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CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenström macroglobulinaemia treated with ibrutinib

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Summary

Ibrutinib is associated with response rate of 90% and median progression-free survival (PFS) in excess of 5 years in Waldenström macroglobulinaemia (WM) patients. *CXCR4* mutations are detected in 30–40% of patients with WM and associate with lower rates of response and shorter PFS to ibrutinib therapy. Both frameshift (*CXCR4*^{FS}) and nonsense (*CXCR4*^{NS}) *CXCR4* mutations have been described. The impact of these mutations on outcomes to ibrutinib have not been evaluated in WM patients. We studied consecutive patients with a diagnosis of WM, on ibrutinib therapy, for the presence of *CXCR4*^{FS} and *CXCR4*^{NS} mutations and evaluated the differences in response and PFS between groups. Of 180 patients, 68 patients (38%) had *CXCR4* mutations; 49 (27%) had *CXCR4*^{NS} and 19 (11%) had *CXCR4*^{FS} mutations. In multivariate models, patients with *CXCR4*^{NS} had lower odds of major response (Odds ratio 0·25, 95% confidence interval [CI] 0·12–0·53; $P < 0·001$) and worse PFS (Hazard ratio 4·02, 95% CI 1·95–8·26; $P < 0·001$) than patients without *CXCR4* mutations. *CXCR4*^{FS} was not associated with worse major response or PFS rates than patients without *CXCR4* mutations. Our results suggest different response and PFS rates to ibrutinib for WM patients with *CXCR4*^{NS} and *CXCR4*^{FS}, and advocate in favour of *CXCR4* mutational testing as well as *CXCR4*-directed therapy.

Keywords: Waldenström macroglobulinaemia, ibrutinib, *CXCR4*, response, survival.

Introduction

Waldenström macroglobulinaemia (WM) is a B-cell lymphoma characterized by the accumulation of malignant IgM-secreting lymphoplasmacytic lymphoma cells. The somatic *MYD88*^{L265P} mutation can be detected in the malignant cells of over 90% of WM patients (Treon *et al*, 2012). *MYD88*^{L265P} signals through the Bruton tyrosine kinase (BTK) pathway, promoting WM cell survival (Yang *et al*, 2013). The oral BTK inhibitor, ibrutinib, is the only approved drug for the treatment of symptomatic WM. Three prospective studies have evaluated the safety and efficacy of single agent ibrutinib at a dose of 420 mg taken orally once daily; two studies in patients with relapsed and/or refractory WM, and one in previously untreated patients (Treon *et al*, 2015a; Dimopoulos *et al*, 2017; Treon *et al*, 2017a). On these studies, ibrutinib therapy was associated with an overall response rates (ORR; minor response or better), ranging between 90% and 100% and a major response rate (partial response or better) ranging between 70% and 80%. The median progression-free survival (PFS) was not reached at 4 years of follow-up in previously treated WM patients (Treon *et al*, 2017b).

CXCR4 mutations are important in the genomic landscape of WM and can be detected in up to 40% of patients with WM (Hunter *et al*, 2014). *CXCR4* mutations have been associated with lower response rates and shorter median PFS in WM patients treated with ibrutinib (Treon *et al*, 2015a; 2015b). However, over 40 *CXCR4* mutation subtypes have been described in WM patients. *CXCR4* mutations can be divided into nonsense (*CXCR4*^{NS}) and frameshift (*CXCR4*^{FS}) mutations (Cao *et al*, 2015a). However, the impact of *CXCR4*

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mutation subtypes on response and survival of patients on ibrutinib therapy needs further study. We carried a retrospective study evaluating the differences, if any, in clinical presentation, response and survival in WM patients with $CXCR4^{NS}$ and $CXCR4^{FS}$ treated with ibrutinib.

Methods

We identified patients seen at our institution between May 2012 and December 2017 who had a diagnosis of WM and received ibrutinib therapy. The clinicopathological diagnosis of WM was made based on the recommendations of the 2nd International Workshop for WM (IWWM) (Owen *et al*, 2003). We included patients who received ibrutinib as primary therapy as well as in the relapsed and/or refractory setting. All patients met criteria for treatment initiation based on the recommendations of the 2nd IWWM (Kyle *et al*, 2003). Patients with Bing-Neel syndrome (i.e. central nervous system involvement by WM) were excluded.

Relevant clinicopathological data were gathered and included age, sex, haemoglobin levels, platelet counts, serum beta-2-microglobulin (B2M) levels, serum IgM levels, bone marrow involvement, treatment indications and presence of extramedullary disease, among others. Extramedullary disease was defined as lymphadenopathy ≥1·5 cm and/or splenomegaly ≥15 cm in largest diameter. *MYD88* and *CXCR4* mutations were identified using allele-specific polymerase chain reaction (AS-PCR) and Sanger sequencing in CD19-selected bone marrow samples, as previously described (Xu *et al*, 2013; Hunter *et al*, 2014). Patients were divided in three groups, according to their *CXCR4* mutational status: wild-type ($CXCR4^{WT}$), $CXCR4^{FS}$ and $CXCR4^{NS}$.

Patients' characteristics are shown using descriptive statistics, and comparisons were made using Chi-square or Rank sum tests, as appropriate. Response was defined based on criteria from the 6th IWWM (Owen *et al*, 2013). Major response was defined as obtaining a partial response or better. Univariate and multivariate logistic regression models were fitted to evaluate the association of variables with major response. The outcome of interest for major response is presented as odds ratio (OR) with 95% confidence interval (CI). PFS was defined as the time between ibrutinib initiation and disease progression or death from any cause. OS on ibrutinib was defined as the time between ibrutinib initiation and death from any cause. Survival curves were estimated using the Kaplan–Meier method, and compared using the log-rank test. Univariate and multivariate survival analyses were performed fitting Cox proportional-hazard regression models. The outcome of interest for survival is presented as hazard ratio (HR) with 95% CI. $P < 0{\cdot}05$ was considered statistically significant for all analyses. All calculations and graphs were obtained using STATA 15.0 (StataCorp, College Station, TX, USA).

Results

Patients' characteristics

Our study included 180 consecutive WM patients treated with ibrutinib. Baseline patients' characteristics, prior to ibrutinib initiation, are shown in Table I. The median age at ibrutinib initiation was 67 years (range 43–93 years). The $MYD88^{L265P}$ mutation was detected in all patients. *CXCR4* mutations were detected in 68 patients (38%), of whom 49 (27%) had $CXCR4^{NS}$ and 19 (11%) had $CXCR4^{FS}$ mutations. A list of *CXCR4* mutations encountered in WM patients and their frequencies has been published (Hunter *et al*, 2014; Cao *et al*, 2015b; Xu *et al*, 2016). Two patients had more than one *CXCR4* mutation and were excluded from this analysis but have not yet progressed at 11 and 22 months on ibrutinib therapy, respectively. Patients with $CXCR4^{NS}$ and $CXCR4^{FS}$ were more likely to have platelet counts $\leq 100 \times 10^9$/l, serum IgM level >70 g/l and to initiate therapy due to symptomatic hyperviscosity, but less likely to present with extramedullary disease than $CXCR4^{WT}$ patients. Between groups, there were no significant differences in age, sex, haemoglobin level, B2M level, level of bone marrow involvement, International Prognostic Scoring System for WM (IPSSWM) and proportion of patients who were previously treated.

Time from WM diagnosis to ibrutinib initiation

The median time from WM diagnosis to ibrutinib initiation for the entire cohort was 41 months (95% CI 28–59 months). There was no significant difference in time to ibrutinib initiation based on *CXCR4* mutational status (log-rank $P = 0{\cdot}37$; Fig. 1A). The median time from WM diagnosis to ibrutinib initiation for patients with $CXCR4^{WT}$, $CXCR4^{NS}$ and $CXCR4^{FS}$ was 46 months (95% CI 30–81 months), 38 months (95% CI 24–62 months) and 10 months (95% CI 5–62 months), respectively. Time to ibrutinib initiation similarly did not differ among the *CXCR4* mutation groups when stratified by patients who were previously treated (log-rank $P = 0{\cdot}13$; Fig. 1B) or previously untreated (log-rank $P = 0{\cdot}53$; Fig. 1C). In previously treated patients, the median time from WM diagnosis to ibrutinib initiation was 62 months (95% CI 45–89 months), and was 83 months (95% CI 49–92 months), 47 months (95% CI 27–91 months) and 12 months (95% CI 4–100 months) for patients with $CXCR4^{WT}$, $CXCR4^{NS}$ and $CXCR4^{FS}$, respectively. In previously untreated patients, the median time from WM diagnosis to ibrutinib initiation was 8·8 months (95% CI 4·7–18·7 months), and was 5 months (95% CI 3–17 months), 19 months (95% CI 2–62 months) and 10 months (95% CI 1 month-not reached) for patients with $CXCR4^{WT}$, $CXCR4^{NS}$, and $CXCR4^{FS}$, respectively.

Table I. Baseline characteristics of 180 patients with Waldenström macroglobulinaemia treated with ibrutinib, according to *CXCR4* mutational status.

Characteristic	Total (n = 180)	*CXCR4*^{WT} (n = 112)	*CXCR4*^{NS} (n = 49)	*CXCR4*^{FS} (n = 19)	P-value
Age> 65 years	102 (57%)	65 (58%)	27 (55%)	10 (53%)	0·88
Male sex	119 (66%)	77 (69%)	27 (55%)	15 (79%)	0·11
Haemoglobin ≤ 115 g/l	125 (70%)	78 (70%)	31 (63%)	16 (84%)	0·24
Platelet count ≤ 100 × 10^9/l	20 (11%)	6 (5%)	8 (17%)	6 (32%)	0·002
Serum B2M> 3 mg/l (n = 157)	107 (68%)	73 (74%)	24 (55%)	10 (71%)	0·07
Serum IgM> 70 g/l	11 (6%)	3 (3%)	6 (12%)	2 (11%)	0·04
Bone marrow ≥ 50%	92 (54%)	52 (50%)	29 (59%)	11 (65%)	0·38
Previously treated	125 (69%)	78 (70%)	33 (67%)	14 (74%)	0·88
IPSSWM (n = 156)					
Low-risk	36 (23%)	23 (23%)	11 (26%)	2 (14%)	0·67
Intermediate-risk	49 (31%)	33 (33%)	13 (30%)	3 (21%)	
High-risk	71 (46%)	43 (43%)	19 (44%)	9 (64%)	
Indications to treat (n = 179)					
Anaemia	101 (56%)	65 (59%)	23 (47%)	13 (68%)	0·21
Constitutional symptoms	62 (35%)	37 (33%)	19 (39%)	6 (32%)	0·77
Hyperviscosity	27 (15%)	10 (9%)	13 (27%)	4 (21%)	0·01
Extramedullary disease	19 (11%)	18 (16%)	1 (2%)	0 (0%)	0·008
Neuropathy	16 (9%)	11 (10%)	4 (8%)	1 (5%)	0·79
Other indications	7 (4%)	6 (5%)	1 (2%)	0 (0%)	0·39

CXCR4^{WT}, *CXCR4* wild type; *CXCR4*^{NS}, *CXCR4* nonsense mutation; *CXCR4*^{FS}, *CXCR4* frameshift mutation; B2M, beta-2-microglobulin; IPSSWM, International Prognostic Score System for Waldenström macroglobulinaemia.

Major response rates to ibrutinib

Categorical responses to ibrutinib in WM patients are shown in Table II. The rates of very good partial response (VGPR), partial response (PR), minor response (MR) and no response were 25%, 51%, 18%, and 6%, respectively; the major response rate (PR or better) was 76%. Response rates did not differ significantly based on treatment status prior to ibrutinib initiation (P = 0·69). Patients with *CXCR4*^{NS} had lower rate of major response than *CXCR4*^{FS} and *CXCR4*^{WT} patients (55%, 79% and 85%, respectively; P < 0·001). In univariate models, patients with *CXCR4*^{NS} had lower odds of major response than patients with *CXCR4*^{WT} (OR 0·25, 95% CI 0·12–0·53; P < 0·001). There was no significant difference in odds of major response between *CXCR4*^{FS} and *CXCR4*^{WT} (OR 0·77, 95% CI 0·23–2·56; P = 0·67). In a multivariate model adjusting for age, sex, haemoglobin level, platelet count, serum B2M level, serum IgM level, bone marrow involvement, time from WM diagnosis to ibrutinib initiation and prior therapy, *CXCR4*^{NS} was independently associated with lower odds of major response than *CXCR4*^{WT} (OR 0·20, 95% CI 0·07–0·57; P = 0·002). There remained no significant difference in odds of major response after adjustment between *CXCR4*^{FS} and *CXCR4*^{WT} (OR 0·42, 95% CI 0·09–2·01; P = 0·28).

Progression-free survival on ibrutinib

The median follow-up time for all patients was 25 months (95% CI 20–32 months), and 33 patients (18%) have progressed. The median PFS to ibrutinib therapy has not yet been reached, and the 3-year PFS rate was 77% (95% CI 67–84%). The median PFS to ibrutinib therapy differed significantly based on *CXCR4* mutational status (log-rank P < 0·001; Fig. 2A). The median PFS in *CXCR4*^{NS} patients was 40 months (95% CI 21–45 months) and was not reached in *CXCR4*^{FS} and *CXCR4*^{WT} patients (log-rank P < 0·001). The 3-year PFS rate was lower for *CXCR4*^{NS} patients (60%; 95% CI 40–76%) than for *CXCR4*^{FS} (76%; 95% CI 30–94%) or *CXCR4*^{WT} patients (83%; 95% CI 72–91%). In the univariate analysis, *CXCR4*^{NS} was associated with a worse PFS (HR 4·02; 95% CI 1·95–8·26; P < 0·001) than *CXCR4*^{WT}, whereas there was no significant difference in PFS between *CXCR4*^{FS} and *CXCR4*^{WT} (HR 1·10; 95% CI 0·25–4·90; P = 0·90). In a multivariate model adjusting for age, sex, haemoglobin level, platelet count, serum B2M level, serum IgM level, bone marrow involvement, time from WM diagnosis to ibrutinib initiation and prior therapy, *CXCR4*^{NS} was independently associated with a worse PFS (HR 6·70; 95% CI 2·70–16·7; P < 0·001) than *CXCR4*^{WT}. There remained no significant difference in PFS after adjustment between *CXCR4*^{FS} and *CXCR4*^{WT} (HR 1·78; 95% CI 0·34–9·27; P = 0·50).

In previously treated patients, the median follow-up time was 35 months (95% CI 31–41 months). The median PFS in *CXCR4*^{NS} patients was 43 months (95% CI 23-not reached) and was not reached in *CXCR4*^{FS} and *CXCR4*^{WT} patients (log-rank P = 0·01). The 3-year PFS rate was lower for *CXCR4*^{NS} patients (64%, 95% CI 41–79%) than for *CXCR4*^{FS}

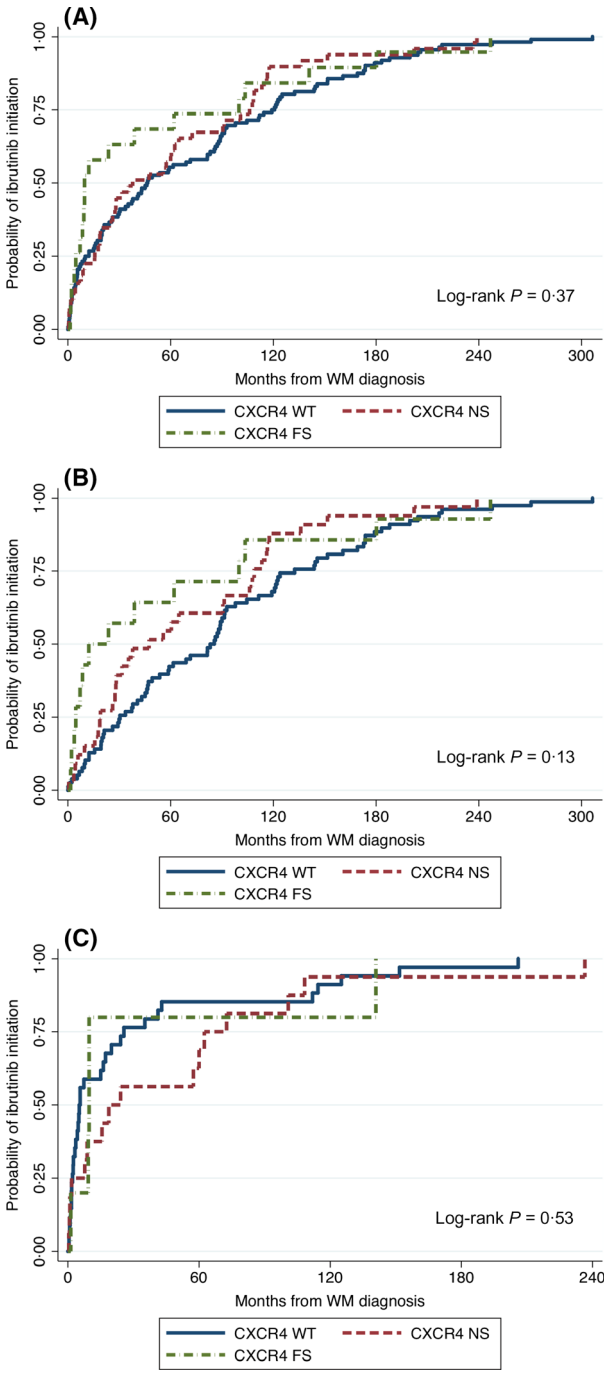

Fig 1. Time from Waldenström macroglobulinaemia diagnosis to ibrutinib initiation estimates, according to *CXCR4* mutational status. Cumulative Kaplan–Meier curves are shown in (A) all patients, (B) previously treated patients and (C) previously untreated patients. CXCR4 FS: *CXCR4* frameshift mutation; CXCR4 NS: *CXCR4* nonsense mutation; CXCR4 WT, *CXCR4* wild type; WM, Waldenström macroglobulinaemia. [Colour figure can be viewed at wileyonlinelibrary.com].

(76%, 95% CI 30–94%) and $CXCR4^{WT}$ patients (81%, 95% CI 68–89%). PFS curves are shown in Fig. 2B. In the univariate analysis, $CXCR4^{NS}$ was associated with worse PFS than $CXCR4^{WT}$ (HR 3·25; 95% CI 1·52–6·95; $P = 0·002$). There was no significant difference in PFS between $CXCR4^{FS}$ and $CXCR4^{WT}$ (HR 1·05; 95% CI 0·24–4·66; $P = 0·95$). In a multivariate analysis adjusting for age, sex, haemoglobin level, platelet count, serum B2M level, serum IgM level, bone marrow involvement and time from WM diagnosis to ibrutinib initiation, $CXCR4^{NS}$ was independently associated with a worse PFS than $CXCR4^{WT}$ (HR 6·77; 95% CI 2·46–18·7; $P < 0·001$). There remained no significant difference in PFS after adjustment between $CXCR4^{FS}$ and $CXCR4^{WT}$ (HR 2·41; 95% CI 0·44–13·1; $P = 0·31$).

In previously untreated patients, the median follow-up time was 13 months (95% CI 12–16 months). The median PFS in $CXCR4^{NS}$ patients was not reached in any group, and the 1-year PFS rate was lower for $CXCR4^{NS}$ patients (77%, 95% CI 44–92%) than for $CXCR4^{FS}$ and $CXCR4^{WT}$ patients, in whom no progression events were seen (log-rank $P = 0·01$). PFS curves are shown in Fig. 2C. Given the absence of progression events in $CXCR4^{FS}$ and $CXCR4^{WT}$ patients treated with ibrutinib, univariate and multivariate analyses were not undertaken.

Overall survival on ibrutinib

At the time of this report, the median OS on ibrutinib was not reached, and the 3-year OS rates on ibrutinib was 89% (95% CI 82–94%). There was no statistical difference in the 3-year OS rates on ibrutinib for $CXCR4^{WT}$, $CXCR4^{NS}$ and $CXCR4^{FS}$ patients at 92% (95% CI 83–97%), 86% (95% CI 66–95%) and 78% (95% CI 44–93%), respectively (log-rank $P = 0·40$).

Discussion

Using whole genome sequencing, somatic *CXCR4* mutations have been identified in 30–40% of patients meeting clinicopathological criteria for WM, and they represent the second most common abnormality identified in these patients after $MYD88^{L265P}$ mutations (Hunter *et al*, 2017). These acquired mutations, located in the C-terminal domain of *CXCR4*, are similar to the germline mutations previously described in patients with the Warts, Hypogammaglobulinaemia, Infections and Myelokathexis (WHIM) congenital syndrome (Dotta *et al*, 2011).

A number of studies have evaluated the clinical impact of CXCR4 mutations in WM. WM patients with *CXCR4* mutations had higher burden of bone marrow involvement, higher serum IgM levels and lower rates of extramedullary disease than patients with $CXCR4^{WT}$ (Treon *et al*, 2014a; Schmidt *et al*, 2015; Poulain *et al*, 2016). Also, WM patients with *CXCR4* mutations have higher risk of hyperviscosity at the time of diagnosis and are more likely to present with acquired von Willebrand disease than $CXCR4^{WT}$ patients (Gustine *et al*, 2017; Castillo *et al*, 2018a). Specifically, on ibrutinib therapy, patients with *CXCR4* mutations have a

Table II. Categorical responses to ibrutinib in patients with Waldenström macroglobulinaemia, according to *CXCR4* mutational status.

	Total	*CXCR4*^{WT}	*CXCR4*^{NS}	*CXCR4*^{FS}	*P*-value
All patients					
Very good partial response	44 (25%)	36 (33%)	3 (6%)	5 (26%)	<0·001
Partial response	90 (51%)	56 (52%)	24 (49%)	10 (53%)	
Minor response	31 (18%)	13 (12%)	16 (33%)	2 (11%)	
No response	11 (6%)	3 (3%)	6 (12%)	2 (11%)	
Major response (≥partial)	134 (76%)	92 (85%)	27 (55%)	15 (79%)	<0·001
Previously treated					
Very good partial response	33 (26%)	28 (36%)	2 (6%)	3 (21%)	0·002
Partial response	61 (49%)	37 (44%)	15 (45%)	19 (64%)	
Minor response	23 (18%)	11 (14%)	11 (33%)	1 (7%)	
No response	8 (6·4)	2 (3%)	5 (15%)	1 (7%)	
Major response (≥partial)	94 (75%)	65 (83%)	17 (51%)	12 (86%)	0·001
Previously untreated					
Very good partial response	11 (20%)	8 (24%)	1 (6%)	2 (40%)	0·28
Partial response	30 (55%)	20 (59%)	9 (56%)	1 (20%)	
Minor response	10 (18%)	4 (12%)	5 (31%)	1 (20%)	
No response	4 (7%)	2 (6%)	1 (6%)	1 (20%)	
Major response (≥partial)	41 (75%)	28 (82%)	10 (63%)	3 (60%)	0·24

CXCR4^{WT}, *CXCR4* wild type; *CXCR4*^{NS}, *CXCR4* nonsense mutation; *CXCR4*^{FS}, *CXCR4* frameshift mutation.

slower time to response, lower VGPR rates and a shorter PFS (Treon *et al*, 2015a; 2015b; 2017a). A previous study from our group evaluated 179 WM patients, of whom 51 (28%) had *CXCR4* mutations, and detected *CXCR4*^{NS} in 25 (49%) and *CXCR4*^{FS} in 26 patients (51%) (Treon *et al*, 2014a). In the present study, the proportions of *CXCR4*^{NS} and *CXCR4*^{FS} were 72% and 28%, respectively. Our previous publication determined frequencies of *CXCR4* mutational status at the time of disease presentation in a series of previously untreated patients. In that study, patients with *CXCR4*^{FS} had the lowest rates of symptomatic disease at presentation versus *CXCR4*^{WT} and *CXCR4*^{NS}. This might account for the relative underrepresentation of *CXCR4*^{FS} patients in the present study, which is evaluating clinical characteristics at the time of ibrutinib initiation. Also, AS-PCR and Sanger sequencing were used for assessment of *CXCR4* mutational status in this study, which could have permitted a more sensitive detection of *CXCR4*^{NS} mutations versus the above-mentioned study, in which only Sanger sequencing was used.

Our study shows that WM patients with *CXCR4*^{NS} have similar ORRs to patients with *CXCR4*^{FS}. However, the rate of major response was lower in *CXCR4*^{NS} when compared to *CXCR4*^{FS} patients (55% vs. 79%, respectively). In multivariate logistic regression models, *CXCR4*^{NS} was associated with 80% lower odds of achieving a major response to ibrutinib than *CXCR4*^{WT}, while no significant difference was identified between *CXCR4*^{FS} and *CXCR4*^{WT}. Also, the median PFS for *CXCR4*^{NS} patients was 40 months while it was not reached in *CXCR4*^{FS}. In the multivariate analysis, *CXCR4*^{NS} was associated with a 7-fold increased risk of progression and/or death than *CXCR4*^{WT}, while no significant difference was identified between *CXCR4*^{FS} and *CXCR4*^{WT}. The results were similar in the stratified analysis in previously untreated and previously treated WM patients. Our results are clinically significant, in the context of the difficulties of performing *CXCR4* mutational testing with Sanger sequencing in routine practice. Our results, if true, would suggest against testing for *CXCR4*^{FS} mutations in WM patients. As 80–90% of *CXCR4*^{NS} mutations in WM are located in S338X, AS-PCR targeting *CXCR4*^{S338X} could inform decision making with regard to ibrutinib use in WM patients (Xu *et al*, 2016). This approach would help to identify the 25% of WM patients who are more likely to have lower rates of major response and shorter PFS to ibrutinib.

The mechanisms by which *CXCR4* mutations promote ibrutinib resistance is a matter of ongoing research. In a natural state, after binding to its ligand CXCL12, CXCR4 is activated and rapidly internalized (Busillo & Benovic, 2007). Upon activation, CXCR4 couples to the G family of protein and activates the Src family of tyrosine kinases, which in turn will promote activation of phosphatidylinositol-3-kinase (PI3K). After internalization, CXCR4 is ubiquitinated and degraded. CXCR4 also activates the JAK/STAT pathway independent of G protein, promoting ERK activation by β-arrestins. As a consequence, CXCR4 activation regulates cell migration and adhesion as well as gene transcription. In WM, *CXCR4* mutations affect receptor internalization, prolonging signalling by G protein and β-arrestins. These result in sustained AKT and ERK signalling and decreased caspase 3 cleavage (Cao *et al*, 2015b). *In vitro*, *CXCR4* mutations have been associated with resistance to alkylating agents, nucleoside analogues, proteasome inhibitors, BTK inhibitors

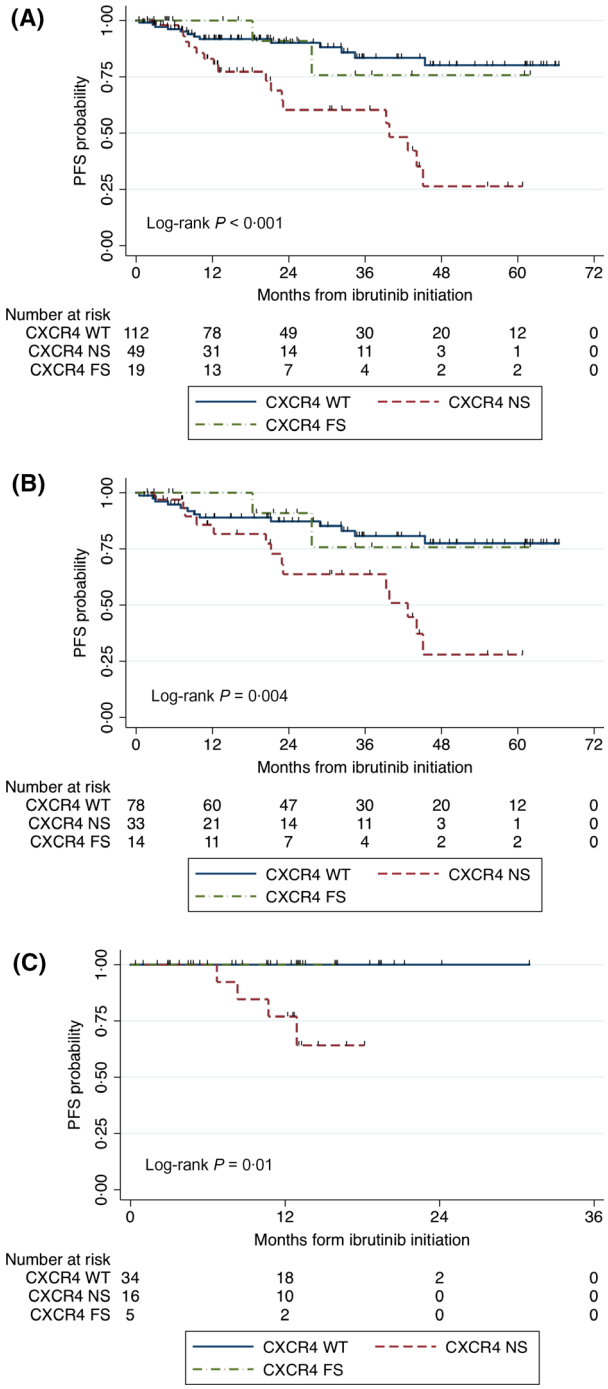

Fig 2. Progression-free survival estimates in patients with Waldenström macroglobulinaemia on ibrutinib therapy, according to CXCR4 mutational status. Kaplan-Meier PFS curves are shown in (A) all patients, (B) previously treated patients and (C) previously untreated patients. CXCR4 FS, *CXCR4* frameshift mutation; CXCR4 NS, *CXCR4* nonsense mutation; CXCR4 WT, *CXCR4* wild type; PFS, progression-free survival. [Colour figure can be viewed at wileyonlinelibrary.com].

and PI3K inhibitors (Roccaro *et al*, 2014; Cao *et al*, 2015b; de Rooij *et al*, 2016). Preclinical studies have also shown that addition of AKT1, MAPK1 or CXCR4 antagonists to ibrutinib reversed CXCR4 signalling and promoted apoptotic changes and tumour reduction in both $CXCR4^{NS}$ and $CXCR4^{FS}$ WM cell models (Roccaro *et al*, 2014; Cao *et al*, 2015b). The biological reasons behind worse outcomes in $CXCR4^{NS}$ patients are less clear. One could hypothesize that the integrity of the transcribed CXCR4 protein could play a biological role in the activity of ibrutinib. $CXCR4^{NS}$ could transcribe a more "aberrant", non-functional protein, which in turn could have a more disruptive role in intracellular processes, than $CXCR4^{FS}$.

Our study is not without limitations. We included patients treated at a specialized centre, *CXCR4* mutational testing was performed using B-cell selected samples and might not be reproducible elsewhere, and the sample size could be considered small. To address these issues, we included consecutive patients with clinical features in line with what has been reported in population-based studies (Olszewski *et al*, 2016), and our sample can be considered appropriate for a rare disease such as WM. The goal of our study was not to validate a research test, but rather provide insights into the biological differences between *CXCR4* mutation subtypes in WM patients.

The impact of *CXCR4* mutations on ibrutinib in combination and treatments other than ibrutinib is evolving. The recently published INNOVATE study showed lower rates of VGPR (34% vs. 19%, respectively) but similar 30-month PFS rates (80% vs. 86%, respectively) in *CXCR4*-mutated versus $CXCR4^{WT}$ WM patients exposed to the combination of ibrutinib and rituximab (Dimopoulos *et al*, 2018). However, at the 36-month update presented at the 2018 American Society of Hematology Annual Meeting, the PFS rate was lower in *CXCR4* mutated than in $CXCR4^{WT}$ patients (64% vs. 84%, respectively) (Buske *et al*, 2018). This finding suggests that *CXCR4* mutations can also adversely impact response and PFS rates of WM patients treated with ibrutinib and rituximab. A recent report suggested that *CXCR4* mutational status did not affect PFS in patients treated with the combination of bortezomib and rituximab (Sklavenitis-Pistofidis *et al*, 2018). Similarly, no significant differences in response and PFS were observed, based on *CXCR4* mutational status, in patients treated with carfilzomib or ixazomib-based regimens in two separate prospective phase II studies (Treon *et al*, 2014b; Castillo *et al*, 2018b). Finally, no clinical data are available on the effect of *CXCR4* mutational status on outcomes of patients treated with chaemoimmunotherapy.

In conclusion, the results of our study suggest that $CXCR4^{FS}$ mutations might not adversely impact major response and PFS rates in WM patients treated with ibrutinib and support the development of PCR-based $CXCR4^{NS}$ mutational testing as well as anti-CXCR4 directed therapy. Our findings would have to be independently validated. A prospective phase II study evaluating the combination of ibrutinib and the anti-CXCR4 monoclonal antibody ulocuplumab in patients with WM and *CXCR4* mutations is ongoing (NCT03225716).

Authorship contribution

JJC, JNG and SPT designed the study and performed the analysis. LX, XL, MGD, AK, NT, JGC, MM, MLG, GGC, CJP, GY and ZRH performed molecular testing in patients' samples. JJC, JNG, AK, KM, TED and SPT took care of patients. JJC drafted the manuscript. All the authors critically reviewed and approved the final manuscript.

Disclosures

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