Inflammation is predictive of outcome in Waldenström macroglobulinemia treated by Bruton tyrosine kinase inhibitors: a multicentric real-life study

Waldenström macroglobulinemia (WM) is a chronic indolent mature B-cell lymphoma characterized by bone marrow infiltration of lymphoplasmacytic cells and a monoclonal immunoglobulin M.[1] The most frequent somatic anomaly in WM is a gain of function mutation of *MYD88* (*MYD88* L265P), present in 90% of WM at diagnosis leading to constitutive activation of NF-kB and JAK/STAT pathways,[1] followed by 6q deletion (del6q, 30-55%)[2,3] and *CXCR4* gain-of-function mutations (30-40%).[3] The therapy is based on chemo-immunotherapy (CIT) or Bruton tyrosine kinase inhibitors (BTKi). The numerous prognostic scores, specific of WM, did not impact the therapeutic choice in clinical practice.[4,5]

Based on WM and inflammatory disorder association, the prognosis impact of inflammation in WM during CIT was recently highlighted by two independent teams.[6,7] In the first cohort, inflammatory syndrome (C-reactive protein [CRP] ≥20 mg/L) was present in one third of the patients.[6] This inflammatory syndrome decreased during treatment but was associated with a shorter time to next treatment (TTNT, 1.6 years *vs*. 4.8 years; *P*<0.001). In the second cohort, inflammation (CRP >5 mg/L) was associated with more frequent del6q and, more frequent need for treatment initiation, inflammation decrease during CIT and a trend towards poorer progression-free survival (PFS) and overall survival (OS).[7]

Importantly, the outcome of inflammatory WM (iWM) was evaluated during CIT, whereas data about BTKi therapy and inflammation were lacking. Therefore, we performed a multicentric cohort to evaluate the impact of BTKi on iWM. In this real-life cohort, the inflammation positively impacts the prognosis of WM with BTKi.

The pooled cohort used in this study was based on published WM cohorts from Saint Louis[6] (n=268) and Pitie-Salpetriere Hospitals (n=270)[7] with the addition of a Necker cohort Hospital (n=110). *MYD88* L265P and *CXCR4* S338X mutations were evaluated by restriction-fragment-length polymorphism and allele-specific polymerase chain reaction or targeted next-generation sequencing, respectively, as previously described.[8]

iWM was defined by the presence of two CRP measures ≥20 mg/L without other causes to explain inflammatory syndrome (e.g., infection, inflammatory complication). We excluded patients without CRP measurement before treatment. The CIT cohort at second line used as control was extracted from the Saint-Louis cohort.[6]

The response was assessed according to the sixth International Workshop on Waldenström's Macroglobulinemia.[9] Patient data were obtained in conformity with the Declaration of Helsinki and registered by the Assistance-Publique-Hôpitaux-de-Paris data protection office.

OS was defined as the time from BTKi initiation to death from all causes. PFS was defined as the time from BTKi initiation and its discontinuation for any reason (progression or toxicity) or death. TTNT was defined as the time from BTKi or CIT initiation to disease progression requiring treatment or death from any cause. All quantitative variables were described using medians (quartiles), while qualitative variables were described by frequencies (percentage). Categorical and quantitative data were compared using Fisher's exact test and Student *t* test, respectively. Kaplan–Meier curves were plotted for survival, and data for the various groups were compared using the log-rank test. Maximally selected-rank statistics were used to select the best cut-off for CRP based on TTNT survival. Statistical analyses were obtained using R 4.0.4.

Among 648 WM patients from the pooled cohort, 474 (73%) WM patients received CIT including 398 (84%) with multiple CRP measures before treatment (*Online Supplementary Table S1A*). We confirmed the inferior outcome after CIT of iWM, defined by CRP ≥20 mg/L, based on TTNT at three levels (*Online Supplementary Figure S1A*) and maximally selected-rank statistics (*Online Supplementary Figure S1B*). *CXCR4* mutation was not associated with TTNT (hazard ratio [HR] =1; 95% confidence interval [CI]: 0.65-1.6; *P*=0.96). iWM was associated with more del6q (49% *vs*. 20%; *P*<0.001; *Online Supplementary Figure S1C*) and less CXCR4 mutation than non-iWM (15% *vs*. 34%; *P*=0.02; O*nline Supplementary Figure S1D*).

Seventy-eight WM patients who received BTKi between 2015 and 2022 were included (Figure 1A). After one exclusion for lack of CRP measurement, 42 (54%) patients were classified as iWM as previously described[6] (Figure 1B). Among iWM, the median CRP before BTKi initiation was 37 mg/L (interquatile range [IQR], 26-60). The median age at WM diagnosis was 65 years (IQR, 56-73; Table 1). Median follow-up after BTKi initiation was 3.3 years (IQR, 1.7-5.9). Thirty-one (40%) patients had full characterization of *MYD88/CXCR4/*del6q. Ninety percent of the patients had *MYD88* mutation. *CXCR4* mutation was less frequent in iWM than in non-iWM (17% [4/24] *vs*. 50% [10/20]; *P*=0.04). Del6q was twice more frequent in iWM than non-iWM without reaching statistical significance (58% [14/24] *vs*. 33% [6/18]; *P*=0.19). No difference was observed for characteristics at

diagnosis, first-line choice or BTKi initiation between iWM and non-iWM. Among BTKi, most patients received Ibrutinib. Half of the patients received BTKi on second line.

Concerning hematologic response, the overall response rate (ORR = partial response/very good partial response/complete response [CR]) was superior in iWM than non-

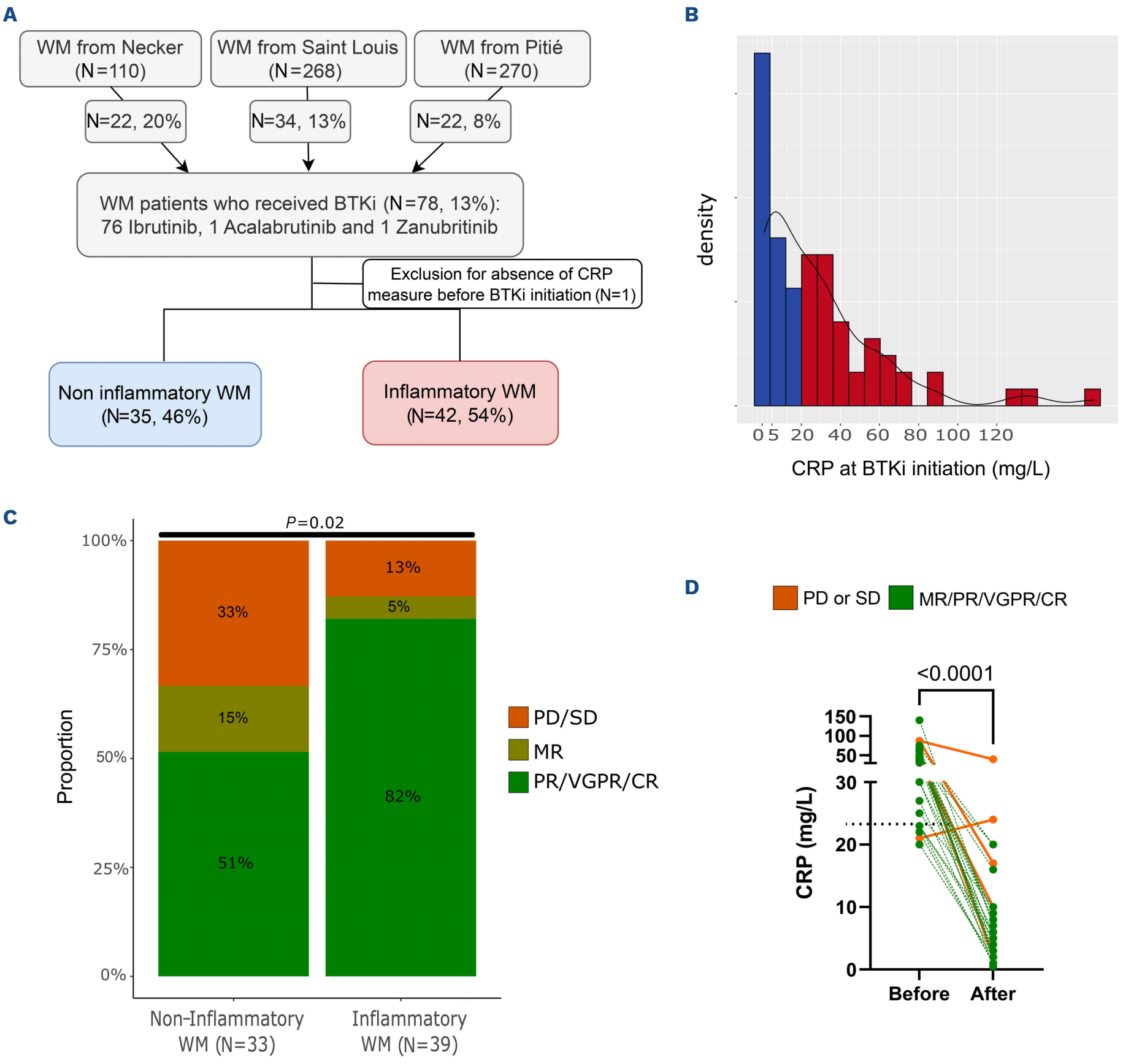

Figure 1. Inflammatory Waldenström macroglobulinemia (iWM) was associated with a higher response to BTK inhibitors than non-iWM. Flowchart of the study. Seventy-eight patients received Bruton tyrosine kinase inhibitors (BTKi) for WM, including 42 (54%) with inflammatory syndrome (C-reactive protein [CRP] ≥20 mg/L). (B) Histogram and density plot for CRP distribution at BTKi initiation with the cutoff 20 mg/L. Two peaks among patients were observed at 0-5 mg/L and at 20 mg/L. (C) Hematologic response based on the sixth International Workshop on Waldenström's Macroglobulinemia criteria between non-iWM and iWM patients. Green bar represents overall response rate (= partial response [PR]/very good partial response [VGPR]/complete response [CR] response), the olive green bar for minimal response (MR) and orange bar for progressive disease (PD)/stable disease (SD). (D) Inflammation evolution in iWM (N=37) during BTKi treatment. Orange lines show patients who had SD/PD. Green lines show patients who had MR/VGPR/PR/CR. For SD/PD patients (N=5) in iWM, two patients had persistent CRP ≥20 mg/L, but received less than 4 months of BTKi. Among patients with clinical signs (N=17), all patients had B-symptom regression except for 2 iWM (1 with hematologic SD and 1 with VGPR but high CRP level at 19 mg/L who had a progression at 9 months) and 1 non-iWM patient who developed diffuse large B-cell lymphoma 3 months after BTKi introduction.

iWM (82% *vs*. 52%; *P*=0.02; Figure 1C). Regarding the inflammation syndrome kinetic, all iWM patients who reached minimal response (MR) or better on BTKi had a nadir of CRP <20 mg/L (Figure 1D). Sixteen patients (43%) obtained normalization of CRP (<5 mg/L). In addition, 91% (34/37) obtained a 50% decrease or more in their CRP level. Among patients with B-symptoms, 82% (14/17) had regression during BTKi treatment.

Furthermore, iWM patients had better PFS than non-iWM upon BTKi treatment (Figure 2A; median: 4 years *vs*. 2.4 years; *P*=0.0025). The leading cause of BTKi discontinuation was progression for non-iWM (n=11, 51%) and BTKi toxicity for iWM (n=8, 47%; Table 1). The cumulative incidence of progression was superior for non-iWM than iWM (4years: 43% *vs*. 21%; *P*=0.05; Figure 2B). There was no difference in discontinuation due to BTKi toxicity between non-iWM and iWM (4 years: 37% *vs*. 34%; *P*=0.11). Also, the TTNT survival was superior for iWM than non-iWM (median: 4 *vs*. 2.6 years; *P*=0.008; Figure 2C). TTNT survival and maximally selected rank statistics confirmed the rel-

Table 1. Initial characteristics of non-inflammatory Waldenström macroglobulinemia and inflammatory Waldenström macroglobulinemia patients.

	Non-iWM N=35	iWM N=42	*P*
At diagnosis			
Men, N (%)	21 (60)	29 (69)	0.556
Age in years at WM diagnosis, median (IQR)	63 (55-75)	66 (59-72)	0.449
Prior inflammatory disease, N (%)	4 (11)[#]	2 (5)[##]	0.402
κ/λ, N (%)	30/5 (85/15)	38/4 (90/10)	0.724
ISSWM score, N (% of the complete case) Low Intermediate High	14/35 (40) 3 (21) 5 (36) 6 (43)	19/42 (45) 1 (5) 3 (16) 15 (79)	0.107
LDH >ULN, N (%)	5/26 (19)	11/31 (36)	0.251
Platelets <100x10^9/L, N (%)	6 (19)	4 (12)	0.511
MYD88 mutation, N=54, 70%, N (%)	21/23 (91)	28/31 (90)	1.000
CXCR4 mutation, N=44, 57%, N (%)	10/20 (50)	4/24 (17)	0.041
Deletion 6q, N=42, 55%, N (%)	6/18 (33)	14/24 (58)	0.190
First line treatment, N (%) Rituximab + alkylating agent Rituximab monotherapy Chlorambucil BTKi Others	 24 (69) 0 6 (17) 1 (3) 4 (11)	 26 (62) 3 (7) 10 (24) 0 3 (7)	0.176
First line response,[$] N (% complete case) PD/SD MR PR/VGPR/CR	33/35 (94) 9 (27) 5 (15) 19 (58)	37/42 (88) 6 (16) 6 (16) 25 (68)	0.526
BTKi initiation			
Age in years at BTKi initiation, median (IQR)	73 (63-82)	75 (70-80)	0.344
B-symptoms before BTKi initiation, N (%)	4 (11)	13 (31)	0.075
Delay in years WM diagnosis - BTKi initiation, median (IQR)	7 (4-11)	8 (4-12)	0.375
Year of BTKi initiation, median (IQR)	2019 (2017-2020)	2018 (2017-2020)	0.580
Line of BTKi initiation, N (%) 1 2 3 4+	 1 (3) 18 (51) 10 (29) 6 (17)	 0 21 (50) 9 (21) 12 (29)	0.457
Ibrutinib/other BTKi, N (%)	34/1* (97/3)	41/1** (98/2)	0.706

*Acalabrutinib; **zanubritinib; [$]based on IMWW-6; [#]2 inactive type 2 cryoglobulinemia, 1 inactive giant cell arteritis, 1 inactive ulcerative colitis; [##]1 inactive bullous dermatosis, 1 scleroderma. BTKi: Bruton tyrosine kinase inhibitors; CR: complete response; ISSWM: International Prognosis Score System for Waldenström Macroglobulinemia; MR: minimal response; PD: progressive disease; PR: partial response; SD: stable disease; VGPR: very good partial response; iWM: inflammatory Waldenström macroglobulinemia; LDH: lactate dehydrogenase; ULN: upper limit of normal; IQR: interquartile range.

evance of the 20 mg/L CRP cutoff (*Online Supplementary Figure 2A, B*). In univariate analysis, TTNT was associated positively with inflammatory syndrome (HR=0.43; 95% CI: 0.22-0.81; *P*=0.01), negatively with *CXCR4* mutations (HR=3.8; 95% CI: 1.5-9.6; *P*=0.01) and platelets <100x10^9/L (HR=2.38; 95% CI: 1.02-5.55; *P*=0.044; *Online Supplementary Table 1B*). Del6q was not associated with TTNT (HR=1.3; 95% CI: 0.49-3.4; *P*=0.60). Multivariate analysis was not performed because of a low number of events (n=18). No difference was observed for OS (4 years: 75% *vs*. 66%; *P*=0.15; *Online Supplementary Figure 2C*).

In order to evaluate the impact of inflammation in the current recommendation of BTKi in second line, TTNT survival analysis was performed with a focus on the second line of treatment (CIT or BTKi) between iWM and non-iWM (Figure 2D; *P*=0.012). No difference was observed for demographic/disease characteristics between BTKi and CIT cohorts (*data not shown*). Among patients receiving CIT at second line, most patients received alkylating agents +/- rituximab (55%) followed by chlorambucil (22%). iWM treated with BTKi (51% at 4 years) had better survival than BTKi treated non-IWM (22%), whereas 4-year survival in patients

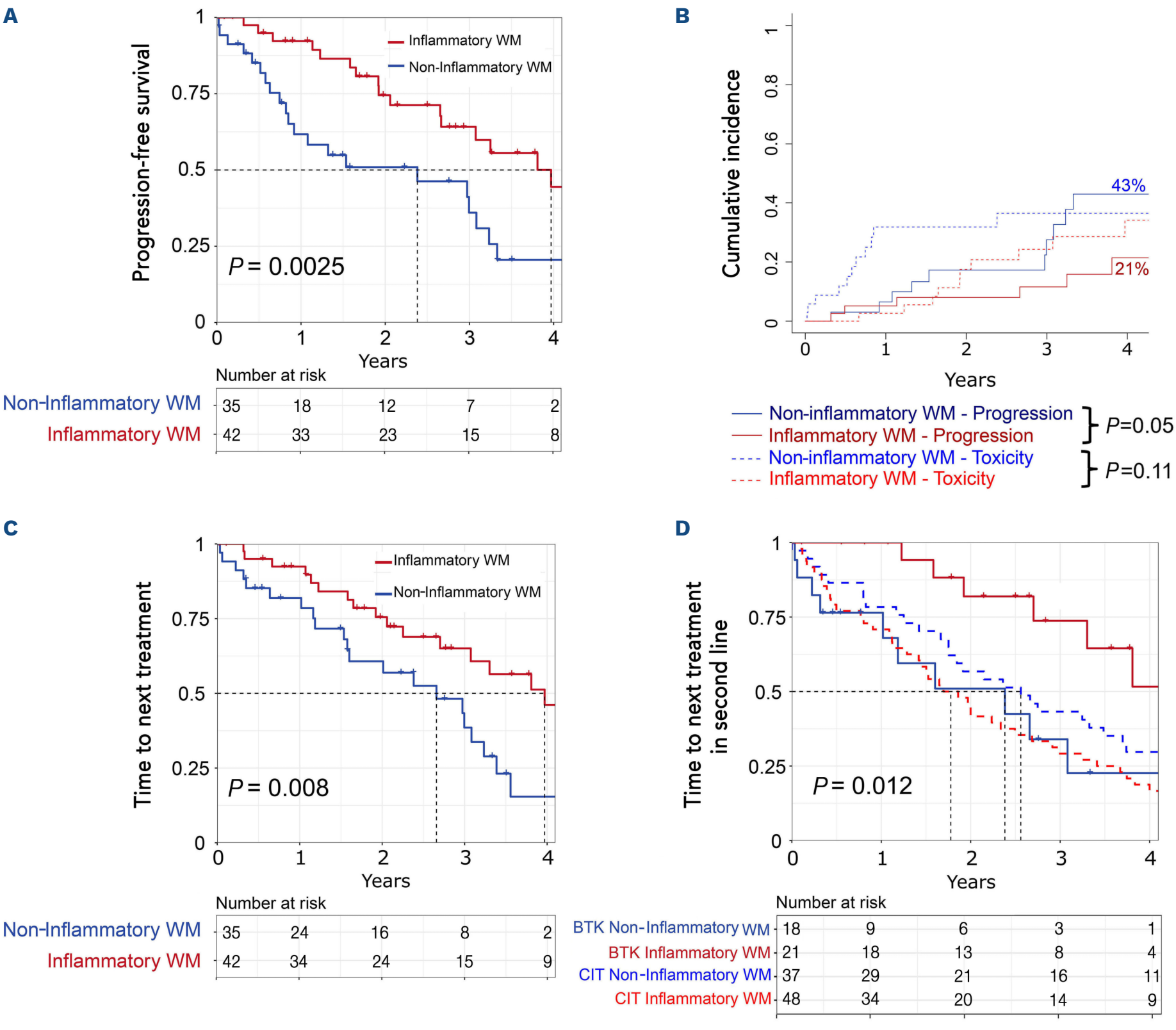

Figure 2. Inflammatory Waldenström macroglobulinemia (iWM) had an improvement of time to next treatment during BTK inhibitor treatment than non-iWM. (A) Progression-free survival (PFS) between non-iWM (blue) and iWM (red). (B) Cumulative incidence of the reason for Bruton tyrosine kinase inhibitor (BTKi) discontinuation (progression in a straight line and toxicity in a dotted line) between non-iWM (blue) and iWM (red). (C) Time to next treatment (TTNT) after BTKi for all cohorts between non-iWM (blue) and iWM (red) (D) TTNT after the 2nd line of treatment (BTKi in a straight line and chemo-immunotherapy [CIT] in dotted line) in non-iWM (blue) and iWM (red).

treated with CIT was 16% in iWM and 29% in non-iWM.
To the best of our knowledge, this is the first study evaluating inflammation and response to BTKi in WM. We validated a cutoff of 20 mg/L of CRP for iWM definition and the association with poorer outcomes after CIT. However, we showed here that iWM with BTKi treatment had better hematological response and TTNT than non-iWM. In addition, the inflammatory syndrome decreased during the hematological response in BTKi-treated iWM. Several explanations could exist for these improved hematological response and survival. First, reduced inflammation via decreased pro-inflammatory cytokines after BTKi was described for SARS-COV-2,[10] chronic graft-*versus*-host disease[11] and Schnitzler syndrome.[12] Second, BTK is expressed by malignant B cells[13] but also by macrophages or monocytes.[14] We can hypothesize that decreased inflammation mediated by BTKi might be related to the action on tumoral cells[15] and/or microenvironment.[14] Also, 6q chromosome contains an inhibitor of BTK.[3] Deletion of 6q, more frequent in iWM, could thus lead to BTK activation, corrected by BTKi treatment. *CXCR4* mutation was less frequent in iWM than non-iWM (17% *vs*. 50%) and could explain a part of differential prognosis to BTKi. Additional study about the inflammation origin in WM would be required to understand BTKi action in iWM. One limit of our study is the small sample size for genetic analysis that limits the evaluation of interaction between *CXCR4* mutation, del6q and inflammation. CXCR4 especially with high clonality (>25%), is the main adverse factor during BTKi therapy in WM.[16,17] Related to the partial evaluation of *CXCR4* mutation (52% of the cohort) without any clonality analysis, additional studies are necessary to evaluate *CXCR4* mutation and inflammation prognosis role independently. Nevertheless, assessment of CRP could be easier, quicker and cheaper to perform than evaluation of *CXCR4* mutational status and clonality analysis on bone marrow samples. Also, the retrospective design of our study is a limit, and we will need to confirm our findings in prospective large multicentric cohorts but also to reanalyze clinical trials of BTKi in WM in light of our results.[16]
In summary, inflammation appears to have a positive impact on the clinical outcome of WM patients on BTKi therapy. Thus, this study supports the use of BTKi in patients with iWM. On the other hand, inflammation could represent a novel biomarker for predicting the effects of BTKi in WM patients that can be easily and quickly evaluated in prospective cohorts and clinical trials.

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Disclosures

No conflicts of interest to disclose.

Contributions

P-ED, BA, DRW, ME and KB developed the concept and designed the study. P-ED, NF, DE, SH, BR, LF, MC, FD, FNK, WC, CB, GL, SK, OH, DRW and BA collected and assembled data. P-ED, BA, AT, DRW, OH, ME and KB analyzed and interpreted data. P-ED, NF, DE, SH, BR, LF, DRW, MC, OH, AT and BA took care of patients. P-ED, AT, DRW, BA, ME and KB wrote the article. All authors read and approved the final version of this manuscript.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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