite rare [Beer *et al.* 2010b]. Some patients contemporarily harbour the *JAK2* V617F and *MPL* W515L/K mutation [Guglielmelli *et al.* 2007; Pardanani *et al.* 2006], and clonal dominance by the *MPL* mutated cells has been demonstrated at least in some instances [Lasho *et al.* 2006]. While seminal studies have shown that the V617F allele is tightly associated with

the growth of erythropoietin-independent erythroid colonies (EECs), some EECs that were wild-type for *JAK2* have been detected at low frequency in PV patients [Nussenzveig *et al.* 2007]. Finally, a variable proportion according to the different studies [Rinaldi *et al.* 2010; Theocharides *et al.* 2007] of blast cells of the AML that developed in patients with a preceding *JAK2* V617F-positive MPN have been found to be *JAK2* V617F-negative. However, the pattern of evolution of a MPN towards the leukaemic phase may differ depending on the characteristics of the chronic phase disease [Beer *et al.* 2010a]. The most likely explanation for the phenomenon of these JAK2 wild-type blasts is that they derived from the transformation of a pre-*JAK2* V617F mutated haematopoietic stem cell that originally expanded during the chronic phase of the MPN after having acquired the V617F allele [Campbell *et al.* 2006a].

On the other hand, at present there is little evidence that differences in clinical phenotype in patients harbouring the *JAK2* V617F mutation originate from a different stem/progenitor cell targeted by the mutational event. The V617F allele has been found in myelolymphoid progenitors in patients with PV or PMF, as well as in flow cytometry purified populations of haematopoietic stem cells and committed progenitors [Delhommeau *et al.* 2007]. Similar observations have been reported for the *MPL*W515L/K mutation [Chaligne *et al.* 2007]. In only a minority of ET cases has a restriction of V617F allele to megakaryocytic lineage been described, although it is unclear whether a very low burden of mutated granulocytes might have escaped detection of the abnormal genotype [Bellosillo *et al.* 2007].

It is also likely that individual-related characteristics contribute to the phenotypic pleiotropy of the MPN [Vannucchi and Guglielmelli, 2008], as originally hypothesized by results obtained in animal models. In fact, in mice transplanted with *JAK2* V617F mutated cells the characteristics of the myeloproliferative disease they developed were dependent on their genetic background; as a matter of fact the *JAK2* V617F allele induced marked leukocytosis and extensive marrow reticulin fibrosis in Balb/c mice, in addition to erythrocytosis, in contrast to C56Bl/6 mice that had a milder disease and no leukocytosis, although they developed erythrocytosis at comparable extent [Wernig *et al.* 2006]. Gender is another genetic-based host modifier that may have relevance [Spivak, 2002], as well as the body availability of iron stores. It was by taking into an account these individual variables that the hypothesis of a 'biological continuum' of *JAK2* V617F mutated ET and PV was formulated [Campbell *et al.* 2005].

Pathophysiology correlates of the *JAK2* V617F allele burden

The possibility that the burden of *JAK2* mutation has an influence on disease phenotype through the level of activation of downstream JAK/STAT signalling pathways is supported by several experimental and clinical observations. First, there are substantial differences in the median burden of V617F allele in peripheral blood granulocytes among individual MPNs; the highest levels are found in PV and the lowest in ET patients [Vannucchi *et al.* 2008b; Passamonti *et al.* 2006], with most PMF patients displaying intermediate–high levels. Most patients with MPN-MF have high levels of the *JAK2* V617F allele. Furthermore, homozygosity for the *JAK2* V617F mutation, which follows mitotic recombination of the short arm of chromosome 9 [Kralovics *et al.* 2002], is displayed by approximately 30% of PV or PMF patients as opposite to 2–4% of ET. Variable proportion of wild-type, heterozygous and homozygous progenitors are present in most patients with PV, while homozygous progenitors are reported as being rare in ET [Scott *et al.* 2006]. Erythroid progenitors harbouring the V617F mutation are more sensitive to erythropoietin than normal erythroid progenitors, and most erythropoietin-independent erythroid colonies are made up of homozygous progenitors [Dupont *et al.* 2007]. Conceivably, duplication of the mutant allele is expected to result in a level of JAK2/STAT activation higher than in cells harbouring one mutant and one wild-type allele and in a greater activation of downstream genes [Vannucchi *et al.* 2006; Kralovics *et al.* 2005b]. This could be due to the loss of competition between normal and mutated allele and/or impaired interaction of mutant JAK2 with cellular regulators such as the suppressor of cytokine signalling-3 (SOCS3) [Hookham *et al.* 2007].

A correlation between mutant allele burden and disease phenotype is indirectly supported also by the phenotype developed by animals expressing the *JAK2* V617F mutation. Retroviral vector

models were characterized by the rapid appearance of polycythemia, leukocytosis and splenomegaly, pointing to a PV-like disease that eventually recapitulated the evolution to a post-PV myelofibrosis [Lacout *et al.* 2006; Wernig *et al.* 2006; Zaleskas *et al.* 2006]. The fact that thrombocytosis was not observed in these mice could have been due to the exceedingly high levels of mutant *JAK2* mRNA after the retroviral infection. As a matter of fact, in transgenic mice obtained with a conditional inverted allele of the human *JAK2*V617F under the human *JAK2* promoter, variable levels of JAK2V617F mRNA versus the wild-type mRNA were obtained in different founders [Tiedt *et al.* 2008]. In some respects, the resulting phenotype closely reflected the ratio between normal and mutated *JAK2* mRNA. In fact, in mice expressing lower levels of mutated *JAK2* significant increases in leukocytes, platelets and hemoglobin were detected; the authors suggested that lower or higher expression of *JAK2*V617F favours an ET or PV phenotype, respectively [Tiedt *et al.* 2008]. Similar findings were reported by others [Shide *et al.* 2008; Xing *et al.* 2008]. More recently, three knock-in murine models were developed through the introduction of the *JAK2* mutation into the host *JAK2* locus to allow physiological expression of the gene. In the two models that used a mouse JAK2 for knocking in, a full MPN phenotype developed with erythrocytosis, thrombocytosis, splenomegaly and later evolution to myelofibrosis [Akada *et al.* 2010; Marty *et al.* 2010]; the phenotype was more evident in homozygous mice and correlated well with a significantly higher level of JAK/STAT signalling [Akada *et al.* 2010]. On the other hand, the group of Tony Green developed a model of mice expressing a conditional human *JAK2*V617F allele; these mice presented a predominant ET-like phenotype with thrombocytosis and moderate erythrocytosis, but very little splenomegaly and no myelofibrosis [Li *et al.* 2010]. Of interest, these mice also presented an impairment of stem cell function. As a whole, and by taking into an account that these models cannot reproduce the situation of human disease exactly in terms of relative expression of mutated allele, concomitant additional mutations, genetic background and balance between normal and mutated stem cells, a general support to the theory that expression level of the *JAK2*V617F allele influences disease phenotype can certainly be derived.

Clinical correlates of the *JAK2*V617F allele burden in myeloproliferative neoplasms

Several studies, although not invariably and with limitations due to their retrospective nature in most cases, have shown that the V617F allele burden correlates with haematologic characteristics and clinical endpoints in MPN patients, although it seems quite unlikely that the burden of mutated allele represents the only mechanism at the basis of MPN pleiotropy [Vannucchi *et al.* 2008b].

The largest study that compared *JAK2*V617F heterozygous and homozygous PV and ET subjects included 323 patients with PV (67.8% heterozygous and 32.2% homozygous) and 639 patients with ET (40.2% wild type, 57.6% heterozygous and 2.2% homozygous) [Vannucchi *et al.* 2007b]. In both diseases, homozygosity was associated with a stimulated erythropoiesis and myelopoiesis, lower platelet count, a higher incidence of splenomegaly, larger spleen size, and a greater proportion of patients requiring cytoreductive therapy. Homozygous PV patients had also a higher incidence of pruritus (42%). The rate of major thrombosis was not increased in homozygous patients with PV compared with heterozygous patients, similar to the finding reported by Tefferi and coworkers in a study that included 45 heterozygote and 13 homozygote PV patients [Tefferi *et al.* 2006]. In contrast, thrombotic events were definitively more frequent among homozygous ET patients, with a hazard ratio 3.97-fold higher than in *JAK2* wild type. The association of homozygosity with cardiovascular events in ET patients was maintained after multivariate analysis, using as covariates other established risk factors (age >60 years and previous history of thrombosis) [Finazzi and Barbui, 2005] as well as leukocytosis (white blood cell counts greater than 8.7×10^9/L) [Carobbio *et al.* 2007]. Furthermore, in both studies from Vannucchi and colleagues and Tefferi and colleagues the number of PV patients who had a fibrotic transformation was significantly higher among homozygous than heterozygous groups (23% *vs.* 2%, $p = 0.009$, in the Mayo study [Tefferi *et al.* 2006] and 11.5% *vs.* 1.4%, $p < 0.001$, in the Italian study [Vannucchi *et al.* 2007b]). Of note, the risk of having a fibrotic transformation was significantly higher also among homozygous ET patients (14.3% of homozygous *vs.* 4.7% of heterozygous *vs.* 1.6% of wild-type patients, $p < 0.001$) in the Italian

study [Vannucchi *et al.* 2007c]. The relationships between the burden of mutant allele and the risk of developing myelofibrosis, that was maintained also on multivariable analysis, is confirmed in a recent prospective series of 320 patients with PV; in contrast, the risk of developing acute leukaemia was not significantly related to mutant allele burden [Passamonti *et al.* 2010b]. As a whole, these data indicate that the burden of *JAK2*V617F allele is associated with the extent of myeloproliferation and with the risk of evolution to myelofibrosis in both PV and ET.

In a prospective study specifically addressing the clinical significance of *JAK2* V617F homozygosity, 173 patients with PV who were genotyped at diagnosis were included. The main finding was that a mutated allele burden greater than 75% was associated with a 3.56-fold higher relative risk (95% CI 1.47–7.1) of total thrombosis [Vannucchi *et al.* 2007a]. Thus, patients with PV who harbour the higher V617F allele burden quartile may represent a subgroup at a particularly higher risk of thrombosis. These observations are supported by recently published, retrospective series of 105 patients with PV. A higher *JAK2* V617F allele burden correlated with more advanced myelofibrosis, greater splenomegaly and higher white blood cell count [Silver *et al.* 2010]. Although a significant correlation with thrombosis risk could not be defined, there was a trend towards increased frequency of thrombosis as the *JAK2* V617F allele burden increased. In contrast, in the series of 320 PV patients published by Passamonti and colleagues, no significant correlation between V617F allele burden and thrombosis could be found [Passamonti *et al.* 2010b]. Finally, in a comparative retrospective study of 415 PV and 867 ET patients the frequency of thrombosis was found to be progressively increased in both diseases according to the amount of *JAK2* V617F allele burden [Carobbio *et al.* 2009]. The highest rate of thrombosis was found among PV patients with greater than 50% mutated allele. However, after 5 years from diagnosis the actuarial probability of arterial and venous thrombosis was similar in PV and *JAK2* V617F-mutated ET, and appeared to be correlated with the progressively increasing V617F allele burden over time. As a matter of fact, 5 years after diagnosis the burden of mutated allele represented the strongest and unique risk factor associated with the rate of subsequent thrombosis, while having a diagnosis of PV or ET was not relevant [Carobbio *et al.* 2009]. In sum, further studies using rigorously designed prospective analysis are necessary to definitively ascertain the predictive role of a high *JAK2*V617F allele burden for the risk of thrombosis in PV. On the other hand, a *JAK2* V617F-mutated status has a defined role in thrombosis in ET, as shown in three independent meta-analyses including more than 2000 patients, with a predicted overall risk of about two. In a study of 260 ET patients, microvessel symptoms were significantly more represented among those with greater than 25% mutated allele and there was a higher frequency of arterial thromboses at diagnosis, confirmed also on multivariate analysis, with a relative risk of 3.0 (95% CI 1.3–6.8; $p = 0.01$) [Antonioli *et al.* 2008].

Primary myelofibrosis is associated with poorer prognosis compared to the other MPN; however, since survival may range from a few months to an excess of decade, the identification of variables associated with prognosis is of considerable importance for driving therapeutic decisions. The International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) developed a new prognostic score system which is capable of discriminated four categories of patients with significantly different median survival in the range of 27 to 135 months [Cervantes *et al.* 2009]. A dynamic International Prognostic Score System (IPSS) has also been validated [Passamonti *et al.* 2010a]. This notwithstanding, the search for biological variables that could function as biomarkers of disease activity and/or evolution is of outmost relevance; some studies have evaluated the correlation of *JAK2* V617F mutational status and V617F mutated allele burden with disease outcome in PMF.

In a series of 168 PMF patients a statistically significant association of the *JAK2* V617F mutated status with a more pronounced myeloproliferative phenotype was found [Guglielmelli *et al.* 2009b], similar to findings reported by Campbell and colleagues [Campbell *et al.* 2006b] and Barosi and colleagues [Barosi *et al.* 2007], but partially different from a Mayo Clinic report [Tefferi *et al.* 2008, 2005]. In the above study, *JAK2* V617F mutated patients were significantly less anaemic than wild type, but neither overall survival nor leukaemia transformation were different in the two categories of patients

defined by their *JAK2* V617F genotype. These results are in line with the study of Tefferi and colleagues [Tefferi *et al.* 2008], but in disagreement with the results of an English study [Campbell *et al.* 2006b] that reported a hazard ratio of shortened survival of 3.3 (95% CI 1.26–8.68) in patients harbouring the V617F allele [Campbell *et al.* 2006b]. Therefore, it seems unlikely that a *JAK2* V617F mutated status *per se* reflect in a poorer prognosis in PMF. By quantifying V617F allele burden in PMF patients, Tefferi and colleagues [Tefferi *et al.* 2008] first described a poorer survival in patients harbouring low mutated allele burden. These findings were confirmed by Guglielmelli and colleagues [Guglielmelli *et al.* 2009b]. In the latter study it was found that patients included in the lower quartile had significantly shorter progression time to anaemia, leukopenia and conversely longer time to large splenomegaly compared with patients in the upper quartiles; furthermore, patients in the lower quartile had a significantly reduced overall survival compared with both upper quartiles and *JAK2* wild-type patients. Reduced survival was mainly due to leukaemic transformation in the Mayo Clinic study [Tefferi *et al.* 2008] and to systemic infections in the Italian study [Guglielmelli *et al.* 2009b]. On the other hand, neither a mutated *JAK2* V617F status nor the mutated allele burden appeared to have clinical relevance in a study of 65 patients with post-PV or PET-MF [Guglielmelli *et al.* 2009a]. In summary, a low *JAK2* V617F allele burden at diagnosis seems to be a strong surrogate marker associated with shortened survival in PMF, although the causes of death as well as the pathobiological mechanisms underlying this correlation remain to be established fully.

In a collaborative study that retrospectively evaluated 707 patients with PMF for the rate and predictive factors for major cardiovascular vascular, Barbui and coworkers [Barbui *et al.* 2010] reported a 7.2% rate of fatal and nonfatal thrombosis. Variables associated with thrombosis in a multivariable model were age greater than 60 and a *JAK2* V617F mutated genotype, producing a hazard ratio of 1.92 (95% CI 1.10–3.34, $p = 0.02$). No information has yet been made available in the literature about the significance of V617F allele burden.

A *JAK2* V617F allele burden exceeding 50% was found in about one quarter of prefibrotic PMF (median value in the 102 patients, 38%) unlike in ET where none of the 90 patients displayed an allele burden greater than 40%, with a median value of 24% [Hussein *et al.* 2009]. Therefore, according to Hussein and colleagues, a *JAK2* V617F allele burden greater than 50% would favour a diagnosis of prefibrotic PMF rather than ET [Hussein *et al.* 2009].

Utility of the *JAK2* V617F allele burden for measuring response to treatment

The occurrence of a specific molecular abnormality in a proportion of MPN patients ranging from 60% to greater than 95% represents a potentially useful biomarker for evaluating the effects induced by different drugs on the mutated clone. Before analyzing in more detail the small amount of information that is currently available regarding this, it is appropriate to take some general considerations into account. First, there are methods validated as yet for measuring the burden of V617F allele, although several methods claiming to be sensitive and specific have been published over the years, and a multicentre evaluation of a number of those assays has been accomplished recently [Lippert *et al.* 2008]. Second, the fact that the *JAK2* V617F mutation can arise in clones harbouring other mutations, potentially more relevant for the initiation of the disease, means that reduction of the V617F allele burden even up to its undetectability does not necessarily signify remission or cure of the underlying MPN [Kiladjian *et al.* 2010].

The effects of hydroxyurea on the burden of V617F allele have been addressed in a few studies with different results. In a retrospective single-centre study in 48 patients the V617F allele burden was not apparently modified over time in most of the patients who were already under hydroxyurea at the time of blood sampling. However, in five of six patients in whom hydroxyurea was started during the follow up, a statistically significant decrease of *JAK2* V617F allele burden was reported after 6 months; halting the treatment caused the level of mutant allele to increase [Theocharides *et al.* 2008]. Hussein and colleagues failed to observe changes in the V617F allele burden after hydroxyurea treatment [Hussein *et al.* 2009], while Girodon and coworkers reported a significant reduction of mutant allele in 36 patients with PV or ET from a median of 43% at diagnosis to 24% at 15 months after starting hydroxyurea [Girodon *et al.* 2008]. In three ET patients the mutation

was no longer detectable after 5–55 months of treatment. Of interest, in both PV and ET, changes in V617F allele burden produced by hydroxyurea treatment were more prominent in women than in males. A significant decrease of *JAK2* V617F burden has been also documented in 72% of 18 patients (9 PV and 9 ET) considered by Ricksten and colleagues after only 4 months of therapy with hydroxyurea [Ricksten *et al.* 2008]. In a study conducted in two Italian institutions that included 172 patients, a minimal (less than 10%), reduction of V617F allele burden was observed only in the group of newly treated ET patients, in accordance with a previous study [Theocharides *et al.* 2008]. A reduction of V617F allele burden consistent with the definition of 'partial response' according to the European LeukemiaNet (ELN) criteria [Barosi *et al.* 2009] involved 15% of ET patients and 12% of PV patients. In summary, the bulk of current evidence seems to suggest that hydroxyurea can produce some reduction in the *JAK2* V617F allele burden in subsets of patients [Girodon *et al.* 2008; Ricksten *et al.* 2008; Theocharides *et al.* 2008], but the clinical relevance of this observation is questionable. On the other hand, it is clinically relevant that patients with PMF who harboured the *JAK2* V617F mutation were more sensitive to hydroxyurea than the wild-type counterpart on multivariable analysis and that in PV the *JAK2* V617F allele burden correlated directly with the response to drug and inversely with the daily dose in responding patients [Sirhan *et al.* 2008].

Kiladjian and colleagues reported a significant reduction in the V617F allele burden, including some documented cases of apparent molecular remission, in a trial that included 37 evaluable patients with PV treated with pegylated interferon alpha 2a. [Kiladjian *et al.* 2008]. In a similar study performed at the MD Anderson Cancer Center in 79 patients, both PV and ET, the molecular response rate was lower than in the French study, with 54% overall response but only 14% of the PV patients having undetectable V617F [Quintas-Cardama *et al.* 2009]. In contrast, modest if any changes in V617F allele burden have been reported by the group of Silver, who pioneered the use of interferon in PV [Jones *et al.* 2006]. Very important, the changes in the *JAK2*V617F allele burden did not mirror the effect of the drug on the frequency of *TET2* mutated haematopoietic progenitors [Kiladjian *et al.* 2010].

The identification of autonomously activated JAK/STAT signalling in MPNs has guided the clinical development of small molecules acting as JAK2 inhibitors [Verstovsek, 2010; Pardanani, 2008]. The first trial with one of these inhibitors, INCB018424, in myelofibrosis has been published recently [Verstovsek *et al.* 2010]; furthermore, a multicentre phase II study in advanced, hydroxyurea-resistant PV or ET has completed enrolment. CEP701, or Lestaurtinib, and TG101348 are also undergoing clinical evaluation [Verstovsek, 2010; Pardanani, 2008]. It is a little disappointing that the appreciable results of INCB018424 in terms of clinical improvement were not mirrored by a significant reduction of the *JAK2* V617F allele burden, pointing to mechanisms other than simple reduction of mutated clonal progenitors load to explain the clinical benefits of the drug [Vannucchi, 2010]. Evidence of a trend towards reduction of the V617F allele burden has been reported in a phase II study in PMF patients treated with Givinostat [Rambaldi *et al.* 2010], a novel orally available histone deacetylases inhibitor that *in vitro* caused significant inhibition of V617F mutated cells through the downregulation of JAK2 mRNA levels [Guerini *et al.* 2008].

In the setting of allogeneic stem cell transplantation for myelofibrosis the quantification of V617F allele burden may have a clinical impact for posttransplant therapeutical strategies, in particular for guiding adoptive immunotherapy [Kroger *et al.* 2009a; Benjamini *et al.* 2008]. In a study performed by Kroger and colleagues, 78% of 21 patients became PCR negative for the *JAK2* V617F mutation at a median of 89 days after allograft [Kroger *et al.* 2009b]. *JAK2* V617F negativity correlated well with the donor-cell chimerism. In one case, residual JAK2-positive cells could be successfully eliminated by donor lymphocyte infusion. In another study, 139 myelofibrosis patients who received a reduced-intensity regimen in preparation to transplantation were considered [Alchalby *et al.* 2010]. It was found that *JAK2* wild-type patients had significantly reduced overall survival on multivariate analysis (HR 2.14, $p = 0.01$) in comparison to *JAK2* mutated patients, while the V617F mutated allele burden, analyzed either as continuous variable or after dividing in quartiles, had no impact on the outcome. Furthermore, patients who cleared *JAK2* mutation level in peripheral blood at 6 months after

transplant had a significant lower risk of relapse (5% *vs*. 35%).

Conclusions

The discovery of the *JAK2* V617F mutation and its association with distinct haematological and clinical characteristics has prompted studies aimed at assessing the predictive value of mutational status and of the burden of V617F allele for the most common complications of PV or ET, i.e. vascular events, for transformation to myelofibrosis or acute leukaemia, and more in general for prognosis and survival, especially survival in PMF. Although most of studies were retrospective in nature, the methods for genotyping and the source of cells were used for genotyping different, and notwithstanding the findings were sometimes conflicting, there is enough evidence to suggest that the burden of mutated allele correlates with defined aspects of the phenotype of MPN and major clinical endpoints. Therefore, these correlative studies support the contention that quantification of *JAK2* V617F allele burden might convey relevant clinical information in well-defined categories of MPN patients.

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Conflict of interest statement

There are no competing financial interests to disclose.

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