

To the editor:

JAK2V617F complete molecular remission in polycythemia vera/essential thrombocythemia patients treated with ruxolitinib

Polycythemia vera (PV) and essential thrombocythemia (ET) are characterized by *JAK2V617F* mutation in 95% and 60% of the patients, respectively.¹ Ruxolitinib is a JAK1/JAK2 inhibitor approved for myelofibrosis (MF) and more recently for hydroxyurea resistant/intolerant PV patients because of its superiority to standard therapy (and placebo in MF) in improving splenomegaly, ameliorating symptoms, and reducing phlebotomies (in PV).² A modest reduction of the *JAK2V617F* allele burden (8% from baseline at 72 weeks) was observed in MF patients in the COMFORT-II study.³ A progressive decrease of the *JAK2V617F* allele burden by a mean of 22% at 36 months was reported in 34 PV patients enrolled in a phase 2 trial (INCB18424-256, ClinicalTrials.gov #NCT00726232) that also included 22 ET patients, with 23.5% of the patients achieving a $\geq 50\%$ reduction; however, no complete molecular remission (CMR) was attained at that time.⁴ Comparably, in the phase 3 RESPONSE study in PV, the mean decrease of *JAK2V617F* allele burden at week 32 (n = 92) and at week 112 (n = 22) was 12.2% and 34.7%, respectively.²

Twenty-two *JAK2V617F*-mutated patients, 11 PV and 11 ET, were enrolled in our center in the INCB18424-256 study, and 19 have been followed for >5 years. We measured the *JAK2V617F* allele burden by 2 reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) assays (sensitivities of 0.8% and 0.08%),⁵ and deep amplicon resequencing (Ion Torrent platform).⁶ Approval was obtained from the Azienda Ospedaliero-Universitaria Careggi institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Overall, *JAK2V617F* allele burden decreased by a mean of 19% and 28% from baseline at 36 and 60 months, respectively, consistent with previous reports.^{2,4} Among 13 patients showing a sustained reduction of the allele burden >25% at 60 months, the mean decrease was 48% (Figure 1A). Notably, 3 patients (1 PV, 2 ET) achieved a $\geq 50\%$ allele burden reduction after 2 years, and progressed to a CMR at 5 years (Figure 1B). Their mean allele burden was 46.6% at baseline and 28.3%, 16.3%, 8.7%, and 0% at 1, 2, 3, and 5 years, respectively (Figure 1B). A *JAK2V617F* CMR status was confirmed by both RT-qPCR assays and deep resequencing 3 months after the first observation. The 3 patients had normal karyotype both at baseline and at 5 years, and the 2 ET patients were *MPL* and *CALR* wild-type. Additionally, a *TET2* Y867H mutation with an allele burden of 48.9% at baseline, remaining unchanged at 5 years (52%), was found in the PV patients.

At the time of CMR, the PV patient was in complete hematologic remission.⁴ However, bone marrow evaluation showed normalization of myeloid and megakaryocyte lineage but persistence of erythroid hyperplasia. Conversely, at the time of CMR, the 2 ET patients were in partial hematologic remission due to their platelet counts of $422 \times 10^9/L$ and $812 \times 10^9/L$; the bone marrow biopsy at 5 years showed slight megakaryocyte hyperplasia without morphologic abnormalities and no fibrosis. To exclude selective persistence of the *JAK2V617F* mutation in the megakaryocyte lineage, we evaluated the mutation in platelet-rich plasma RNA but found no evidence of it.⁷

Until now, *JAK2V617F* CMR has been reported in 14% to 24.1% of PV patients and 6% to 17% of ET patients receiving interferon⁸

and in 9% of MF patients treated with imetelstat.⁹ Reported effects of hydroxyurea were variable, with some series reporting CMR in 12% to 26% of ET patients and in 8% to 17% of PV patients, whereas other studies showed only modest allele burden decrease in few patients.^{10,11}

Our data confirmed the overall modest *JAK2V617F* allele burden reduction seen in previous studies in patients receiving ruxolitinib but indicated that some (16% in this series) may attain a *JAK2V617F* CMR

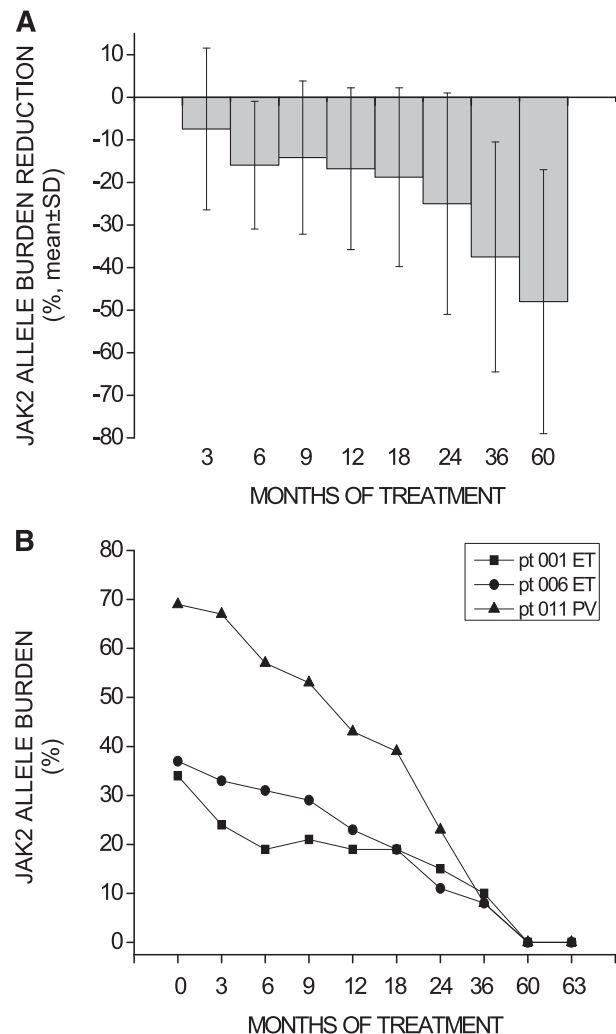


Figure 1. *JAK2V617F* allele burden decrease in patients achieving >25% reduction at 60 months and details of patients attaining complete molecular remission. (A) The percentage decline over time (mean \pm SD) of the *JAK2V617F* allele burden in the 13 patients who presented a >25% allele burden reduction at 60 months. *JAK2V617F* allele burden decreased by a mean of 7%, 11%, 19%, and 28% at 1, 2, 3, and 5 years, respectively. (B) The absolute level of *JAK2V617F* allele burden in the 3 patients (pt) who finally achieved a CMR at 5 years (confirmed 3 months later) is presented. Measurement of the *JAK2V617F* allele burden was performed in peripheral blood granulocytes by RT-qPCR. The attainment of CMR was further confirmed by both a high-sensitivity RT-qPCR assay (detection limit, 0.08%) and deep resequencing at 5 years and at +3-month time points. SD, standard deviation.

with prolonged treatment. The persistence of other mutations, such as *TET2*, may suggest biclonal disease or a single ancestral *TET2*-mutated founder clone later acquiring *JAK2V617F*; we could not distinguish between these 2 possibilities, lacking viable cells for clonal analysis.

The attainment of *JAK2V617F* CMR occurring notwithstanding the persistence of some PV- and ET-associated features might reflect either additional clone(s) with unknown mutations insensitive to *JAK2* inhibition or different kinetics of normalization of histologic and hematologic parameters. Larger studies are required to establish the frequency of CMR with *JAK2* inhibitors and its relevance for the management of these chronic diseases.

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