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Modulation of *JAK2 V617F* allele burden dynamics by hydroxycarbamide in polycythaemia vera and essential thrombocythaemia patients

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Received 3 June 2010; accepted for publication 16 September 2010

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Summary

The modulation of *JAK2 V617F* allele burden dynamics was prospectively analysed in 47 patients (26 polycythaemia vera [PV] and 21 essential thrombocythaemia [ET]) treated with first-line hydroxyurea (HU) and compared with the *JAK2 V617F* dynamics of a control group of 45 PV and ET patients. A partial molecular response (PMR), according to European Leukaemia Net criteria, was observed in 27/47 (57%) patients. Median time to PMR was 14 months (3–66) with a probability of PMR at 3 years of 57%. A significant decrease in *JAK2 V617F* allele load was observed at 36 months both in PV and ET patients, being the reduction in PV higher than in ET patients ($P = 0{\cdot}01$). A haematocrit ≥0·45 L/L was associated with a higher probability of attaining a PMR (HR:3·4; 95%CI:1·02–11·6, $P = 0{\cdot}04$). Control group showed a slight increase of *JAK2 V617F* allele burden over time. The reduction in the mutated allele load comparing treated patients versus controls was highly significant both in PV and ET, demonstrating a clear effect of HU on the *JAK2 V617F* allele burden. In conclusion, first-line HU can attain PMR in more than 50% of newly diagnosed PV and ET patients, with a continuous decrease of the *JAK2 V617F* allele burden in PV patients during treatment.

Keywords: *JAK2 V617F*, allele burden, hydroxyurea, polycythaemia vera, essential thrombocythaemia.

Polycythaemia vera (PV) and essential thrombocythaemia (ET) are myeloproliferative neoplasms (MPN) characterised by thrombotic and haemorrhagic events during their clinical course. A somatic activating mutation resulting in a valine to phenylalanine substitution at position 617 (V617F) in Janus kinase 2 (*JAK2*) has been identified in more than 90% of patients with PV and in 40 to 60% patients with ET and primary myelofibrosis (PMF)(Campbell & Green, 2006; Tefferi *et al*, 2009).

The main goal of cytoreductive treatment in high-risk PV and ET patients is to control major vascular complications without increasing the risk of clonal evolution. Hydroxyurea (HU) is currently considered the treatment of choice for PV and ET patients older than 60 years and for those presenting severe thrombohaemorrhagic complications. This drug has shown efficacy in controlling increased blood cell counts and splenomegaly, as well as in reducing the incidence of major vascular events in randomised clinical trials and in retrospective and non-controlled historical cohorts of patients with MPN (Finazzi & Barbui, 2008).

The *JAK2 V617F* allele load has been correlated with a high frequency of vascular complications and myelofibrotic transformation in PV patients with a high amount of mutant alleles (Vannucchi *et al*, 2007a; Álvarez-Larrán *et al*, 2009) as well as with a more aggressive clinical course in homozygous ET patients (Vannucchi *et al*, 2007b). These features highlight the importance of achieving a molecular response in the treatment of *JAK2 V617F*-positive MPN. However, the clinical or biological variables associated to the modulation of *JAK2 V617F* allele load by chemotherapy in PV and ET patients or the type of response are still unclear. In this regard, a European Leukaemia Net (ELN) consensus conference has recently developed a proposal in order to define clinico-haematologic (CHR), molecular (MR), and histologic response categories in ET and PV patients treated with cytoreductive drugs (Barosi *et al*, 2009).

First published online 11 January 2011
doi:10.1111/j.1365-2141.2010.08467.x

JAK2 V617F modulation has been demonstrated in PV and ET patients treated with pegylated-interferon-alfa (Kiladjian *et al*, 2008; Quintás-Cardama *et al*, 2009) as well as in the setting of haematopoietic blood cell transplantation in PMF (Kroger *et al*, 2007). In addition, the effect of HU on the *JAK2 V617F* allele burden in PV, ET, and PMF patients (Girodon *et al*, 2008; Ricksten *et al*, 2008; Sirhan *et al*, 2008; Theocharides *et al*, 2008; Larsen *et al*, 2009; Spanoudakis *et al*, 2009; Antonioli *et al*, 2010) has been reported with contradictory results.

We have performed a prospective single-centre study to analyse the modulation of *JAK2 V617F* allele burden by first-line HU in newly diagnosed high-risk PV and ET patients, assessing MR according to ELN criteria. We have also investigated the variables predicting MR achievement and compared *JAK2 V617F* allele load modulation by HU with the natural *JAK2 V617F* allele dynamics in a cohort of ET and PV patients who had never received cytoreductive treatment.

Methods

Patients

A total of 92 *JAK2 V617F*-positive patients (47 PV and 45 ET) consecutively diagnosed at the Haematology Department from the University Hospital del Mar were prospectively included in the study. Patients were diagnosed according to World Health Organisation criteria (Vardiman *et al*, 2002). The study was approved by the local Ethics Committee and informed consent was provided according to the Declaration of Helsinki.

Forty-seven patients (26 PV and 21 ET) received cytoreductive treatment with HU following standard guidelines. HU dose was established according to blood cell counts and clinical response criteria.

The control group included 45 patients (21 PV and 24 ET) who had never received cytoreductive therapy. PV patients not receiving cytoreductive therapy underwent phlebotomy in order to maintain haematocrit below 0·45 and 0·42 L/L for males and females, respectively, plus 100 mg aspirin daily. ET patients were clinically observed without any treatment or received aspirin.

Analysis of JAK2 V617F allele burden and sample chronology

In all treated *JAK2 V617F*-positive patients, the first sample was taken immediately before starting treatment and then every 3–6 months during all treatment period. In PV and ET control patients, *JAK2 V617F* allelic burden was determined at diagnosis and every 6–12 months during clinical follow-up. Analysis of *JAK2 V617F* in cDNA from purified granulocytes was performed in duplicate by real-time allele-specific reverse transcription polymerase chain reaction with probes specific for the mutated and the wild type forms, as previously described (Levine *et al*, 2006; Bellosillo *et al*, 2007). In our hands, this technique reached a sensitivity of 0·5% when homozygous *JAK2 V617F* cells are diluted in normal cells. Patients with allele burden higher than 50% were defined as homozygous. Determination of the relative change in the allelic ratios between the pretreatment sample and the last blood sample in each patient was calculated as follows: *JAK2 V617F* variation = (% last *JAK2 V617F* – % pretreatment *JAK2 V617F*)/(% pretreatment *JAK2 V617F*) × 100. Whenever the *JAK2 V617F* allele burden became undetectable or below 5%, quantification was repeated.

Molecular responses were defined according to ELN criteria as follows: (i) Complete response: reduction of any specific molecular abnormality to undetectable levels; (ii) Partial response (applying only to patients with a baseline value of mutant allele burden greater than 10%): a reduction of ≥50% from baseline value in patients with <50% mutant allele burden at baseline or a reduction of ≥25% from baseline value in patients with >50% mutant allele burden at baseline, and (iii) No response: any response that does not satisfy partial response (Barosi *et al*, 2009).

Statistical analysis

Categorical variables were expressed as frequencies and percentages and continuous variables as mean and standard deviation (SD) or medians and range. To compare continuous variables between the study groups, we used the Mann–Whitney U-test, and to compare continuous variables over the time in the same group, Wilcoxon test for repeated measures was used. The probability of MR was calculated by the Kaplan–Meier method, with the log-rank test being used for the comparisons. Since no patient died during the study period, patients not achieving a molecular response were censored at last follow-up. The following initial variables were analysed at univariate level for their possible association with molecular response: age, gender, platelet counts, haematocrit level, leukocyte count, serum LDH, spleen size, *JAK2 V617F* allelic burden, and achievement of complete haematological response. Variables attaining a significant level at the univariate analysis were included in a Cox proportional hazards model for assessing their independent association with molecular response. Because type of diagnosis (PV or ET) is associated with blood cell count and *JAK2 V617F* allele burden (Vannucchi *et al*, 2008) this variable was also included in the multivariate model. All independent variables were forced to stay in the model. Additional analysis were performed including known prognostic factors like age and leukocyte count. Significance was considered for P values <0·05. The statistical analysis was performed using the SPSS 16.0 package (SPSS, Chicago, IL, USA).

Results

Baseline Characteristics

The main clinical and biological characteristics of the patients are shown in Table I. Treated patients had a higher median age

Table I. Clinical and biological characteristics of 92 PV and ET patients.

	HU-treated group		Control group	
	PV	ET	PV	ET
Number of patients	26	21	21	24
Age (years)*	70 (34–87)	74 (48–86)	52 (25–67)	44·5 (29–78)
Gender (M/F)	12/14	7/14	12/9	8/16
Palpable splenomegaly	10/26	1/21	6/21	1/24
Haematological values at diagnosis				
Haematocrit (%)*	44·6 (29·7–70·9)	43·7 (37·2–47·6)	48·7 (42·5–61·8)	43·4 (38·9–53·4)
Leukocyte count ($\times 10^9$/l)*	12·5 (5·7–38·5)	7·4 (5·7–17·0)	9·3 (5·5–18·2)	8·7 (5·5–12·7)
Platelet count ($\times 10^9$/l)*	776 (207–1546)	606 (464–1303)	599·5 (229–1347)	620 (417–946)
Haematological values at last sample				
Haematocrit (%)*	39·8 (34·4–50·2)	39·1 (31·5–44·1)	46·6 (42·7–57·6)	45·4 (40·7–51·3)
Leukocyte count ($\times 10^9$/l)*	5·4 (2·8–12·3)	4·4 (3·3–6·6)	11·6 (4·4–21·5)	8·9 (4·8–11·8)
Platelet count ($\times 10^9$/l)*	260·5 (135–507)	312·5 (147–378)	820·5 (204–1600)	590 (419–1152)
JAK2 V617F positive	26/26 (15 homozygous)	21/21	21/21 (5 homozygous)	24/24
Treatment duration*	29·5 (6·4–73·7)	31·5 (7·0–71·5)	N.A.	N.A.
Interval between first and last sample (months)	30·8 (6·4–74·6)	32·8 (7·0–72·5)	48·2 (13·7–82·1)	51·6 (16·5–78·2)
Mean number of samples per patient	7	7	6	5
HU daily dose (mg, mean ± SD)	958 ± 206 mg	968 ± 251 mg	N.A.	N.A.
% *JAK2* V617F at first sample*	69·1 (21·7–100)	27·5 (10·0–43·2)	38·3 (9·0–83·1)	26·1 (10·6–37·8)
% *JAK2* V617F at 24 months*	24·4 (5·5–100)	17·2 (1·7–34·5)	41·9 (12·4–100)	26·9 (9·1–37·8)
% *JAK2* V617F at 36 months*	20·3 (7·8–100)	17·0 (1·1–34·9)	43·2 (12·9–100)	27·7 (9·1–55·9)
% *JAK2* V617F at last sample*	21·6 (2·6–100)	15·1 (0·7–39·9)	40·7 (10·1–100)	29·3 (6·4–66·5)
% *JAK2* V617F allele variation at 24 months*	−40·1 (−96·9 to 67·0)	−32·8 (−93·5 to 118·7)	14·1 (−15·9 to 121·9)	5·5 (−22·3 to 53·1)
% *JAK2* V617F allele variation at 36 months*	−50·4 (−90·9 to 12·5)	−35·9 (−95·7 to 103·9)	21·5 (−12·5 to 123·0)	10·9 (−19·0 to 53·3)
% *JAK2* V617F allele variation at last sample*	−54·1 (−98·1 to 67·0)	−42·0 (−97·1 to 77·4)	15·0 (−15·9 to 212·6)	5·51 (−40·0 to 131·6)

*Median (range).
N.A., not applicable.

than untreated patients ($P < 0{\cdot}001$ for both PV and ET patients). As expected, the baseline percentage of *JAK2 V617F* alleles was higher in PV than in ET with a significant difference between both patients receiving HU (69·1% in PV vs. 27·5% in ET, $P < 0{\cdot}001$) and control patients (38·2% in PV versus 26·1% in ET, $P < 0{\cdot}001$). PV patients receiving HU had a significantly higher percentage of baseline *JAK2 V617F* alleles than those managed only with phlebotomies (69·1% vs. 38·2%, $P = 0{\cdot}003$), whereas no significant difference was observed in the initial *JAK2 V617F* allele burden between treated and control ET patients.

Dynamics of JAK2 V617F allele burden in control patients

With a mean of six and five samples per patient for PV and ET patients, respectively, the spontaneous dynamics of *JAK2 V617F* allele burden is shown in Table I and Fig 1. Median interval between the first and last sample was 48·2 months (13·7–82·1) and 51·6 months (16·5–78·2) for PV and ET patients, respectively. As can be seen, the majority of patients experienced a slight increase in the *JAK2 V617F* allele load, although the difference was not statistically significant. No significant differences were seen between PV and ET patients at 24 and 36 months (Table I and Table SI). However, an increase higher than 50% of the initial values was observed in five PV and in two ET patients (Fig 1).

Dynamics of JAK2 V617F allele burden in HU-treated patients

The median treatment duration at the time of the last sample assessment was 29·5 months (range 6·4–73·7) in PV patients and 31·5 months (range 7–71·5) in ET patients. The mean HU daily dose was 958 ± 206 mg for PV patients and 968 ± 251 mg for ET patients (Table I). With a mean of 7 samples per patient analysed in 47 *JAK2 V617F*-positive patients (26 PV and 21 ET), treatment with HU resulted in a median variation of the *JAK2 V617F* allele percentage of −54·1% and −42% for PV and ET patients, respectively (Table I). The *JAK2 V617F* allele burden variation in each patient treated with HU is shown in Fig 1.

According to ELN criteria, a partial MR was observed in 27 (17 PV and 10 ET) out of 47 (57%) assessable patients. Median time to partial MR was 14 months (range 3–66). The majority of MR was durable since only 7 out of the 27 responders lost their MR (3 PV and 4 ET) at a median time of 12 months (range 4–18). In 3 of them (1 PV and 2 ET) a new partial MR was obtained 7, 10 and 6 months after, respectively.

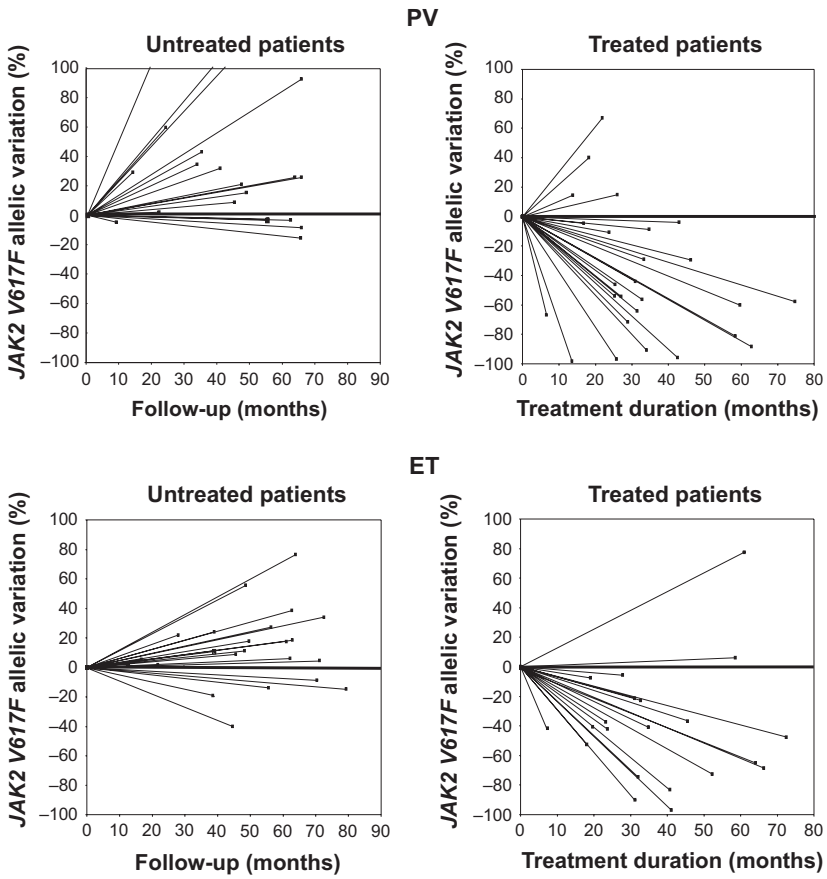

Fig 1. Variation in the *JAK2 V617F* allele burden in PV patients (upper panels) and ET patients (lower panels) who had never received cytorreductive treatment (left) and who had been treated with hydroxyurea (right). The figure shows the variation in the percentage of *JAK2 V617F* in the last sample analysed and is referred to the first sample obtained from each patient.

Twenty-two patients (43%), classified as non-responders, did not present any type of MR in the different samples analysed during the follow-up. Moreover, in three of these patients (two PV and one ET) an increase >20% in *JAK2 V617F* allele burden with respect to the initial level was observed. The cumulative probability of achieving a partial MR to initial HU therapy in the whole treated group is shown in Fig 2A. As can be seen, MR at 24 and 36 months was 46% and 57%, respectively. According to diagnosis, the probability of obtaining a MR for PV and ET patients was 46% and 40% at 24 months, and 73% and 40% at 36 months, respectively; $P = 0{\cdot}09$ (Fig 2B). Variables at diagnosis significantly associated with a higher probability of obtaining a partial MR in the whole group were: *JAK2 V617F* allele percentage ≥50% (probability of response at 36 months: 67% vs. 50%, $P = 0{\cdot}003$), thrombocytosis $<600 \times 10^9$/l (65% vs. 51%, $P = 0{\cdot}02$) and haematocrit ≥0·45 L/L (71% vs. 29%, $P < 0{\cdot}001$). Age, gender, type of diagnosis (PV versus ET), palpable splenomegaly, leukocyte count, LDH and complete haematological response were not associated with the likelihood of obtaining a partial MR. In multivariate analysis, the only variable related with a higher probability of achieving a partial MR was haematocrit ≥0·45 L/L (estimated hazard ratio [HR], 3·4; 95% CI, 1·02 to 11·6, $P = 0{\cdot}04$). Multivariate analysis including age and leukocyte count was also performed obtaining similar results (HR for haematocrit >0·45 L/L 3·36 (95% CI, 0·97–11·6) $P = 0{\cdot}055$ and not significant for the remaining variables).

The reduction in *JAK2 V617F* allele load respect to baseline (pre-treatment values) was analysed separately in PV and ET patients at 6, 12, 18, 24 and 36 months (Table SII). As shown in Fig 3, the differences were statistically significant both in PV and in ET at any time point respect to baseline values, indicating a clear decrease of *JAK2 V617F* allele load by HU. In addition, we compared the dynamics of *JAK2 V617F* allele reduction respect to basal values at different time points in PV versus ET treated patients. A progressive decrease of the *JAK2 V617F* allele load in PV versus ET patients over time was observed, with the difference from baseline levels being marginally significant at 24 months (median difference from baseline values in PV: −17·2% [range: −94·5 to 2·8] and in ET : −10% [range: −26·1 to 18·5]; $P = 0{\cdot}06$) and statistically significant at 36 months (median difference from baseline values in PV: −22·9% [range: −85·6 to 2·8] and in ET : −9·55% [range: −26·2 to 16·2]; $P = 0{\cdot}01$).

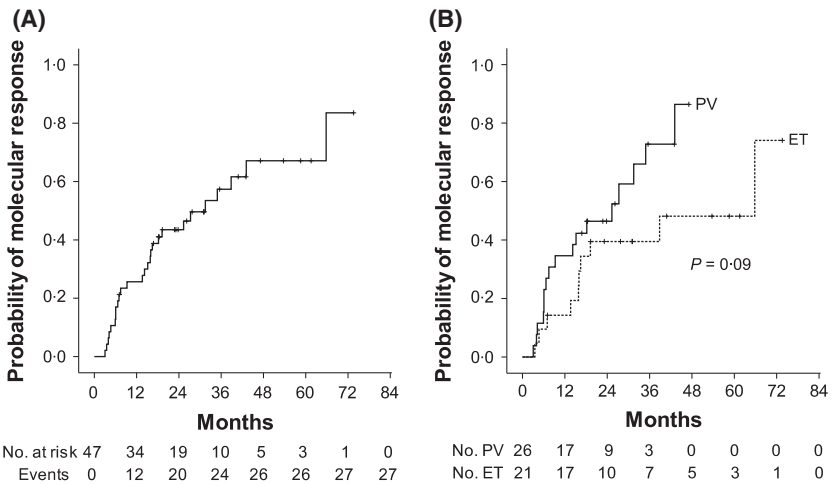

Fig 2. Kaplan–Meier estimate of the cumulative partial molecular response to initial hydroxyurea therapy. (A) Cumulative probability of achieving a partial molecular response in the whole cohort. (B) Cumulative probability of achieving a partial molecular response according to diagnosis .

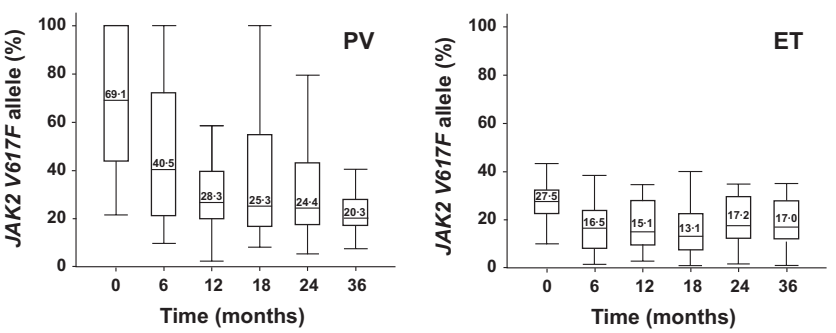

Fig 3. Dynamics of *JAK2 V617F* allele burden in PV and ET patients treated with hydroxyurea at different time points.

When the variation in the mutated allele load in the HU-treated group was compared with the variation of the *JAK2 V617F* allele burden in control patients, a highly significant decrease in the HU-treated group at 24 and 36 months was observed both in PV (median difference from baseline values in untreated patients at 24 months: 4·54% [range: −4·66 to 40·41] vs. treated patients −17·2% [range: −94·5 to 2·8] and at 36 months: 5·77% [range: −4·09 to 41·46] vs. −22·9% [range: −85·6 to 2·8]; $P < 0·001$) and ET patiens (median difference from baseline values in untreated patients at 24 months: 1·6% [range:−7·2 to 9·1] vs. treated patients −10% [range: −26·1 to 18·5]; and at 36 months: 2·8% [range:−4·1 to 18·1] vs. −9·6% [range: −26·2 to 16·2]; $P < 0·001$).

Discussion

This prospective study from a single institution assessed at several time points the modulation of *JAK2 V617F* allele burden dynamics by HU in the largest group of PV and ET patients reported to date, as well as the molecular response according to the newly reported ELN criteria (Barosi *et al*, 2009). The spontaneous dynamics of *JAK2 V617F* mutated allele load was also studied in a control group of PV and ET patients who had never received any cytoreductive treatment.

This study demonstrates a reduction of *JAK2 V617F* allele burden by HU in a substantial proportion (57%) of therapy-naive PV and ET patients. The ability of HU to reduce the *JAK2 V617F* allelic load in PV and ET patients has been previously reported with contradictory results. Whereas four prospective studies have shown a variable decrease in *JAK2 V617F* allele burden by HU in a limited number of patients (Girodon *et al*, 2008; Ricksten *et al*, 2008; Theocharides *et al*, 2008; Spanoudakis *et al*, 2009), others have not confirmed this effect (Larsen *et al*, 2009; Antonioli *et al*, 2010). The heterogeneity in the population reported in these studies, including newly treated and already on-treatment patients, makes an accurate interpretation of the results difficult. In addition, the criteria used for molecular response have also been different among studies, varying from a reduction ≥30% of the *JAK2 V617F* allelic load (Girodon *et al*, 2008) to non-quantitative analysis of *JAK2 V617F* mutation (Spanoudakis *et al*, 2009). Another factor that may account for the divergences among the published reports is the different sensitivity of the techniques used and the different types of cell population analysed. In our hands, the sensitivity of

the quantitative PCR assay for cDNA *JAK2 V617F* in purified granulocytes is around 0·5% allelic ratio, providing a sensitive tool for detecting small amounts and variations of mutant allele load. However, in spite of the differences found concerning the nature of the studies (prospective or retrospective), type of population analysed (newly versus already treated), definition of molecular response, and technique sensitivity, there is some agreement in that HU barely modifies the amount of mutated alleles in patients already on-treatment (Theocharides *et al*, 2008; Larsen *et al*, 2009; Antonioli *et al*, 2010), whereas a variable reduction in the clonal cell population can be observed in newly treated patients (Girodon *et al*, 2008; Ricksten *et al*, 2008; Theocharides *et al*, 2008). Moreover, a continuous decrease of the *JAK2 V617F* allelic load in PV patients after 36 months of treatment, in contrast to ET patients who remain in a relatively steady mutated allelic load was also observed. To our knowledge, this finding has not been previously described.

An important finding of our study refers to the variables predictive for attaining a molecular response in PV and ET patients. It is noteworthy that in multivariate analysis, haematocrit ≥0·45 L/L was the only variable predictive for obtaining a molecular response, suggesting that *JAK2 V617F*-positive MPN with an erythrocytosic phenotype are more likely to achieve a molecular response.

Finally, we observed no significant variation in *JAK2 V617F* allele load in a group of 45 PV and ET control patients. The natural dynamics of *JAK2 V617F* allele burden in PV and ET patients not receiving chemotherapy has been reported previously by some groups (Girodon *et al*, 2008; Theocharides *et al*, 2008) and recently in a retrospective study (Antonioli *et al*, 2010). Our results, with a longer follow-up than the above reported studies, confirm the absence of a time-dependent increase of *JAK2 V617F* allele burden in the majority of non-treated PV and ET patients. However, in a small number of ET and PV cases a remarkable increase higher than 50% of the initial values was observed.

In conclusion, first-line HU significantly decreased the *JAK2 V617F* allelic ratio both in PV and ET patients, with more than 50% of patients achieving a molecular response according to the ELN criteria especially in those cases with an erythrocytosic phenotype. The effect of HU in reducing *JAK2 V617F* allele burden in PV patients was maintained over the treatment period. These data show the ability of HU to modulate *JAK2 V617F* allele burden in PV and ET patients, which may be useful to compare the efficacy of other cytoreductive agents, such as pegylated interferon or new JAK2 inhibitors. The clinical effect of the reduction in *JAK2 V617F* allele load in the control of vascular complications is unknown and should be explored in future studies.

Acknowledgements

This work was supported by grants from the Spanish Health Ministry ''Fondo de Investigación Sanitaria EC07/90791 and from Mútua Madrileña 2007. LMA is recipient of a fellowship FI2008 from AGAUR.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table SI. JAKV617 allele percentage at different time points in untreated PV and ET patients.

Table SII. JAKV617 allele percentage at different time points in treated PV and ET patients.

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References

Álvarez-Larrán, A., Bellosillo, B., Martínez-Avilés, L., Saumell, S., Salar, A., Abella, E., Gimeno, E., Serrano, S., Florensa, L., Sánchez, B., Pedro, C. & Besses, C. (2009) Postpolycythaemic myelofibrosis: frequency and risk factors for this complication in 116 patients. *British Journal of Haematology*, **146,** 504–509.

Antonioli, E., Carobbio, A., Pieri, L., Pancrazzi, A., Guglielmelli, P., Delaini, F., Ponziani, V., Bartalucci, N., Tozzi, L., Bosi, A., Rambaldi, A., Barbui, T. & Vannucchi, A. (2010) Hydroxyurea does not appreciably reduce JAK2 V617F allele burden in patients with polycythemia vera or essential thrombocythemia. *Haematologica*, **95,** 1435–1438.

Barosi, G., Birgegard, G., Finazzi, G., Griesshammer, M., Harrison, C., Hasselbalch, H.C., Kiladjian, J.J., Lengfelder, E., McMullin, M.F., Passamonti, F., Reilly, J.T., Vannucchi, A.M. & Barbui, T. (2009) Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. *Blood*, **113,** 4829–4833.

Bellosillo, B., Martínez-Avilés, L., Gimeno, E., Florensa, L., Longarón, R., Navarro, G., Salar, A., Espinet, B., Sole, F., Serrano, S. & Besses, C. (2007) A higher JAK2 V617F-mutated clone is observed in platelets than in granulocytes from essential thrombocythemia patients, but not in patients with polycythemia vera and primary myelofibrosis. *Leukemia*, **21,** 1331–1332.

Campbell, P.J. & Green, A.R. (2006) The myeloproliferative disorders. *The New England Journal of Medicine*, **355,** 2452–2466.

Finazzi, G. & Barbui, T. (2008) Evidence and expertise in the management of polycythemia vera and essential thrombocythemia. *Leukemia*, **22,** 1494–1502.

Girodon, F., Schaeffer, C., Cleyrat, C., Mounier, M., Lafont, I., Santos, F.D., Vidal, A., Maynadie, M. & Hermouet, S. (2008) Frequent reduction or absence of detection of the JAK2-mutated clone in JAK2 V617F-positive patients within the first years of hydroxyurea therapy. *Haematologica*, **93,** 1723–1727.

Kiladjian, J.J., Cassinat, B., Chevret, S., Turlure, P., Cambier, N., Roussel, M., Bellucci, S., Grandchamp, B., Chomienne,

C. & Fenaux, P. (2008) Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. *Blood*, **112,** 3065–3072.

Kroger, N., Badbaran, A., Holler, E., Hahn, J., Kobbe, G., Bornhauser, M., Reiter, A., Zabelina, T., Zander, A.R. & Fehse, B. (2007) Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. *Blood*, **109,** 1316–1321.

Larsen, T.S., Pallisgaard, N., de Stricker, K., Moller, M.B. & Hasselbalch, H.C. (2009) Limited efficacy of hydroxyurea in lowering of the JAK2 V617F allele burden. *Hematology*, **14,** 11–15.

Levine, R.L., Belisle, C., Wadleigh, M., Zahrieh, D., Lee, S., Chagnon, P., Gilliland, D.G. & Busque, L. (2006) X-inactivation-based clonality analysis and quantitative JAK2 V617F assessment reveal a strong association between clonality and JAK2 V617F in PV but not ET/MMM, and identifies a subset of JAK2 V617F-negative ET and MMM patients with clonal hematopoiesis. *Blood*, **107,** 4139–4141.

Quintás-Cardama, A., Kantarjian, H., Manshouri, T., Luthra, R., Estrov, Z., Pierce, S., Richie, M.A., Borthakur, G., Konopleva, M., Cortes, J. & Verstovsek, S. (2009) Pegylated interferon alfa-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. *Journal of Clinical Oncology*, **27,** 5418–5424.

Ricksten, A., Palmqvist, L., Johansson, P. & Andreasson, B. (2008) Rapid decline of JAK2 V617F levels during hydroxyurea treatment in patients with polycythemia vera and essential thrombocythemia. *Haematologica*, **93,** 1260–1261.

Sirhan, S., Lasho, T.L., Hanson, C.A., Mesa, R.A., Pardanani, A. & Tefferi, A. (2008) The presence of JAK2 V617F in primary myelofibrosis or its allele burden in polycythemia vera predicts chemosensitivity to hydroxyurea. *American Journal of Hematology*, **83,** 363–365.

Spanoudakis, E., Bazdiara, I., Kotsianidis, I., Margaritis, D., Goutzouvelidis, A., Christoforidou, A., Tsatalas, C. & Bourikas, G. (2009) Hydroxyurea (HU) is effective in reducing JAK2 V617F mutated clone size in the peripheral blood of essential thrombocythemia (ET) and polycythemia vera (PV) patients. *Annals of Hematology*, **88,** 629–632.

Tefferi, A., Skoda, R. & Vardiman, J.W. (2009) Myeloproliferative neoplasms: contemporary diagnosis using histology and genetics. *Nature Reviews. Clinical Oncology*, **6,** 627–637.

Theocharides, A., Passweg, J.R., Medinger, M., Looser, R., Li, S., Hao-Shen, H., Buser, A.S., Gratwohl, A., Tichelli, A. & Skoda, R.C. (2008) The allele burden of JAK2 mutations remains stable over several years in patients with myeloproliferative disorders. *Haematologica*, **93,** 1890–1893.

Vannucchi, A.M., Antonioli, E., Guglielmelli, P., Longo, G., Pancrazzi, A., Ponziani, V., Bogani, C., Ferrini, P.R., Rambaldi, A., Guerini, V., Bosi, A. & Barbui, T. (2007a) Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. *Leukemia*, **21,** 1952–1959.

Vannucchi, A.M., Antonioli, E., Guglielmelli, P., Rambaldi, A., Barosi, G., Marchioli, R., Marfisi, R.M., Finazzi, G., Guerini, V., Fabris, F., Randi, M.L., De Stefano, V., Caberlon, S., Tafuri, A., Ruggeri, M., Specchia, G., Liso, V., Rossi, E., Pogliani, E., Gugliotta, L., Bosi, A. & Barbui, T. (2007b) Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*, **110,** 840–846.

Vannucchi, A.M., Antonioli, E., Guglielmelli, P., Pardanani, A. & Tefferi, A. (2008) Clinical correlates of JAK2 V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. *Leukemia*, **22,** 1299–1307.

Vardiman, J.W., Harris, N.L. & Brunning, R.D. (2002) The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*, **100,** 2292–2302.