

# *CXCR4*<sup>S338X</sup> clonality is an important determinant of ibrutinib outcomes in patients with Waldenström macroglobulinemia

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## Key Points

- *CXCR4*<sup>S338X</sup> clonality  $\geq 25\%$  is associated with lower very good partial response and shorter progression-free survival to ibrutinib.
- *CXCR4*<sup>S338X</sup> clonality assessment represents a novel biomarker to predict outcomes to ibrutinib in Waldenström macroglobulinemia patients.

## Introduction

Waldenström macroglobulinemia (WM) is an immunoglobulin M (IgM)–secreting lymphoplasmacytic lymphoma.<sup>1</sup> Activating somatic mutations in *MYD88* and *CXCR4* are present in 95% to 97% and 30% to 40% of WM patients, respectively.<sup>2–5</sup> Mutated *MYD88* triggers prosurvival NF- $\kappa$ B signaling via Bruton tyrosine kinase (BTK) and hematopoietic cell kinase, both direct targets of ibrutinib.<sup>6,7</sup> *MYD88*<sup>WT</sup> patients harbor NF- $\kappa$ B pathway mutations downstream of BTK, and derive minimal benefit from ibrutinib.<sup>8,9</sup> *CXCR4* mutations occur nearly exclusively with mutated *MYD88* and promote enhanced AKT and extracellular signal-regulated kinase 1/2 activation.<sup>5,10,11</sup> *CXCR4* mutations also confer both in vitro and clinical resistance to ibrutinib, particularly nonsense variants such as *CXCR4*<sup>S338X</sup>.<sup>10–16</sup> In WM patients, *CXCR4*<sup>S338X</sup> constitutes the most common *CXCR4* mutation identified.<sup>3,5</sup> *CXCR4*<sup>S338X</sup> is primarily subclonal to mutated *MYD88*, but shows a highly variable clonal distribution.<sup>5</sup> These findings prompted us to examine the impact of *CXCR4*<sup>S338X</sup> clonality on outcomes to ibrutinib in WM patients.

## Methods

We identified consecutive patients seen at our institution between May 2012 and January 2018 who met the consensus criteria for WM and received ibrutinib monotherapy.<sup>1</sup> The presence of *MYD88* and *CXCR4* mutations was assessed by allele-specific polymerase chain reaction (AS-PCR) and Sanger sequencing in sorted CD19 cells derived from bone marrow (BM) aspirates.<sup>4,5,17</sup> Cancer cell fraction (CCF) analysis was performed for patients with *CXCR4*<sup>S338X</sup> using synchronous, parallel quantitative AS-PCR analyses for *MYD88*<sup>L265P</sup> and *CXCR4*<sup>S338X</sup>, as previously described.<sup>5</sup> The CCF was determined as the ratio of cells expressing *CXCR4*<sup>S338X</sup>/*MYD88*<sup>L265P</sup>. Patients with non-S338X *CXCR4* mutations unamenable to AS-PCR were excluded, and their outcomes to ibrutinib are provided in supplemental Table 1. Time-dependent receiver operator curve estimation with area under the curve (AUC) analysis was used to determine the optimal cutoff for *CXCR4*<sup>S338X</sup> clonality. Time to events was estimated using the Kaplan-Meier method, and comparisons were made using the log-rank test. A multivariate model for progression-free survival (PFS) was not pursued given the lack of statistically significant covariates in univariate analysis. *P* values were considered to be statistically significant if  $< .05$ . Calculations were performed with R (R Foundation for Statistical Computing, Vienna, Austria).

## Results and discussion

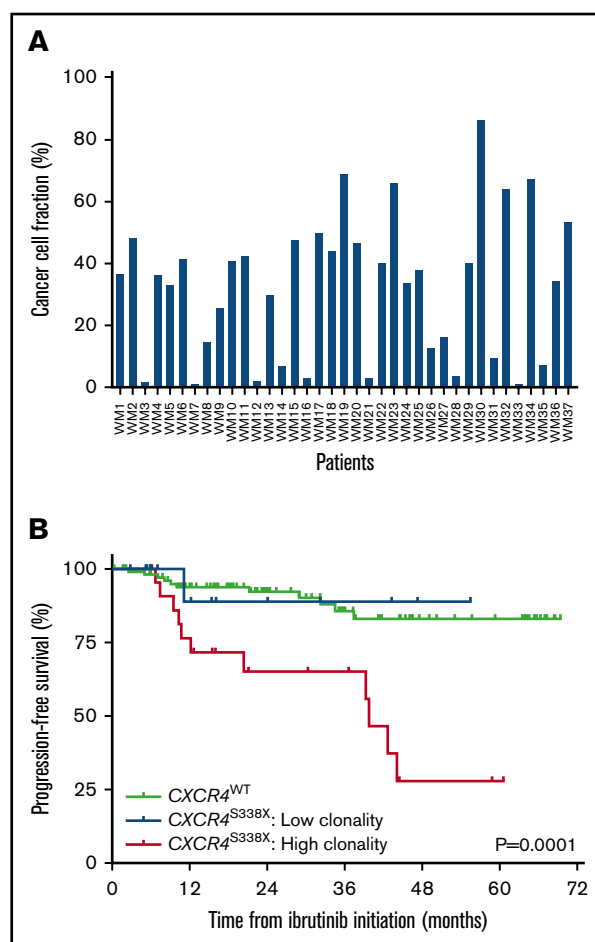
A total of 147 patients with WM met inclusion criteria for this analysis. The *MYD88*<sup>L265P</sup> and *CXCR4*<sup>S338X</sup> mutations were identified in 147 patients (100%) and 37 patients (25%), respectively. Baseline clinical characteristics at the time of ibrutinib initiation are shown in supplemental Table 2. The median treatment duration of ibrutinib was 21.1 months (range, 0.3–69 months). Patients with *CXCR4*<sup>S338X</sup> had lower rates of major response (62% vs 85%; *P* = .001) and very good partial response (11% vs 35%; *P* = .006) vs *CXCR4*<sup>WT</sup>, as well as delayed attainment of both minor (1.8 vs 1.1 months; *P* < .001) and major responses (7.4 vs 1.8 months; *P* < .001). No difference in overall

response rate was observed (92% vs 96%;  $P = .27$ ). At the time of this report, 23 patients (16%) have progressed on ibrutinib therapy.  $CXCR4^{S338X}$  was the only variable associated with worse progression-free survival (PFS) (hazard ratio [HR], 5.03; 95% confidence interval [CI], 1.91-13.2;  $P = .001$ ) with a significantly shorter PFS compared with  $CXCR4^{WT}$  (44.1 months vs not reached [NR]; supplemental Table 3).

Among the 37 patients with  $CXCR4^{S338X}$ , the median clonality was 35.3% (range, 0.94% to 86.2%; Figure 1A). By receiver operator curve analysis, a  $CXCR4^{S338X}$  clonality of 25% was calculated as the optimal cutoff for disease progression within 12 months (sensitivity, 71%; specificity, 72%; AUC, 0.73) or 24 months (sensitivity, 72%; specificity, 69%; AUC, 0.69) of ibrutinib initiation. Patients were stratified into 2 groups based on  $CXCR4^{S338X}$  clonality: high clonality ( $n = 23$ ; 62%) defined as  $\geq 25\%$  and low clonality ( $n = 14$ ; 38%) defined as  $< 25\%$ . Baseline clinical characteristics as well as response rates and kinetics to ibrutinib therapy according to  $CXCR4^{S338X}$  clonality are shown in supplemental Table 4 and Table 1, respectively. Patients with high  $CXCR4^{S338X}$  clonality were more likely to have a baseline BM involvement  $\geq 50\%$  (87%, 57%, and 50%;  $P = .005$ ), serum IgM level  $> 7000$  mg/dL (17%, 7%, and 3%;  $P = .02$ ), and platelet count  $\leq 100 \times 10^9/L$  (17%, 14%, and 4%;  $P = .03$ ) vs patients with low  $CXCR4^{S338X}$  clonality and  $CXCR4^{WT}$ , respectively. In addition, patients with high  $CXCR4^{S338X}$  clonality had lower rates of very good partial response (4%, 21%, and 35%;  $P = .01$ ) and delayed major response attainment (9.7, 7.4, and 1.9 months;  $P < .001$ ) to ibrutinib. Compared with patients with  $CXCR4^{WT}$ , high  $CXCR4^{S338X}$  clonality was associated with significantly worse PFS (HR, 10.44; 95% CI, 3.43-31.8;  $P < .0001$ ), whereas low  $CXCR4^{S338X}$  clonality did not impact PFS (HR, 0.90; 95% CI, 0.13-6.44;  $P = .92$ ). Patients with high  $CXCR4^{S338X}$  clonality also had a significantly shorter median PFS vs patients with low  $CXCR4^{S338X}$  clonality and  $CXCR4^{WT}$  (39.9 months, NR, NR, respectively;  $P = .0001$ ; Figure 1B).

These findings demonstrate that  $CXCR4^{S338X}$  mutations adversely impact clinical outcomes to ibrutinib monotherapy in WM patients. Our data are consistent with preclinical studies showing that WM cells transduced with  $CXCR4^{S338X}$  have increased CXCL12-triggered AKT and extracellular signal-regulated kinase 1/2 activation, and decreased in vitro ibrutinib-related apoptosis.<sup>10,11</sup> Prospective studies have also reported that  $CXCR4$  mutations are associated with lower response rates, delayed response attainment, and shorter PFS on ibrutinib.<sup>12-15</sup> However, these studies included heterogeneous groups of  $CXCR4$ -mutated patients, and were not large enough to discriminate the effect of individual somatic variants.

An important finding was the identification of  $CXCR4^{S338X}$  clonality as a predictor of long-term outcomes to ibrutinib therapy. Our study shows that WM patients with both low and high  $CXCR4^{S338X}$  clonality have evidence of intrinsic resistance to ibrutinib (ie, lower response rates, delayed response attainment), but only high clonality was associated with inferior PFS, suggesting that ibrutinib was unable to overcome the pejorative impact of  $CXCR4^{S338X}$  in these patients. Although the mechanism for this finding remains to be delineated, tumors with higher  $CXCR4^{S338X}$  clonality may be less amenable to eradication by ibrutinib due to enhanced prosurvival signaling. Likewise, it is possible that tumors with high  $CXCR4^{S338X}$  clonality could have an increased tropism for the BM



**Figure 1. Clonality assessment of  $CXCR4^{S338X}$ .** (A) CCF analysis for  $CXCR4^{S338X}$  clonality in CD19-selected BM cells from WM patients. (B) Kaplan-Meier curves for PFS on ibrutinib in WM patients stratified by  $CXCR4^{S338X}$  mutant clonality.

stroma, which provides a permissive niche for drug resistance as well as malignant cell survival and IgM release.<sup>10,11,18-20</sup> This may account for the higher baseline BM involvement and serum IgM levels observed herein for patients with high  $CXCR4^{S338X}$  clonality, as well as the lower incidence of ibrutinib-triggered peripheral lymphocytosis in  $CXCR4$ -mutated WM patients previously reported.<sup>12</sup> Prospective longitudinal studies as well as in vitro signaling studies are needed to further elucidate these hypotheses. A phase 2 trial

**Table 1. Response rates and kinetics on ibrutinib in WM patients according to  $CXCR4^{S338X}$  mutational status and clonality**

|                                     | $CXCR4^{S338X}$ clonality |               | $CXCR4^{WT}$ ,<br>$n = 110$ | $P$   |
|-------------------------------------|---------------------------|---------------|-----------------------------|-------|
|                                     | High, $n = 23$            | Low, $n = 14$ |                             |       |
| Overall response, $n$ (%)           | 22 (96)                   | 12 (86)       | 106 (96)                    | .19   |
| Major response, $n$ (%)             | 15 (65)                   | 8 (57)        | 94 (85)                     | .004  |
| Very good partial response, $n$ (%) | 1 (4)                     | 3 (21)        | 38 (35)                     | .01   |
| Time to minor response, mo          | 2.1                       | 1.8           | 1.1                         | .0001 |
| Time to major response, mo          | 9.7                       | 7.4           | 1.9                         | .0003 |

evaluating ibrutinib in previously untreated WM patients with serial whole-genome sequencing is now fully enrolled (NCT02604511).

Our study highlights the importance of developing novel strategies to overcome ibrutinib resistance in *CXCR4*<sup>S338X</sup>-mutated WM patients. The use of *CXCR4* inhibitors has been shown to restore the sensitivity of *CXCR4*<sup>S338X</sup>-mutated cells to ibrutinib,<sup>10</sup> and a phase 1/2 study investigating the *CXCR4*-blocking antibody ulocuplumab and ibrutinib is ongoing in *CXCR4*-mutated WM patients (NCT03225716). Moreover, the iNNOVATE study recently evaluated the combination of ibrutinib and rituximab, and updated results demonstrate shorter 36-month PFS among *CXCR4*<sup>MUT</sup> vs *CXCR4*<sup>WT</sup> WM patients (64% vs 84%).<sup>15,21</sup> Preliminary data suggest that the second-generation BTK inhibitor zanubrutinib has some activity in *MYD88*<sup>WT</sup> WM patients, but the impact of *CXCR4* mutations is currently unknown.<sup>22</sup>

The present study is not without limitations. Despite the largest cohort of WM patients on ibrutinib with *CXCR4*<sup>S338X</sup> clonality determined, the results are based on a limited number of patients and a clonality cutoff with modest sensitivity and specificity. In addition, we were unable to evaluate the impact of *CXCR4* clonality on ibrutinib for non-S338X mutations unamenable to AS-PCR. Larger studies are needed to provide external validation for our preliminary findings.

In summary, high *CXCR4*<sup>S338X</sup> clonality adversely impacts clinical outcomes to ibrutinib therapy in WM patients. Clonality assessment represents a novel biomarker for predicting outcomes on ibrutinib in WM patients carrying *CXCR4*<sup>S338X</sup> nonsense mutations.

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## Acknowledgments

J.J.C. was supported by the WMR Fund.

J.N.G. was awarded Young Investigator Awards for this research at the 10th International Workshop for Waldenström's Macroglobulinemia, New York, NY (October 2018), and at the 17th International Myeloma Workshop, Boston, MA (September 2019).

## Authorship

Contribution: J.N.G., L.X., Z.R.H., S.P.T., and J.J.C. conceived and designed the experiments, performed the data analysis, and wrote the manuscript; N.T., M.G.D., A. Kofides, and L.X. performed the sequencing studies; J.G.C., X.L., M.M., M.L.G., G.G.C., C.J.P., and G.Y. prepared samples; and K.M., A. Keezer, T.D., J.N.G., J.J.C., and S.P.T. provided patient care, obtained consent, and were responsible for sample collection.

Conflict-of-interest disclosure: J.J.C. has received honoraria and/or research funds from AbbVie, BeiGene, Janssen, Millennium, Pharmacyclics, and TG Therapeutics. S.P.T. has received research funding and consulting fees from Pharmacyclics and Janssen. The remaining authors declare no competing financial interests.

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