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To cite this article: Jorge J. Castillo, David F. Moreno, Maria I. Arbelaez, Zachary R. Hunter & Steven P. Treon (2019) CXCR4 mutations affect presentation and outcomes in patients with Waldenström macroglobulinemia: A systematic review, Expert Review of Hematology, 12:10, 873-881, DOI: [10.1080/17474086.2019.1649132](https://doi.org/10.1080/17474086.2019.1649132)

To link to this article: <https://doi.org/10.1080/17474086.2019.1649132>



Published online: 30 Jul 2019.



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REVIEW



CXCR4 mutations affect presentation and outcomes in patients with Waldenström macroglobulinemia: A systematic review

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ABSTRACT

Introduction: The genomic landscape of Waldenström macroglobulinemia (WM) is characterized by recurrent *MYD88* (*MYD88*^{L265P}) and *CXCR4* mutations (*CXCR4*^{MUT}), detected in 90% and 30% of cases, respectively. The role of *CXCR4*^{MUT} in clinical features and outcomes to therapy in WM patients is evolving.

Areas covered: We performed a systematic review aimed at evaluating the prevalence of *CXCR4*^{MUT} in WM patients, and at assessing differences in clinical features and outcomes to therapy between WM patients with and without *CXCR4*^{MUT}. Seventeen studies were included in our analysis. The pooled prevalence of *CXCR4*^{MUT} in WM patients was 31%; 34% in *MYD88*^{L265P} and 5% in *MYD88*^{WT} patients. *CXCR4*^{MUT} were associated with higher serum IgM levels and higher risk of hyperviscosity than *CXCR4*^{WT} patients. Very good partial response (VGPR) and progression-free survival (PFS) rates to ibrutinib, with and without rituximab, appeared lower in *CXCR4*^{MUT} than in *CXCR4*^{WT} patients. Response and PFS rates were not affected by *CXCR4*^{MUT} status on patients treated with proteasome inhibitors.

Expert opinion: Our systematic review shows that WM patients with *CXCR4*^{MUT} have specific clinical features and have lower response and PFS rates to BTK inhibitors. Our findings support standardization of *CXCR4* testing and development of *CXCR4*-directed therapy.

ARTICLE HISTORY

Received 5 June 2019

Accepted 24 July 2019

KEYWORDS

Waldenström macroglobulinemia; *cxcr4*; ibrutinib; rituximab; outcomes

1. Introduction

Waldenström macroglobulinemia (WM) is an indolent and incurable lymphoma, characterized by the accumulation of malignant IgM-secreting lymphoplasmacytic cells in the bone marrow and other organs [1]. The genomic landscape of WM is defined by recurrent somatic mutations in the *MYD88* gene, which have been identified in over 90% of patients with WM [2]. The most common *MYD88* mutation detected is *MYD88*^{L265P}. However, other non-L265P mutations have rarely been reported in WM patients [3]. The presence of *MYD88*^{L265P} has diagnostic, therapeutic and prognostic implications in patients with WM [2,4,5].

Recent studies have detected recurrent somatic mutations in the carboxyl terminal of the C-X-C chemokine receptor type 4 (*CXCR4*) gene in patients with WM [6]. These *CXCR4* mutations (*CXCR4*^{MUT}) are similar to the ones described in the congenital WHIM (Warts, Hypogammaglobulinemia, Immunodeficiency, and Myelokathexis) syndrome [7]. Over 30 somatic *CXCR4* mutations have been described in patients with WM and can be grouped in frameshift (*CXCR4*^{F5}) and nonsense (*CXCR4*^{N5}) mutations [8]. Emerging data suggest that WM patients with *CXCR4* mutations not only can have specific clinical features but also different outcomes to standard therapies. Therefore, *CXCR4* mutations might need to be

considered at the time of the initial evaluation and/or at the time of treatment initiation in patients with WM.

The purpose of this systematic review is to provide the current understanding on the biology of *CXCR4* mutations, as well as the potential impact these might have in clinical presentation and outcomes to therapy in patients with WM.

2. Methods

Three authors (JJC, DFM, MIA) performed a literature search independently using PubMed through 31 December 2018. The key terms used in the search were: (*CXCR4 AND (Waldenström* OR lymphoplasmacytic)*). An article (retrospective or prospective) was considered relevant if it contained original data on clinical presentation and/or outcomes in patients with WM. The titles and abstracts were reviewed, and full-text articles were selected based on our inclusion criteria. The reference list of each selected study was reviewed to search for additional studies. Abstracts from the American Society of Hematology, the American Society of Clinical Oncology, the European Hematology Association and the European Society of Medical Oncology Annual Meetings were searched only looking for updated results of previously published studies. Any discrepancies on inclusion or exclusion of a study were resolved through consensus in all cases.

Article highlights

- We performed a systematic review to evaluate the prevalence of *CXCR4* mutations and their impact in clinical presentation and outcomes to therapy in patients with Waldenstrom macroglobulinemia (WM)
- Prevalence: Activating mutations in *CXCR4* can be detected in 34% of WM patients.
- Clinical presentation: *CXCR4* mutations are associated with higher serum IgM levels, lower β 2-microglobulin levels and lower rates of lymphadenopathy.
- Impact on therapy outcomes: *CXCR4* mutations are associated with shorter time to first therapy, and longer time to response, lower rates of major and very good partial response, as well as shorter progression-free survival in WM patients treated with Bruton tyrosine kinase inhibitors.
- Survival: *CXCR4* mutations are not associated with worse or better overall survival.

3. Search results

Our search returned 85 publications. Of these, 27 were pre-clinical studies (i.e. laboratory and/or basic science studies), 24 were clinical studies (16 retrospective studies, case reports and/or case series, and 8 were clinical trials), 27 were reviews and 7 were other types of publications (3 editorials, 2 guidelines, and 2 position papers).

4. The *CXCR4* pathway

In a physiological state, after binding to its ligand CXCL12, *CXCR4* is activated and rapidly internalized [9]. Upon activation,

CXCR4 couples to the G family of protein and activates the Src family of tyrosine kinases, which in turn promotes 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.

Somatic *CXCR4* mutations were initially described in a study in which whole genome sequencing was performed 30 WM patients [6]. Germline and tumor sequencing were performed in 10 of these patients. *CXCR4* mutations affected receptor internalization, prolonging signaling by G protein and β -arrestins. These anomalies would result in sustained AKT and ERK signaling and decreased caspase 3 cleavage [10]. A second study validated these findings by associating *CXCR4* mutations with significant tumor proliferation [11]. Therefore, the *CXCR4* pathway could represent an activation pathway complementary to MYD88 in WM cells (Figure 1).

In addition, a number of laboratory studies have associated *CXCR4* mutations with varying levels of resistance to alkylating agents, nucleoside analogues, proteasome inhibitors, BTK inhibitors and PI3K inhibitors in WM cells lines and/or primary WM cells [10,11,13]. In an early study, *CXCR4* knockdown or the use of *CXCR4* inhibitors (e.g. AMD3100) inhibited migration and adhesion of WM cells to the bone marrow microenvironment [14]. Other pre-clinical studies have also shown that addition of AKT1, MAPK1 or *CXCR4* antagonists to ibrutinib reversed *CXCR4*

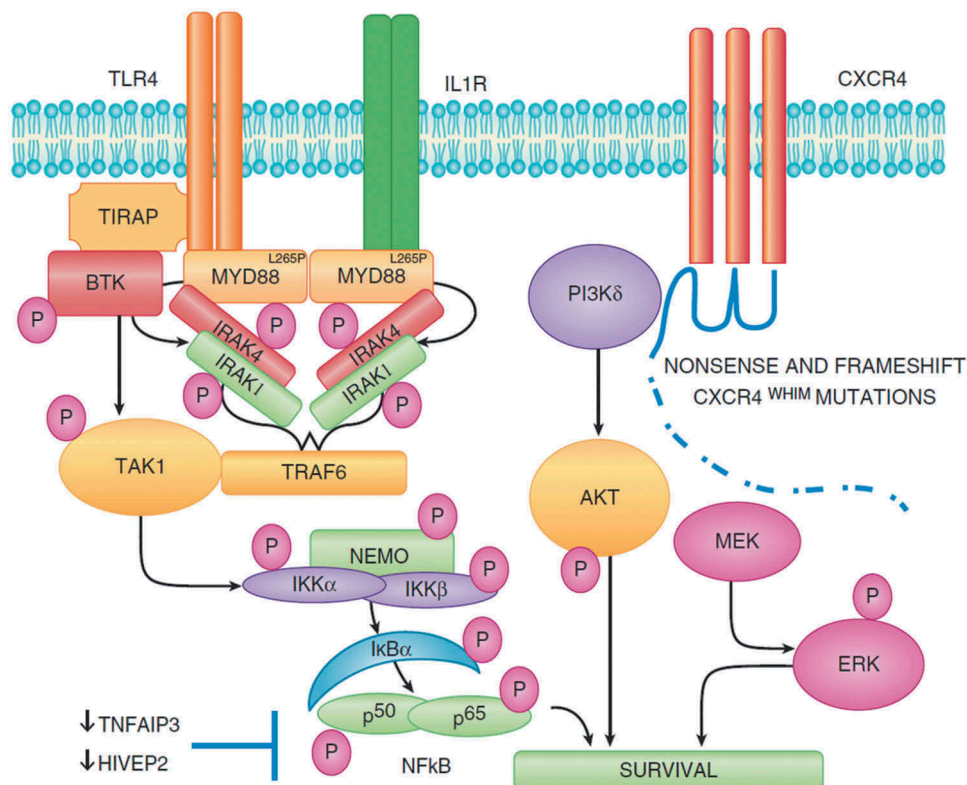


Figure 1. The *CXCR4* pathway provides a complementary activation mechanism to the MYD88 pathway in Waldenstrom macroglobulinemia cells. Reproduced with permission from [12].

signaling and promoted apoptotic changes and tumor reduction in both *CXCR4^{NS}* and *CXCR4^{FS}* WM cell models [10,11].

5. Prevalence of CXCR4 mutations in WM

Seventeen studies have reported on the prevalence of *CXCR4* mutations in WM patients [5,6,15–29]. Seven studies were from Europe, seven studies from the United States, two studies were multinational and one study from China. According to the method of detection of *CXCR4* mutations, six studies used polymerase chain reaction (PCR) techniques in addition to Sanger sequencing, six studies used next-generation sequencing (NGS), four studies used only Sanger sequencing, and one study used Sanger sequencing in addition to whole genome sequencing (WGS). The pooled prevalence of *CXCR4* mutations was 31% (364 of 1161 patients tested were *CXCR4^{MUT}*). The pooled prevalence of *CXCR4* mutations in WM patients with *MYD88^{L265P}* was 34% (359 of 1058 patients tested were *CXCR4^{MUT}*) and 5% in *MYD88^{WT}* patients (5 of 103 patients tested were *CXCR4^{MUT}*). Data from individual studies are shown in Table 1.

6. Impact of CXCR4 mutations in clinical presentation

A summary on the impact of *CXCR4* mutations on the clinical features of patients with WM is shown on Table 2.

6.1. Symptomatic hyperviscosity and serum IgM levels

About 10–15% of WM patients develop symptomatic hyperviscosity, which has been directly related with high serum IgM levels [30]. An association between higher serum IgM levels and *CXCR4* mutations has been described in several studies in WM patients.

One study examined a group of 50 patients harboring *CXCR4* mutations divided by nonsense ($n = 26$) or frameshift ($n = 24$) and were compared with patients without *CXCR4* mutations (*CXCR4* wildtype or *CXCR4^{WT}*) [4]. In this study, *CXCR4^{MUT}* patients had a median serum IgM level of

5,200 mg/dl versus <3,000 mg/dl in *CXCR4^{WT}*. A second study found similar results and reported a rate of serum IgM level >3,000 mg/dl of 30% and 12% in *CXCR4^{MUT}* and *CXCR4^{WT}* patients, respectively [21]. A recent Spanish study confirmed statistically significant higher serum IgM levels in *CXCR4^{MUT}* than in *CXCR4^{WT}* patients (4,400 versus 2,687 mg/dl, respectively) [20]. A prospective study evaluating ibrutinib in 30 previously untreated WM patients also showed higher serum IgM levels in *CXCR4^{MUT}* than in *CXCR4^{WT}* patients (5,295 versus 3,928 mg/dl, respectively) [24].

A relatively large study evaluated the risk of symptomatic hyperviscosity in 825 previously untreated patients with WM [31]. Of these, 224 patients had genotyping and 106 (47%) had *CXCR4* mutations. Among patients who developed symptomatic hyperviscosity and had genotyping available, 78% were *CXCR4^{MUT}*. *CXCR4^{MUT}* patients had 5-fold higher odds of presenting with symptomatic hyperviscosity than *CXCR4^{WT}* patients (OR 4.9, 95% CI 2.1–11.4; $p < 0.001$). The odds of symptomatic hyperviscosity were 9-fold higher in patients with *CXCR4^{NS}* than *CXCR4^{WT}* patients (OR 9.4, 95% CI 2.9–22.5; $p < 0.001$). However, the odds of symptomatic hyperviscosity were not significantly increased in *CXCR4^{FS}* patients when compared to *CXCR4^{WT}* patients (OR 0.8, 95% CI 0.2–3.9; $p = 0.77$).

6.2. Extramedullary disease

Extramedullary disease is identified in 20–80% of patients with WM, typically manifested as lymphadenopathy and/or

Table 2. Impact of *CXCR4* mutations in clinical features of patients with Waldenstrom macroglobulinemia.

	CXCR4 mutated	CXCR4 wild type
Serum IgM level	+++	+
Risk of hyperviscosity	+++	+
Lymphadenopathy	+	+++
Splenomegaly	+	+
Serum beta-2-microglobulin level	+	++
Thrombocytopenia	++	+
Leukopenia	+	+
Anemia	+	+
Bone marrow involvement	++	+
Acquired von Willebrand disease	+++	+
Time to therapy initiation	Shorter	Longer

Table 1. Prevalence of *CXCR4* mutations in patients Waldenstrom macroglobulinemia, based on published studies.

Author (year)	Country	Method	CXCR4 ^{MUT} MYD88 ^{MUT}	CXCR4 ^{MUT} MYD88 ^{WT}
Hunter (2014)	USA	WGS/Sanger	50/160 (31%)	1/17 (6%)
Treon (2014)	USA	Sanger	11/30 (37%)	0/1 (0%)
Schmidt (2015)	Germany	Sanger	17/47 (36%)	NR
Treon (2015)	USA	Sanger/PCR	21/62 (34%)	0/5 (0%)
Ballester (2016)	Spain	Sanger/PCR	7/23 (30%)	1/10 (10%)
Xu (2016)	USA	Sanger/PCR	64/152 (42%)	1/12 (8%)
Baer (2017)	Germany	NGS	17/69 (25%)	0/9 (0%)
Cao (2017)	China	Sanger	9/39 (23%)	1/3 (33%)
Dimopoulos (2017)	Multinational	NGS	7/25 (28%)	0/1 (0%)
Poulain (2017)	France	NGS	14/43 (33%)	0/10 (0%)
Varettoni (2017)	Italy	NGS	14/53 (26%)	0/9 (0%)
Castillo (2018)	USA	Sanger/PCR	15/26 (58%)	0/0 (0%)
Dimopoulos (2018)	Multinational	NGS	49/163 (36%)	0/20 (0%)
Hiemcke-Jiwa (2018)	Netherlands	NGS	9/30 (30%)	NR
Jimenez (2018)	Spain	Sanger	24/63 (38%)	1/6 (17%)
Sklavetinis-Pistofidis (2018)	USA	Sanger/PCR	17/43 (40%)	NR
Treon (2018)	USA	Sanger/PCR	14/30 (47%)	NR

WGS: Whole genome sequencing; PCR: polymerase chain reaction; NGS: next-generation sequencing; NR: not reported

splenomegaly [32]. Higher rates of extramedullary disease are seen as the disease progresses and the patients are exposed to therapy [33]. Other rare extramedullary sites of involvement include the pleural space, soft tissues, the kidneys and the central nervous system [34–37].

The rate of lymphadenopathy was evaluated in a study that included 109 WM patients who were $CXCR4^{WT}$ and 50 who were $CXCR4^{MUT}$. The frequency of lymphadenopathy was 54% and 30%, respectively ($p = 0.01$) [4]. There was no statistical difference in the rate of lymphadenopathy between patients with $CXCR4^{NS}$ and $CXCR4^{FS}$ (30% vs. 29%, respectively). A second study showed similar results with higher rates of lymphadenopathy in $CXCR4^{WT}$ than in $CXCR4^{MUT}$ patients, with rates of 38% and 20%, respectively [22]. In a prospective study evaluating ibrutinib in 63 previously treated patients with WM, $CXCR4^{MUT}$ patients had lower rates of lymphadenopathy than $CXCR4^{WT}$ patients (33% vs. 68%, respectively) [5]. Lower rates of lymphadenopathy were also seen in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients (14% vs. 53%, respectively) in the prospective study evaluating ibrutinib in 31 patients with WM who were rituximab-refractory [29]. Similar findings were seen in a prospective study on 30 previously untreated WM patients in which $CXCR4^{MUT}$ patients had lower rates of lymphadenopathy than $CXCR4^{WT}$ patients (14% vs. 50%, respectively) [24].

There were no significant differences between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients with regard to rates of splenomegaly in three separate studies [4,21,22]. One prospective study showed a trend towards lower rates of splenomegaly in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients (7% vs. 25%, respectively) [24].

6.3. Laboratory parameters

Several studies analyzed blood counts and other biochemistry parameters in relation with $CXCR4$ mutational status. High serum beta-2-microglobulin (B2M) levels, hemoglobin levels, and platelet counts are components of the International Prognostic Scoring System for WM (IPSSWM) [38].

One study from the US showed lower serum beta-2-microglobulin (B2M) levels in $CXCR4^{MUT}$ patients when compared with $CXCR4^{WT}$ patients (median, 2.3 mg/l, and 3.1 mg/l, respectively) [4]. A Spanish study also reported significantly lower serum B2M levels in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients (2.8 mg/l and 3.7 mg/l, respectively) [20]. A prospective study on ibrutinib in 30 previously untreated WM patients showed a trend towards lower serum B2M levels in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients (3.4 mg/l and 4.2 mg/l, respectively) [24].

With regard to blood counts, a retrospective study from the US showed no differences in absolute neutrophil count, absolute lymphocyte count (ALC) and hemoglobin levels and a trend towards lower platelet counts (251 K/uL and 274 K/uL, respectively) between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients [4]. A French retrospective study found that $CXCR4^{MUT}$ patients had higher rates of platelets count <100 K/uL than $CXCR4^{WT}$ patients (7% vs. 27%, respectively), while there were no differences in ALC between the groups [21]. A German retrospective study found

lower platelet and white blood cell count (WBC) as well as hemoglobin levels in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients [22]. In this study, $CXCR4^{FS}$ patient had lower WBC count, and $CXCR4^{NS}$ had lower platelet and WBC count, than $CXCR4^{WT}$ patients.

6.4. Bone marrow involvement

A retrospective US study showed no differences between level of bone marrow involvement between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients (50% vs. 40%, respectively) [4]. A German study suggested a higher level of bone marrow involvement in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients, without a discernible difference between $CXCR4^{FS}$ and $CXCR4^{NS}$ patients [22]. A retrospective Italian study showed a higher degree of bone marrow involvement in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients (50% vs. 30%, respectively) [26]. A prospective study in previously untreated WM patients showed no differences in degree of bone marrow involvement between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients (70% vs. 60%, respectively).

6.5. Acquired Von Willebrand disease

Acquired von Willebrand disease (VWD) has been associated to WM and could have prognostic value [39]. There is only one study that analyzed the relation between acquired VWD and $CXCR4$ mutational status [40]. In that study, 57 out of 125 genotyped patients (46%) were $CXCR4^{MUT}$. Patients with VWD markers (i.e. VW antigen, VW ristocetin cofactor, and factor VIII) levels less than 30% were more likely to be $CXCR4^{MUT}$ than patients with normal levels of VWD markers (77% vs. 37%, respectively). $CXCR4^{MUT}$ patients had 5-times higher odds of having levels of VWD markers less than 30% than $CXCR4^{WT}$ patients. $CXCR4^{NS}$ patients had 8-times higher odds of low levels of VWD markers than $CXCR4^{WT}$ patients, while there was no statistical difference between $CXCR4^{FS}$ and $CXCR4^{WT}$ patients.

6.6. Conclusions

- (1) Current data support a relation between $CXCR4^{MUT}$ status and high serum IgM levels as well as symptomatic hyperviscosity. The risk of hyperviscosity appears higher in $CXCR4^{NS}$ patients.
- (2) Studies have showed consistently lower rates of lymphadenopathy in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients. There is no evidence of an association between $CXCR4$ mutational status and splenomegaly.
- (3) $CXCR4^{MUT}$ are associated with lower serum B2M levels and a trend towards lower platelet counts than $CXCR4^{WT}$ patients. The relation between $CXCR4$ mutational status and hemoglobin level, ANC, ALC or WBC count is less clear.
- (4) Data are not consistently associating a higher degree of bone marrow involvement in $CXCR4^{MUT}$ patients.

- (5) One study has suggested higher odds for acquired VWD in $CXCR4^{MUT}$ patients, especially $CXCR4^{NS}$, when compared to $CXCR4^{WT}$ patients.

7. Impact of CXCR4 mutations in outcomes to therapy

7.1. Time to first therapy

Based on current guidelines, asymptomatic patients with WM (also called inactive or smoldering) should not be treated, as standard therapies are not curative and may have toxic effects. Data are currently limited with regard to factors that would increase the risk of progressing from inactive to active WM. One study evaluated the treatment-free survival of 81 WM patients who were inactive at diagnosis [26]. After a median follow-up time of 43 months, 26 asymptomatic patients required treatment. The median treatment-free survival was 51 months in $CXCR4^{MUT}$ patients, which was shorter than a median time to treatment survival that was not reached in $CXCR4^{WT}$ patients ($p = 0.007$).

7.2. Bruton tyrosine kinase (BTK) inhibitors

Ibrutinib, an oral BTK inhibitor, is approved in the US and Europe for the treatment of patients with symptomatic WM. Four prospective studies have reported response and survival outcomes to ibrutinib, alone or in combination with rituximab, in WM patients with $CXCR4$ mutations [5,24,28,29]. In the pivotal trial that evaluated ibrutinib in previously treated WM patients, 21 of the 62 (34%) evaluated patients had $CXCR4$ mutations, of which 18 were $CXCR4^{NS}$ and three were $CXCR4^{FS}$ [5]. $CXCR4$ mutational status was evaluated using Sanger sequencing of CD19-selected bone marrow cells. After a median follow-up of 19 months, the overall and major response rates (ORR and MRR, respectively) in $CXCR4^{MUT}$ patients were 86% and 62%, respectively, in comparison to 100% and 91%, respectively, in $CXCR4^{WT}$ patients. Results were updated with a median follow-up time of 47 months [41]. ORR, MRR and very good partial response (VGPR) rates in $CXCR4^{MUT}$ patients were 86%, 67%, and 10%, respectively, which were lower than in $CXCR4^{WT}$ patients, who had rates of 100%, 97% and 44%, respectively. In the updated results, the median PFS in $CXCR4^{MUT}$ patients was 45 months, while the median PFS was not yet reached in $CXCR4^{WT}$ patients.

Similar results were observed in a study in 31 rituximab-refractory WM patients treated with ibrutinib [29]. In this study, 7 of 24 patients (29%) were $CXCR4^{MUT}$. $CXCR4$ mutational status was assessed using the Personalis ACE Extended Cancer Panel. After a median follow-up of 18 months, the MRR and VGPR rate in $CXCR4^{MUT}$ patients were 71% and 0%, respectively. $CXCR4^{WT}$ patients had MRR and VGPR rate of 83% and 18%, respectively. The 18-month PFS rates for $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients were 86% and 94%, respectively.

A phase II study evaluated single-agent ibrutinib in previously untreated WM patients [24]. $CXCR4$ mutational status was evaluated using Sanger sequencing and allele-specific

PCR of CD19-selected bone marrow cells. The median follow-up on this study was 18 months, and 14 out of 30 patients (47%) had $CXCR4$ mutations. The median time to response was longer in $CXCR4^{MUT}$ patients at 1.7 months, when compared to 0.9 months in $CXCR4^{WT}$ patients. Similarly, the time to major response was longer at 7.3 months versus 1.8 months, respectively. MRR and VGPR rates were lower in $CXCR4^{MUT}$ patients at 71% and 7%, when compared to $CXCR4^{WT}$ patients, who had rates at 94% and 31%, respectively. Interestingly, improvements in hemoglobin concentration had similar kinetics over time for $CXCR4^{WT}$ and $CXCR4^{MUT}$ patients.

A phase III randomized study evaluated the combination of ibrutinib and rituximab versus placebo and rituximab in 150 patients with WM [28]. In this study, 75 patients, 34 previously untreated and 41 previously treated, were randomized to the ibrutinib and rituximab arm, and 26 out of 69 (38%) tested patients were $CXCR4^{MUT}$. The technique used for assessment of $CXCR4$ mutational status was not provided in the manuscript. With a median follow-up of 30 months, MRR and VGPR (or better) rates in $CXCR4^{MUT}$ patients were 73% and 19%, respectively, while the rates in $CXCR4^{WT}$ patients were 78% and 34%, respectively. The 30-month PFS rates to the ibrutinib and rituximab combination were 80% and 86% for $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients, respectively. However, the 36-month PFS rates showed a 20% difference at 64% and 84% for $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients, respectively [42].

7.3. Proteasome inhibitors

Three proteasome inhibitors are commercially available in the US (i.e. bortezomib, carfilzomib, and ixazomib), which have also been prospectively evaluated in WM patients.

A post-hoc study evaluated the effect of $CXCR4$ mutations in 43 patients with previously treated WM who participated in a prospective phase II study evaluating weekly bortezomib and rituximab [23]. The original study included 64 patients [43], but 21 patients were not evaluated given lack of material for genotyping. In the post-hoc study, the $CXCR4$ mutational status was evaluated by Sanger sequencing, and 17 out of 43 patients (40%) were $CXCR4^{MUT}$. After a median follow-up time of 91 months, $CXCR4^{MUT}$ status did not associate with better or worse PFS (Log-rank $p = 0.99$). Time to response and categorical responses based on $CXCR4$ mutational status were not reported.

A prospective phase II study evaluated the combination of twice-weekly carfilzomib, rituximab, and dexamethasone in 31 patients with previously untreated WM [25]. $CXCR4$ mutations were evaluated using Sanger sequencing of CD19-selected bone marrow cells. $CXCR4^{MUT}$ were detected in 11 of 30 evaluated patients (37%). There were no statistical differences between $CXCR4^{WT}$ and $CXCR4^{MUT}$ patients with regard to ORR (85% and 91%, respectively) and MRR (65% and 73%, respectively). Time to response and PFS according to $CXCR4$ mutational status were not reported.

A third prospective phase II study evaluated the combination of weekly ixazomib, dexamethasone, and rituximab in 26 patients with previously untreated WM [18]. $CXCR4$ mutations were evaluated using Sanger sequencing of CD19-selected bone marrow cells. $CXCR4^{MUT}$ were detected in 15 of 26

patients (58%). The time to response was longer in $CXCR4^{MUT}$ than in $CXCR4^{WT}$ patients at 12 weeks and 8 weeks, respectively (Log-rank $p = 0.03$). The MRR between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients were not different at 74% and 82%, respectively, although the rates of VGPR were 7% and 27%, respectively. The rates of PFS at 22 months were not different between $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients.

7.4. mTOR inhibitors

One prospective phase II study evaluated single-agent everolimus in 33 previously untreated patients with WM [44]. The $CXCR4$ mutational status was evaluated by Sanger sequencing and allele-specific PCR of CD19-selected bone marrow cells, and $CXCR4^{MUT}$ were detected in 4 of 21 genotyped patients (19%). The ORR in $CXCR4^{MUT}$ and $CXCR4^{WT}$ patients were 50% and 81%, respectively, and the MRR were 25% and 63%, respectively. These differences were not statistically different, probably due to the small sample size. Time to response and PFS according to $CXCR4$ mutational status were not reported.

Another prospective phase I/II study enrolled 46 previously WM patients, who went on to receive the combination of everolimus and rituximab ($n = 6$ patients) or the combination of everolimus, bortezomib and rituximab (RVR; $n = 40$) [45]. The $CXCR4$ mutational status was evaluated by allele-specific PCR for $CXCR4^{S338X}$ in bone marrow cells. $CXCR4^{MUT}$ were detected in 1 of 20 genotyped patients (5%). The only $CXCR4^{MUT}$ patient attained stable disease to RVR.

7.5. Conclusions

- (1) One study has suggested a shorter time from diagnosis to first therapy in $CXCR4^{MUT}$ when compared to $CXCR4^{WT}$ patients.
- (2) When treated with single-agent ibrutinib, $CXCR4^{MUT}$ status was associated with longer time to major response, lower MRR and VGPR rates and also shorter median PFS when compared to $CXCR4^{WT}$ in WM patients.
- (3) $CXCR4^{MUT}$ status was associated with lower rates of VGPR and 36-month PFS than $CXCR4^{WT}$ in WM patients treated with ibrutinib and rituximab.
- (4) $CXCR4^{MUT}$ status was not associated with PFS in WM patients treated with proteasome inhibitors in combination with dexamethasone and rituximab.
- (5) MRR and VGPR rates appeared lower in $CXCR4^{MUT}$ when compared with $CXCR4^{WT}$ WM patients treated with single-agent everolimus, although the sample size was small and the difference non-significant.

8. Impact of $CXCR4$ mutations in OS

Two studies had evaluated the prognostic value of $CXCR4$ mutations in WM patients. In one study, 175 WM patients were genotyped, and $CXCR4$ mutations were detected in 50 patients (29%) [4]. There was no difference in OS in $CXCR4^{MUT}$ when compared with $CXCR4^{WT}$ WM patients (HR 0.96, 95% CI 0.16–6.7; $p = 0.96$). In a more recent study, 286 WM patients

were genotyped, and $CXCR4$ mutations were detected in 101 patients (35%) [46]. In a multivariate analysis adjusting for age, sex, hemoglobin level, serum IgM level and bone marrow involvement, there was no difference in OS in $CXCR4^{MUT}$ when compared with $CXCR4^{WT}$ WM patients (HR 1.07, 95% CI 0.36–3.12; $p = 0.91$).

8.1. Conclusions

- (1) $CXCR4^{MUT}$ status does not seem to be associated with a better or worse OS than $CXCR4^{WT}$ WM patients.

9. Discussion

Recurrent mutations in $MYD88$ and $CXCR4$ can be detected in over 90% and 30–40% of patients with WM, respectively. Prior studies have suggested clinical differences in WM patients with difference genomic profiles, but this has not yet been evaluated systematically. In this systematic review, we aimed at increasing our understanding of the prevalence of $CXCR4$ mutations and the impact of these mutations in the clinical presentation and outcomes to therapy in patients with WM. Specifically, clinical features evaluated included serum IgM level and risk of hyperviscosity, laboratory markers, prevalence of extramedullary disease, degree of bone marrow involvement, risk of acquired VWD and time to first treatment initiation. Clinical outcomes of interest included MRR, VGPR rate, PFS and OS.

Our study shows that the prevalence of $CXCR4$ mutations in WM patients was 31%. $CXCR4$ mutations were more frequently detected in patients with $MYD88$ mutations than in patients without $MYD88$ mutations with prevalence rates of 34% and 5%, respectively. The actual number of WM patients with $CXCR4$ mutations and without $MYD88$ mutations was too small, however, to allow deeper analyses for clinical features, response or survival on these patients. Therefore, most of the data and analysis reported below would pertain to patients with concurrent $MYD88$ and $CXCR4$ mutations.

Current evidence supports that WM patients with $CXCR4$ mutations have a distinct clinical profile than patients without $CXCR4$ mutations, as the former can present with higher serum IgM levels, as well as lower serum B2M levels and lower rates of lymphadenopathy. Serum IgM level is a prognostic marker for survival after first treatment initiation (SAFTI), as shown by the IPSSWM [38], and could imply that $CXCR4$ mutated patients might have a worse SAFTI than patients without $CXCR4$ mutations. However, $CXCR4$ mutated patients can also present with lower serum B2M levels, which have been associated with better SAFTI in previous studies [38,47]. Our study, however, does not provide a direct analysis of the association between $CXCR4$ mutations and SAFTI. The lower rates of lymphadenopathy in $CXCR4$ mutated patients are of interest as extramedullary disease was suggested to be of poor prognosis in WM in a retrospective study [33]. However, a previous population-based study showed no OS differences between WM patients with and without extramedullary disease [48,49].

The results of our review show that *CXCR4* mutations affect time to response to ibrutinib and the combination of ixazomib, dexamethasone and rituximab, as well as response rates to ibrutinib and everolimus, and rate of VGPR to ibrutinib and the combination of ibrutinib and rituximab. We believe these findings could help for better counseling WM patients, when ibrutinib or ibrutinib and rituximab are treatment considerations. Data on the effect of *CXCR4* mutations on response to novel BTK inhibitors are limited. In the ongoing phase I/II study evaluating zanubrutinib in WM patients, the VGPR rate appeared lower in *CXCR4*^{MUT} than in *CXCR4*^{WT} patients (33% vs. 48%, respectively) [50]. However, these data have not been formally published. No data are available on the effect of *CXCR4* mutations in WM patients on acalabrutinib. Our study supports the outcomes of WM patients treated with proteasome inhibitors are not adversely affected by *CXCR4* mutations. There are no data evaluating outcomes of WM patients treated with chemotherapy or chemoimmunotherapy according to *CXCR4* mutational status.

In conclusion, current evidence supports distinct clinical features in WM patients with and without *CXCR4* mutations, and also an adverse impact on response and PFS in WM patients treated with ibrutinib alone or in combination with rituximab. Additional research is needed to further understand the biology of *CXCR4* mutations in WM and how to overcome its adverse effect on outcomes of WM patients treated with ibrutinib.

10. Expert opinion

Based on the results of our systematic review, *CXCR4* has emerged as a biomarker and a driver of disease in WM. However, the testing for the detection of *CXCR4* mutations needs further refinement. The testing utilized to detect *CXCR4* mutations has not been standardized. In our study, several techniques were used for the assessment of *CXCR4* mutational status and involved use of CD19-selected cells. WGS was initially used in the discovery study by Hunter and colleagues [6]. However, the routine clinical use of WGS is not practical for routine clinical use. Over 40 different types of *CXCR4* mutations have been identified in WM patients and include *CXCR4*^{NS} and *CXCR4*^{FS} mutations [4,21]. While allele-specific PCR can identify common *CXCR4*^{NS} mutations that are produced by point mutations such as *CXCR4*^{S338X} or *CXCR4*^{R334X}, FS mutations are missed by this technique. Targeted next-generation sequencing, which is becoming routinely used in clinical molecular diagnostic laboratories, could be used to identify both *CXCR4*^{NS} and *CXCR4*^{FS} mutations. To further complicate things, not only these tests might have sensitivity limitations, but the burden of disease is also of importance, as the rate of false negative results increases with lower burden of disease in the bone marrow or tissue tested. Ideal conditions for detection of *CXCR4* mutations by Sanger sequencing, allele-specific PCR and/or NGS would include untreated patients, selection of CD19 cells for genomic testing, and use of bone marrow mononuclear cells [27]. The identification of *CXCR4*^{NS} and *CXCR4*^{FS} mutations could have clinical implications, as a recent study reported lower odds of major response and worse PFS to

ibrutinib monotherapy in *CXCR4*^{NS} than in *CXCR4*^{FS} WM patients [51].

Based on our observations, *CXCR4* could serve as a therapeutic target in WM. The *CXCR4* antagonist plerixafor has shown to be safe and effective in patients with WHIM syndrome [52–54]. On plerixafor therapy, WHIM patients experienced improvement in leukopenia, anemia, and thrombocytopenia, as well as decline in wart burden and human papillomavirus-related lesions. Of most importance, the patients' quality of life improved with the intervention. Preclinical experiences have shown the anti-*CXCR4* monoclonal antibody ulocuplumab-induced apoptosis, at nanomolar levels, in chronic lymphocytic leukemia and myeloma cell lines [55,56]. A phase I/II study evaluating ibrutinib in combination with the anti-*CXCR4* monoclonal antibody ulocuplumab in symptomatic WM patients with *CXCR4* mutations is undergoing accrual (NCT03225716). Other anti-*CXCR4* small molecules such as EPI-X4 and X4P-001 are currently at early stages of clinical development.

With the careful design and execution of prospective clinical trials focusing exclusively in patients with *CXCR4* mutations, we envision a broad use of *CXCR4*-targeted therapy in the near future, not only for patients with WM, but also for patients with other hematologic and solid malignancies with *CXCR4* overexpression. *CXCR4* appear to play an important role on tumorigenesis as well as metastasis in breast, colon, lung and prostate cancer, as well as leukemia and myeloma [55–69]. We foresee *CXCR4*-targeted therapy as a potential future standard on the treatment of human cancer.

Authors contributions

JJC and SPT designed the study. JJC, DFM, and MIA performed the literature search, study selection and data gathering, and wrote the initial draft. All the authors critically reviewed and approved the final manuscript.

Funding

This paper is not funded.

Declaration of interest

JJ Castillo received research funds from AbbVie, Beigene, Janssen, Millennium, Pharmacyclics and TG Therapeutics, and honoraria from Beigene, Janssen and Pharmacyclics. SP Treon received research funding from Bristol-Myers Squibb and Pharmacyclics, and honoraria from Pharmacyclics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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