

## CORRESPONDENCE



# Effectiveness of ibrutinib as first-line therapy for chronic lymphocytic leukemia patients and indirect comparison with rituximab-bendamustine: Results of study on 486 cases outside clinical trials

To The Editor:

Ibrutinib (IB) has been initially approved for naïve patients' treatment with chronic lymphocytic leukemia (CLL) based on its superiority over chlorambucil.<sup>1</sup> Two subsequent large phase 3 randomized trials demonstrated a longer progression-free survival (PFS) in IB treated cases compared to those receiving rituximab (R) combined with fludarabine (F) and cyclophosphamide (C, FCR)<sup>2</sup> or with bendamustine (BR).<sup>3</sup>

Explorative analyses, demonstrating the superiority of IB over chemotherapy<sup>1</sup> or chemoimmunotherapy<sup>2,3</sup> in cases with high-risk features, including unmutated *IGHV* (*IGHV*-UM) genes, *del*(17p) and/or *TP53* mutations (*TP53*mut) and *del*(11q), justifies the attitude to limit FCR and BR only for low-risk and fit cases. However, these biomarkers' practical prognostic relevance in the era of new drugs remains an open issue.<sup>4</sup> Results investigating the clinical impact of IB in the current clinical practice mainly focused so far on relapsed-resistant (RR) patients.<sup>5</sup>

Here, we conducted a multicenter, retrospective study to ascertain the predictive and prognostic relevance of well-known biological and clinical indicators in 165 patients treated with IB as first-line. In the same setting, we assessed the relative usefulness of IB versus BR, comparing the IB cohort with an additional retrospective multicenter cohort of 321 CLL cases treated with BR as first-line therapy outside clinical trials.

The baseline characteristics of the IB cases are listed in Table S1. The majority of patients were Binet stages B and C (89.7%). The median age was 71.8 years, 104 cases (63%) were males, and 70.2% of cases were *IGHV*-UM; moreover, *del*(17p) and *TP53*mut were observed in 43.6% and 38.8% of cases, respectively. Of note, patients with *del*(17p) were not included in the Resonate-2 trial<sup>1</sup> or in FCR versus IB study,<sup>2</sup> and only 5% to 6% of cases with *del*(17p) were accrued in the IB arms of the BR versus IB trial.<sup>3</sup> Here, the incidence of *del*(17p) or *TP53*mut cases, due to IB prescription indications, supported a reliable explorative *TP53* disruption sub-analysis. Forty-three patients (26.7%) discontinued IB, 24/43 for

disease progression, including Richter's transformation (nine cases), and 19/43 for toxicity.

After a median follow-up of 31.6 months, 36 patients progressed or died, and 88.4% remained progression-free and alive at 1 year. On univariate Cox regression analysis, patients with anemia [Hazard ratio (HR) 2.0, 95% CI 1.0–3.8,  $p = .042$ ], Binet C (HR 2.0, 95% CI 1.0–3.8,  $p = .043$ ), *del*(17p) (HR 3.4, 95% CI 1.7–6.9,  $p = .001$ ), and *TP53* mutation (HR 2.4, 95% CI 1.1–5.1,  $p = .025$ ) had a significantly higher risk of progression or death (Table S2). We performed two different multivariate Cox analyses in which either *del*(17p) (model 1) or a *TP53* mutation (model two) were introduced together with anemia and Binet stage (Table S2). Notably, *del*(17p) (HR 3.1 95% CI 1.5–6.5,  $p = .002$ ) in model one and *TP53* mutations (HR 2.4 95% CI 1.1–5.2,  $p = .025$ ) in model two, remained unique predictors independently associated with PFS (Table S2). Moreover, we tested the hypothesis of whether the concomitant presence of *del*(17p) and *TP53* mutations, the latter representing 70.5% of *del*(17p) cases, could provide a more precise risk assessment. A Cox regression analysis adjusted for anemia and Binet stage showed a significantly inferior PFS (HR 4.5, 95% CI 1.7–11.7,  $p = .002$ ) for cases with both *TP53* mutation and *del*(17p) compared with those with a wild-type *TP53* status, while the single *TP53* gene alteration, either mutation or deletion, failed to significantly increase the risk of progression (HR 1.5, 95% CI 0.5–4.6,  $p = .4$ ) (Figure S1).

Note, OS data revealed that 15/165 patients died, and 91.4% of cases were still alive at 2.5 years. Notably, none of the variables depicted in Table S2 were significantly associated with OS except that *del*(17p) (HR 4.1, 95% CI 1.3–13.3,  $p = .016$ ). Again, cases with *TP53* mutation and *del*(17p) disclosed a significant higher death risk (HR 5.5, 95% CI 1.7–25.8,  $p = .031$ ) than the remaining groups of patients with unaltered or partially altered *TP53* gene. Overall, our results suggest that the degree of *TP53* function disruption [i.e., *del*(17p) or *TP53*mut versus *del*(17p) and *TP53*mut] appears to affect the response to IB in term of shorter PFS and possibly shorter OS. Our findings are germane to those of the largest cohort of IB-treated patients with *del*(17p),<sup>6</sup> demonstrating an inferior PFS in cases with this chromosome abnormality.<sup>6</sup> Notably, a landmark analysis evidenced that drug withdrawal predicted a significantly shorter OS than patients still on IB therapy, irrespective of whether discontinuation was driven by toxicity, disease progression, or Richter transformation (Figure S2). This finding is in keeping with previous reports indicating a poor outcome after IB discontinuation.<sup>7</sup> The baseline characteristics of all patients who discontinued IB therapy for toxicity are listed in Table S3. A significantly higher rate of older patients was documented in this subset of patients (73.7% versus 54.1%,  $p = .02$ ). New treatment regimens were initiated for 13/19 (68.4%) of the patients that discontinued IB, most commonly VEN-based ( $n = 9$ ) or IDELA-based ( $n = 4$ ) treatments.

Another aim of this study was to compare the IB cohort with an additional cohort of 321 cases treated with BR as first-line therapy outside clinical trials. In the BR cohort, we found a significantly higher proportion of cases with abnormal levels of both  $\beta$ 2-microglobulin ( $\beta$ 2-M) and lactic dehydrogenase (LDH), while *IGHV*-UM and *del*(17p) cases were more frequently observed in the IB cohort (Table S4).

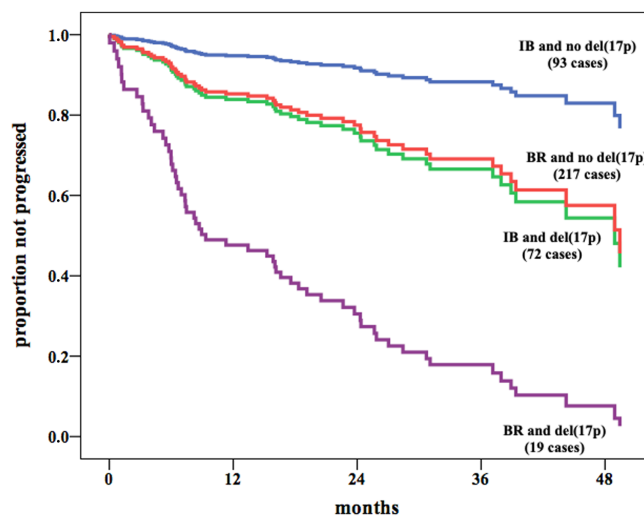
An unadjusted Cox analysis performed in the combined cohort showed that IB was significantly more effective than BR in decreasing the risk of disease progression in treatment-naïve patients with CLL (Table S5). However, this analysis poses a hypothetical hitch of confounding by indication, that is, a bias that distorts the comparison between two treatments by the presence of an indication tilting the prescription toward a drug rather than another (herein, IB versus BR). To minimize the confounding by indication we adjusted the relationship between allocation therapy (IB versus BR) and disease progression for all the variables which resulted differently distributed between the two cohorts at study inception (see Table S4), as well as for all variables significantly associated with PFS at Cox univariate analysis, as described in Table S5. After jointly introducing these variables as covariates into a multiple Cox regression model, the protective effect of IB versus BR in terms of risk of disease progression (HR = 0.31, 95% CI 0.14–0.66,  $p = .002$ ) was fully confirmed independently of a series of potential confounders (Table S6). Notably, *del*(17p) remained the only independent predictor of PFS (HR, 3.52; 95% CI, 1.83–6.78,  $p < .001$ ) together with therapy allocation (Table S6).

We also investigated the interaction between the treatments under investigation, the presence/absence of *del*(17p), and PFS. In an unadjusted Cox regression analysis, patients treated with BR and harboring *del*(17p) had an HR of progression or death higher than that expected in the absence of interaction under the additive model, with a synergy index of 2.6 (Figure S3A). It means that the risk due to BR and *del*(17p) interaction was 2.6 times higher than that expected as a simple sum of the two risk factors' effects (i.e., by considering no interaction). These results did not change when the same analysis was carried out by adjusting for a series of potential confounders (Figure S3B).

To visually compare the IB versus BR PFS benefit consistently observed also for patients bearing *del*(17p), we constructed an additional multiple Cox model in which the variable representing the combination of the type of therapy and the *del*(17p) status was introduced together with Binet stage,  $\beta$ 2-M, anemia, LDH, and *IGHV* mutational status. This analysis showed a clear overlap of PFS curves of *del*(17p) cases treated with IB with no-*del*(17p) cases treated with BR, while patients bearing *del*(17p) treated with BR experienced the worst outcome (Figure 1).

Finally, both the unadjusted and adjusted analysis of our cohort showed no significant differences between IB and BR in OS (data not shown).

Altogether, our retrospective multicenter analysis involving patients treated outside clinical trials, confirmed the superiority of IB over BR in terms of PFS, but not when OS was considered. Similarly, the ALLIANCE trial, while demonstrating a superior



**FIGURE 1** Cox regression-derived progression-free survival curves (adjusted for Binet stage,  $\beta$ 2-M, anemia, LDH, and *IGHV* mutational status) according to the combined variable *del*(17p) and therapy allocation. IB, ibrutinib; BR, bendamustine–rituximab

PFS in IB treated cases compared to cases treated with BR, fails to validate such superiority in the OS setting.<sup>3</sup> Accordingly, less intensive chemotherapy with a combination of anti-CD20 and bendamustine given as front-line therapy has remained an additional choice for low-risk *IGHV* mutated (*IGHV*-MUT) fit cases without *TP53* disruption.<sup>3</sup> This short-term therapy could be envisaged during oncological counseling for a shared optimal clinical management of CLL low-risk patients in countries where this choice is allowed.

In conclusion, the results of this current clinical practice study demonstrated that IB therapy provides a superior PFS compared to BR, particularly in patients with *del*(17p). However, *TP53* disruption still maintains its prognostic power in treatment-naïve patients with CLL treated with IB.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

F.M., M.G., M.F., G.D.P., F.R.M., D.R., F.D.R., G.G., L.T., A.P., R.F., and V.G. designed the study, analyzed and interpreted data, and wrote the manuscript; M.G., G.T., G.D., and F.M. performed statistical analysis; S.B., G.C., G.F., P.M., P.Me., F.M.R., A.Z., I.D.G., R.B., A.N., and M.F. performed central laboratory tests; G.R., P.S., L.L., M.C., Y.H., M.V., R.M., A.Ch., A.Co., R.Mo., A.V., D.P., G.L., U.C., I.S., E.V., E.A.M., R.C., A.R., I.A., A.B., S.G., H.A., and J.O. provided the patients and collected clinical data; and all authors gave final approval for the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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# PF-07059013: A non-covalent hemoglobin modulator favorably impacts disease state in a mouse model of sickle cell disease

To the Editor:

Sickle cell disease (SCD) is a severe genetic disorder that impacts approximately 20 million people worldwide.<sup>1</sup> The causative  $\beta 6$  Glu-

Val substitution is a gain of function mutation; in the deoxygenated state, the mutant protein, Hb S, can form polymers, leading to red blood cell sickling and precipitating downstream consequences, including vaso-occlusion (pain crisis), hemolytic anemia, stroke and related pathophysiology.<sup>2,3</sup> Polymerization is exponentially dependent on deoxy Hb S concentration.<sup>4</sup> Thus, relatively small changes in deoxyHb S concentration will significantly impact polymerization, red blood cell sickling, and ultimately the clinical course of the disease. Pharmacologic evidence for the benefit of reducing the concentration of deoxyHb S arises from studies of covalent modification of Hb S, where stabilization of the oxygenated conformation increases Hb O<sub>2</sub> affinity, reduces RBC sickling, extends RBC half-life, and ultimately reduces the frequency of vaso-occlusive crisis (VOC).<sup>5-8</sup> In clinical trials, ex vivo carbamylation of patient blood led to improvements in hemolytic anemia; treated patients exhibited a 2.7 g/dl increase in hemoglobin, a 58% decrease in reticulocytes, a 65% decrease in irreversibly sickled cells, and an 80% decrease in frequency of painful crises.<sup>8</sup> Subsequent oxyHb S stabilizing molecules were developed based on the observation that benzaldehyde derivatives formed stable, covalent Schiff bases with hemoglobin. The most advanced of this class of molecules is the covalent compound Voxelotor (GBT 440, Oxbryta), which was approved by the FDA in 2019 for the treatment of SCD. In the pivotal study, 59% of patients in the higher dose group (1500 mg/day) had increases of 1 g/dl or greater in hemoglobin, with a mean hemoglobin modification of 26.5%.<sup>9</sup> While the impact of covalent Hb S modifiers on hemolytic anemia is well established, the rise in hemoglobin observed for voxelotor falls short of the effects observed by ex vivo carbamylation, and was not accompanied by a significant effect on the frequency of VOC. This suggests that the therapeutic potential of hemoglobin modification has not been fully realized.

PF-7059013 is a non-covalent modifier of hemoglobin that stabilizes the oxygenated state (see Gopalsamy et al.). Here we present the impact this molecule has on a well-established mouse model of sickle cell disease. Treatment with PF-7059013 demonstrated robust changes in key markers of hemolytic anemia in the Townes mouse model,<sup>10</sup> suggesting it has the potential to be a potent and efficacious therapy for SCD.

PF-07059013 was orally administered to Townes SCD model mice twice daily at a dose of 200 mg/kg for 15 days. This dose was selected as it was expected to result in approximately 25% hemoglobin coverage.<sup>11</sup> At 30 minutes post the initial dose, total blood concentrations for individual animals were 2–4 mM, consistent with the low total blood clearance observed in the single dose administration studies. These values translate to approximately 40%–60% hemoglobin coverage, based on measured hemoglobin concentrations. The high total blood concentrations observed following the initial dose were maintained for the duration the 15-day dose period (Supporting Information S1).

Animals treated with PF-07059013 show a significant stabilization of the oxygenated state. The average p50 decreased by 53.7% ( $\pm 21.2\%$ ) in the treated group, relative to vehicle, and the average p20, a more sensitive marker of compound occupancy, decreased by