## JAK2$^{V617F}$ allele burden is reduced by busulfan therapy: a new observation using an old drug

Busulfan, a highly effective and established drug for treating polycythemia vera (PV) produces lasting clinical and hematologic responses.[1] The frequency of its use as therapy for PV has recently diminished owing to concerns of increased leukemogenicity.[1] However, in a pivotal phase III trial of busulfan *versus* $P^{32}$ in patients with PV, treatment with busulfan resulted in long-term clinical and hematologic responses and superior 10-year overall survival (70% vs. 55%).[1,2] Toxicity was minimal and there was no increase in early leukemia as seen with chlorambucil.[1] The long-term incidence of acute leukemia was also considered "very low" (1% at 8 years).[1]

Since nearly all patients with PV carry the JAK2$^{V617F}$ mutation,[3] several groups, including the European Leukemia Net (ELN), have incorporated molecular response (decrease in JAK2$^{V617F}$ allele burden) as a measure of treatment efficacy.[4] The hematologic and molecular responses to recombinant interferon alpha (rIFNα) and hydroxyurea (HU) in PV have been characterized.[5-8] Sustained molecular responses following pegylated-rIFNα therapy have been observed by some clinicians[5,6] but not by others.[8] Similar variability in molecular response has been observed with HU.[8] Owing to its reduced use in the JAK2$^{V617F}$ era, clinical and laboratory data for busulfan are limited. Therefore, we call attention to the effectiveness of busulfan in PV patients refractory to rIFNα and HU, and its efficacy in reducing the JAK2$^{V617F}$ allele burden in these patients.

We treated 6 PV patients with busulfan, all of whom were refractory to multiple drugs including (HU), rIFNα, imatinib, dasatinib, and anagrelide. Starting doses ranged from 2 mg to 4 mg daily; the dose was then titrated based on clinical response and toxicity. Clinical and hematologic response was graded according to PVSG criteria and molecular response according to ELN criteria.[4] JAK2$^{V617F}$ allele burdens were determined by pyrosequencing, which quantifies mutant alleles more than 5%.3 If negative by pyrosequencing, we used an ARMS-PCR assay with a sensitivity of 0.1%.[3] Phlebotomy was performed to maintain the hematocrit (Hct) less than 45% for men and 42% for women. Treatment with busulfan was discontinued if patients experienced adverse side effects attributable to busulfan (respiratory or dermatological problems) and/or had platelet counts less than 100x10$^9$/L while in clinical remission.

Median duration of therapy for all 6 patients prior to starting busulfan was 56.5 months (range 12-82 months). Within three months of starting busulfan, all had complete hematologic responses (CHR) (Table 1). The 5 patients who had significant molecular response on busulfan had pre-treatment JAK2$^{V617F}$ allele burdens of 100%, 26%, 83%, 77%, and 27%, respectively. Of these, 2 had complete molecular response (CMR) after six and eight months respectively, and 3 had partial molecular response (PMR) after three, six, and 18 months, respectively. The patient with a pre-treatment allele burden of 100% attained CMR within six months of starting busulfan, at which point therapy was discontinued owing to a decrease in platelet count to less than 100 x 10$^9$/L. She has since maintained CHR and CMR for three years without any treatment. The other patient with CHR and CMR had a pre-treatment allele burden of 26%; he has maintained complete molecular response on busulfan 2 mg weekly without any toxicity to date. Of the 3 patients with PMR, 2 continue to have significant decline in JAK2$^{V617F}$ allele burdens from pre-treatment values of 83% and 27%, respectively (Table 1 and Figure 1). Treatment of the third patient with PMR was discontinued after three months owing to his preference to discontinue all cytoreductive therapy. He attained CHR and PMR while on treatment (decrease in JAK2$^{V617F}$ allele burden from 77% to 24%) with subsequent increase in the JAK2$^{V617F}$ allele burden to 71% over a period of 13 months after busulfan was discontinued, while still in clinical remission. The remaining patient had no change in her JAK2$^{V617F}$ allele burden after 60 months of treatment, despite maintaining CHR. The only grade 3 adverse event, observed over a median treatment duration of 15 months (range 3-60 months) was thrombocytopenia, requiring treatment discontinuation in one patient.

The rapid and dramatic reduction in JAK2$^{V617F}$ allele burden along with complete hematologic response, in both homozygous and heterozygous disease states is notewor-

**Table 1.** Demographics and treatment results of 6 patients treated with busulfan for PV.

| Patient | Age (year)-Gender (M/F) | Prior treatments -duration (year) | Busulfan dose | Rx duration (mos) | Adverse effects | Hematologic response/time to response (mos) | Molecular response/time to response (mos) | JAK2$^{V617F}$ allele burden Pre-busulfan | JAK2$^{V617F}$ allele burden Post-busulfan |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 76-F | HU-4 | 4 mg q.d. | 3 | Thrombocytopenia 594 to 64 (x10$^8$/L) | CHR/3 | CMR/6 | 100.0% | 0.0% |
| 2 | 82-M | HU-5 | 2 mg q.d. | 12 | None | CHR/2 | CMR/8 | 26.1% | 0.0% |
| 3 | 82-M | Anagrelide-1 | 2 mg t.i.w. | 6 | None | CHR/3 | PMR/6 | 77.4% | 24.2% |
| 4 | 70-F | HU+anagrelide-1 Imatinib-2 Dasatinib-2 IFNa-1 | 2 mg t.i.w. | 24 | None | CHR/1 | PMR/6 | 83.2% | 22.2% |
| 5 | 84-F | HU-2 Dasatinib-3 Imatinib-1 | 2 mg q.i.w. | 13 | None | CHR/3 | PMR/18 | 27.4% | 9.7% |
| 6 | 81-F | HU-1 | 2 mg t.i.w. | 60 | None | CHR/3 | None | 45.5% | 36.8% |

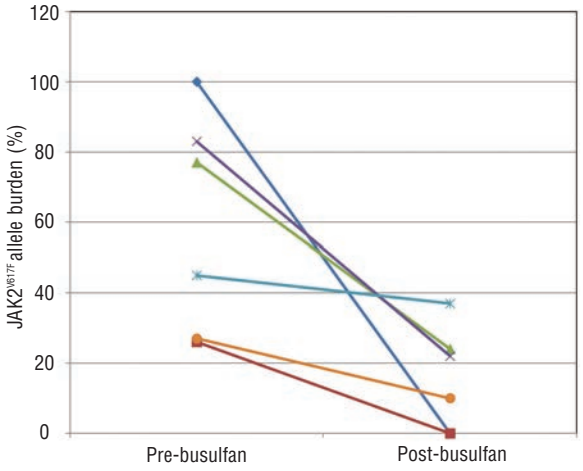


**Figure 1.** Change in JAK2$^{V617F}$ allele burden with busulfan treatment.

thy. That such responses did not occur in all patients treated with busulfan may relate to disease heterogeneity involving other mutations in PV[8,9] which may similarly contribute to the variability in disease response seen with rIFNα and HU. Our patients were not screened for other known mutations in PV.[9]

Despite its excellent response rates, busulfan is not commonly used as first-line therapy for PV because of concerns regarding leukemogenicity. However, there is little substantive data in the literature that mechanistically validate this. As reported previously, several differences in its mechanism of action may render busulfan less leukemogenic than other alkylating drugs such as chlorambucil, including its lower DNA binding capacity, decreased ability to form inter- or intra-strand linkages, and its preferential activity on cells in the G0 phase of the cell cycle.[2,10-12]

In several clinical studies of busulfan in PV and other diseases, clinicians have raised concerns regarding the risk of leukemia with busulfan treatment,[2,13-15] but only a few reported the actuarial risk.[2,13] In a prospective analysis of the ECLAP study, those patients treated with alkylating agents including busulfan, P32, and pipobroman had a higher risk of AML/MDS (HR, 5.46; 95%CI: 1.84-16.25; *P*=0.0023) compared to those treated with phlebotomy or interferon at a median follow up of 8.4 years from diagnosis.[16] The dosage and duration of treatment with busulfan were not specified. In another prospective study of patients with essential thrombocythemia (ET), sequential treatment with busulfan and HU was associated with a 5.4% annual incidence of AML/MDS, compared to 0.6% and 0.0% for HU and no treatment, respectively.[17] The dose of busulfan in this study was 4 mg daily for two weeks followed by 2 mg daily.[17] In the EORTC study, which reported a 1% incidence of leukemia in busulfan-treated patients over a median follow up of eight years, the authors also emphasized the importance of low dosage,[2] suggesting that leukemogenicity may be a dose-related risk. While this notion requires validation with long-term prospective studies, our own clinical observations thus far suggest that low-dose busulfan, as we used here, is a safe and effective treatment strategy in refractory PV, and importantly, is associated with an excellent quality of life.

In summary, of 6 patients with multidrug refractory PV, all had rapid and sustained hematologic responses to busulfan at low dose, while 5 had significant and lasting molecular responses. In addition to confirming its known clinical efficacy in PV, we make the new observation that busulfan also dramatically decreases the JAK2$^{V617F}$ allele burden in treatment refractory patients. In the light of the data showing increased risk of secondary leukemia with standard dose busulfan,[16,17] longer follow up is needed to establish the safety of a low-dose treatment strategy with respect to this parameter. Given its low cost and relatively favorable toxicity profile at low doses, we suggest that low-dose busulfan should be used as a comparator in large-scale studies of newer, more costly drugs in PV that are refractory to multiple other drugs, including HU.

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