Genomic Landscape of CXCR4 Mutations in Waldenström Macroglobulinemia

Stéphanie Poulain1,2,3, Christophe Roumier2,3, Aurélie Venet-Caillault2, Martin Figeac4, Charles Herbaux5,6, Guillelmette Marot5, Emmanuelle Doye2, Elisabeth Bertrand3, Sandrine Geffroy2, Frédéric Lepretre4, Olivier Nibourel2,3, Audrey Decambron1, Eileen Mary Boyle3,5, Aline Renneville2, Sabine Tricot1, Agnès Daudignon1, Bruno Quesnel3,4, Patrick Duthilleul1, Claude Preudhomme2,3, and Xavier Leleu3,5

Abstract

Purpose: Whole-genome sequencing has revealed MYD88 L265P and CXCR4 mutations (CXCR4mut) as the most prevalent somatic mutations in Waldenström macroglobulinemia. CXCR4 mutation has proved to be of critical importance in Waldenström macroglobulinemia, in part due to its role as a mechanism of resistance to several agents. We have therefore sought to unravel the different aspects of CXCR4 mutations in Waldenström macroglobulinemia.

Experimental Design: We have scanned the two coding exons of CXCR4 in Waldenström macroglobulinemia using deep next-generation sequencing and Sanger sequencing in 98 patients with Waldenström macroglobulinemia and correlated with SNP array landscape and mutational spectrum of eight candidate genes involved in TLR, RAS, and BCR pathway in an integrative study.

Results: We found all mutations to be heterozygous, somatic, and located in the C-terminal domain of CXCR4 in 25% of the Waldenström macroglobulinemia. CXCR4 mutations led to a truncated receptor protein associated with a higher expression of CXCR4. CXCR4 mutations pertain to the same clone as to MYD88 L265P mutations but were mutually exclusive to CD79A/CD79B mutations (BCR pathway). We identified a genomic signature in CXCR4mut Waldenström macroglobulinemia, traducing a more complex genome. CXCR4 mutations were also associated with gain of chromosome 4, gain of Xq, and deletion 6q.

Conclusions: Our study panned out new CXCR4 mutations in Waldenström macroglobulinemia and identified a specific signature associated to CXCR4mut, characterized with complex genomic aberrations among MYD88L265P Waldenström macroglobulinemia. Our results suggest the existence of various genomic subgroups in Waldenström macroglobulinemia. Clin Cancer Res; 1–9.

Introduction

Whole-genome sequencing has revealed CXCR4 as the second most frequent somatic mutation, identified in approximately 30% of Waldenström macroglobulinemia, similar to germline mutations found in the WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome (1–3). MYD88 L265P mutations remain the most frequent mutation reported in Waldenström macroglobulinemia to date, in nearly 90% of Waldenström macroglobulinemia (4, 5). Interestingly, MYD88 L265P may act as a founder mutation because of its high frequency in Waldenström macroglobulinemia; and thus it is suspected that a second hit may accelerate development and progression of the malignant clones leading to full-blown in Waldenström macroglobulinemia. Although the mechanism of deregulation of CXCR4 axis is not fully understood in Waldenström macroglobulinemia, it is possible that CXCR4 mutation might represent one of these secondary events.

C-X-C chemokine receptor type 4 (CXCR4) is a G-protein-coupled receptor that plays an important role in lymphopoiesis and cell trafficking (2, 6), along with its ligand, the stromal cell–derived factor-1 (CXCL12/SDF-1). The SDF1/CXCR4 axis promotes activation of several pathways including RAS, Akt, and NF-κB and interplays with BCR pathway (6–8). The CXCR4 gene is located on the long arm of chromosome 2 at position 21 and codes for a chemokine receptor that promotes migration and survival of various B lymphoid malignancies (8–10).

CXCR4 mutations were identified using Sanger sequencing by Treon and colleagues in nearly 25% of Waldenström macroglobulinemia, and the CXCR4 C1013G mutation was described as the most frequent recurrent CXCR4 mutation in 7% (11). However, the occurrence of potential subclonal of CXCR4 mutation and the proportion of CXCR4 mutation over the other known mutations was not available; in other words, how this mutation lies in the architecture of the MYD88-mutated clone. On contrary, Roccaro and colleagues have reported 30% incidence rate of the CXCR4

1Service d’Hématologie-Immunologie-Cytogénétique, Centre Hospitalier de Valenciennes, France. 2Laboratoire d’Hématologie, Centre de Biologie et Pathologie, CHRU de Lille, France. 3INSERM UMR 1172, IRL, Lille, France. 4IFR114, Plateforme de Génomique, Lille, France. 5Service des Maladies du Sang, Hôpital Huriez, CHRU, Lille, France. 6Université de Lille, UDSL, EA2694 Biostatistics/Inria Lille Nord Europe, MODAL, Lille, France.

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Corresponding Author: Xavier Leleu, Hôpital Huriez, CHRU, Rue Michel Polonovski, Lille 59037, France. Phone: 33-3-20446883; Fax: 33-3-2044-4094; E-mail: xavier.leleu@chu-lille.fr
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Translational Relevance

CXCR4 mutation has proved to be of critical importance in Waldenström macroglobulinemia, in part due to its role as a mechanism of resistance to several agents. We have therefore sought to unravel the different aspects of CXCR4 mutations in Waldenström macroglobulinemia and to characterize the genetic background using targeted next-generation sequencing (NGS) and SNP arrays. Mutational spectrum of eight candidate genes involved in TLR, RAS, and BCR pathway was studied in an integrative study. About 25% of Waldenström macroglobulinemia displays CXCR4 mutation in C-terminal domain responsible for higher CXCR4 expression. CXCR4 mutations pertain to the same clone as to MYD88 L265P mutations, with a clear-cut genomic signature traducing a more complex genome using SNP arrays but were mutually exclusive to BCR pathway mutations (CD79A/B mutations). CXCR4 mutation identified a genomic subgroup of MYD88L265P Waldenström macroglobulinemia.

C1013G mutation using qPCR; however, they have solely studied this mutation (12).

In this study, we characterized the genomic landscape of CXCR4 somatic mutation in a large cohort of 98 patients with Waldenström macroglobulinemia, combining deep sequencing of target genes allowing study of the clonal architecture in the Waldenström macroglobulinemia cells, including subclonal analysis, and to confirm the incidence rate of these mutations and single polymorphism nucleotide array (SNPa) to decipher the genomic landscape of CXCR4-mutated Waldenström macroglobulinemia.

Patients and Methods

Patients

Ninety-eight patients diagnosed with Waldenström macroglobulinemia were included in this study (63 males, 35 females). The diagnosis and treatment initiation criteria of Waldenström macroglobulinemia were included in this study (63 males, 35 females). Among the cohort with available clinical follow up (n = 93), 68 patients were treated. First-line treatment was initiated at diagnosis in 41% of the patients. Front-line therapy included chloraminophene alone in 12 cases, dexamethasone-R-cyclophosphamide in 19 cases, bortezomib-rituximab alone in 3 cases or in association with chemotherapy in 51 cases (Supplementary Tables S1 and S2). The PGSEA package (17) was performed on DSP30 arrays (Affymetrix) for seven of the patients with Waldenström macroglobulinemia. Total RNA was extracted from purified B cells using QiAmp kit (Sigma-Aldrich Co.). MYD88 L265P, CD79A, and CD79B mutations were analyzed as previously described (16) in 98 patients. C-terminal CXCR4 gene was amplified from gDNA by PCR as previously described (n = 98 patients; ref. 5). All the exons of CXCR4 gene were analyzed using targeted next-generation sequencing (NGS) in a cohort of 53 patients. NGS was analyzed using the Ion Torrent PGM platform (Life Biotecnologies). Amplicons covering the regions of interest were designed with an amplicon length of 150 to 250 bp. Libraries were sequenced with 200-bp read length on a 318 chip. Mutation calling was performed using the Ion Torrent suite variant caller under the low stringency somatic settings (TS4.0). We have studied CXCR4 mutations along with MYD88 L265P (exon 5), CD79A (ITAM domain), CD79B (ITAM domain), CARD11 (exons 5–9), N-RAS (exons 2 and 3), K-RAS (exons 2 and 3), BRAF6 (exon 15), and PSEN (exon 5–7) using NGS (n = 53). To quantify the mutated clone, we have analyzed the VAF (variant allele frequency) defined as the number of reads that mapped each studied to this position, cover the variant base and show the reference allele, divided by all fragments covering the site. VAF was corrected by the percentage of tumor cells in B cell selected sample. Mutation calls were considered positive when called at by least 20 variants reads. Complete sequence data of the coding and splice site regions of CXCR4 were generated at a mean depth coverage of 2,000× per nucleotide. NGS assay also allowed a better assessment of the percentage of CXCR4 mutant allele whose sensitivity is of 1%.

Gene expression profiling

Gene expression profiling (GEP) was performed using U133A arrays (Affymetrix) for seven of the patients with Waldenström macroglobulinemia. Total RNA was extracted from purified B tumoral cells population isolated from bone marrow using the TRIzol method. Expression data were normalized using the Robust Multi-array Average (RMA) algorithm. Differential gene expression was analyzed with the Bioconductor R package “limma,” which is well-known to improve variance modeling when the number of replicates is small. All probes with an adjusted P value under 0.05 were considered differentially expressed. Their normalized expression values were then represented in a heatmap, whose colors vary according to the standardized by row values of the input data. In parallel, a gene set enrichment analysis (GSEA) was performed for several gene sets among them the C2 collection of the MSigDB (Supplementary Tables S1 and S2). The PGSEA package (17) was used to calculate z scores for all gene sets, and P values were adjusted for multiple testing (18).

Cytogenetic analysis, FISH, SNP arrays, and immunophenotypic studies

Genome-wide detection of copy number alteration (CNA) and LOH was performed using the Genome-Wide Human SNP Array 6.0 (Affymetrix). Conventional cytogenetic analysis was performed on DSP30 + IL2 stimulated bone marrow cells. FISH was performed to detect chromosomal aberrations: 6q23 deletion, 17p12, 11q22 deletions, 13q14 deletion, trisomy 12,
trisomy 4 (n = 90; ref. 15). Immunophenotypic expression of CXCR4 (R et D systems, UK), CD49d, CD27, CD80, CD86, CD138 gating on CD19+ cells was performed along with CD38 (ImmuneTech) and determination of the Matutes’s score by flow cytometry. The ratio of mean fluorescence intensity (MFR) was calculated as the ratio of specific fluorescence with an isotype control.

The relationships between the clinical, biologic, and molecular parameters were determined using a nonparametric test (Mann–Whitney), a t test, a χ², or Fisher exact tests when appropriate. Correlations were tested through Spearman correlation coefficient. The differences between the results of the comparative tests were considered statistically significant if P < 0.05. All statistical analyses were done with the SPSS 15.0 software. Survival was assessed by the Kaplan–Meier test, comparisons were made with the log-rank test, and extended to the entire cohort of 98 patients with Waldenström macroglobulinemia (including the 53 done by NGS). We identified CXCR4 mutations (CXCR4 mut) for a total of 24 of 98 (24.5%), and we have characterized several new mutations across the entire coding exons of CXCR4 (Fig. 1).

Overall, 17 different mutations were identified in CXCR4, including 12 that we have described for the first time in Waldenström macroglobulinemia. Interestingly, only one type of CXCR4 mutation was observed in either patient, and all mutations identified were located in the 45 amino acid intracytoplasmic carboxy-terminal tail. These mutations were never observed in the paired T lymphocytes and thus confirmed their somatic feature. All mutations were heterozygous, and no UPDAs (acquired uniparental disomy, LOH without variation of copy number) was observed at CXCR4 locus nor variation of copy gene number (gain or deletion) in our cohort, using SNP array.

Results

New CXCR4 mutations identified in Waldenström macroglobulinemia

We have first used NGS (deep sequencing) to screen the entire sequence of CXCR4 (n = 53 Waldenström macroglobulinemia samples) with a greater sensitivity and to quantify the allelic frequency of the variant of CXCR4 mutations in B selected cells. We have identified 14 of 53 (26.4%) Waldenström macroglobulinemia with CXCR4 mutations. We then confirmed the observed mutations using Sanger sequencing (SaS) and extend to the entire cohort of 98 patients with Waldenström macroglobulinemia (including the 53 done by NGS). We identified CXCR4 mutations (CXCR4 mut) for a total of 24 of 98 (24.5%), and we have characterized several new mutations across the entire coding exons of CXCR4 (Fig. 1).

High allelic frequency of CXCR4 mutation in Waldenström macroglobulinemia

NGS also allowed the quantification the allelic frequency of the variant of CXCR4 mutations. This quantification allows to

![Diagram of CXCR4 structure with highlighted mutations](image)

Figure 1.
Somatic mutations in C terminus of CXCR4 identified by Sanger sequencing and targeted NGS in patients with Waldenström macroglobulinemia (WM).
List of CXCR4 mutations observed in our study. n, number of patients. The mutations previously reported in WM by Treon et al. are noted with *. In the schematic structure of CXCR4, sites of mutation are highlighted in blue (frameshift mutation) or gray (nonsense mutation). The S338 hotspot was represented in black.
conclude for the percentage of mutation in the clonal population for a given patient and thus to differentiate between clonal (dominant clonal) and subclonal. The mutation load of CXCR4 varied from 13.5% to 47.58% (mean, 35.2%) using the VAF \( n = 53 \). Among the \( CXCR4^{\text{mut}} \) cases, there was a significant greater expression of \( CXCR4 \) protein in Waldenström macroglobulinemia with \( CXCR4^{\text{mut}} \) \( \left( P = 0.003; \text{Fig. 3} \right) \). This increased expression was observed independently of the type of mutation (data not shown).

**Phenotypic signature associated to \( CXCR4^{\text{mut}} \) genotype**

\( CXCR4 \) directly interacts with CD49d in regulating migration and adhesion of Waldenström macroglobulinemia cells to the bone marrow microenvironment (10). CD49d favors lymphocyte homing by cooperating with CXCR4 in stromal cell adhesion and extracellular matrix (9). We then studied the impact of \( CXCR4 \) mutation on the expression of the integrin VLA4 (CD49d) using flow cytometry \( (n = 53) \). CD49d was expressed in all Waldenström macroglobulinemia cases; but no difference in CD49d expression was observed according to \( CXCR4 \) mutation. In contrast, a higher expression of CD49d was observed in Waldenström macroglobulinemia with \( MYD88 \) L265P mutation \( (P = 0.048; \text{Fig. 3}) \). Similarly, we found no difference in CD38 expression according to \( CXCR4 \) mutation, despite of the known functional link between \( CXCR4, \) CD49d, and CD38 (20). CD138 mediates cell adhesion to the extracellular matrix and is a marker of plasmacytic differentiation (21). Interestingly, we found a significant lower expression of CD138 (syndecan1) in the \( CXCR4^{\text{mut}} \) group \( (P = 0.034) \). We found no difference in the CD27, CD23, CD10, K/L expression and the Matutes’ score between \( CXCR4^{\text{mut}} \) and \( CXCR4^{\text{wild}} \) subgroups.

**Genomic signature of \( CXCR4^{\text{mut}} \) Waldenström macroglobulinemia**

We then sought to study whether a specific high-throughput SNPa signature was associated to \( CXCR4^{\text{mut}} \) Waldenström macroglobulinemia in a series of 53 Waldenström macroglobulinemia including 12 \( CXCR4^{\text{mut}} \). For this analysis, we have considered...
The presence of one genomic abnormality including gain, loss, and/or copy number without LOH (CN-LOH), identified using SNP arrays. In this first analysis, we have compared presence versus absence of genomic abnormality according to presence of CXCR4 mutation. We found a relationship between CXCR4mut and a greater frequency of genomic aberrations in Waldenström macroglobulinemia compared with CXCR4wild (91% vs. 68%, P = 0.010).

We then evaluated the number of genomic abnormality as a reflection of the genomic complexity on the basis of the number of SNP abnormalities. We found that CXCR4-mutated Waldenström macroglobulinemia had a greater mean number of abnormality (5.8 vs. 2.8 per patient, P = 0.046). No significant difference was observed between Waldenström macroglobulinemia with clonal or subclonal CXCR4 mutation.

Furthermore, we analyzed the relationship between CXCR4 mutation and certain type of SNP aberration. We have observed that Waldenström macroglobulinemia with CXCR4mut had a greater incidence rate of trisomy 4 (complete or partial), 58% versus 12% respectively (P = 0.002); a greater frequency of gain of Xq, including MYD88 exon 5, known to be interconnected with CXCR4 pathway, using targeted NGS (6).

When looking at the incidence rate of the CXCR4 mutations with presence of MYD88 L265P mutation in the cohort of 98 patients, it appeared that 23 of 24 (95.8%) of the CXCR4mut were MYD88 L265P mutated, except one Waldenström macroglobulinemia with a CXCR4FS mutation. The high correlation between MYD88 L265P and CXCR4 mutational loads tends to suggest the coexistence of the 2 mutations in the same clone (Fig. 2). However, in four patients with Waldenström macroglobulinemia, a lower VAF of CXCR4 in comparison to MYD88 suggested a subclonal CXCR4 mutation in the dominant clone harboring MYD88 mutation.

Regarding the presence of mutations in the RAS pathway in Waldenström macroglobulinemia, including PTEN, K-Ras, and N-Ras, crucial regulators of RAS pathway, we have not identified any mutation in our cohort (n = 53).

BCR pathway can be constitutively activated, either by signals from the microenvironment or by genetic aberration (22). Several mutations of key signaling molecules and regulators of the BCR pathway were reported overtime, particularly in the activated B-cell–like (ABC) diffuse large B-cell lymphoma (DLBCL) and in...
Waldenström macroglobulinemia (15, 23), including the immunoreceptor tyrosine-based activation motif (ITAM) signaling modules of the CD79A and CD79B BCR coreceptors and the coiled-coil domain of CARD11 (22). Mutations of CD79B and CD79A were observed in 12 of 98 (12.2%) of Waldenström macroglobulinemia. Interestingly, we found no coexistence of CXCR4mut and CD79A/CD79B mutations, but in one patient who had CXCR4mut at the subclonal level. No mutation of CARD11 was observed in our studied group (n = 53). One might conclude that there is no unique somatic mutation in the BCR pathway, but instead a wide variety of molecules that could be altered through molecular alterations in the BCR pathway, likely mutually exclusive in the same clone, such as CXCR4mut and CD79A/CD79B mutations in the present situation in our study.

Transcriptional signature related to presence of CXCR4 mutation

We finally studied the gene expression profile of Waldenström macroglobulinemia on 7 patients with Waldenström macroglobulinemia to understand whether the CXCR4 mutation impacted the gene expression and to identify a potential gene signature of Waldenström macroglobulinemia with CXCR4mut. The differential analysis allowed to identify a gene signature attached to the CXCR4mut molecular profile, corresponding to 32 probes and 27 genes. We found several genes among these, such as MMP8, CRISP3, or BRCC3 involved in cell death and survival or cell movement (ARG1, LYST). These probes were all underexpressed in the five CXCR4wild Waldenström macroglobulinemia compared with the two CXCR4mut Waldenström macroglobulinemia (Fig. 5 and Supplementary Tables S1 and S2). We then sought to identify deregulated pathways using Ingenuity analysis software. We found CXCR4mut to be related to several functional network networks such as cell cycle and DNA replication or cell-cell signaling and interaction, cellular growth, and proliferation. Using GSEA for targeted gene sets previously associated with CXCR4 mutation (12), we also identified deregulation of proteasome pathway and BCR/B lymphocyte pathway in CXCR4mut Waldenström macroglobulinemia (P = 0.01 and P = 0.04, respectively; Supplementary Table S1).

Clinical and biologic features of CXCR4mut

We thought to identify clinical–biologic characteristics of Waldenström macroglobulinemia according to CXCR4mut features. The median age was 67 years (range, 36–92 years) in our series. We found that CXCR4mut was associated to a higher IgM monoclonal component (r = 0.309; P = 0.006) and thrombocytopenia (r = –0.226; P = 0.048), markers of adverse prognosis in Waldenström macroglobulinemia. Indeed, 35% and 12% of patients had serum IgM level greater than 30 g/L (P = 0.023) and 27% and 6.5% of patients had low platelet count lower than 100 g/L (P = 0.018), respectively, in patients with CXCR4mut versus CXCR4wild. There was a trend for CXCR4mut patients to have more often IPSS Waldenström macroglobulinemia stage 3 (P = 0.086). No association was found with regard to other markers of tumoral syndrome, such as adenopathy and splenomegaly, extramedullary localization, or lymphoedema (one would consider a marker of progression of tumoral cells from the bone marrow; Supplementary Table S3). With a median follow-up of 6 years, 21 (22%) patients in our series had died with a median OS (95% confidence interval) not reached and estimated at 63% at 10 years. No significant difference in type of treatment or number of treatment line was observed (data not shown). The estimated overall survival (OS) at 10 years according to CXCR4mut status was lower for CXCR4mut patients, 50% versus 65.5% for patients with CXCR4wild (P = ns, Fig. 6). Interestingly, the difference in OS between CXCR4mut versus CXCR4wild became significant in patients with indolent Waldenström macroglobulinemia (estimated 5-year OS rate at 50% and 93%, respectively, P = 0.019); the CXCR4mut patients required therapy more often and much earlier than the CXCR4wild.

Discussion

We have described 12 new CXCR4 mutations for a total of 17 patients with Waldenström macroglobulinemia so far, observed in 25% of patients with Waldenström macroglobulinemia, using targeted deep sequencing. These data suggest a highly heterogeneous pattern of CXCR4 mutations in Waldenström macroglobulinemia and confirms using a more sensitive technique that was reported by Treon and colleagues using Sanger (11). We have also confirmed CXCR4 1013C>G, followed by 1013C>A, mutations to be the most frequent CXCR4 mutations in Waldenström macroglobulinemia, although in a lesser frequency as previously reported, 12.5% (for the 2 mutations using Sanger sequencing in CD19 selected cells) and 30% (only 1013C>A using allele-specific PCR in unselected tumoral samples) in Treon and colleagues and Roccaro and colleagues, respectively (11–12). Overall the high frequency of CXCR4 Whim-like mutation is a new hallmark of Waldenström macroglobulinemia (12, 24).
Interestingly, all mutations, either nonsense or frameshift, occurred in the C-terminus of CXCR4. It was suggested that the truncation of the distal amino acid region of the C-terminus known to regulate signaling of CXCL12, might deregulate the CXCR4/SDF1 axis signaling pathway, and thus participate in the pathogenesis of Waldenström macroglobulinemia (2, 11, 12, 25). One might propose that the type of mutation does not matter much in Waldenström macroglobulinemia, on the contrary to the loss of C-terminus domain of CXCR4 protein which modified function might play a role in Waldenström macroglobulinemia pathogenesis (2). Functional studies may confirm this hypothesis in the near future.

We found a greater CXCR4 expression on tumor cells of Waldenström macroglobulinemia carrying CXCR4 mutation, independently of the type of mutation, similar to that was described in WHIM syndrome. It is suggested that the increase of CXCR4 receptor expression would induce an altered migration profile in response to SDF1 (25), resulting in an increased egress of tumor cells as demonstrated in mouse model (12). In our study, we also found a decreased expression of CD138, a cell surface syndecan-1 that mediates cancer cell adhesion to the extracellular matrix, and alteration of expression of several genes involved in matrix degradation such as MMP8 or cell migration on gene expression profiling. Taking together, CXCR4 mutation might alter the ability of the tumor cells to interact with the bone marrow microenvironment in Waldenström macroglobulinemia, as shown in CXCR4 S338X cells (12).

Nearly all patients with Waldenström macroglobulinemia with CXCR4 mutations harbored the MYD88 L265P mutation, as initially described by Treon and colleagues suggesting a potential cooperation between these pathways (11). An important dataset obtained in our series came from the quantification of the allelic frequency of the variant (VAF) of CXCR4 mutations into tumoral cells using NGS that may identify minor subclones of less than 1%
in our study. Targeted deep sequencing allows to conclude for the percentage of mutations in the clonal population for a given patient and thus to differentiate between clonal (dominant clonal) and subclonal mutations. Combining analysis of VAF of several genes, CXCR4 mutation was expressed in the same clone as MYD88 L265P, as we have identified a high allelic frequency of CXCR4 mutation MYD88 L265P in Waldenström macroglobulinemia tumor cells; but for 4 patients who had CXCR4 mutation present only at the subclonal level. Overall, these data may suggest the following hypothesis, where MYD88 L265P mutation is a founder mutation in Waldenström macroglobulinemia, a first genetic hit that would promote NF-κB and JAK/STAT3 signaling (4, 26). Direct inhibition of MYD88 L265P signaling overcomes CXCL12-triggered survival effects in CXCR4-mutated cells, supporting a primary role for MYD88 signaling in Waldenström macroglobulinemia (25). CXCR4 could then be one of the secondary events in some patients who showed subclonal mutation of CXCR4 compared with MYD88 L265P mutation present in the main clone. However, longitudinal analysis studies are needed to explore the dynamic of clonal architecture to identify driver or additional mutations in Waldenström macroglobulinemia that could contribute to clinical progression or chemoresistance. Indeed, it was shown that a diminished clinical activity of ibritinib was observed in patients with Waldenström macroglobulinemia with CXCR4 mutations (25-30).

In addition, we also found absence of co-occurrence of CD79A/CD79B and CXCR4 mutations in Waldenström macroglobulinemia. These data may suggest that these types of activating mutations of BCR and CXCR4 pathway were mutually exclusive in MYD88 L265P Waldenström macroglobulinemia (6). A cooperation between CXCR4 and TLR signaling activated by MYD88L265P mutation was described in B cells (6). The functional role of mutations in the BCR pathway is not fully described in Waldenström macroglobulinemia, but CD79B mutation was shown to alter the BCR response in diffuse large B lymphoma (15, 23). We might thus suggest the existence of two subgroups of MYD88 L265P Waldenström macroglobulinemia, one with CXCR4 mutations and one with BCR pathway mutations that may involve in cooperation with the aforementioned driver mutation which primarily affect TLR signaling. These data further may advance the concept that a complex rather than a unique deregulation of the TLR/NF-κB pathway characterizes MYD88 L265P Waldenström macroglobulinemia with others interconnected pathways.

We have observed that CXCR4 mutation was more frequently associated to a complex genomic signature, including gain of chromosome 4, deletion 6q, and gain of chromosome X using SNPa. High-throughput genomic studies have identified multiple mechanisms of genetic changes in Waldenström macroglobulinemia including several recurrent CNA such as deletion 6q, deletion 13q, or gain of chromosome 4 and CN-LOH (15, 27). This genomic pattern segregates further Waldenström macroglobulinemia among the others B- cell tumors. The prognostic role of these genomic alterations is not fully understood, but the presence of more than 3 SNPa abnormalities was associated with symptomatic status (15, 27, 28). Taking together, this finding suggests an inter- and intraclonal heterogeneity and the potential selective advantage of specific combinations of genetic lesions in Waldenström macroglobulinemia, in particular in the subset of CXCR4mutant Waldenström macroglobulinemia. The understanding of these genomic abnormalities, including gene mutations and CNA, described herein might help deciphering the pathogenesis of Waldenström macroglobulinemia to determine associated risk of progression, relapse, and drug resistance.

Overall, Waldenström macroglobulinemia with CXCR4 mutations had a specific clinicobiologic and genomic signature associated to features characterized with adverse prognosis in Waldenström macroglobulinemia, including high IgM M- spike and thrombocytopenia (part of the iPS Waldenström macroglobulinemia score adverse features) and greater frequency of complex genomic aberrations pattern using SNP array. Our data therefore may suggest a worse prognosis for CXCR4 mutation subgroup of asymptomatic Waldenström macroglobulinemia, but we did not observe significant impact on overall survival in our cohort as previously described (11). Further studies are needed to explore the prognosis value of CXCR4 mutation in Waldenström macroglobulinemia in clinical trials. Interestingly, the potential clinical impact of Waldenström macroglobulinemia with CXCR4 mutation has already been suggested by Roccaro and colleagues and Treon and colleagues on the basis of their in vitro studies showing drug resistance in CXCR4-mutated Waldenström macroglobulinemia cells exposed to BTK, mTOR, and PI3K inhibitors but not proteasome inhibitors (29, 30). This study, along with other, thus led us to propose that it is primetime for a systematic evaluation of CXCR4 mutation as part of the pretreatment package of Waldenström macroglobulinemia, to identify patients with CXCR4 mutation that might benefit more from proteasome inhibitors as compared with other options, such a BTK inhibitors.

In conclusion, approximately 25% of Waldenström macroglobulinemia harbor CXCR4 mutation, leading to altered CXCR4 protein at the C-terminal end, irrespective of the pleiotropic pattern of mutations. The study of CXCR4 mutations showed existence of intraclonal (variation in co-expression of MYD88 and CXCR4 mutations) and interclonal (BCR and CXCR4 mutations in MYD88L265P Waldenström macroglobulinemia) heterogeneity when analyzing the molecular landscape of CXCR4 mutation in Waldenström macroglobulinemia. Patients with CXCR4 mutation displayed a specific clinical—biologic—genomic and transcriptomic signature, possibly related to specific physiopathologic mechanism related to CXCR4 mutation. This dataset further suggests the genomic heterogeneity of Waldenström macroglobulinemia, beyond the current indolent versus symptomatic classification.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: S. Poulain, M. Figeac, F. Lepretre, X. Leleu
Development of methodology: S. Poulain, A. Venet-Caillault, M. Figeac, S. Geoffroy, X. Leleu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Poulain, C. Roumier, M. Figeac, C. Herbaux, G. Marot, E. Bertrand, A. Decambron, E.M. Boyle, A. Rennesville, S. Tricot, B. Quesnel, X. Leleu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Poulain, C. Roumier, M. Figeac, G. Marot, E. Doye, B. Quesnel, X. Leleu
Writing, review, and/or revision of the manuscript: S. Poulain, C. Roumier, C. Prenaudhomme, X. Leleu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Poulain, C. Roumier, E. Doye, F. Lepretre, O. Nibourel, B. Quesnel, X. Leleu
Study supervision: S. Poulain, E. Doye, P. Duthilleul, X. Leleu
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**References**


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Stéphanie Poulain, Christophe Roumier, Aurélie Venet-Caillault, et al.

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