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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Ibrutinib plus rituximab versus ibrutinib monotherapy in patients with Waldenström macroglobulinemia: A pooled analysis

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Alberto Guijosa (Dana Farber Cancer-Institute, United States) Andres Ramirez-Gamero (Roger Williams Medical Center, United States) Shayna Sarosiek (Dana Farber Cancer Institute, United States) Andrew Branagan (Massachusetts General Hospital, United States) Gottfried von Keudell (Beth Israel Deaconess Medical Center, United States) Steven Treon (Dana Farber Cancer Institute, United States) Jorge Castillo (Dana-Farber Cancer Institute, United States)

Abstract:
Ibrutinib monotherapy (I) and ibrutinib plus rituximab (I+R) are highly efficacious in treating Waldenström Macroglobulinemia (WM). However, the benefit of adding rituximab remains unclear, and a prospective trial to compare these two regimens has not been undertaken. Therefore, we performed a pooled analysis of prospective studies to compare their effectiveness. Patient-level demographic, response, and survival data were pooled from three prospective studies (NCT01614821, NCT02604511, NCT02165397). We included patients treated with I+R or I, excluding those without MYD88 mutations. Among 174 patients (58 I+R, 116 I), very good partial response (VGPR) rates were comparable across treatment groups (38% I+R vs. 28% I, p=0.21). The 48-month progression-free survival (PFS) rate was 74% with I+R versus 61% with I (p=0.22), and the 48-month overall survival (OS) rate was 94% versus 89% (p=0.45). In patients with CXCR4 mutations, I+R trended toward higher VGPR (27% vs. 11%; p=0.10) and had a superior 48‐month PFS rate (72% vs. 43%; p=0.03). CXCR4 mutations were associated with inferior VGPR (42% vs. 17%; p=0.001) and 48-month PFS in the I arm (43% vs. 72%; p=0.002). In the I+R arm, CXCR4 mutations were associated with numerically inferior VGPR (47% vs. 27%; p=0.12), but the 48-month PFS rate was not impacted (74% vs. 72%; p=0.96). I+R significantly improved PFS over I in patients with CXCR4-mutated WM, along with a non-significant increase in VGPR in this subgroup. These results support routine CXCR4 testing in patients with WM and clinical trials of rituximab with covalent or non‐covalent BTK inhibitors.

Conflict of interest: COI declared - see note

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Authors:

Alberto Guijosa (1, 2); Andres Ramirez-Gamero (3); Shayna Sarosiek (1, 2); Andrew R. Branagan (4, 2); Gottfried von Keudell (2); Steven P. Treon (1, 2); Jorge J. Castillo (1, 2)

Affiliations:

(1) Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA

(2) Department of Medicine, Harvard Medical School, Boston, MA, USA

(3) Department of Medicine, Roger Williams Medical Center, Providence, RI, USA

(4) Division of Hematology and Oncology, Massachusetts General Hospital, Boston, MA, USA

(5) Division of Hematology and Oncology, Beth Israel-Deaconess Medical Center, Boston, MA, USA

Corresponding author:

Jorge J. Castillo, MD

Address: 450 Brookline Ave, Boston, MA 02215

Phone: 617-632-6045. Fax: 617-582-8608. E-mail: jorgej_castillo@dfci.harvard.edu

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Key Points

- Patients with Waldenström macroglobulinemia and CXCR4 mutations have improved outcomes with ibrutinib + rituximab vs. ibrutinib monotherapy.
- In patients with CXCR4 wild-type disease, adding rituximab to ibrutinib showed no clear additional benefit.

Abstract

Ibrutinib monotherapy (I) and ibrutinib plus rituximab (I+R) are highly efficacious in treating Waldenström Macroglobulinemia (WM). However, the benefit of adding rituximab remains unclear, and a prospective trial to compare these two regimens has not been undertaken. Therefore, we performed a pooled analysis of prospective studies to compare their effectiveness. Patient-level demographic, response, and survival data were pooled from three prospective studies (NCT01614821, NCT02604511, NCT02165397). We included patients treated with I+R or I, excluding those without MYD88 mutations. Among 174 patients (58 I+R, 116 I), very good partial response (VGPR) rates were comparable across treatment groups (38% I+R vs. 28% I, p=0.21). The 48-month progression-free survival (PFS) rate was 74% with I+R versus 61% with I (p=0.22), and the 48-month overall survival (OS) rate was 94% versus 89% (p=0.45). In patients with *CXCR4* mutations, I+R trended toward higher VGPR (27% vs. 11%; p=0.10) and had a superior 48-month PFS rate (72% vs. 43%; p=0.03). *CXCR4* mutations were associated with inferior VGPR (42% vs. 17%; p=0.001) and 48-month PFS in the I arm (43% vs. 72%; p=0.002). In the I+R arm, *CXCR4* mutations were associated with numerically inferior VGPR (47% vs. 27%; p=0.12), but the 48-month PFS rate was not impacted (74% vs. 72%; p=0.96). I+R significantly improved PFS over I in patients with CXCR4-mutated WM, along with a non-significant increase in VGPR in this subgroup. These results support routine *CXCR4* testing in patients with WM and clinical trials of rituximab with covalent or non-covalent BTK inhibitors.

Introduction

Bruton tyrosine kinase (BTK) inhibitors are standard for treating Waldenström macroglobulinemia (WM). Ibrutinib was the first-in-class BTK inhibitor and is safe and highly effective in WM [1-4]. Based on the results of a seminal prospective phase II study, the Food & Drug Administration (FDA) approved ibrutinib monotherapy for treating WM in 2015. Somatic mutations in *CXCR4* are highly recurrent in WM, detected in 30-40% of tested patients [5-8], and are associated with lower rates of very good partial response (VGPR) and inferior progression-free survival (PFS) in treatment-naïve patients and patients with relapsed disease [3,4,9].

The INNOVATE study compared ibrutinib plus rituximab to a placebo plus rituximab [10,11]. This study showed that the combination was associated with superior outcomes, prompting the FDA approval for treating WM in 2018. *CXCR4* mutations were associated with lower VGPR rates in patients treated with ibrutinib plus rituximab, but an impact on PFS was less evident.

The National Comprehensive Cancer Network (NCCN) endorses ibrutinib monotherapy and ibrutinib plus rituximab as preferred treatment options for WM patients [12]. However, it is unclear whether ibrutinib plus rituximab provides additional benefits over ibrutinib monotherapy in WM, and prospective clinical trials to answer this question will unlikely be undertaken.

Therefore, we performed a comparative pooled analysis of prospective studies to evaluate the response and survival outcomes of ibrutinib plus rituximab versus ibrutinib monotherapy in patients with WM. A secondary objective was to assess the impact of *CXCR4* mutational status in patients with WM treated with ibrutinib plus rituximab and ibrutinib monotherapy.

Methods

Patients

We collected demographic, response, and survival patient-level data from three prospective studies evaluating ibrutinib monotherapy or in combination with rituximab in WM patients. NCT01614821 was a single-arm study that included 63 patients with relapsed or refractory (RR) WM treated with ibrutinib monotherapy [1,3]. NCT02604511 was a single-arm study that included 30 patients with treatment-naïve (TN) WM treated with ibrutinib monotherapy [2,4]. NCT02165397 included three arms: Arm A included 75 patients with RR and TN WM treated with ibrutinib plus rituximab, Arm B included 75 patients with RR and TN WM treated with R, and Arm C included 31 patients with RR, rituximab-refractory WM treated with ibrutinib monotherapy [10,11,13]. Responses in all three trials were assessed using the modified consensus criteria established by the 6th International Workshop on Waldenström Macroglobulinemia (IWWM-6) [14]. Data from NCT02165397 were obtained through the Yale University Open Data Access (YODA) project (ID #2022-4928).

For this analysis, we included patients treated with ibrutinib plus rituximab or ibrutinib monotherapy. We excluded patients without *MYD88* mutations (n=25) and those treated with rituximab monotherapy (n=75). **Figure 1** shows the CONSORT diagram.

Statistical design

Clinicopathological data were categorized as follows: age (>65 and ≤65 years), sex (male and female), hemoglobin level (<115 and ≥115 g/l), platelet count (<100 and ≥100 k/ul), serum IgM level (>70 and ≤70 g/l), serum beta-2-microglobulin level (>3.5 and ≤3.5 mg/l), treatment history (previously treated and treatment naïve), and *CXCR4* mutational status (mutated and wildtype). The International Prognostic Scoring for WM (IPSSWM) was calculated as previously described [15]. Data on hemoglobin level (n=6; 3.4%), platelet count (n=3; 1.7%), and IPSSWM (n=5; 2.9%) were missing. Categorical responses were assessed based on the modified 6th International Workshop for WM criteria [14] and divided into very good partial response (VGPR) or better, partial response (PR), minor response (MR), and no response (NR).

Categorical variables were reported as frequencies and proportions. Continuous variables were analyzed in two ways: (1) they were dichotomized based on clinically relevant thresholds (as described above) and reported as frequencies and proportions, and (2) they were also analyzed in their continuous form and summarized using medians and interquartile ranges (IQR), given the relatively small sample size.

Differences between groups were assessed using Fisher's exact or Chi-square tests for categorical variables, including dichotomized continuous variables. For continuous variables analyzed in their original form, differences between groups were assessed using the Wilcoxon rank-sum test.

Univariate and multivariate logistic regression models were fitted to evaluate the association between treatment arms and VGPR attainment. The outcome measure for the logistic regression was the odds ratio (OR) with a 95% confidence interval (CI). Progression-free survival (PFS) was defined as the time between treatment initiation and disease progression, death, or last follow-up. Overall survival (OS) was defined as the time between treatment initiation and death or last follow-up. PFS and OS curves were estimated using the Kaplan-Meier method for incomplete observations and compared using the log-rank test. Univariate and multivariate proportional-hazard Cox regression models were fitted to assess the prognostic value of the treatment arms for PFS and OS. The outcome measure for the proportional-hazard Cox regression was the hazard ratio (HR) with a 95% CI. Multivariate analyses included age, sex, hemoglobin, platelet count, serum beta-2-microglobulin (or the composite IPSSWM score for PFS to avoid overadjustment), treatment status, and *CXCR4* mutational status, based on their established prognostic relevance in large WM cohorts. [15-17] LDH and albumin (components of the MSS-WM) were not available in the pooled trials. [16]

P-values <0.05 were considered significant. Correction for multiple testing was performed using the Benjamini-Hochberg false discovery rate (FDR) method: across 7

subgroups for the I+R vs. I comparison (CXCR4-mutated, CXCR4-wild-type, treatment-naïve, previously treated, low, intermediate, and high IPSSWM), and across 2 subgroups in the CXCR4 mutation analysis (ibrutinib monotherapy vs. ibrutinib plus rituximab). All calculations and graphics were obtained using STATA version 17 (College Station, TX, USA).

Results

Patients' characteristics

The final analytical cohort included 174 patients: 58 patients treated with ibrutinib plus rituximab and 116 with ibrutinib monotherapy. Baseline characteristics according to treatment are shown in **Table 1**. There was a higher proportion of men (75% vs. 59%; p=0.03) and previously treated patients (72% vs. 47%; p=0.001) in the ibrutinib monotherapy group than in the ibrutinib plus rituximab group. The median beta-2-microglobulin level was higher in the ibrutinib monotherapy group (5.2 vs. 3.8 mg/l; p=0.001). The proportion of patients with serum IgM ≥70 g/dl was higher in the ibrutinib monotherapy than in the ibrutinib plus rituximab group (7% vs. 0%; p=0.04). There were no differences between groups in the other variables (p>0.05 for all remaining variables). Baseline differences by *CXCR4* mutational status were also evaluated and are shown in **Table 2**. Patients with *CXCR4* mutations had a higher frequency of platelet count <100 k/ul (17% vs. 4%) and a higher median serum IgM level (43.1 vs. 33.6 g/L; p=0.01). No other significant differences were observed (p>0.05).

Response to therapy

Figure 2A shows categorical responses for ibrutinib plus rituximab and ibrutinib monotherapy. The VGPR rate for ibrutinib plus rituximab was 38% versus 28% for ibrutinib monotherapy, with an OR of VGPR of 1.54 (95% CI 0.79-2.99; p=0.21) favoring ibrutinib plus rituximab. In a multivariate model adjusting for categorical groups of age, sex, hemoglobin level, platelet count, serum beta-2-microglobulin level, treatment status, and *CXCR4* mutations, ibrutinib plus rituximab was associated with an OR of VGPR of 1.54 (95% CI 0.71-3.35; p=0.27) compared with ibrutinib monotherapy. In this model, *CXCR4* mutations were independently associated with lower odds of VGPR (OR 0.31, 95% CI 0.14-0.70; p=0.005).

There were numerically higher odds of VGPR for ibrutinib plus rituximab over ibrutinib monotherapy in patients with *CXCR4* mutations (27% vs. 11%; **Figure 2B**) with an OR of 2.87 (95% CI 0.81-10.3; p=0.10). In a multivariate model adjusting for categorical groups of age, sex, hemoglobin level, platelet count, serum beta-2-microglobulin level, and treatment status, ibrutinib plus rituximab had numerically higher odds of VGPR with OR 3.61 (95% CI 0.83-15.7; p=0.09) than ibrutinib monotherapy.

There were numerically higher odds of VGPR with ibrutinib plus rituximab over ibrutinib monotherapy in patients with high IPSSWM scores (55% vs. 30%; **Figure 2C**), with an OR of VGPR of 2.74 (95% CI 0.96-7.82; p=0.06) compared with ibrutinib monotherapy.

In a multivariate model adjusting for treatment status and *CXCR4* mutations, ibrutinib plus rituximab was associated with an OR of VGPR of 2.75 (95% CI 0.87-8.76; p=0.09) compared with ibrutinib monotherapy.

There were no significant differences in VGPR rates between ibrutinib plus rituximab and ibrutinib monotherapy in patients without *CXCR4* mutations, treatment-naive, previously treated patients, and patients with low or intermediate IPSSWM scores (p>0.05 for all subgroups).

Patients with *CXCR4* mutations had lower odds of VGPR than patients without *CXCR4* mutations (17% vs. 42%; OR 0.29, 95% CI 0.14-0.61; p=0.001; **Figure 2D**). Patients with *CXCR4* mutations had numerically lower odds of VGPR in the ibrutinib plus rituximab arm (27% vs. 47%; OR 0.42, 95% CI 0.14-1.27; p=0.12; **Figure 2E)** and lower odds of VGPR than patients without *CXCR4* mutations in the ibrutinib monotherapy group (11% vs. 39%; OR 0.20, 95% CI 0.07-0.57; p=0.003; **Figure 2F**). In multivariate models adjusting for categorical groups of age, sex, hemoglobin level, platelet count, serum beta-2-microglobulin level, and treatment status, *CXCR4* mutations were independently associated with lower odds of VGPR in the entire cohort (OR 0.31, 95% CI 0.14-0.70; p=0.005) and in patients treated with ibrutinib monotherapy (OR 0.20, 95% CI 0.07-0.63; p=0.007) and had numerically lower odds of VGPR in patients treated with ibrutinib plus rituximab (OR 0.43, 95% CI 0.11-1.72; p=0.24).

Figure 3 presents the forest plot of all subgroup analyses assessing the effect of ibrutinib plus rituximab on VGPR. No subgroups demonstrated a statistically significant benefit after adjustment for multiple testing. The only significant association after correction was the adverse impact of *CXCR4* mutations on VGPR in patients receiving ibrutinib monotherapy (adjusted and corrected p=0.01), an effect not observed with I+R.

No significant differences in VGPR rates were observed between previously treated and untreated WM patients (p=0.98).

Survival analysis

The median follow-up time for the ibrutinib monotherapy group was longer at 54.6 months (95% CI 50.8-57.1) than at 49.7 months (95% CI 48.1-50.2) for the ibrutinib plus rituximab group(p<0.001). There were 58 progression events (42 in the ibrutinib monotherapy and 16 in the ibrutinib plus rituximab arm), including 15 deaths (11 in the ibrutinib monotherapy and 4 in the ibrutinib plus rituximab arm).

The median PFS was not reached for both treatment arms. The 48-month PFS rate was 61% (95% CI 51-70) for ibrutinib monotherapy and 74% (95% CI 60-83%) for ibrutinib plus rituximab for an HR of 0.70 (95% CI 0.39-1.24; p=0.22; **Figure 4A**). In a multivariate model adjusting for IPSSWM, treatment status, and *CXCR4* mutations, ibrutinib plus rituximab had an HR of 0.70 (95% CI 0.37-1.31; p=0.26) when compared with ibrutinib monotherapy. In this model, *CXCR4* mutations were associated with an

HR of 2.24 (95% CI 1.31-3.84; p=0.003) compared to *CXCR4* wild-type status. IPSSWM and treatment status were not statistically associated with PFS (p>0.05 for all subgroups).

In subgroup analyses, ibrutinib plus rituximab was associated with a superior PFS in patients with *CXCR4* mutations with a 48-month PFS rate of 72% (95% CI 51-86%) versus 43% (95% CI 27-58%) for ibrutinib monotherapy with an HR of 0.40 (95% CI 0.17-0.94; p=0.03; **Figure 4B**). In a multivariate model adjusting for treatment status and IPSSWM scores, ibrutinib plus rituximab was associated with an HR of 0.41 (95% CI 0.16-1.02; p=0.06) when compared with ibrutinib monotherapy in patients with *CXCR4* mutations.

There were no statistical differences in PFS between arms in patients without *CXCR4* mutations (**Figure 4C**), treatment-naïve or previously treated patients, and patients with low IPSSWM, intermediate IPSSWM, or high IPSSWM scores (p>0.05 for all subgroups).

CXCR4 mutations were associated with an inferior PFS in the entire cohort with a 48-month PFS rate of 54% vs. 73% (HR 1.94, 95% CI 1.16-3.26; p=0.01; **Figure 4D**). *CXCR4* mutations did not impact PFS in patients treated with ibrutinib plus rituximab with a 48-month PFS rate of 72% vs. 74% (HR 0.97, 95% CI 0.36-2.61; p=0.96; **Figure 4E**) and were associated with inferior PFS in patients treated with ibrutinib monotherapy with a 48-month PFS rate of 43% vs. 72% (HR 2.69, 95% CI 1.46-4.97; p=0.002; **Figure**

4F). In multivariate models adjusting for treatment status and IPSSWM scores, *CXCR4* mutations were associated with an inferior PFS in the entire cohort (HR 2.24, 95% CI 1.31-3.84; p=0.003), not associated with an inferior PFS in patients treated with ibrutinib plus rituximab (HR 1.04, 95% CI 0.35-3.03; p=0.95), and associated with an inferior PFS in patients treated with ibrutinib monotherapy (HR 3.00; 95% CI 1.60-5.62; p=0.001).

Figure 5 presents the forest plot of all subgroup analyses assessing the effect of ibrutinib plus rituximab on PFS. No subgroups demonstrated a statistically significant benefit after adjustment for multiple testing. The only significant association after correction was the adverse impact of *CXCR4* mutations on PFS in patients receiving ibrutinib monotherapy (adjusted and corrected p=0.002), an effect not observed with I+R.

The median OS was not reached for both treatment arms. The 48-month OS rate was 89% (95% CI 80-94) for ibrutinib monotherapy and 94% (95% CI 82-98%) for ibrutinib plus rituximab for an HR of 0.64 (95% CI 0.20-2.02; p=0.45). There were no detectable differences in OS between arms in patients with or without *CXCR4* mutations, treatment-naïve or previously treated patients, and patients with low IPSSWM, intermediate IPSSWM, or high IPSSWM scores (p>0.05 for all subgroups).

No differences were seen in PFS (p=0.08) or OS (p=0.18) across patients with previously treated and untreated WM

Discussion

Without prospective data evaluating the effect of adding rituximab to ibrutinib in patients with WM, we compared the efficacy of rituximab plus ibrutinib versus ibrutinib monotherapy in WM using individual patient-level data from three prospective studies. Given the incidence of rituximab-related infusion reactions and flare, which can transiently worsen symptoms and limit its use in patients with serum IgM levels >4,000 mg/dl despite concomitant ibrutinib, as well as the logistical challenges of rituximab infusion versus single-agent oral ibrutinib, its routine addition to ibrutinib warrants thoughtful consideration [18,19].

Notably, in the subgroup of patients with *CXCR4* mutations, adding rituximab to ibrutinib demonstrated a significant PFS benefit. Pre-clinically, *CXCR4* mutations have been shown to confer ibrutinib resistance through AKT and ERK activation despite effective BTK inhibition [20], with demonstrated clinical relevance [1,2]. The near twofold increase in the 48-month PFS rate observed with rituximab-ibrutinib compared to ibrutinib alone in this study suggests a synergistic effect, supporting a potential role of rituximab in mitigating or delaying resistance in this subset of patients. Recent studies in chronic lymphocytic leukemia show that BTK inhibitors downregulate immunosuppressive molecules transcriptionally (e.g., CTLA-4, CD200), enhancing T-cell cytotoxicity when combined with bispecific antibodies. These immune-modulatory effects of BTK inhibitor

therapy may complement rituximab's antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity [21,22].

Still, the numerically lower VGPR rates of ibrutinib plus rituximab in patients with *CXCR4* mutations compared to their wild-type counterparts, along with the independent association of *CXCR4* mutations with lower odds of VGPR and inferior PFS in our pooled analysis, underscore a potential unmet therapeutic need. While ibrutinib plus rituximab outperforms ibrutinib monotherapy in this subgroup, its role relative to alternative regimens in *CXCR4*-mutated disease remains unclear.

The phase III ASPEN trial demonstrated a numerically improved efficacy of zanubrutinib monotherapy over ibrutinib monotherapy in patients with WM [23,24]. In patients with *CXCR4* mutations, the VGPR rates for zanubrutinib and ibrutinib were 21% vs. 10%, respectively (p=0.35). However, patients with *CXCR4* mutations had numerically lower VGPR rates than patients with *CXCR4* wild-type disease treated with zanubrutinib at 21% vs. 45%, respectively (p-value not provided). In a recent biomarker study from ASPEN [25], *CXCR4* mutations were also associated with a 50% higher risk of disease progression or death in patients treated with zanubrutinib with an HR of 1.5 (95% 0.8-2.0; p=0.20).

The 21% VGPR rate with zanubrutinib in patients with *CXCR4* mutations was only marginally lower than the 27% observed with ibrutinib plus rituximab. In comparison, the 48-month PFS rate on zanubrutinib in patients with *CXCR4* mutations, though not

reported, appears lower than the 74% on ibrutinib plus rituximab reported here. The advantage of the lower rates of arrhythmia and other adverse events with zanubrutinib over ibrutinib and the absence of rituximab should be considered when counseling patients.

Acalabrutinib, tirabrutinib, and orelabrutinib are covalent BTK inhibitors, and pirtobrutinib is a non-covalent BTK inhibitor used to treat patients with WM [26-30]. However, data on the impact of *CXCR4* mutations on the outcomes of patients treated with these agents are unavailable.

Other highly active WM treatments, such as bendamustine plus rituximab and proteasome inhibitor-based therapies, are independent of *CXCR4* status in their efficacy [31,32]. However, bendamustine plus rituximab has the drawback of stem cell toxicity associated with a small risk of secondary myeloid neoplasms, while proteasome inhibitors exhibit lower overall efficacy in retrospective studies with an increased incidence of peripheral neuropathy [33,34].

Our results show that rituximab provides additional benefits in patients with WM and *CXCR4* mutations. Adding rituximab to zanubrutinib could also improve outcomes in this patient subgroup. Preclinical lymphoma models suggest a synergistic advantage of newer-generation BTK inhibitors in enhancing rituximab-induced ADCC [35]. Thus, the combination of BTK inhibitors plus rituximab is a particularly interesting approach for patients with *CXCR4*-mutated disease, and it should be evaluated in prospective clinical

trials. Ibrutinib plus rituximab remains a viable option for *CXCR4*-mutated disease alongside other standard treatments, such as zanubrutinib and bendamustine plus rituximab. However, the benefits of adding rituximab in patients without *CXCR4* mutations were not evident, and the added toxicity and logistics associated with rituximab would make this approach less preferred in this patient subgroup. Still, the possibility of a modest benefit in *CXCR4* wild-type patients cannot be excluded given the limited sample size in this subgroup, although any such effect is likely to be less pronounced than in *CXCR4*-mutated disease.

Our findings advocate for *CXCR4* mutational testing in WM, especially when considering therapy with covalent BTK inhibitors. Bone marrow aspirate is the preferred source of malignant cells for DNA isolation for *CXCR4* mutational testing purposes. Multiple next-generation sequencing platforms are commercially available for *CXCR4* mutation detection. Our study did not evaluate *CXCR4* mutation subgroups (i.e., nonsense and frameshift), as these data were unavailable in the INNOVATE study. Nonsense *CXCR4* mutations were associated with worse outcomes than frameshift mutations in patients with WM treated with ibrutinib [9]. Furthermore, patients with a higher mutational burden of CXCR4 S338X treated with ibrutinib appeared to have worse outcomes than WM patients with lower mutational burden [36].

This study has inherent limitations due to its pooled design. These include significant variation in follow-up durations across studies, which is important given the potential for response deepening over time; a lack of formal randomization, which increases the risk

of selection bias and may have contributed to imbalances in baseline characteristics such as prior treatment status; and differences in *MYD88* and *CXCR4* testing methodologies (such as polymerase chain reaction versus next-generation sequencing). In addition, limitations in the available pooled data prevented adjustment for key prognostic markers such as LDH and albumin, and did not allow for a detailed analysis of causes of death. This limited the ability to assess cause-specific survival, which is particularly relevant since most WM patients die from non-WM-related causes [37]. Despite these limitations, the baseline characteristics of the pooled cohort were consistent with those of typical WM populations and enabled a meaningful comparison that is unlikely to be achieved through a prospective clinical trial.

Our findings hint at the benefit of BTK inhibitors plus chemoimmunotherapy combinations, which are under investigation and have shown deep responses thus far. However, the specific patient subsets most likely to benefit remain unclear. The ongoing BRAWM study, evaluating 12 months of acalabrutinib plus six cycles of bendamustine and rituximab, recently reported a 64% VGPR-or-better rate [38]. A phase II trial of zanubrutinib plus bendamustine and rituximab showed a 71% VGPR-or-better rate [39]. The ZEBRA trial (NCT06561347), evaluating four cycles of bendamustine and rituximab combined with 15 months of zanubrutinib to reduce toxicity while maintaining efficacy, is ongoing.

Conclusion

Based on this pooled analysis of patient-level data from three prospective studies, adding rituximab to ibrutinib was associated with numerical improvements in VGPR and 48-month PFS rates compared to ibrutinib monotherapy in patients with *CXCR4* mutations. Our findings support *CXCR4* mutational testing in WM and the development of clinical trials adding rituximab to regimens with covalent and non-covalent BTK inhibitors.

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Authors contributions

JJC designed the study. AG and JJC analyzed the data and wrote the initial manuscript. All the authors critically reviewed the manuscript and accepted the final manuscript.

ORCID numbers

AG: 000-0002-8205-6643

ARG: 0009-0008-9637-4221

SS: 0000-0002-0075-6735

ARB: 0000-0002-3868-9267

GVK: 0000-0001-7254-5374

SPT: 0000-0001-6393-6154

JJC: 000-0001-9490-7532

References

1. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med*. 2015;372(15):1430-1440.

2. Treon SP, Gustine J, Meid K, et al. Ibrutinib Monotherapy in Symptomatic, Treatment-Naive Patients With Waldenstrom Macroglobulinemia. *J Clin Oncol*. 2018;36(27):2755-2761.

3. Treon SP, Meid K, Gustine J, et al. Long-Term Follow-Up of Ibrutinib Monotherapy in Symptomatic, Previously Treated Patients With Waldenstrom Macroglobulinemia. *J Clin Oncol*. 2021;39(6):565-575.

4. Castillo JJ, Meid K, Gustine JN, et al. Long-term follow-up of ibrutinib monotherapy in treatment-naive patients with Waldenstrom macroglobulinemia. *Leukemia*. 2022;36(2):532-539.

5. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*. 2014;123(11):1637-1646.

6. Roccaro AM, Sacco A, Jimenez C, et al. C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. *Blood*. 2014;123(26):4120-4131.

7. Schmidt J, Federmann B, Schindler N, et al. MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. *Br J Haematol*. 2015;169(6):795-803.

8. Ballester LY, Loghavi S, Kanagal-Shamanna R, et al. Clinical Validation of a CXCR4 Mutation Screening Assay for Waldenstrom Macroglobulinemia. *Clin Lymphoma Myeloma Leuk*. 2016;16(7):395-403 e391.

9. Castillo JJ, Xu L, Gustine JN, et al. CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenstrom macroglobulinaemia treated with ibrutinib. *Br J Haematol*. 2019;187(3):356-363.

10. Dimopoulos MA, Tedeschi A, Trotman J, et al. Phase 3 Trial of Ibrutinib plus Rituximab in Waldenstrom's Macroglobulinemia. *N Engl J Med*. 2018;378(25):2399-2410.

11. Buske C, Tedeschi A, Trotman J, et al. Ibrutinib Plus Rituximab Versus Placebo Plus Rituximab for Waldenstrom's Macroglobulinemia: Final Analysis From the Randomized Phase III iNNOVATE Study. *J Clin Oncol*. 2022;40(1):52-62.

12. Kumar SK, Callander NS, Adekola K, et al. Waldenström Macroglobulinemia/Lymphoplasmacytic Lymphoma, Version 2.2024, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network*. 2024;22(1D):e240001.

13. Trotman J, Buske C, Tedeschi A, et al. Single-Agent Ibrutinib for Rituximab-Refractory Waldenstrom Macroglobulinemia: Final Analysis of the Substudy of the Phase III Innovate(TM) Trial. *Clin Cancer Res*. 2021;27(21):5793-5800.

14. Owen RG, Kyle RA, Stone MJ, et al. Response assessment in Waldenstrom macroglobulinaemia: update from the VIth International Workshop. *Br J Haematol*. 2013;160(2):171-176.

15. Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for Waldenström macroglobulinemia. *Blood*. 2009;113(18):4163-4170.

16. Zanwar S, Le-Rademacher J, Durot E, et al. Simplified Risk Stratification Model for Patients With Waldenstrom Macroglobulinemia. *J Clin Oncol*. 2024;42(21):2527-2536.

17. Castillo JJ, Sarosiek SR, Gustine JN, et al. Response and survival predictors in a cohort of 319 patients with Waldenstrom macroglobulinemia treated with ibrutinib monotherapy. *Blood Adv*. 2022;6(3):1015-1024.

18. Castillo JJ, Kanan S, Meid K, Manning R, Hunter ZR, Treon SP. Rituximab intolerance in patients with Waldenström macroglobulinaemia. *British Journal of Haematology*. 2016;174(4):645-648.

19. Ghobrial IM, Fonseca R, Greipp PR, et al. Initial immunoglobulin M 'flare' after rituximab therapy in patients diagnosed with Waldenstrom macroglobulinemia: an Eastern Cooperative Oncology Group Study. *Cancer*. 2004;101(11):2593-2598.

20. Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4S338X somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. *Leukemia*. 2015;29(1):169-176.

21. Mhibik M, Gaglione EM, Eik D, et al. BTK inhibitors, irrespective of ITK inhibition, increase efficacy of a CD19/CD3-bispecific antibody in CLL. *Blood*. 2021;138(19):1843-1854.

22. Mhibik M, Gaglione EM, Eik D, et al. Cytotoxicity of the CD3xCD20 bispecific antibody epcoritamab in CLL is increased by concurrent BTK or BCL-2 targeting. *Blood Adv*. 2023;7(15):4089-4101.

23. Dimopoulos MA, Opat S, D'Sa S, et al. Zanubrutinib Versus Ibrutinib in Symptomatic Waldenstrom Macroglobulinemia: Final Analysis From the Randomized Phase III ASPEN Study. *J Clin Oncol*. 2023;41(33):5099-5106.

24. Tam CS, Opat S, D'Sa S, et al. A randomized phase 3 trial of zanubrutinib vs ibrutinib in symptomatic Waldenstrom macroglobulinemia: the ASPEN study. *Blood*. 2020;136(18):2038-2050.

25. Tam CS, Opat S, D'Sa S, et al. Biomarker analysis of the ASPEN study comparing zanubrutinib with ibrutinib for patients with Waldenstrom macroglobulinemia. *Blood Adv*. 2024;8(7):1639-1650.

26. Owen R, McCarthy H, Rule S, et al. P1130: ACALABRUTINIB IN TREATMENT-NAIVE OR RELAPSED/REFRACTORY WALDENSTRÖM MACROGLOBULINEMIA: 5-YEAR FOLLOW-UP OF A PHASE 2, SINGLE-ARM STUDY. *HemaSphere*. 2022;6(S3):1020-1021.

27. Owen RG, McCarthy H, Rule S, et al. Acalabrutinib monotherapy in patients with Waldenstrom macroglobulinemia: a single-arm, multicentre, phase 2 study. *Lancet Haematol*. 2020;7(2):e112-e121.

28. Sekiguchi N, Rai S, Munakata W, et al. Two-year outcomes of tirabrutinib monotherapy in Waldenstrom's macroglobulinemia. *Cancer Sci*. 2022;113(6):2085-2096.

29. Cao XX, Jin J, Fu CC, et al. Evaluation of orelabrutinib monotherapy in patients with relapsed or refractory Waldenstrom's macroglobulinemia in a single-arm, multicenter, open-label, phase 2 study. *EClinicalMedicine*. 2022;52:101682.

30. Palomba ML, Patel MR, Eyre TA, et al. Efficacy of Pirtobrutinib, a Highly Selective, Non-Covalent (Reversible) BTK Inhibitor in Relapsed / Refractory Waldenström Macroglobulinemia: Results from the Phase 1/2 BRUIN Study. *Blood*. 2022;140(Supplement 1):557-560.

31. Castillo JJ, Gustine JN, Meid K, et al. CXCR4 mutational status does not impact outcomes in patients with Waldenstrom macroglobulinemia treated with proteasome inhibitors. *Am J Hematol*. 2020;95(4):E95-E98.

32. Laribi K, Poulain S, Willems L, et al. Long-term results of Waldenstrom macroglobulinaemia treatment by bendamustine and rituximab: A study on behalf of the French Innovative Leukemia Organization (FILO). *Br J Haematol*. 2024;204(6):2233-2236.

33. Abeykoon JP, Zanwar S, Ansell SM, et al. Assessment of fixed-duration therapies for treatment-naive Waldenstrom macroglobulinemia. *Am J Hematol*. 2021;96(8):945-953.

34. Leleu X, Soumerai J, Roccaro A, et al. Increased incidence of transformation and myelodysplasia/acute leukemia in patients with Waldenstrom macroglobulinemia treated with nucleoside analogs. *J Clin Oncol*. 2009;27(2):250-255.

35. Yu H, Wang X, Li J, et al. Addition of BTK inhibitor orelabrutinib to rituximab improved anti-tumor effects in B cell lymphoma. *Mol Ther Oncolytics*. 2021;21:158-170.

36. Gustine JN, Xu L, Tsakmaklis N, et al. CXCR4 (S338X) clonality is an important determinant of ibrutinib outcomes in patients with Waldenstrom macroglobulinemia. *Blood Adv*. 2019;3(19):2800-2803.

37. Dalal NH, Dores GM, Curtis RE, Linet MS, Morton LM. Cause-specific mortality in individuals with lymphoplasmacytic lymphoma/Waldenstrom macroglobulinaemia, 2000-2016. *Br J Haematol*. 2020;189(6):1107-1118.

38. Berinstein N. A phase II trial of Bendamustine, Rituximab and Acalabrutinib in previously untreated Waldenström's Macroglobulinemia (BRAWM). The 12th International Workshop on Waldenström's Macroglobulinemia (IWWM-12). Prague, Czech Republic; 2024.

39. Wenjie X, Yan Y, Yu Y, et al. Phase II Clinical Study of Zanubrutinib Combined with Bendamustine and Rituximab (ZBR) for Time-Limited Treatment of Waldenstrom Macroglobulinemia. *Blood*. 2024;144(Supplement 1):6324-6324.

Table 1. Patients' baseline characteristics according to treatment group

Variable	Ibrutinib monotherapy (n=116)	Ibrutinib + rituximab (n=58)	p-value
Median age, years	66 (56-71)	66 (55-77)	0.74
Age >65 years	61 (53%)	36 (62%)	0.24
Male sex	87 (75%)	34 (59%)	0.03
Median hemoglobin, g/l	105 (96-119)	103 (92-116)	0.50
Hemoglobin <115 g/l	81 (70%)	41 (73%)	0.71
Median platelet count, k/ul	215 (150-282)	242 (166-340)	0.15
Platelet count <100 k/ul	11 (10%)	4 (8%)	0.67
Median beta-2-microglobulin, mg/l	5.2 (3.3-7.5)	3.8 (3.0-5.0)	0.001
Beta-2-microglobulin >3.5 mg/l	96 (83%)	43 (74%)	0.18
Median serum IgM, g/l	38.4 (27.4-55.6)	35.8 (18.2-51.4)	0.17
Serum IgM >70 g/l	8 (7%)	0 (0%)	0.04
Previously treated	84 (72%)	27 (47%)	0.001
CXCR4 mutations	44 (38%)	26 (45%)	0.38
Low IPSSWM	21 (18%)	7 (13%)	0.67
Intermediate IPSSWM	48 (42%)	25 (46%)	
High IPSSWM	46 (40%)	22 (41%)	

IPSSWM: International Prognostic Scoring System for Waldenström Macroglobulinemia

Table 2. Patients' baseline characteristics according to *CXCR4* mutational status

Variable	***CXCR4 WT* (n=104)**	***CXCR4 MUT* (n=70)**	**p-value**
Median age, years	66 (55-71)	66 (56-71)	0.48
Age >65 years	56 (54%)	41 (59%)	0.54
Male sex	75 (72%)	46 (66%)	0.37
Median hemoglobin, g/l	102 (94-116)	108 (99-120)	0.10
Hemoglobin <115 g/l	76 (73.8%)	46 (67.6%)	0.38
Median platelet count, k/ul	252 (174-329)	184 (121-247)	0.001
Platelet count <100 k/ul	4 (4%)	11 (17%)	0.004
Median beta-2-microglobulin, mg/l	5.0 (3.3-7.2)	4.0 (3.0-7.0)	0.082
Beta-2-microglobulin >3.5 mg/l	88 (85%)	51 (73%)	0.058
Median serum IgM, g/l	33.6 (20.8-51.3)	43.1 (32.5-58.7)	0.011
Serum IgM >70 g/l	5 (5%)	3 (4%)	0.87
Previously treated	70 (67%)	41 (59%)	0.24
Ibrutinib + Rituximab	32 (31%)	26 (37%)	0.38
Low IPSSWM	19 (18%)	9 (14%)	0.63
Intermediate IPSSWM	42 (41%)	31 (47%)	
High IPSSWM	42 (41%)	26 (39%)	

IPSSWM: International Prognostic Scoring System for Waldenström Macroglobulinemia; WT: Wild-type; MUT: Mutated

Figure legends

Figure 1. CONSORT diagram

Figure 2. Categorical responses to ibrutinib plus rituximab (I+R) and ibrutinib monotherapy (I) in the entire cohort (I:118, I+R:58) (A), patients with *CXCR4* mutations (I:44, I+R:26), showing numerical improvements in VGPR (B), and patients with high IPSSWM scores (I:46, I+R:22) (C). Categorical responses according to *CXCR4* mutational status in the entire cohort (Wild-Type: 104, Mutated: 70) (D), in patients treated with I+R (Wild-Type: 32, Mutated: 26) (E), and in patients treated with I alone (Wild-Type: 72, Mutated: 44) (F). VGPR: very good partial response; PR: partial response; mR: minor response; NR: no response

Figure 3. Subgroup analyses of very good partial response (VGPR) rates comparing ibrutinib plus rituximab versus ibrutinib monotherapy (above), and the impact of *CXCR4* mutations on VGPR within each treatment arm (below). False discovery rate (FDR) correction was applied for multiple comparisons: 7 subgroup comparisons in (A) and 2 in (B).

Figure 4. Kaplan-Meier survival estimates comparing progression-free survival (PFS) of ibrutinib plus rituximab versus ibrutinib monotherapy for the whole cohort (A), patients with *CXCR4* mutations (B), and patients without *CXCR4* mutations (C). PFS according to *CXCR4* mutational status for the whole cohort (D), patients treated with I+R (E), and patients treated with I alone (F).

Figure 5. Subgroup analyses of progression-free survival (PFS) comparing ibrutinib plus rituximab versus ibrutinib monotherapy (above), and the impact of *CXCR4* mutations on VGPR within each treatment arm (below). False discovery rate (FDR) correction was applied for multiple comparisons: 7 subgroup comparisons in (A) and 2 in (B).

Figure 1

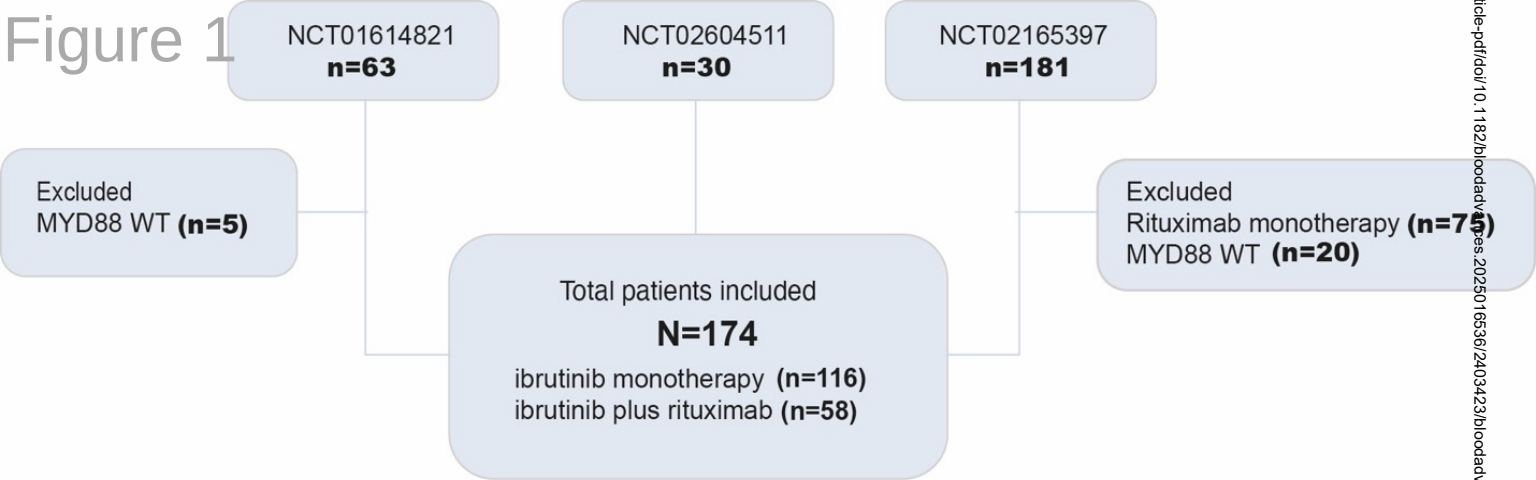


Figure 2

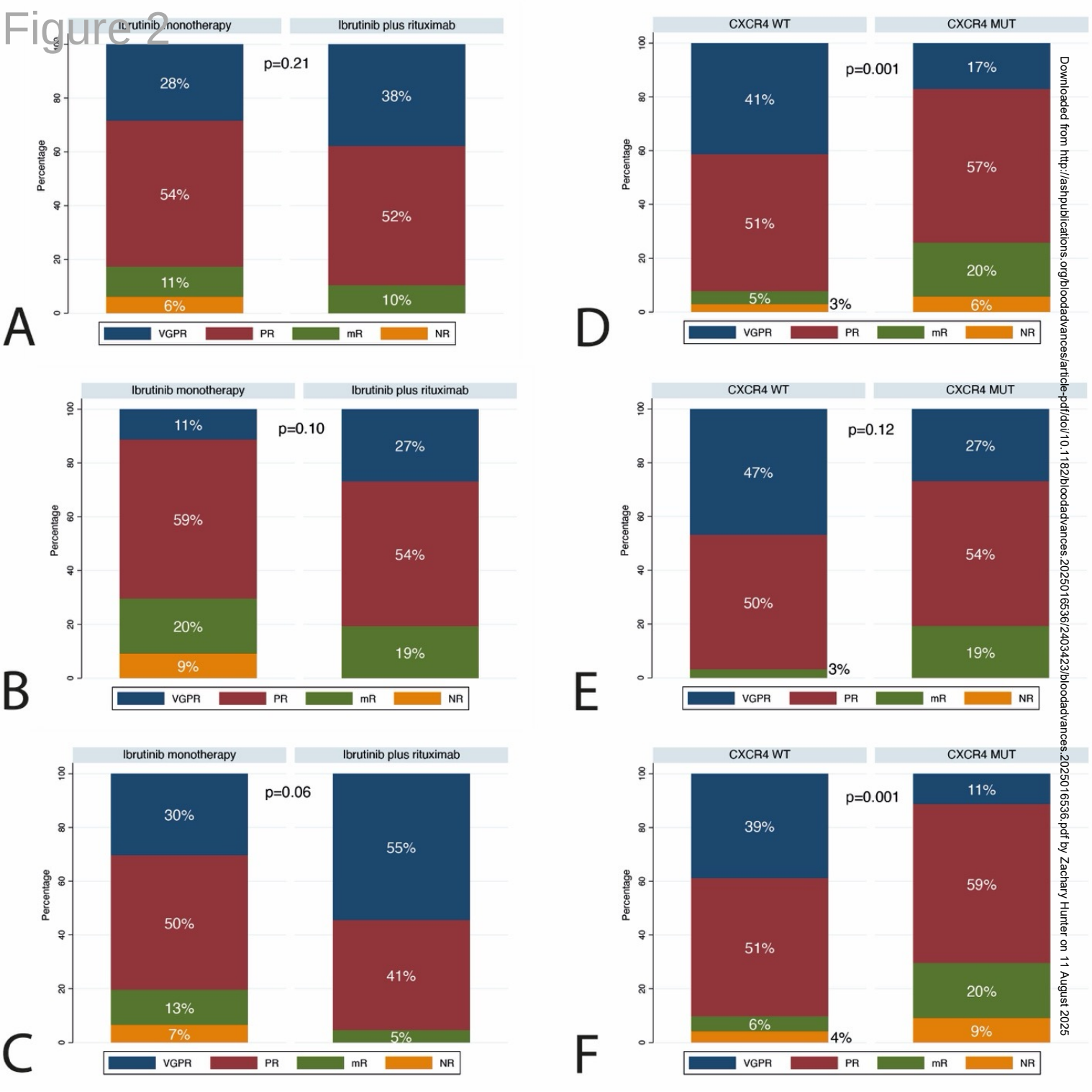
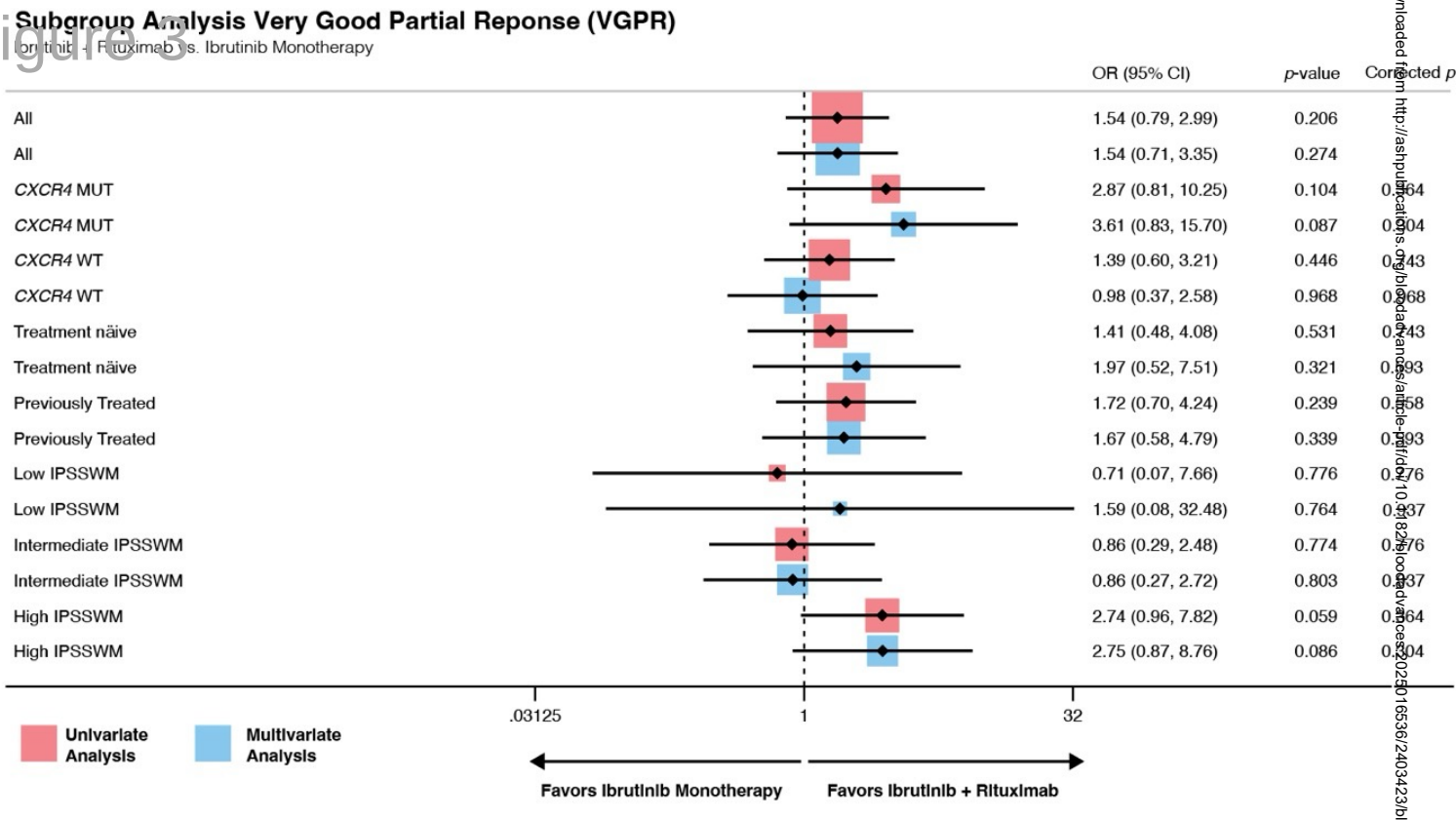


Figure 3



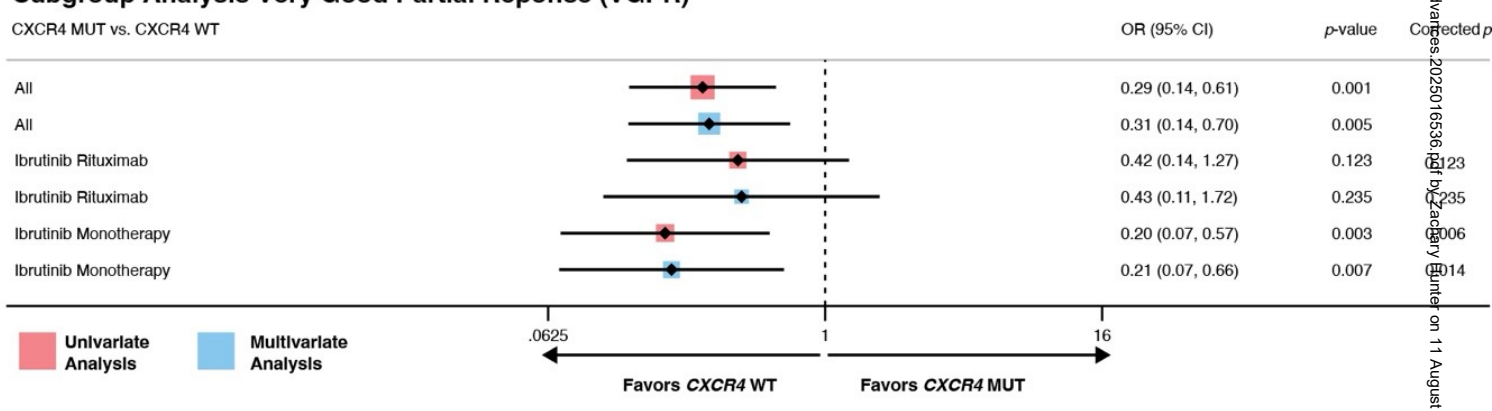


Figure 4

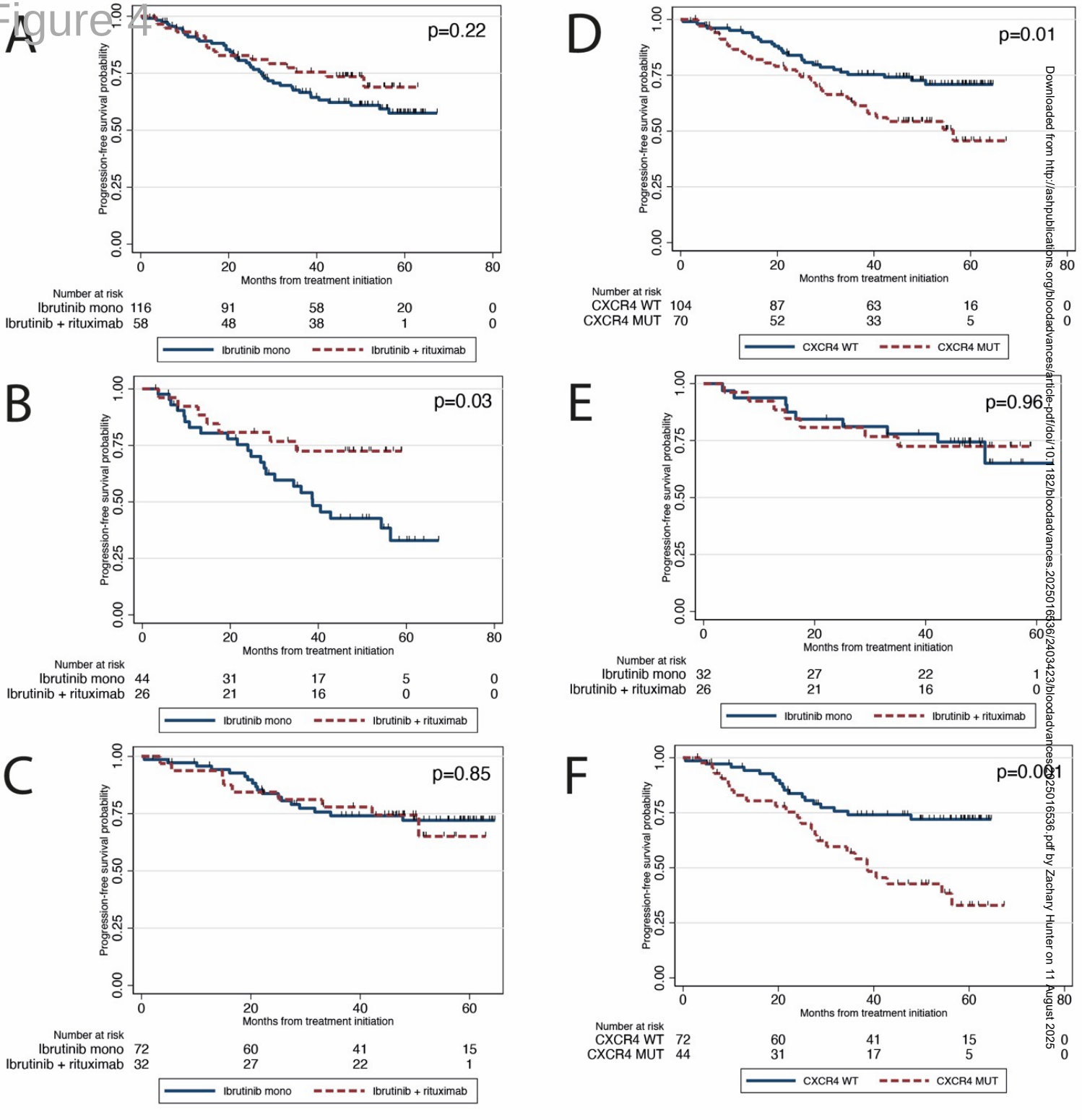


Figure 5