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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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CXCR4 mutational status does not impact outcomes in patients with Waldenström macroglobulinemia treated with proteasome inhibitors

To the Editor:

Waldenström macroglobulinemia (WM) is rare subtype of non-Hodgkin lymphoma characterized by the malignant accumulation of IgM-secreting lymphoplasmacytic cells in the bone marrow and other

organs. The genomic landscape of WM is characterized by somatic mutations in *MYD88*, and *CXCR4*, detected in 90-95% and 30-40% of WM patients, respectively.^{1,2} Waldenström macroglobulinemia patients with *CXCR4* mutations seem to have longer time to response, lower rates of major response and shorter progression-free survival (PFS), when treated with the oral Bruton Tyrosine Kinase (BTK) inhibitor ibrutinib.³ The impact of *CXCR4* mutations in the response and survival outcomes of WM patients treated with other therapies is less understood. A recent study has suggested that *CXCR4* mutations do not adversely affect PFS in WM patients treated with bortezomib.⁴ We conducted a pooled analysis from three prospective studies aimed at evaluating the impact of *CXCR4* mutations in response and survival outcomes of WM patients treated with proteasome inhibitor-based regimens.

All patients were participants on three prospective clinical trials evaluating the combinations of bortezomib, dexamethasone and rituximab (BDR; NCT00250926), carfilzomib, dexamethasone and rituximab (CaRD; NCT01470196), and ixazomib, dexamethasone and rituximab (IDR; ID NCT02400437) in previously untreated patients with WM. All patients signed informed consent for participation in their respective clinical trial. All studies were reviewed and approved by the Institutional Review Board at our institution. All patients had a clinicopathological diagnosis of WM and met criteria to treat, according to second International Workshop for WM (IWWM) guidelines. The design of the studies has been previously published.⁵⁻⁷ Pertinent clinical and laboratory data were collected. The *MYD88* and *CXCR4* mutations were assessed using allele-specific PCR and Sanger sequencing assays performed in CD19-selected bone marrow samples. Response to therapy was assessed using modified sixth IWWM response criteria, in which a decrease in extramedullary disease was not required for partial (PR) or very good partial response (VGPR), but was required for complete response (CR) attainment. Progression-free survival (PFS) was defined as the time from treatment initiation until disease progression, death or last follow-up. Patients' characteristics are presented using descriptive statistics. Group comparisons were made using the Fisher's exact test. Age >65 years, male sex, hemoglobin level ≤ 11.5 g/dL, platelet count ≥ 100 K/ μ L, serum IgM level ≥ 4000 mg/dL, serum $\beta 2$ -microglobulin level > 3 mg/L, serum albumin level ≤ 3.5 g/dL, bone marrow involvement $> 60\%$, International Prognostic Scoring System for WM. And, *MYD88* as well as *CXCR4* mutational status were variables included in the logistic and Cox proportional-hazard regression models. Univariate logistic regression models were fitted to identify predictive factors for response, and the results presented as odds ratio (OR) with 95% confidence interval (CI). The PFS curves were obtained using the Kaplan-Meier method and compared using the log-rank test. Univariate Cox proportional-hazard regression models were fitted to evaluate prognostic factors for PFS, and the results presented as hazard ratios (HR) with 95% CI. *P* values $< .05$ were statistically significant. Calculations and graphs were obtained using STATA version 15 (StataCorp, College Station, TX, USA).

A total of 76 patients were included in this analysis, of which 19 (25%) received BDR, 31 (41%) received CaRD and 26 (34%) received

IDR. Thirty-six patients (55%) did not have *CXCR4* mutations (*CXCR4*^{WT}), and 29 (45%) had a *CXCR4* mutation (*CXCR4*^{MUT}). The *CXCR4* mutational status was not determined in 11 patients. The median age at treatment initiation was 63 years (range 46-83 years), median hemoglobin level was 10.3 g/dL (range 6.9-17.1 g/dL), median serum IgM level was 4048 mg/dL (range 345-9550 mg/dL), median serum $\beta 2$ -microglobulin level was 3.5 mg/L (range 1.0-10.8 mg/L), serum albumin was 3.7 g/dL (range 2.4-4.8 g/dL) and median bone marrow involvement was 55% (range 5-95%). There was a higher proportion of serum IgM levels ≥ 4000 mg/dL (62% vs 39%) and lower proportion of serum albumin levels ≤ 3.5 g/dL (21% vs 47%) in *CXCR4*^{MUT} than in *CXCR4*^{WT} patients. There was no detectable difference on the rate of *CXCR4*^{MUT} status between treatment groups (*P* = .12).

At 6 months, the rates of CR, VGPR, PR and minor response (mR) were 1%, 11%, 42% and 29%, respectively, for an overall response rate of 91% and a major response rate of 59%. There was no detectable difference in categorical responses at 6 months between treatment regimens (*P* = .13). Also, there was no detectable difference in categorical responses at 6 months between *CXCR4*^{WT} and *CXCR4*^{MUT} patients (Figure 1A; *P* = .56). Univariate logistic regression analysis showed that *CXCR4*^{MUT} status was not associated with major response rate attainment at 6 months (OR 0.77, 95% CI 0.27-2.22; *P* = .63). Serum albumin level ≤ 3.5 g/dL was the only factor associated with higher odds of major response attainment at 6 months (OR 5.40, 95% CI 1.59-18.3; *P* = .007). At 12 months, the rates of CR, VGPR, PR and mR were 5%, 25%, 52% and 16%, respectively, for an overall response rate of 98% and a major response rate of 83%. There was no detectable difference in categorical responses at 12 months between treatment regimens (*P* = .30). Also, there was no detectable difference in categorical responses at 12 months *CXCR4*^{WT} and *CXCR4*^{MUT} patients (Figure 1B; *P* = .73). Univariate logistic regression analysis showed that *CXCR4*^{MUT} status was not associated with major response rate attainment at 12 months (OR 0.76, 95% CI 0.18-3.23; *P* = .71). Serum $\beta 2$ -microglobulin level > 3 mg/L was the only factor associated with lower odds of major response at 12 months (OR 0.11, 95% CI 0.01-0.91; *P* = .04).

At the time of this report, 40 patients (53%) have progressed and five patients (7%) have died. The median follow-up time for all patients was 6 years (95% CI 4.7-6.6 years). The median follow-up times for patients who received BDR, CaRD and IDR were 11.7 years (95% CI 9-12.1 years), 6.3 years (6-6.8 years) and 2.9 years (95% CI 2.8-3.2 years), respectively. The median PFS for all patients was 4.8 years (95% CI 3.3-6.5 years; Figure 1C). There was no detectable difference in PFS between treatment regimens (log-rank *P* = .73). The median PFS for *CXCR4*^{WT} patients was 3.6 years (95% CI 1.7-5.9 years) vs 6.5 years (95% CI 2.7-not reached) for *CXCR4*^{MUT} patients (log-rank *P* = .12; Figure 1D). In the univariate analysis, *CXCR4*^{MUT} status was not associated with better or worse PFS (HR 0.61, 95% CI 0.30-1.23; *P* = .16). Serum $\beta 2$ -microglobulin level > 3 mg/L was the only factor associated with a worse PFS (HR 2.03, 95% CI 1.03-4.00; *P* = .04). There were statistical trends towards worse PFS in men (HR 1.79, 95% CI 0.91-3.53, *P* = .09) and

patients with bone marrow involvement $\geq 60\%$ at baseline (HR 1.74, 95% CI 0.94-3.25; $P = .08$). No other factors were associated with PFS.

The interest in our study stemmed from previously published conflicting preclinical data, in which *CXCR4* mutations were associated with decreased cell killing in WM cells treated with alkylating agents, nucleoside analogues, BTK inhibitors, phosphatidylinositol three kinase (PI3K) inhibitors and proteasome inhibitors.⁸ Another study associated *CXCR4* mutations with resistance to BTK inhibitors, mammalian target of rapamycin inhibitors, and PI3K inhibitors, but not to proteasome inhibitors.⁹ In our study, there were no detectable differences in the rates of major response at 6 and 12 months to proteasome inhibitor-based therapy between patients with and without *CXCR4* mutations. Similarly, there were no detectable differences in median PFS between groups. Overall, the results of our study support a *CXCR4*-agnostic response and PFS in WM patients treated with proteasome inhibitor-containing regimens. Therefore, bortezomib, carfilzomib and ixazomib-based regimens are appropriate frontline treatment options for WM

patients with *CXCR4* mutations. Proteasome inhibitors are used for the treatment of patients with symptomatic WM, and a number of prospective clinical trials have shown these agents to be safe and highly effective in these patients.⁵⁻⁷ Additionally, large retrospective and population-based studies from the United States and Europe have shown proteasome inhibitors are increasingly being used for the treatment of WM in both frontline and relapsed settings. Fixed-duration therapy and lack of risk of secondary myeloid neoplasms are some of the favorable features of proteasome inhibitor-based therapy in WM patients, which would make these agents desirable for younger patients, for example, who might be less inclined towards indefinite therapy, or in whom one would like to minimize the risk of myeloid neoplasms. Our study is not without limitations, however. The relatively small number of patients, who were treated exclusively with proteasome inhibitor-based regimens and were participants in prospective clinical trials at a tertiary institution, could have potentially introduced patient selection bias. The patients' clinical characteristics and laboratory values appear in line with prior

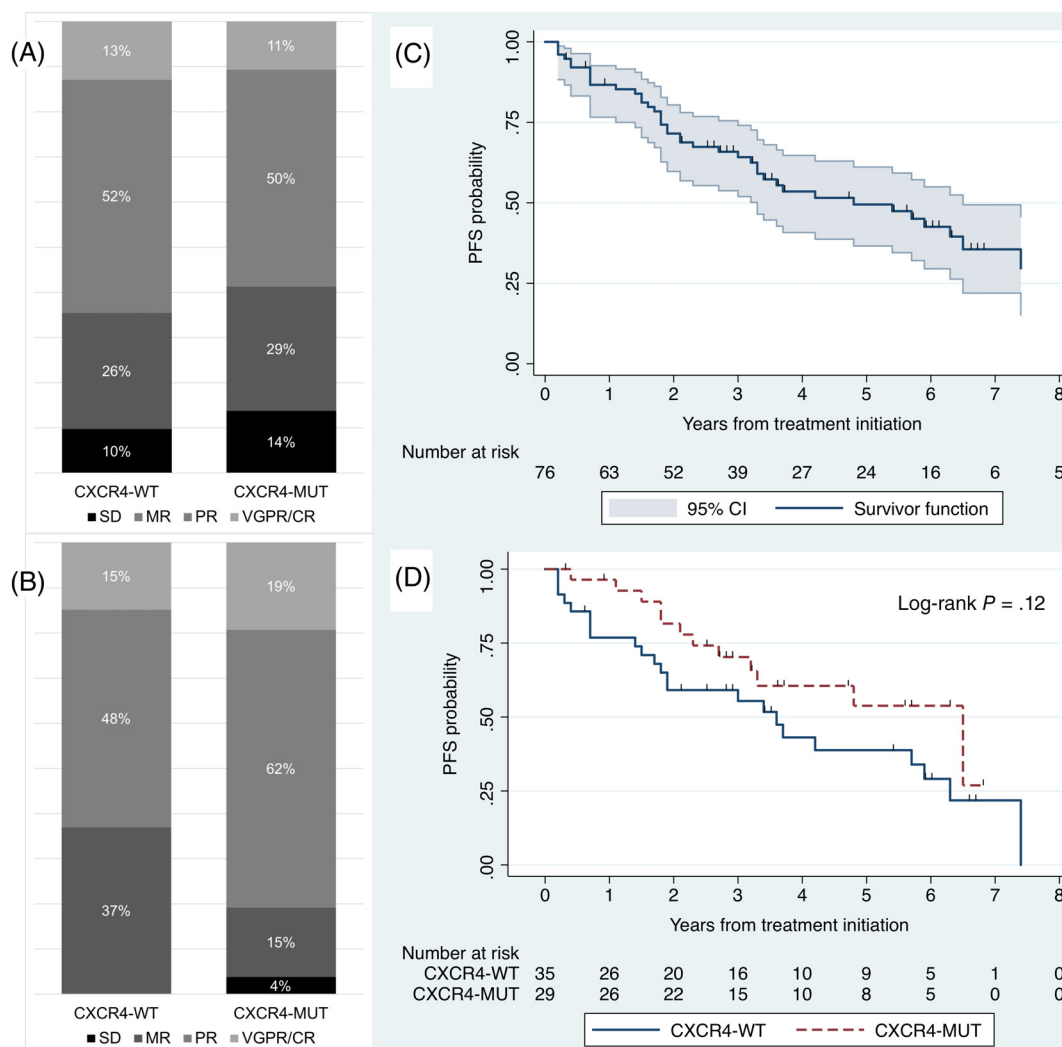


FIGURE 1 Categorical responses at 6 months A, and at 12 months B, and progression-free survival estimates for the entire cohort C, and according to *CXCR4* mutational status D, of 76 patients with Waldenström macroglobulinemia treated with proteasome inhibitor-based primary therapy

prospective and retrospective studies in WM patients. Additionally, the rate of missing clinical and laboratory data was minimal, and the follow-up time is relatively long. Despite the limitations, our study provides valuable insights for personalization of therapy. Additional studies are needed to further confirm our findings, and to evaluate the impact of CXCR4 mutations in the outcomes of patients treated with chemoimmunotherapy and other targeted agents. Our study, however, does not detract from the adverse impact of CXCR4 mutations in WM patients treated with BTK inhibitors, the value of CXCR4 mutations as driver mutations in WM, and the potential importance of anti-CXCR4 directed therapy in WM and other malignancies.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

JJC designed the study and performed the analysis. JJC, JNG and KM collected the data. JJC, CAF and SPT provided clinical care to patients. MD, MLG, CJ, AKo, XL, MM, NT, CJP, LX, GY and ZRH performed the genomic analysis. JJC and JNG wrote the initial draft. All authors reviewed the initial manuscript, provided feedback and approved the final manuscript.

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Hematologic findings in a large sample of patients with anorexia nervosa and bulimia nervosa

To the Editor:

Patients with anorexia nervosa (AN) and bulimia nervosa (BN) suffer from many medical complications as a result of their eating disorders. The Diagnostic and Statistical Manual of Mental Disorders Fourth