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 (BESREM®, 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 (#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 Supplemental Table 4 and Supplementary Fig. 2. At database lock, 70/
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Mutant JAK2

interferon alfa-2b whereas in the control arm, only 1 patient (1.4%) decreased to <1% in 18/92 patients (19.6%) receiving 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 ≥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 ≥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 ≥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 <1%, sustained complete hematologic response [hematocrit <45% without phlebotomy in the last 3 months, platelet count <400 × 10^9/L, and leukocyte count <10 × 10^9/L; drop-outs considered non-responders] for ≥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 alfa 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 are not fully understood.
REFERENCES

1. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M, et al. Pegylated interferon-alpha-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood. 2008;112:3065–72.
2. Verger E, Soret-Duply J, Maslah N, Roy L, Rey J, Grrieb Z, et al. Peginterferon alpha-2b targets JAK2V617F-positive polycythemia vera cells in vitro and in vivo. Blood Cancer J. 2018;8:94.
3. Kiladjian JJ, Giraudier S, Cassinat B. Interferon-alpha for the therapy of myeloproliferative neoplasms: targeting the malignant clone. Leukemia. 2016;30:734–54.
4. Utke Rank C, Weis Bjerrum O, Larsen TS, Kjaer L, de Stricker K, Riley CH, et al. Minimal residual disease after long-term interferon-alpha2 treatment: a report on hematological, molecular and histomorphological response patterns in 10 patients with essential thrombocythemia and polycythemia vera. Leuk Lymphoma. 2016;57:348–54.
5. Hasselbalch HC, Holmstrom MO. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: minimal residual disease and cure? Semin Immunopathol. 2019;41:5–19.
6. Daltro De Oliveira R, Soret-Duply J, Zhao L-P, Marcault C, Gauthier N, Verger E, et al. Interferon-alpha (IFN) therapy discontinuation is feasible in myeloproliferative neoplasm (MPN) patients with complete hematological remission. Blood. 2020;136:35–6.
7. Pedersen RK, Andersen M, Knudsen TA, Sajid Z, Gudmand-Hoeyer J, Dam MJ, et al. Data-driven analysis of JAK2V617F kinetics during interferon-alpha2 treatment of patients with polycythemia vera and related neoplasms. Cancer Med. 2020;9:2039–51.
8. Ginslinger H, Klade C, Georgiev P, Krochmalczyk D, Gercheva-Kychukhova L, Egyed M, et al. Peginterferon alfa-2b versus standard therapy for polycythemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. Lancet Haematol. 2020;7:e196–e208.
9. Barosi G, Birgegard G, Finazzi G, Gianelli U, Harrison C, Hasselbalch HC, et al. Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. Blood. 2009;113:4829–33.
10. Mosca M, Hermange G, Tisserand A, Noble R, Marzuc R, Marty C, et al. Inferring the dynamics of mutated hematopoietic stem and progenitor cells induced by IFNa in myeloproliferative neoplasms. Blood. 2021;138:2231–43.
11. Ginslinger H, Zagriffischku C, Buxhofer-Ausch V, Thaler J, Schlögl E, Gastl GA, et al. Peginterferon alfa-2b, a novel IFNalpha-2b, induces high response rates with low toxicity in patients with polycythemia vera. Blood. 2015;126:1762–9.
12. Karantanos T, Molterno AR. The roles of JAK2 in DNA damage and repair in the myeloproliferative neoplasms: opportunities for targeted therapy. Blood Rev. 2018;32:426–32.
13. Alvarez-Larrán A, Bellasio B, Pereira A, Kerguelen A, Hernández-Boluda JC, Martinez-Avilés L, et al. JAK2V617F monitoring in polycythemia vera and essential thrombocythemia: clinical usefulness for predicting myelofibrotic transformation and thrombotic events. Am J Hematol. 2014;89:517–23.
14. Shirane S, Araki M, Morishita S, Edahiro Y, Sunami Y, Hironaka Y, et al. Consequences of the JAK2V617F allele burden for the prediction of transformation
into myelofibrosis from polycythemia vera and essential thrombocythemia. Int J Hematol. 2015;101:148–53.
15. Abu-Zeinah G, Krichevsky S, Cruz T, Hoberman G, Jaber D, Savage N, et al. Interferon-alpha for treating polycythemia vera yields improved myelofibrosis-free and overall survival. Leukemia. 2021;35:2592–601.

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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
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