

## LETTER OPEN



## CHRONIC MYELOPROLIFERATIVE NEOPLASMS

## Long-term outcomes of polycythemia vera patients treated with ropeginterferon Alfa-2b

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## TO THE EDITOR:

Interferon alfa not only restores normal blood cell counts in patients with polycythemia vera (PV) but can diminish the mutant *JAK2V617F* allele burden [1–3]. After discontinuing long-term interferon therapy, hematologic responses may persist [4, 5], which is more likely in patients achieving *JAK2V617F* allele burden <10% before stopping treatment [6]. Allele burden declines gradually during interferon treatment [7]; however, no data from large, prospective, clinical studies of long-term treatment with pegylated alfa interferons are available.

Results from 3 years' treatment in the phase 3, open-label, randomized clinical trial PROUD-PV and its phase 3b extension trial CONTINUATION-PV [8] led to regulatory approval of ropeginterferon alfa-2b (BESREMi®), a novel, monopegylated interferon alfa-2b with an extended administration interval of 2–4 weeks, in the European Union. The compound was subsequently approved by the US FDA for first-line treatment of PV. Here we report hematologic and molecular responses and safety results after 5 years' treatment in PROUD-PV and CONTINUATION-PV, which compared the efficacy and safety of ropeginterferon alfa-2b with hydroxyurea in the first year, and with best available treatment (BAT) thereafter.

Study design and methods were published previously [8]; selection criteria, dosing and endpoints are described in the supplement (Supplementary Tables 1–3 and Supplementary Fig. 1). *JAK2V617F*-positive patients with PV who were hydroxyurea naïve or hydroxyurea pre-treated for <3 years without complete response, resistance or intolerance were randomized 1:1 (stratified by age, history of thromboembolic events and hydroxyurea pretreatment) in PROUD-PV to receive ropeginterferon alfa-2b or hydroxyurea for 12 months. Dosing increased until blood counts

normalized. Patients completing PROUD-PV were invited to enter CONTINUATION-PV: the ropeginterferon alfa-2b arm continued the same treatment with individualized dosing every 2, 3, or 4 weeks, and patients allocated to hydroxyurea in PROUD-PV received BAT (hydroxyurea or another standard first-line treatment with individualized dosing) in CONTINUATION-PV.

Assessment visits were performed 3–6 monthly to determine efficacy (*JAK2V617F* allelic burden [*JAK2V617F* ipsogen® *JAK2* MutaQuant® kit, QIAGEN GmbH, Hilden, Germany; limit of background: 0.014%], hematocrit, platelet, leukocyte and erythrocyte counts, phlebotomy requirement, spleen size, and quality of life) and safety parameters (including clinical chemistry, immunological parameters, urinalysis, Hospital Anxiety and Depression Scale score and adverse events).

The studies were conducted in accordance with the Declaration of Helsinki, received ethics committee approval, and were registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (#NCT01949805; #NCT02218047). All patients gave informed consent.

Efficacy data up to Month 60 were analyzed in patients who entered CONTINUATION-PV ( $N = 171$ ; full analysis set, treatment as assigned). Safety data up to database lock (29 May 2020) were analyzed for all patients treated ( $N = 254$ ). Efficacy between treatment groups was compared using a log binomial regression model. Rate ratios (RR) of responders between arms and 95% CI were calculated from estimates of regression coefficients. Last observation carried forward was imputed for molecular parameters. Safety was analyzed descriptively. Statistical Analysis System® software was used (version 9.3 or higher, SAS Institute, Cary, NC, USA).

Patient characteristics and disposition are reported in Supplementary Table 4 and Supplementary Fig. 2. At database lock, 70/

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95 patients in the ropeginterferon alfa-2b arm and 57/76 in the BAT arm remained in the extension study, while 25/95 (26.3%) and 19/76 (25.0%), respectively had discontinued. Most patients in the BAT arm received only hydroxyurea, but at 60 months, 5 had switched to interferon therapy (ropeginterferon alfa-2b, pegylated interferon alfa-2a, or recombinant interferon alfa-2a) and 2 had switched to ruxolitinib.

After 5 years' treatment, 53/95 patients (55.8%) in the ropeginterferon alfa-2b arm and 33/75 (44.0%) in the control arm achieved complete hematologic response (CHR) according to modified European LeukemiaNet (ELN) criteria [9] (RR: 1.30 [95% CI: 0.95 to 1.77];  $p = 0.0974$ ; discontinued patients were considered non-responders). Response rates in a prospectively planned sensitivity analysis of CHR with imputation of the last observation carried forward were 69/95 (72.6%) in the ropeginterferon alfa-2b arm versus 40/76 (52.6%) in the control arm at Month 60 (RR: 1.43 [95% CI: 1.12–1.81];  $p = 0.004$ ).

The molecular response rate at 5 years according to ELN criteria [9] was significantly higher in the ropeginterferon alfa-2b arm than in the control arm (65/94 [69.1%] versus 16/74 [21.6%], respectively; RR: 3.04 [95% CI: 1.96–4.71];  $p < 0.0001$ ). The median *JAK2V617F* allele burden declined continuously during ropeginterferon alfa-2b treatment, from 37.3% at baseline (before treatment in PROUD-PV) to 8.5% at 60 months (Fig. 1). In contrast, the median *JAK2V617F* allele burden in the control arm decreased from 38.1% at baseline to 18.2% at 12 months but rebounded to 44.4% by 60 months (comparison of treatment arms:  $p < 0.0001$ ). *JAK2V617F* allele burden  $< 10\%$ , achieved in 50/92 (54.3%) ropeginterferon alfa-2b-treated patients at Month 60 (Supplementary Table 5), correlated with lower age and lower *JAK2V617F* allele burden at baseline (Supplementary Table 6) and was associated with a higher complete hematologic response rate at Month 60 (Supplementary Table 7). Notably, allele burden decreased to  $< 1\%$  in 18/92 patients (19.6%) receiving ropeginterferon alfa-2b whereas in the control arm, only 1 patient (1.4%) achieved an allele burden  $< 1\%$  at 60 months ( $p = 0.0002$ ). Mutant *JAK2V617F* allele burden routinely assessed in peripheral blood yields only a reflection of the effect in the bone marrow. Ropeginterferon alfa-2b has been shown to target the malignant cancer stem cell clone in patients [2]; its sustained effect on allele burden aligns with the hypothesis of gradual clonal exhaustion [10].

Disease progression (secondary myelofibrosis or leukemic transformation) and major thromboembolic events were assessed

over a cumulative exposure period of 499 and 401 patient years in the ropeginterferon alfa-2b and control arms, respectively. The incidence of disease progression among ropeginterferon alfa-2b treated patients was 0.2%-patient years (1 case of myelofibrosis) versus 1.0%-patient years in the control treatment arm (2 cases of myelofibrosis and 2 cases of acute leukemia). Regarding major thromboembolic events, 5 events in 4 patients in the ropeginterferon alfa-2b arm and 5 events in 5 patients in the control arm occurred, incidence rates of 1.0%-patient year and 1.2%-patient year respectively. Thromboembolic events did not appear to correlate with higher hematocrit levels or greater phlebotomy need. All affected patients were aged  $\geq 60$  years and thus considered at high risk; no marked difference between the treatment arms was observed regarding other cardiovascular risk factors.

Rates of adverse events, serious adverse events and treatment-related adverse events over the entire treatment period were balanced between the study arms (Table 1). The most common adverse events (in  $> 10\%$  of patients) in the ropeginterferon alfa-2b arm regardless of causality were thrombocytopenia, anemia, leukopenia, elevated hepatic enzymes, arthralgia, fatigue, headache, dizziness, splenomegaly, pyrexia, and back pain. In control-treated patients the most frequent adverse events were thrombocytopenia, anemia, leukopenia, fatigue, headache, nausea, diarrhea, influenza, and nasopharyngitis. Most adverse events were of grade 1–2 intensity. Treatment-related adverse events of grade  $\geq 3$  occurred in 21/127 patients (16.5%) in each arm; these included only 1 grade 4 event (increased gamma-glutamyltransferase in the ropeginterferon-alfa-2b arm) and 1 grade 5 event (acute leukemia with fatal outcome in the control arm). In total, 13/127 patients (10.2%) in the ropeginterferon-alfa-2b arm and 4/127 (3.1%) in the control arm discontinued treatment due to drug-related adverse events, approximately half of which occurred during the first year (in 6/13 and 2/4 patients, respectively).

Overall, results of the present analysis over  $\geq 5$  years' treatment further indicate that ropeginterferon alfa-2b is an effective and safe option for longer-term treatment that induces a sustained molecular response, confirming data from an earlier phase I/II study also using ropeginterferon alfa-2b [11]. The majority of ropeginterferon alfa-2b-treated patients (54.3%) achieved a *JAK2V617F* allele burden  $< 10\%$  at 5 years and might be potential candidates for treatment discontinuation. Applying more stringent criteria (allele burden  $< 10\%$ , sustained complete hematologic response [hematocrit  $< 45\%$  without phlebotomy in the last 3 months, platelet count  $< 400 \times 10^9/L$  and leukocyte count  $< 10 \times 10^9/L$ ; drop-outs considered non-responders] for  $\geq 2$  years, and no disease progression, thromboembolic events, or worsening of disease-related signs or symptoms over the entire treatment period), 30.4% of patients who received ropeginterferon alfa-2b (versus 4.2% in the control arm;  $p < 0.0001$ ) might be considered for treatment discontinuation according to previous findings [5, 6]. However, prerequisites for treatment stop remain a subject of research.

Disease transformation of *JAK2V617F*-positive MPNs may arise from accumulating genomic instability promoted by the expanding pool of homozygous *JAK2V617F* clones [12]. High *JAK2V617F* allele burden is a risk factor for progression to secondary myelofibrosis in patients with PV [13, 14]. Since alpha interferons reduce the *JAK2V617F* allele burden, this finding is congruent with an improved myelofibrosis-free survival rate reported among interferon-treated patients with PV compared to those receiving hydroxyurea or phlebotomy in a long-term retrospective study [15]. In the PROUD-PV/CONTINUATION-PV trials, conducted in an early-stage PV population, a fivefold lower incidence rate of disease progression (including myelofibrosis and leukemic transformation) was observed in ropeginterferon alfa-2b treated patients compared with the control arm, although these events



**Fig. 1 Median *JAK2V617F* allele burden (%).** Figure 1 shows the median *JAK2V617F* allele burden (%) during a period of 5 years of treatment. *JAK2V617F* allele burden was determined every 6 months. Medians were calculated for the last observation carried forward at each timepoint assessed. \* Denotes a statistically significant ( $p < 0.05$ ) difference between the treatment arms.

**Table 1.** Summary of all adverse events in PROUD-PV and CONTINUATION-PV (Safety set).

Type of event	Intensity grade	Ropeginterferon alfa-2b		Control	
		(499 patient years)		(401 patient years)	
		Adverse events	Number of patients <sup>a</sup> n (%) N = 127	Adverse events	Number of patients <sup>a</sup> n (%) N = 127
Adverse events		1767	116 (91.3%)	1267	117 (92.1%)
Serious adverse events		56	30 (23.6%)	56	32 (25.2%)
Treatment-related serious adverse events		4	4 (3.1%)	7	5 (3.9%)
Adverse events related to polycythemia vera		171	48 (37.8%)	135	48 (37.8%)
Treatment-related adverse events		758	100 (78.7%)	458	100 (78.7%)
Intensity of treatment-related adverse events <sup>b</sup>	Grade 1	477	73 (57.5%)	277	79 (62.2%)
	Grade 2	241	75 (59.1%)	152	62 (48.8%)
	Grade 3	39	21 (16.5%)	28	20 (15.7%)
	Grade 4	1	1 (0.8%)	0	0
	Grade 5	0	0	1	1 (0.8%)
	Grade ≥3	40	21 (16.5%)	29	21 (16.5%)
Treatment-related adverse events leading to discontinuation		15	13 (10.2%)	4	4 (3.1%)
Disease progression (myelofibrosis and leukemic transformation) <sup>c</sup>		1	1 (0.8%)	4	4 (3.1%)
Any neoplasm		16	12 (9.4%)	18	15 (11.8%)
Skin cancers related to treatment (basal cell carcinoma and malignant melanoma)		0	0	3	3 (2.4%)
Major thromboembolic adverse events <sup>d</sup>		5	4 (3.1%)	5	5 (3.9%)

<sup>a</sup>Number of patients in whom the event was reported throughout the entire study period.

<sup>b</sup>Intensity grading according to CTCAE 4.0.

<sup>c</sup>One patient in the ropeginterferon alfa-2b arm developed myelofibrosis; in the control arm, 2 patients developed myelofibrosis and leukemic transformation occurred in 2 patients.

<sup>d</sup>Ropeginterferon alfa-2b arm: splenic infarction/truncus coeliacus thrombosis, intracardiac thrombus, hemorrhagic transformation stroke, and 2 cases of ischemic stroke. Control arm: embolism, femoral artery occlusion, superficial thrombophlebitis, venous thrombosis of the limb, and cerebrovascular accident.

were too rare to allow statistical comparison, which would require longer follow-up.

These new results provide further evidence for the possible disease modifying capability of ropeginterferon alfa-2b, which may alter the natural course of PV. In a sizable proportion of ropeginterferon alfa-2b-treated patients, treatment discontinuation (a goal that cannot be achieved in PV with any other currently available therapy) could potentially be considered, and warrants further study.

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## AUTHOR CONTRIBUTIONS

JK, CK, KK, and HG contributed to the study design, analysis, interpretation of data and preparation of the manuscript. VE contributed to the analysis and interpretation of data and wrote the manuscript. PG, DK, LGK, ME, VR, PD, AI, HP, LS, JM, VY, and JK and the PROUD-PV Study Group collected clinical data. RK provided critical analytical tools, oversaw analyses and interpreted the data. HH contributed to interpretation of the data and preparation of the manuscript. All authors had full access to the primary study data and reviewed the manuscript.

## COMPETING INTERESTS

JJK reports grants and personal fees from AOP Orphan, and personal fees from Novartis, BMS/Celgene, Abbvie and PharmaEssentia. CK, KK, and VE report that they are employees of AOP Orphan. JM reports grants from AOP Orphan. RK reports personal fees from AOP Orphan Pharmaceuticals, PharmaEssentia, Qiagen and Novartis and stock ownership in MyeloPro Diagnostics and Research. HH reports Data Monitoring Board honoraria from AOP Orphan and grants from Novartis. HG reports grants and personal fees from AOP Orphan, grants and personal fees from Novartis and personal fees from PharmaEssentia, MyeloPro Diagnostics and Research, Janssen-

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## ADDITIONAL INFORMATION

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