Hydroxyurea dose impacts hematologic parameters in polycythemia vera and essential thrombocythemia but does not appreciably affect *JAK2*-V617F allele burden

A recent publication by Antonioli *et al.* shows that the continuous use of hydroxyurea (HU) does not appreciably reduce *JAK2* V617F allele burden in patients with polycythemia vera (PV) or essential thrombocythemia (ET).[1] Our results confirm and extend these data. To assess the effects of a cytoreductive treatment on the *JAK2*-V617F allelic ratio, a single center retrospective study in myeloproliferative neoplasm (MPN) patients was performed. The effect of HU on the *JAK2*-V617F allele burden was evaluated in at least two sequential samples of 21 patients with PV or ET submitted to HU therapy and referred for molecular diagnosis. The *JAK2*V617F allele load was measured by an allele-specific PCR with fluorescent primers in DNA of density gradient purified granulocytes according to Jones *et al.*[2] with slight modifications.[3] As standards, serial dilutions of plasmids carrying the wild-type and V617F mutated *JAK2* sequences were used. *JAK2*-V617F allele burden was determined based on availability of serial samples of genomic DNA, laboratory data and clinical records of the 8 PV and 13 ET (one of them transforming to acute leukemia) patients, as shown in Figure 1. The *JAK2*-V617F allele burden was evaluated before and/or after initiation of HU therapy and compared to the last sample that was available during treatment.

We herein correlate changes in the *JAK2*-V617F allele burden with HU dose variation; hematocrit (Ht) and platelet counts during HU treatment for PV (Figure 2A) and ET patients (Figure 2B). Hydroxyurea usage was consistently associated with hematologic changes over time (Table 1). However, while HU dosage importantly impacted Ht (PV patients 3, 7, and 63) and platelet count (ET patients 4, 6, 10, 11 and 31), *JAK2*-V617F allele burden did not necessarily vary according to changes in HU doses. Although 3 PV patients (1, 2, and 3) and 5 ET patients (18, 31, 34, 51 and 54) evolved with *JAK2* fluctuations, these oscillations did not significantly impact allele burden over time. In general, the percentage of *JAK2*-V617F allele burden tended to remain stable through HU treatments.

While fluctuations with reductions of *JAK2*-V617F allele burden were seen when comparing pre-treatment *JAK2*-V617F allele load with the latest sample, patients with longer follow ups evolved with a stable allelic burden despite variations in hematologic counts and HU

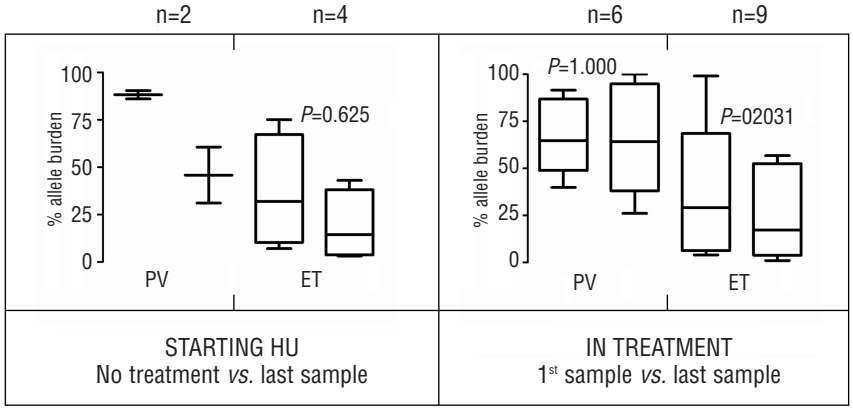

Figure 1. *JAK2*-V617F allele burden of patients with PV and ET. Patient samples were analyzed using the paired Wilcoxon's non-parametric test with *P*=0.05. Left panel represents a comparison of *JAK2*-V617F burden for patient's sample before or after HU initiation. Right panel shows *JAK2*-V617F allele burden for patients already in HU treatment, comparing the first *versus* the last patient's sample. HU: hydroxyurea; PV: polycythemia vera; ET: essential thrombocythemia.

Table 1.

Patient	Disease	Hematologic response to HU dose modifications	JAK2-V617F response to HU dose modifications
1	PV	HU independent oscillation*	JAK2 increase unrelated to HU
2	PV	HU induced oscillation*	JAK2 oscillation unrelated to HU
3	PV	HU induced oscillation*	JAK2 oscillation unrelated to HU
9	PV	HU induced discrete oscillation*	JAK2 unchanged (4th quartile)
53	PV	HU induced discrete oscillation*	JAK2 unchanged (4th quartile)
63	PV	HU induced oscillation*	JAK2 independent variation
6	ET	HU induced oscillation#	JAK2 unchanged (2nd quartile)
17	ET	HU induced oscillation#	JAK2 unchanged (1st quartile)
18	ET→AL	No variation#	JAK2 discrete variation (3rd → 2nd → 3rd quartile)
31	ET	HU induced oscillation#	HU induced JAK2 oscillation? (1st → 2nd → 1st quartile)
34	ET	HU induced oscillation#	JAK2 unchanged (1st quartile)
51	ET	HU induced oscillation#	JAK2 oscillation unrelated to HU
54	ET	HU induced oscillation#	JAK2 discrete variation (1st quartile)

**hematocrit value; # platelet counts; HU: hydroxyurea; PV: polycythemia vera; ET: essential thrombocythemia, AL: acute leukemia.*

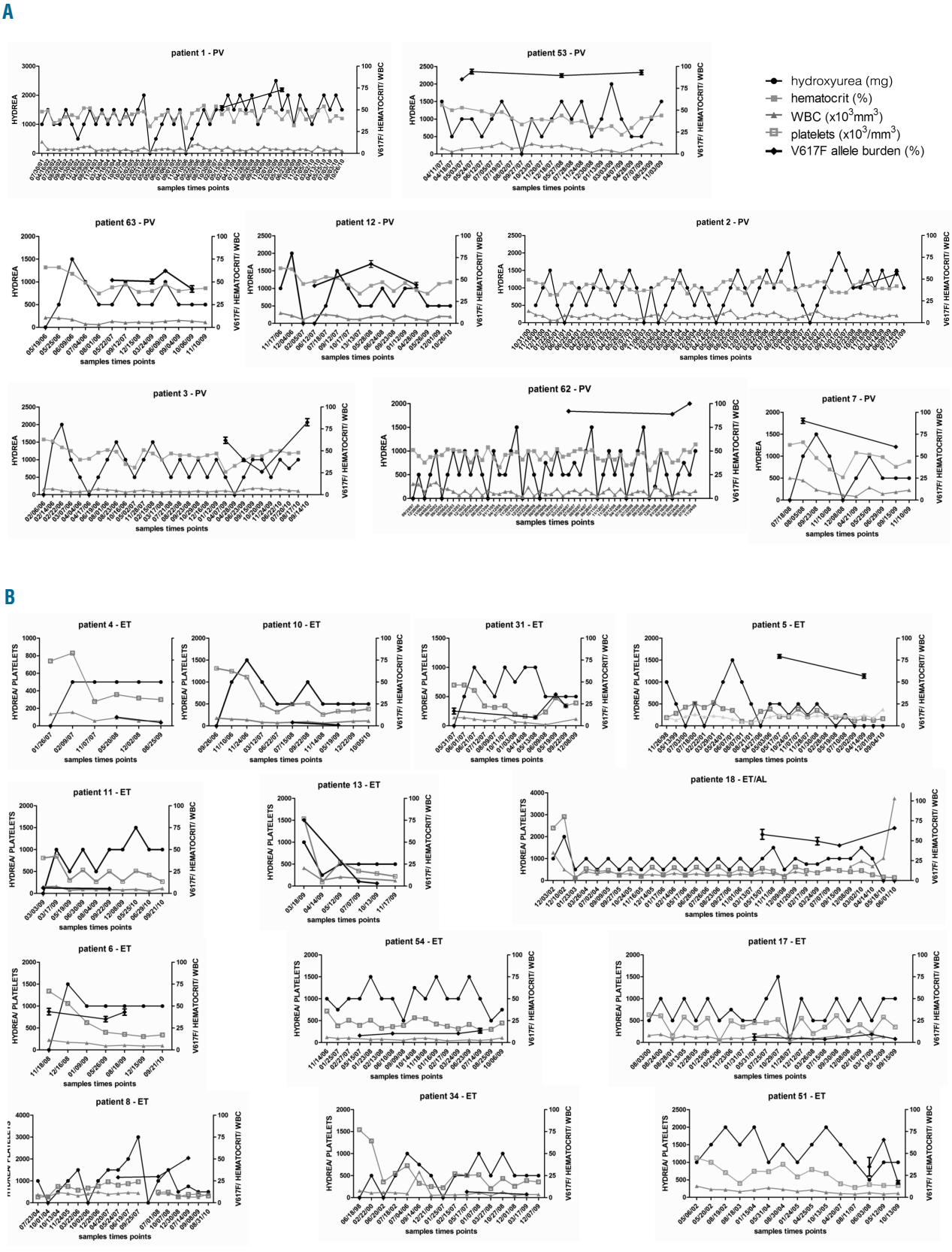

Figure 2. Graphic illustration of the dynamics of the *JAK2*-V617F allele burden, HU dose and hematologic parameters in patients during treatment. From the original 65 patients analyzed in our study, the 21 patients evaluated in at least two sequential samples once submitted to HU therapy are represented in this figure. (A) represents the evolution of single PV patients. (B) represents the evolution of single ET patients.

doses (median period of follow up of 20 months for PV and 21 months for ET).

The relationship between the kinetics of HU-mediated response and clone size of *JAK2*-V617V in MPN is still not clear. Although changes in the *JAK2*-V617F allele burden were reported at HU initiation or termination for individual PV or ET patients,[4,5,6,7] and during short follow ups,[4,6] it appears that after a certain threshold of *JAK2* allele burden is achieved, only slight fluctuations are seen.[4,6] Reports on long-term evaluation of patients receiving HU treatment, which show slight variations or stabilization of a certain value of *JAK2*-V617F allele load variable from patient to patient seem to reinforce this concept.[4,6,7]

It is noteworthy that patients who presented broader fluctuations of *JAK2*-V617F allele burden (those changing from a former assigned quartile to another) were those with intermediate ratios of *JAK2*-V617F allele burden (2nd and 3rd quartile). In contrast, patients situated in the boundaries of a *JAK2*-V617F mutation ratio scale (1st and 4th quartile) showed no major modification of *JAK2* V617F allele burden in response to HU variations (Figure 2 and Table 1).

Our study has limitations owing to the small size of the sample and the short follow up. Nevertheless, the results reinforce the idea that hematologic parameters are deeply impacted by HU dosage, as evidenced by the decrease in platelet and red blood cell counts in response to HU dose elevation and the increase under HU dose reduction. Moreover, it suggests that, in the lower and upper extremes (1st or 4th quartile) of allele burden detection, the variation of *JAK2*-V617F allele burden is less evident.

The findings herein lead to interesting questions regarding the effect of HU in hematologic compartments (as those seen in Ht or platelet counts in our data) as compared to its effect on *JAK2*-V617F positive cells. The kinetics of *JAK2*-V617F *versus* Ht in PV or platelet counts in ET suggest that *JAK2*-V617F allele burden is, at least to some extent, dissociated from HU effects on the hematologic parameters. Since pharmacological JAK inhibition has been shown to impact spleen size in MPN patients,[8] it will be of great interest to observe the effect of JAK inhibitor dose oscillations on *JAK2*-V617F allele burden.

Ilana Renault Zalcberg,[1] Jackline Ayres-Silva,[1,2] Alexandre Mello de Azevedo,[3] Cristiana Solza,[3] Adelmo Daumas,[4] and Martin Bonamino[2]

[1]Bone Marrow Transplantation Center (CEMO) Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; [2]Division of Experimental Medicine, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; [3]Universidade do Estado do Rio de Janeiro (UERJ); [4]Universidade Federal Fluminanse (UFF)

Correspondence: Martín H. Bonamino, Divisão de Medicina Experimental, INCA, Rua Andre Cavalcanti 37/6º andar Centro, 20231-050, Rio de Janeiro - Brasil. Phone: +55 21 32076547; Fax: +55 21 32076536; E-mail:mbonamino@inca.gov.br

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