

## Predictors of response and survival in a large cohort of 319 Waldenström macroglobulinemia patients treated with ibrutinib monotherapy

Tracking no: ADV-2021-006106R2

Jorge Castillo (Dana-Farber Cancer Institute, United States) Shayna Sarosiek (Dana-Farber Cancer Institute, United States) Joshua Gustine (Dana-Farber Cancer Institute, United States) Catherine Flynn (Dana-Farber Cancer Institute, United States) Carly Leventoff (Dana-Farber Cancer Institute, United States) Timothy White (Dana-Farber Cancer Institute, United States) Kirsten Meid (Dana-Farber Cancer Institute, United States) Maria Luisa Guerrero (Dana-Farber Cancer Institute, United States) Amanda Kofides (Dana Farber Cancer Institute, United States) Xia Liu (DANA-FARBER cancer institute, United States) Mani Munshi (Dana-Farber Cancer Institute, United States) Nicholas Tsakmaklis (Dana Farber, United States) Zachary Hunter (Dana-Farber Cancer Institute, United States) Christopher Patterson (Dana Farber Cancer Institute, United States) Andrew Branagan (Massachusetts General Hospital, United States) Steven Treon (Dana Farber Cancer Institute, United States)

### Abstract:

Bruton tyrosine kinase (BTK) inhibitors are the only FDA-approved treatments for Waldenström macroglobulinemia (WM). Factors prognostic of survival and predictive of response to BTK inhibitors remained to be clarified. We evaluated 319 patients with WM to identify predictive and prognostic factors on ibrutinib monotherapy. Logistic and Cox proportional-hazard regression models were fitted for response and survival. Multiple imputation analyses were used to address bias associated with missing data. Major (partial response or better) and deep responses (very good partial response or better) were attained in 78% and 28% of patients. *CXCR4* mutations were associated with lower odds of major (OR 0.2, 95% CI 0.1–0.5;  $p < 0.001$ ) and deep response (OR 0.3, 95% CI 0.2–0.6;  $p = 0.001$ ). *CXCR4* mutations (HR 2.0, 95% CI 1.2–3.4;  $p = 0.01$ ) and platelet count 100 K/uL or less (HR 2.5, 95% CI 1.3–4.9;  $p = 0.007$ ) were associated with worse progression-free survival (PFS). We proposed a scoring system using these two factors. The median PFS for patients with zero, one and two risk factors were not reached, 5 years and 3 years ( $p < 0.001$ ). Patients with two risk factors had HR 2.2 (95% CI 1.3–3.8;  $p = 0.004$ ) compared with one factor, and patients with one factor had HR 2.3 (95% CI 1.1–5.1;  $p = 0.03$ ) compared with zero factors. Age 65 years or older was the only factor associated with overall survival (HR 3.2, 95% CI 1.4–7.0;  $p = 0.005$ ). Multiple imputation analyses did not alter our results. Our study confirms the predictive and prognostic value of *CXCR4* mutations in patients with WM treated with ibrutinib monotherapy.

**Conflict of interest:** COI declared - see note

**COI notes:** JJC received research funds and/or honoraria from Abbvie, BeiGene, Janssen, Millennium, Pharmacyclics, Roche, and TG Therapeutics. ARB received research funds and/or honoraria from Adaptive, Beigene, CSL Behring, Genzyme, Karyopharm, Pharmacyclics and Sanofi. SPT received research funds and/or honoraria from Beigene, BMS, Eli Lilly, Janssen, Pharmacyclics, and X4 Pharmaceuticals. All other authors have no conflicts of interest to disclose.

**Preprint server:** No;

**Author contributions and disclosures:** JJC designed the study, performed the analysis, and wrote the initial draft of the manuscript. JJC, SS, CAF, CRL, TPW and SPT provided clinical care to the patients. JJC, JNG, SS and KM collected the data. MLG, AK, XL, MM, NT, ZRH and CJP run the genomic studies. JJC, JNG, SS, ARB and SPT analyzed the data. All the authors approved the final version of the manuscript.

**Non-author contributions and disclosures:** No;

**Agreement to Share Publication-Related Data and Data Sharing Statement:** Data are available upon request to jorgej\_castillo@dfci.harvard.edu.

**Clinical trial registration information (if any):**

# Predictors of response and survival in a large cohort of 319 Waldenström macroglobulinemia patients treated with ibrutinib monotherapy

## Short title

Prognostic factors to ibrutinib in Waldenstrom

## Authors

Jorge J. Castillo (1, 2); Shayna R. Sarosiek (1, 2); Joshua N. Gustine (1, 3); Catherine A. Flynn (1); Carly R. Leventoff (1); Timothy P. White (1); Kirsten Meid (1); Maria L. Guerrero (1); Amanda Kofides (1); Xia Liu (1); Mani Munshi (1); Nicholas Tsakmaklis (1); Zachary R. Hunter (1, 2); Christopher J. Patterson (1); Andrew R. Branagan (2, 4); Steven P. Treon (1, 2)

## Affiliation

- (1) Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA
- (2) Department of Medicine, Harvard Medical School, Boston, MA, USA
- (3) Department of Medicine, Boston University School of Medicine, Boston, MA, USA
- (4) Division of Hematology and Oncology, Massachusetts General Hospital, Boston, MA, USA

## Corresponding author

Jorge J. Castillo, MD  
450 Brookline Ave, Mayer 221, Boston, MA 02215  
Phone: 617-632-6045. Fax: 617-582-8608. E-mail: [jorgej\\_castillo@dfci.harvard.edu](mailto:jorgej_castillo@dfci.harvard.edu)

|                              |      |
|------------------------------|------|
| Abstract word count          | 250  |
| Manuscript word count        | 3791 |
| Number of figures and tables | 6    |
| Number of references         | 36   |

## KEY POINTS

- *CXCR4* mutations are associated with lower rates of major (67% v 86%) and deep responses (16% v 35%) in ibrutinib-treated WM patients.
- *CXCR4* mutations and platelet count 100 K/uL or less were associated with worse PFS, and a scoring system using these 2 factors is proposed.

## ABSTRACT

Bruton tyrosine kinase (BTK) inhibitors are the only FDA-approved treatments for Waldenström macroglobulinemia (WM). Factors prognostic of survival and predictive of response to BTK inhibitors remained to be clarified. We evaluated 319 patients with WM to identify predictive and prognostic factors on ibrutinib monotherapy. Logistic and Cox proportional-hazard regression models were fitted for response and survival. Multiple imputation analyses were used to address bias associated with missing data. Major (partial response or better) and deep responses (very good partial response or better) were attained in 78% and 28% of patients. *CXCR4* mutations were associated with lower odds of major (OR 0.2, 95% CI 0.1-0.5;  $p < 0.001$ ) and deep response (OR 0.3, 95% CI 0.2-0.6;  $p = 0.001$ ). *CXCR4* mutations (HR 2.0, 95% CI 1.2-3.4;  $p = 0.01$ ) and platelet count 100 K/uL or less (HR 2.5, 95% CI 1.3-4.9;  $p = 0.007$ ) were associated with worse progression-free survival (PFS). We proposed a scoring system using these two factors. The median PFS for patients with zero, one and two risk factors were not reached, 5 years and 3 years ( $p < 0.001$ ). Patients with two risk factors had HR 2.2 (95% CI 1.3-3.8;  $p = 0.004$ ) compared with one factor, and patients with one factor had HR 2.3 (95% CI 1.1-5.1;  $p = 0.03$ ) compared with zero factors. Age 65 years or older was the only factor associated with overall survival (HR 3.2, 95% CI 1.4-7.0;  $p = 0.005$ ). Multiple imputation analyses did not alter our results. Our study confirms the predictive and prognostic value of *CXCR4* mutations in patients with WM treated with ibrutinib monotherapy.

## INTRODUCTION

Waldenström macroglobulinemia (WM) is an indolent lymphoma composed of malignant IgM-secreting lymphoplasmacytic cells that accumulate in the bone marrow and other organs [1]. The identification of recurrent somatic mutations in *MYD88* and *CXCR4*, detected in 90% and 40% of WM patients, respectively, have helped our understanding of the biology of the disease [2, 3]. Bruton tyrosine kinase (BTK) activation is apparent in *MYD88* mutated WM cells [4], supporting the development of BTK inhibitors in WM patients, while *CXCR4* signaling suggested resistance mechanisms in WM cells despite effective *MYD88* inhibition [5]. Mounting evidence supports an impact of genomic profile in the clinical features of WM patients as well as response to BTK inhibitors [6-9].

Ibrutinib, a first-in-class oral BTK inhibitor, was approved by the United States Food & Drug Administration and the European Medicines Agency in 2015 for the treatment of symptomatic WM patients, based on a response rate of 90% observed in a prospective investigator-initiated phase II study evaluating 63 previously treated WM patients [10]. Similar response rates to ibrutinib monotherapy were reported in rituximab-refractory and in treatment-naïve WM patients [11, 12]. A recent study from our group reported similar outcomes to ibrutinib in WM patients on and off clinical trials [13], but long-term data on the safety and efficacy of ibrutinib monotherapy are limited [14]. The prognostic value of the International Prognostic Scoring System for WM (IPSSWM) has not yet been validated in patients treated with ibrutinib.

We designed a retrospective study to evaluate the long-term safety and efficacy of ibrutinib monotherapy in WM patients. The main objective of our study was to evaluate factors predictive of response and prognostic of progression-free and overall survival.

## **PATIENT AND METHODS**

### **Patient Selection**

The analytical cohort was composed of patients treated with ibrutinib monotherapy who participated in two prospective clinical trials (NCT01614821 and NCT02604511) and consecutive patients from a prospectively maintained database at our institution. The study inclusion period ran from January 2012 through December 2018 with follow-up through December 2020. Data on the individual cohorts of patients in clinical trials have been previously published [10, 12]. All patients met clinicopathological criteria for the diagnosis of WM and for treatment initiation based on 2<sup>nd</sup> International Workshop on WM (IWWW-2) guidelines [15, 16]. All patients provided consent to having blood and marrow samples and data collected for research. Patients with central nervous system involvement by WM (Bing-Neel syndrome), non-IgM lymphoplasmacytic lymphoma, or who received ibrutinib in combination with other agents were excluded from the study.

### **Data Collection**

Pertinent data were collected at the time of ibrutinib monotherapy initiation. Data were categorized as follows: age ( $>65$ ,  $\leq 65$  years), sex (male, female), hemoglobin level ( $<11.5$ ,  $\geq 11.5$  g/dl), platelet count ( $<100$ ,  $\geq 100$  K/uL), serum IgM level ( $>4,000$ ,  $\leq 4,000$  mg/dl, and  $>7,000$ ,  $\leq 7,000$  mg/dl), serum  $\beta 2$ -microglobulin level ( $>3$ ,  $\leq 3$  mg/l), serum albumin level ( $\leq 3.5$ ,  $>3.5$  g/dl), bone marrow involvement ( $\geq 60\%$ ,  $<60\%$ ), IPSSWM (low, intermediate, high), *CXCR4* mutational status (mutated, wildtype), *CXCR4* mutational status subtype (nonsense, frameshift), and previously treated (yes, no). Responses were assessed using modified criteria from the 6<sup>th</sup> International Workshop on WM [17]. A decrease of 25-49%, 50-89%, and  $\geq 90\%$  in serum IgM levels denoted minor (MR), partial (PR), and very good partial (VGPR) responses. Normalization of serum IgM level; no monoclonal IgM spike, BM disease involvement or pathological adenopathy or splenomegaly was required for complete response (CR). Overall response rate (ORR) included MR or better, major response included PR or better, and deep response included VGPR or better. Disease progression was defined as an increase in serum IgM level of  $\geq 25\%$  with an increase of  $\geq 500$  mg/dl from the lowest serum IgM level attained on therapy. Transient increases in serum IgM level while on a temporary hold of ibrutinib therapy were not considered disease progression. At our institution, *MYD88* and *CXCR4* mutations were assessed using the Rapid Heme Panel, a custom next-generation sequencing (NGS) panel for hematologic malignancies [18]. Additionally, *MYD88 L265P* and nonsense *CXCR4* mutations were assessed using allele-specific polymerase chain reaction (PCR) assays, and frameshift *CXCR4* mutations by Sanger sequencing, in CD19-selected bone marrow samples, as previously reported [2, 3, 19, 20]. Progression-free survival (PFS) was defined as the time between ibrutinib initiation

and disease progression, last follow-up or death from any cause, and overall survival after ibrutinib (OS) as the time between ibrutinib initiation and last follow-up or death from any cause. The IPSSWM was estimated as previously reported [21]. Missing data had a random distribution and rates were as follows: hemoglobin level (n=4; 1%), platelet count (n=6; 2%), serum  $\beta$ 2-microglobulin level (n=68; 21%), serum albumin level (n=16; 5%), bone marrow involvement (n=41; 13%), IPSSWM (n=51; 16%), *MYD88* mutational status (n=46; 14%), *CXCR4* mutational status (n=75; 24%).

## Statistical analysis

Patients' characteristics and response rates are presented using descriptive statistics. Differences between categorical variables were assessed using Fisher exact test or *Chi-square* test, depending on the number of events. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate logistic regression models were fitted to identify predictive factors of major and deep response to ibrutinib. Logistic regression outcomes are reported using odds ratio (OR) with 95% confidence interval (CI). Univariate and multivariate Cox proportional-hazard regression models were fitted to identify prognostic factors of PFS and OS. Cox regression outcomes are reported using hazard ratio (HR) with 95% CI. Only factors with p-values <0.05 in the univariate analysis were included in the multivariate analysis. We handled missing data by performing multiple imputation analyses in addition to complete case analyses. Multiple imputation analyses for logistic and Cox proportional-hazard regression models were performed after generating 20

imputed data sets using the chained equations method. P-values <0.05 were considered statistically significant. Calculations were obtained using STATA 17 (StataCorp, College Station, TX, USA).

## RESULTS

### Patients' characteristics

A total of 380 patients were identified for this study. After excluding 29 patients who received ibrutinib in combination with other agents, 25 patients with Bing-Neel syndrome and 7 patients with non-IgM lymphoplasmacytic lymphoma, 319 patients with WM were included in the analysis. The patients' baseline characteristics are shown in **Table 1**. Indications for ibrutinib monotherapy initiation are shown in **Supplemental Table S1**. *CXCR4* mutations were detected in 89 patients, of which 56 carried a nonsense mutation, 31 a frameshift mutation, and two had concurrent nonsense and frameshift mutations. Given the small sample size of the latter group, we excluded these patients from subsequent analyses. A higher proportion of platelet count <100 K/uL was observed in patients with versus without *CXCR4* mutations (24% vs. 6%;  $p<0.001$ ). Moreover, non-significantly higher proportion of patients with serum IgM level  $\geq 4,000$  mg/dl (48% vs. 37%;  $p=0.10$ ) and bone marrow involvement  $\geq 60\%$  (64% vs. 52%;  $p=0.08$ ) were observed in patients with versus without *CXCR4* mutations. Patients' characteristics according to *CXCR4* mutational status are shown in **Supplemental Table S2**. There was a trend towards a lower proportion of hemoglobin  $\leq 11.5$  g/dl (68%

vs. 82%;  $p=0.10$ ) and bone marrow involvement  $\geq 60\%$  (58% vs. 79%;  $p=0.07$ ) in patients with nonsense versus frameshift *CXCR4* mutations. Patients' characteristics according to *CXCR4* mutation subtypes are shown in **Supplemental Table S3**.

The median follow-up time for the entire cohort was 4.2 years (95% CI 3.9-4.5). No difference was observed between patients with and without *CXCR4* mutations (4.1 years, 95% CI 3.6-4.4 vs. 3.9 years, 95% CI 3.3-4.5;  $p=0.54$ ).

#### Response to ibrutinib monotherapy

At best response, 48 patients (15%) attained MR, 161 (50%) attained PR, 87 (27%) attained VGPR and 1 (0.3%) attained CR. An overall response was attained in 297 patients (93%), major response in 249 (78%) and deep response in 88 (28%) (**Supplemental Figure 1A**). The rates of major response for patients with and without *CXCR4* mutations were 67% and 86%, respectively ( $p<0.001$ ). The rates of deep response for patients with and without *CXCR4* mutations were 16% and 35%, respectively ( $p=0.001$ ). Categorical response distribution by *CXCR4* mutational status is shown in **Supplemental Figure 1B**. The rates of major response for patients with nonsense and frameshift *CXCR4* mutations were 59% and 81%, respectively ( $p=0.04$ ). The rates of deep response for patients with nonsense and frameshift *CXCR4* mutations were 13% and 23%, respectively ( $p=0.22$ ). Categorical response distribution by *CXCR4* mutation subtype is shown in **Supplemental Figure 1C**.

Logistic regression models for major and deep response are shown in **Table 2**. Univariate regression analyses showed that hemoglobin level  $<11.5$  g/dl,  $\beta 2$ -microglobulin level  $>3$  mg/l and bone marrow involvement  $\geq 60\%$  were associated with higher odds, while nonsense *CXCR4* mutations were associated with lower odds of attaining major response to ibrutinib monotherapy. The IPPSWM was not predictive of major response ( $p=0.40$ ). In the multivariate analysis evaluating these four variables, *CXCR4* mutations were independently associated with lower odds of attaining a major response (OR 0.20, 95% CI 0.09-0.45;  $p<0.001$ ). In addition, hemoglobin  $<11.5$  g/dl was independently associated with higher odds of major response (OR 2.72, 95% CI 1.25-5.95:  $p=0.01$ ). Univariate analyses showed that hemoglobin  $\leq 11.5$  g/dl was associated with higher, and *CXCR4* mutations were associated with lower odds of attaining deep response to ibrutinib monotherapy. The IPPSWM was not predictive of deep response ( $p=0.29$ ). A multivariate model evaluating hemoglobin and *CXCR4* mutations, showed that *CXCR4* mutations were independently associated with lower odds of attaining a deep response (OR 0.32, 95% CI 0.15-0.55;  $p=0.001$ ). Hemoglobin  $<11.5$  g/dl was independently associated with higher odds of deep response (OR 2.34, 95% CI 1.14-4.80:  $p=0.02$ ). The multiple imputation analysis did not modify our results (data not shown).

In addition, we fitted univariate and multivariate models for major and deep response using continuous variables for age at ibrutinib initiation, hemoglobin level, platelet count, serum  $\beta 2$ -microglobulin, albumin, and IgM levels, and percentage of bone marrow involvement, in addition to categorical variables for sex, previous treatment and *CXCR4*

mutational status, which confirmed the predictive value of *CXCR4* mutations, especially nonsense *CXCR4* mutations, for lower odds of major and deep responses to ibrutinib monotherapy (**Supplemental Table S4**).

### PFS analysis and risk stratification

At the time of this report, 93 patients (30%) had progressed or died. The median PFS was 6.5 years (95% CI 6.0-not reached) and the estimated 5-year PFS rate was 60% (95% CI 51-68%; **Figure 1A**). Univariate and multivariate regression models for PFS are shown in **Table 3, top**. In the univariate analysis for PFS, platelet count  $\leq 100$  K/uL and *CXCR4* mutations were associated with worse PFS. The IPPSWM was not prognostic of PFS, as there were not differences between intermediate and low risk ( $p=0.19$ ), and high and intermediate risk ( $p=0.13$ ). In the multivariate analysis, platelet count  $\leq 100$  K/uL and *CXCR4* mutations were independent factors associated with worse PFS. There was a poor correlation between platelet count  $\leq 100$  K/uL and presence of *CXCR4* mutations ( $r=0.28$ ). Platelet count  $\leq 100$  K/uL was associated with HR 2.51 (95% CI 1.28-4.89;  $p=0.007$ ) and *CXCR4* mutations with HR 1.98 (95% CI 1.17-3.36;  $p=0.01$ ). The multiple imputation analysis did not change our results. For patients with platelet count  $\leq 100$  K/uL and  $>100$  K/uL, median PFS were 4.4 years (95% CI 0.9-not reached) and 6.6 years (95% CI 6.0-not reached), respectively, and the estimated 5-year PFS rates were 34% (95% CI 8-63%) and 63% (95% CI 54-71%), respectively (log-rank  $p=0.001$ ; **Figure 1B**). For patients with and without *CXCR4* mutations, median PFS were 4.4 years (95% CI 3.3-6.8) and not reached, respectively,

and the estimated 5-year PFS rates were 39% (95% CI 23-54%) and 71% (95% CI 59-80%), respectively (log-rank  $p < 0.001$ ; **Figure 1C**). There was no statistically significant difference in PFS between patients with nonsense and frameshift *CXCR4* mutations ( $p = 0.10$ ).

We then evaluated a PFS scoring system in WM patients on ibrutinib monotherapy using platelet count  $\leq 100$  K/uL and *CXCR4* mutations as adverse prognostic factors. This analysis included 241 WM patients with available data; 144 patients (60%) had zero adverse factors, 76 patients (32%) had 1 adverse factor and 21 patients (9%) had 2 adverse factors (**Table 4**). Patients with zero, 1 and 2 adverse factors, had median PFS not reached, 4.8 years (95% CI 3.6-not evaluable) and 3.3 years (95% CI 1.0-not evaluable), respectively, and estimated 5-year PFS rates of 72% (95% CI 60-82%), 44% (95% CI 27-60%) and 20% (95% CI 1-55%), respectively (log-rank  $p < 0.001$ ; **Figure 1D**). In a univariate Cox proportional-hazard regression model, patients with 1 adverse factor had a HR of 2.21 (95% CI 1.29-3.80;  $p = 0.004$ ), and patients with 2 adverse factors had HR 4.70 (95% CI 2.18-10.1;  $p < 0.001$ ) when compared with patients without adverse factors. Patients with 2 adverse factors had a HR of 2.33 (95% CI 1.07-5.07;  $p = 0.03$ ) compared with patients with 1 adverse factor. The multiple imputation analysis did not alter our results.

The proposed PFS scoring system was independently significant after adjusting for age, sex, serum IgM level, hemoglobin level, serum  $\beta 2$ -microglobulin level, serum albumin level, bone marrow involvement and prior therapy status ( $p < 0.05$  in all instances). PFS

estimates according to the proposed PFS scoring system stratified by age, serum IgM level, hemoglobin level, and prior therapy status are shown in **Supplemental Figure S2**.

### OS survival analysis

Fifty-three patients (16%) in our cohort have died. Causes of death are listed on **Supplemental Table S5**. The median OS was not reached, and the estimated 5-year OS rate was 75% (95% CI 67-81%; **Figure 2A**). There was no statistically significant difference in OS between patients with and without *CXCR4* mutations ( $p=0.27$ ), or between patients with nonsense and frameshift *CXCR4* mutations ( $p=0.91$ ). Univariate and multivariate Cox proportional-hazard regression models for OS are shown in **Table 3**. In the univariate regression analysis, age  $>65$  years, hemoglobin level  $<11.5$  g/dl, platelet count  $<100$  K/uL, serum  $\beta 2$ -microglobulin  $>3$  mg/l and serum albumin level  $<3.5$  g/dl were associated with a worse OS. In the multivariate analysis, age  $>65$  years was the only independent factor associated with a worse OS (HR 3.15, 95% CI 1.41-7.04;  $p=0.005$ ). The multiple imputation analysis did not change our results. For patients age  $>65$  (median age 73, range 66-96) and  $\leq 65$  years (median age 59, range 40-65), the median OS was 6.9 years (95% CI 5.2-not evaluable) and not reached, and the estimated 5-year OS rates were 63% (95% CI 50-73%) and 87% (95% CI 78-93%), respectively (**Figure 2B**). Given the strong prognostic value of age in OS, which could have masked the prognostic value of other factors, we fitted a separate multivariate model including the variables associated with worse OS in the univariate analysis but

excluding age. In this model, platelet count  $\leq 100$  K/uL (HR 2.21, 95% CI 1.01-4.85;  $p=0.047$ ) and serum albumin level  $<3.5$  g/dl (HR 2.06, 95% CI 1.08-3.93;  $p=0.03$ ) were independently associated with a worse OS.

We then evaluated the IPSSWM as prognostic tool in 89 patients with WM who received ibrutinib as primary therapy and had IPSSWM data available, of which 8 patients (9%) have died. The median follow-up for these patients was 3 years (95% CI 2-3.5). The 3-year OS rates for patients with low, intermediate, and high-risk disease were 92% (54-99%), 96% (73-99%) and 87% (71-94%), respectively ( $p=0.74$ ; **Figure 2C**). When compared to patients with low-risk disease, patients with intermediate and high-risk disease had HR of death of 1.73 (95% CI 0.16-19.1;  $p=0.65$ ) and 2.24 (95% CI 0.26-19.2;  $p=0.46$ ), respectively. High-risk patients had HR 1.29 (95% CI 0.25-6.67;  $p=0.76$ ) versus intermediate-risk patients. The multiple imputation analysis did not change our results. The proposed PFS scoring system was not prognostic of OS in patients who received ibrutinib as primary therapy ( $p=0.54$  for the entire model).

In addition, we fitted univariate and multivariate models for PFS and OS using continuous variables for age at ibrutinib initiation, hemoglobin level, platelet count, serum  $\beta 2$ -microglobulin, albumin, and IgM levels, and percentage of bone marrow involvement, in addition to categorical variables for sex, previous treatment and *CXCR4* mutational status, which confirmed the adverse prognostic value of *CXCR4* mutations, especially nonsense *CXCR4* mutations, in PFS but no effect in OS on ibrutinib monotherapy (**Supplemental Table S6**).

## DISCUSSION

In the present study, we aimed at evaluating factors predictive of response and prognostic of survival in WM patients treated with ibrutinib monotherapy. To the best of our knowledge, this is the largest effort to study these factors in WM patients treated with BTK inhibitors.

Our study showed having a *CXCR4* mutation was the only independent factor associated with lower odds of attaining deeper responses on ibrutinib monotherapy. In multivariate models adjusted for relevant clinical factors, the odds of a major response were 80% lower, and the odds of a deep response were 65% lower in patients with than in patients without *CXCR4* mutations. Our findings are consistent with published experience in patients with WM treated with ibrutinib on and off clinical trials [9, 10, 12, 13, 22]. In the recently published ASPEN study, in which patients with WM were randomized to ibrutinib or zanubrutinib, approximately 10% of patients with *CXCR4* mutations attained a VGPR while the rate of VGPR in patients without *CXCR4* mutations was 20-30% [23]. Furthermore, in the INNOVATE study, which randomized patients with WM to ibrutinib plus rituximab and placebo plus rituximab, the VGPR rate or better to ibrutinib plus rituximab was lower in patients with *CXCR4* mutations (19% and 34%, respectively). Therefore, *CXCR4* mutations associate with lower rates of deep response in patients with WM treated with ibrutinib (alone and in combination with rituximab) or with other covalent BTK inhibitors.

Platelet count  $\leq 100$  K/uL and *CXCR4* mutations were independently associated with worse PFS. We proposed a risk stratification scoring system for PFS in which patients with no risk factors (low risk), 1 risk factor (intermediate risk) and 2 risk factors (high risk) had 5-year PFS rates of 72%, 45% and 19%, respectively. Thrombocytopenia has previously been reported as an adverse prognostic factor for OS in patients with WM [21], but thrombocytopenia has not previously been reported as an adverse marker for PFS on ibrutinib. Thrombocytopenia could represent either a higher bone marrow burden of disease or a bone marrow microenvironment with a specific cytokine profile that portends a mechanism of resistance to ibrutinib effect.

*CXCR4* mutations have been previously associated with shorter PFS in WM patients treated with ibrutinib monotherapy [22]. In the INNOVATE study, that evaluated ibrutinib plus rituximab combination therapy, there were no detectable differences in 30-month PFS rates between patients with and without *CXCR4* mutations [24]. However, at the 50-month follow-up update, the PFS rate appeared numerically higher in patients without *CXCR4* mutations at approximately 80% versus approximately 62% in patients with *CXCR4* mutations [25]. It is possible that the addition of rituximab would partially revert the adverse impact of *CXCR4* mutations when combined with ibrutinib in patients with WM. However, other interventions might be needed to optimize the treatment of patients with WM who harbor *CXCR4* mutations.

Preclinical studies in WM cell lines from our group showed that frameshift and nonsense *CXCR4* mutations were associated with a reduction of CXCR4 receptor internalization following stimulation with the CXCR4 ligand CXCL12 [26]. Upon ibrutinib exposure, cell death induction was decreased in *CXCR4* mutation-harboring cells thought to be mediated by AKT and ERK activation. CXCR4 internalization, and subsequent, cell death was partially regained by exposing these cells to CXCR4 antagonists. Our findings support targeting CXCR4 as a valid therapeutic mechanism in WM. A phase I/II study evaluated ibrutinib in combination with the anti-CXCR4 monoclonal antibody ulocuplumab in 13 patients with WM and CXCR4 mutations; nine patients were previously untreated and seven had a nonsense mutation [27]. Major responses were attained in 100% of the patients with a median time to major response of 1.2 months, and the 2-year PFS rate was 90%. By comparison, ibrutinib therapy associated with major response rates of 60%, a median time to major response of 4-6 months and 2-year PFS rates of 30-70% in patients with WM and *CXCR4* mutations [12, 22, 25]. NCT04274738 is a phase I study evaluating ibrutinib in combination with the small-molecule CXCR4 inhibitor mavorixafor [28]. The study is currently accruing participants in centers in the United States and Europe.

Based on population-based studies, the survival of patients with WM has improved in recent decades [29-31]. This improvement is likely multifactorial and associated with improved supportive therapies, increased patient disease-related education levels, higher medical practitioner awareness, and the advent of novel treatment options. The IPSSWM included age >65 years, hemoglobin level  $\leq 11.5$  g/dl, platelet count  $\leq 100$

K/uL, serum beta-2-microglobulin level >3 mg/l, and monoclonal IgM level >7,000 mg/dl and divided patients with low, intermediate, and high risk of overall mortality, though it was developed in a cohort of patients with WM with a low rate of exposure to non-chemotherapeutic approaches [21]. The IPSSWM was later validated in patients treated with rituximab-containing regimens [32]. However, current data do not support a prognostic value of the IPSSWM in patients treated with BTK inhibitors. The INNOVATE study did not show a prognostic value for PFS in patients with WM treated with ibrutinib plus rituximab, but OS was not evaluated [24]. Our study showed that the IPSSWM may be prognostic of OS in patients with WM treated with ibrutinib. However, the main driver of the prognosis appears to be the age at ibrutinib initiation, as age >65 years was the only adverse prognostic factor in a multivariate model. Additionally, the IPSSWM was not prognostic of OS in patients who received ibrutinib as primary therapy. However, the sample size was small, and the follow-up was relatively short. A larger sample size and longer follow-up are needed to better evaluate the prognostic value of the IPSSWM in patients with WM treated with BTK inhibitors as primary therapy.

Our study identified that a hemoglobin level  $\leq 11.5$  g/dl was associated with higher rates of major and deep responses to ibrutinib monotherapy, independent of *CXCR4* mutational status. The rationale behind this finding is not well understood but may be associated with the role of CXCL13 in the biology of WM. CXCL13 is a chemokine produced by WM cells and can act as a chemotactic for mast cells into the bone marrow microenvironment [33, 34]. In a prior study from our group, high serum CXCL13 levels were inversely correlated with hemoglobin levels in patients with WM and directly

associated with higher response rates to ibrutinib monotherapy [35]. The role of CXCL13 in WM needs further investigation.

Our study is not without limitations. All the patients included were evaluated, at least at one point in time, at a clinical center for national reference. However, the patients' characteristics from our cohort are representative of the general population of patients with WM, with a median age at treatment initiation of 68 years and a male-to-female ratio of 1.9:1. Given the retrospective nature of our study, variable proportions of missing data are expected. To minimize the bias introduced by missing data, we performed multiple imputation analyses, in addition to complete-case analysis. In all cases, the analyses of imputed data did not alter our results or resulted in stronger associations than the ones estimated by the complete-case analyses. Finally, the high sensitivity *CXCR4* mutational testing performed at our institution, which combines NGS assays for all mutations, PCR assays for nonsense and Sanger sequencing for frameshift mutations, might not be replicated elsewhere. However, our study permitted an evaluation of the true biological effect of *CXCR4* mutations on ibrutinib outcomes. As previously reported, NGS can be associated with a high rate of false negative results in patients with WM, especially in patients with low tumor burden in the bone marrow [36]. Standardization of *CXCR4* mutational testing is warranted.

The US FDA recently approved zanubrutinib, a second-generation, covalent BTK inhibitor for the treatment of patients with WM [37]. Other covalent BTK inhibitors, such as acalabrutinib and tirabrutinib have also shown efficacy in WM [38, 39]. Finally, non-

covalent BTK inhibitors, such as pirtobrutinib, are under clinical evaluation in WM [40]. We believe the present scoring system could serve to predict responses and prognosticate PFS in patients with WM treated with zanubrutinib. Additional studies are needed, however, to confirm the prognostic value of our proposed score in patients with WM receiving other covalent and non-covalent BTK inhibitors.

We present a robust prognostic tool for PFS in patients with WM treated with ibrutinib, which could serve to guide prognostic discussions between practitioners, patients, and family members.

### **Data sharing statement**

Data are available upon request to [jorgej\\_castillo@dfci.harvard.edu](mailto:jorgej_castillo@dfci.harvard.edu).

### **Authors' contributions**

JJC designed the study, performed the analysis, and wrote the initial draft of the manuscript. JJC, SS, CAF, CRL, TPW and SPT provided clinical care to the patients. JJC, JNG, SS and KM collected the data. MLG, AK, XL, MM, NT, ZRH and CJP run the genomic studies. JJC, JNG, SS, ARB and SPT analyzed the data. All the authors approved the final version of the manuscript.

### **Disclosures**

JJC received research funds and/or honoraria from Abbvie, BeiGene, Janssen, Millennium, Pharmacyclics, Roche, and TG Therapeutics. ARB received research funds and/or honoraria from Adaptive, Beigene, CSL Behring, Genzyme, Karyopharm, Pharmacyclics and Sanofi. SPT received research funds and/or honoraria from Beigene, BMS, Eli Lilly, Janssen, Pharmacyclics, and X4 Pharmaceuticals. All other authors have no conflicts of interest to disclose.

## REFERENCES

1. Swerdlow SH, Cook JR, Sohani AR, Pileri SA, Harris NL, Jaffe ES, et al, *Lymphoplasmacytic lymphoma*, in *WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues*, S.H. Swerdlow, et al., Editors. 2017, IARC: Lyon. p. 232-235.
2. Hunter ZR, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*. 2014. 123(11): 1637-46.
3. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. *N Engl J Med*. 2012. 367(9): 826-33.
4. Yang G, Zhou Y, Liu X, Xu L, Cao Y, Manning RJ, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton

- tyrosine kinase in Waldenstrom macroglobulinemia. *Blood*. 2013. 122(7): 1222-32.
5. Cao Y, Hunter ZR, Liu X, Xu L, Yang G, Chen J, et al. CXCR4 WHIM-like frameshift and nonsense mutations promote ibrutinib resistance but do not supplant MYD88(L265P) -directed survival signalling in Waldenstrom macroglobulinaemia cells. *Br J Haematol*. 2015. 168(5): 701-7.
  6. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. *Blood*. 2014. 123(18): 2791-6.
  7. Schmidt J, Federmann B, Schindler N, Steinhilber J, Bonzheim I, Fend F, et al. MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. *Br J Haematol*. 2015. 169(6): 795-803.
  8. Poulain S, Roumier C, Venet-Caillault A, Figeac M, Herbaux C, Marot G, et al. Genomic Landscape of CXCR4 Mutations in Waldenstrom Macroglobulinemia. *Clin Cancer Res*. 2016. 22(6): 1480-8.
  9. Castillo JJ, Xu L, Gustine JN, Keezer A, Meid K, Dubeau TE, et al. CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenstrom macroglobulinaemia treated with ibrutinib. *Br J Haematol*. 2019. 187(3): 356-363.
  10. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med*. 2015. 372(15): 1430-40.

11. Dimopoulos MA, Trotman J, Tedeschi A, Matous JV, Macdonald D, Tam C, et al. Ibrutinib for patients with rituximab-refractory Waldenstrom's macroglobulinaemia (iINNOVATE): an open-label substudy of an international, multicentre, phase 3 trial. *Lancet Oncol*. 2017. 18(2): 241-250.
12. Treon SP, Gustine J, Meid K, Yang G, Xu L, Liu X, et al. Ibrutinib Monotherapy in Symptomatic, Treatment-Naive Patients With Waldenstrom Macroglobulinemia. *J Clin Oncol*. 2018. 36(27): 2755-2761.
13. Castillo JJ, Gustine JN, Meid K, Flynn CA, Demos MG, Guerrero ML, et al. Response and Survival Outcomes to Ibrutinib Monotherapy for Patients With Waldenstrom Macroglobulinemia on and off Clinical Trials. *Hemasphere*. 2020. 4(3): e363.
14. Abeykoon JP, Zanwar S, Ansell SM, Gertz MA, Kumar S, Manske M, et al. Ibrutinib monotherapy outside of clinical trial setting in Waldenstrom macroglobulinaemia: practice patterns, toxicities and outcomes. *Br J Haematol*. 2020. 188(3): 394-403.
15. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. *Semin Oncol*. 2003. 30(2): 110-5.
16. Kyle RA, Treon SP, Alexanian R, Barlogie B, Bjorkholm M, Dhodapkar M, et al. Prognostic markers and criteria to initiate therapy in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second

- International Workshop on Waldenstrom's Macroglobulinemia. *Semin Oncol*. 2003. 30(2): 116-20.
17. Owen RG, Kyle RA, Stone MJ, Rawstron AC, Leblond V, Merlini G, et al. Response assessment in Waldenstrom macroglobulinaemia: update from the VIth International Workshop. *Br J Haematol*. 2013. 160(2): 171-6.
  18. Kluk MJ, Lindsley RC, Aster JC, Lindeman NI, Szeto D, Hall D, et al. Validation and Implementation of a Custom Next-Generation Sequencing Clinical Assay for Hematologic Malignancies. *J Mol Diagn*. 2016. 18(4): 507-15.
  19. Xu L, Hunter ZR, Tsakmaklis N, Cao Y, Yang G, Chen J, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenstrom Macroglobulinaemia. *Br J Haematol*. 2016. 172(5): 735-44.
  20. Xu L, Hunter ZR, Yang G, Zhou Y, Cao Y, Liu X, et al. MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. *Blood*. 2013. 121(11): 2051-8.
  21. Morel P, Duhamel A, Gobbi P, Dimopoulos MA, Dhodapkar MV, McCoy J, et al. International prognostic scoring system for Waldenstrom macroglobulinemia. *Blood*. 2009. 113(18): 4163-70.
  22. Treon SP, Meid K, Gustine J, Yang G, Xu L, Liu X, et al. Long-Term Follow-Up of Ibrutinib Monotherapy in Symptomatic, Previously Treated Patients With Waldenstrom Macroglobulinemia. *J Clin Oncol*. 2021. 39(6): 565-575.

23. Tam CS, Opat S, D'Sa S, Jurczak W, Lee HP, Cull G, et al. A randomized phase 3 trial of zanubrutinib vs ibrutinib in symptomatic Waldenstrom macroglobulinemia: the ASPEN study. *Blood*. 2020. 136(18): 2038-2050.
24. Dimopoulos MA, Tedeschi A, Trotman J, Garcia-Sanz R, Macdonald D, Leblond V, et al. Phase 3 Trial of Ibrutinib plus Rituximab in Waldenstrom's Macroglobulinemia. *N Engl J Med*. 2018. 378(25): 2399-2410.
25. Buske C, Tedeschi A, Trotman J, García-Sanz R, MacDonald D, Leblond V, et al. Five-Year Follow-Up of Ibrutinib Plus Rituximab Vs Placebo Plus Rituximab for Waldenstrom's Macroglobulinemia: Final Analysis From the Randomized Phase 3 INNOVATETM Study. *Blood*. 2020. 136(Supplement 1): 24-26.
26. Cao Y, Hunter ZR, Liu X, Xu L, Yang G, Chen J, et al. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. *Leukemia*. 2015. 29(1): 169-76.
27. Treon SP, Meid K, Hunter ZR, Flynn CA, Sarosiek SR, Leventoff CR, et al. Phase I study of Ibrutinib and the CXCR4 antagonist Ulocuplumab in CXCR4 mutated Waldenstrom Macroglobulinemia. *Blood*. 2021.
28. Treon S, Buske C, Thomas S, Branagan A, Dimopoulos M, Castillo JJ, et al. PRELIMINARY CLINICAL DATA FROM A PHASE 1B STUDY OF MAVORIXAFOR AND IBRUTINIB IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA WITH MYD88 AND CXCR4 MUTATIONS. *Hematological Oncology*. 2021. 39(S2).

29. Castillo JJ, Olszewski AJ, Cronin AM, Hunter ZR, Treon SP. Survival trends in Waldenstrom macroglobulinemia: an analysis of the Surveillance, Epidemiology and End Results database. *Blood*. 2014. 123(25): 3999-4000.
30. Castillo JJ, Olszewski AJ, Kanan S, Meid K, Hunter ZR, Treon SP. Overall survival and competing risks of death in patients with Waldenstrom macroglobulinaemia: an analysis of the Surveillance, Epidemiology and End Results database. *Br J Haematol*. 2015. 169(1): 81-9.
31. Kristinsson SY, Eloranta S, Dickman PW, Andersson TM, Turesson I, Landgren O, et al. Patterns of survival in lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia: a population-based study of 1,555 patients diagnosed in Sweden from 1980 to 2005. *Am J Hematol*. 2013. 88(1): 60-5.
32. Kastiris E, Morel P, Duhamel A, Gavriatopoulou M, Kyrtsonis MC, Durot E, et al. A revised international prognostic score system for Waldenstrom's macroglobulinemia. *Leukemia*. 2019. 33(11): 2654-2661.
33. Hunter ZR, Xu L, Yang G, Tsakmaklis N, Vos JM, Liu X, et al. Transcriptome sequencing reveals a profile that corresponds to genomic variants in Waldenstrom macroglobulinemia. *Blood*. 2016. 128(6): 827-38.
34. Tripodo C, Gri G, Piccaluga PP, Frossi B, Guarnotta C, Piconese S, et al. Mast cells and Th17 cells contribute to the lymphoma-associated pro-inflammatory microenvironment of angioimmunoblastic T-cell lymphoma. *Am J Pathol*. 2010. 177(2): 792-802.
35. Vos JM, Tsakmaklis N, Patterson CJ, Meid K, Castillo JJ, Brodsky P, et al. CXCL13 levels are elevated in patients with Waldenstrom macroglobulinemia,

- and are predictive of major response to ibrutinib. *Haematologica*. 2017. 102(11): e452-e455.
36. Gustine JN, Xu L, Yang G, Liu X, Kofides A, Tsakmaklis N, et al. Bone marrow involvement and subclonal diversity impairs detection of mutated CXCR4 by diagnostic next-generation sequencing in Waldenstrom macroglobulinaemia. *Br J Haematol*. 2021.
  37. FDA approves zanubrutinib for Waldenström's macroglobulinemia [website]. Available at <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-zanubrutinib-waldenstroms-macroglobulinemia>. Accessed on December 5, 2021.
  38. Owen RG, McCarthy H, Rule S, D'Sa S, Thomas SK, Tournilhac O, et al. Acalabrutinib monotherapy in patients with Waldenstrom macroglobulinemia: a single-arm, multicentre, phase 2 study. *Lancet Haematol*. 2020. 7(2): e112-e121.
  39. Sekiguchi N, Rai S, Munakata W, Suzuki K, Handa H, Shibayama H, et al. A multicenter, open-label, phase II study of tirabrutinib (ONO/GS-4059) in patients with Waldenstrom's macroglobulinemia. *Cancer Sci*. 2020. 111(9): 3327-3337.
  40. Mato AR, Shah NN, Jurczak W, Cheah CY, Pagel JM, Woyach JA, et al. Pirtobrutinib in relapsed or refractory B-cell malignancies (BRUIN): a phase 1/2 study. *Lancet*. 2021. 397(10277): 892-901.



**Table 1.** Patients' baseline characteristics

| <b>Characteristic</b>                       | <b>N (%) or median (range)</b> |
|---------------------------------------------|--------------------------------|
| Age at WM diagnosis, years                  | 61 (35-91)                     |
| Age at ibrutinib initiation, years          | 68 (40-96)                     |
| Age >65 years                               | 190 (60%)                      |
| Male sex                                    | 206 (65%)                      |
| Hemoglobin level, g/dl                      | 10.3 (4-17)                    |
| Hemoglobin <11.5 g/dl                       | 226 (72%)                      |
| Platelet count, K/uL                        | 211 (9-639)                    |
| Platelet count <100 K/uL                    | 42 (13%)                       |
| Serum IgM level, mg/dl                      | 3,400 (88*-10,321)             |
| Serum IgM level >4,000 mg/dl                | 129 (40%)                      |
| Serum IgM level >7,000 mg/dl                | 15 (5%)                        |
| Serum $\beta$ 2-microglobulin level, mg/l   | 3.6 (1.4-18.3)                 |
| Serum $\beta$ 2-microglobulin level >3 mg/l | 164 (65%)                      |
| Serum albumin level, g/dl                   | 3.7 (2.1-5)                    |
| Serum albumin level $\leq$ 3.5 g/dl         | 92 (30%)                       |
| Bone marrow involvement                     | 60% (5-100%)                   |
| Bone marrow involvement $\geq$ 60%          | 154 (55%)                      |
| IPSSWM                                      |                                |
| Low                                         | 64 (24%)                       |
| Intermediate                                | 92 (34%)                       |
| High                                        | 112 (42%)                      |
| <i>MYD88</i> mutated                        | 265 (97%)                      |
| <i>CXCR4</i> mutated                        | 89 (36%)                       |
| Previously untreated                        | 100 (31%)                      |
| Previously treated                          | 219 (69%)                      |
| Median number of prior lines                | 2 (1-8)                        |
| $\geq$ 2 prior lines                        | 115 (53%)                      |

\*Six patients with normal serum IgM levels had an IgM monoclonal spike in the serum protein electrophoresis.

IPSSWM: International Prognostic Scoring System for Waldenström Macroglobulinemia

**Table 2.** Univariate and multivariate logistic regression analyses for major and deep response in 319 patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy.

| Major response           | Univariate analysis |                  | Multivariate analysis |                  |
|--------------------------|---------------------|------------------|-----------------------|------------------|
| Variables                | OR (95% CI)         | p                | OR (95% CI)           | p                |
| Age >65 years            | 1.53 (0.90-2.61)    | 0.12             |                       |                  |
| Male sex                 | 1.19 (0.69-2.06)    | 0.53             |                       |                  |
| Hemoglobin <11.5 g/dl    | 2.25 (1.28-3.93)    | <b>0.005</b>     | 2.72 (1.25-5.95)      | <b>0.01</b>      |
| Platelet <100 k/ul       | 0.50 (0.25-2.45)    | 0.05             |                       |                  |
| β2-microglobulin >3 mg/l | 2.41 (1.31-4.43)    | <b>0.005</b>     | 1.54 (0.68-3.45)      | 0.30             |
| Albumin <3.5 g/dl        | 1.69 (0.88-3.24)    | 0.12             |                       |                  |
| Serum IgM >4,000 mg/dl   | 1.29 (0.74-2.24)    | 0.36             |                       |                  |
| Serum IgM >7,000 mg/dl   | 0.40 (0.14-1.17)    | 0.09             |                       |                  |
| Bone marrow >60%         | 2.03 (1.14-3.62)    | <b>0.02</b>      | 2.26 (0.98-5.21)      | 0.06             |
| Previously treated       | 0.92 (0.52-1.64)    | 0.78             |                       |                  |
| <i>CXCR4 mutated</i>     | 0.33 (0.17-0.61)    | <b>&lt;0.001</b> | 0.20 (0.09-0.45)      | <b>&lt;0.001</b> |
| <i>CXCR4 WT</i>          | 1.00                |                  | 1.00                  |                  |
| <i>CXCR4 NS</i>          | 0.24 (0.12-0.48)    | <b>&lt;0.001</b> | 0.19 (0.01-0.37)      | <b>&lt;0.001</b> |
| <i>CXCR4 FS</i>          | 0.69 (0.25-1.87)    | 0.47             | 0.40 (0.13-1.23)      | 0.11             |
| Deep response            | Univariate analysis |                  | Multivariate analysis |                  |
| Variables                | OR (95% CI)         | p                | OR (95% CI)           | p                |
| Age >65 years            | 1.04 (0.63-1.71)    | 0.88             |                       |                  |
| Male sex                 | 1.08 (0.65-1.82)    | 0.76             |                       |                  |
| Hemoglobin <11.5 g/dl    | 1.94 (1.07-3.53)    | <b>0.03</b>      | 2.34 (1.14-4.80)      | <b>0.02</b>      |
| Platelet <100 k/ul       | 1.03 (0.50-2.11)    | 0.94             |                       |                  |
| β2-microglobulin >3 mg/l | 1.46 (0.81-2.64)    | 0.21             |                       |                  |
| Albumin <3.5 g/dl        | 1.70 (1.01-2.88)    | 0.05             |                       |                  |
| Serum IgM >4,000 mg/dl   | 0.60 (0.36-1.01)    | 0.05             |                       |                  |
| Serum IgM >7,000 mg/dl   | 0.64 (0.18-2.34)    | 0.50             |                       |                  |
| Bone marrow >60%         | 1.65 (0.96-2.83)    | 0.07             |                       |                  |
| Previously treated       | 1.31 (0.76-2.25)    | 0.33             |                       |                  |
| <i>CXCR4 mutated</i>     | 0.34 (0.18-0.66)    | <b>0.001</b>     | 0.32 (0.15-0.55)      | <b>0.001</b>     |
| <i>CXCR4 WT</i>          | 1.00                |                  | 1.00                  |                  |
| <i>CXCR4 NS</i>          | 0.26 (0.11-0.61)    | <b>0.002</b>     | 0.25 (0.11-0.60)      | <b>0.002</b>     |
| <i>CXCR4 FS</i>          | 0.53 (0.21-1.31)    | 0.17             | 0.47 (0.19-1.16)      | 0.11             |

WT: wild type; NS: nonsense; FS: frameshift

MYD88 mutational status was not included as sample size for *MYD88* wild type status was small (n=8)

**Table 3.** Univariate and multivariate Cox proportional-hazard regression analyses for progression-free survival and overall survival in 319 patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy.

| Progression-free survival | Univariate analysis |                  | Multivariate analysis |              |
|---------------------------|---------------------|------------------|-----------------------|--------------|
| Variables                 | HR (95% CI)         | p                | HR (95% CI)           | p            |
| Age >65 years             | 1.21 (0.77-1.89)    | 0.41             |                       |              |
| Male sex                  | 1.09 (0.67-1.78)    | 0.72             |                       |              |
| Hemoglobin <11.5 g/dl     | 1.40 (0.84-2.32)    | 0.19             |                       |              |
| Platelet <100 k/ul        | 2.55 (1.42-4.59)    | <b>0.002</b>     | 2.51 (1.28-4.89)      | <b>0.007</b> |
| β2-microglobulin >3 mg/l  | 1.48 (0.84-2.61)    | 0.17             |                       |              |
| Albumin <3.5 g/dl         | 1.57 (0.97-2.57)    | 0.07             |                       |              |
| Serum IgM >4,000 mg/dl    | 0.90 (0.58-1.41)    | 0.64             |                       |              |
| Serum IgM >7,000 mg/dl    | 1.90 (0.76-4.73)    | 0.17             |                       |              |
| Bone marrow >60%          | 0.75 (0.47-1.22)    | 0.25             |                       |              |
| Previously treated        | 1.40 (0.77-2.53)    | 0.27             |                       |              |
| <i>CXCR4</i> mutated      | 2.31 (1.41-3.79)    | <b>0.001</b>     | 1.98 (1.17-3.36)      | <b>0.01</b>  |
| <i>CXCR4</i> WT           | 1.00                |                  |                       |              |
| <i>CXCR4</i> NS           | 2.93 (1.71-5.00)    | <b>&lt;0.001</b> | 2.56 (1.45-4.50)      | <b>0.001</b> |
| <i>CXCR4</i> FS           | 1.49 (0.68-3.23)    | 0.32             | 1.22 (0.55-2.74)      | 0.63         |
| Overall survival          | Univariate analysis |                  | Multivariate analysis |              |
| Variables                 | HR (95% CI)         | p                | HR (95% CI)           | p            |
| Age >65 years             | 3.03 (1.61-5.70)    | <b>0.001</b>     | 3.15 (1.41-7.04)      | <b>0.005</b> |
| Male sex                  | 1.55 (0.81-2.95)    | 0.18             |                       |              |
| Hemoglobin <11.5 g/dl     | 2.36 (1.15-4.84)    | <b>0.02</b>      | 2.00 (0.81-4.93)      | 0.13         |
| Platelet <100 k/ul        | 3.10 (1.61-5.97)    | <b>0.001</b>     | 2.05 (0.94-4.48)      | 0.07         |
| β2-microglobulin >3 mg/l  | 3.01 (1.27-7.18)    | <b>0.01</b>      | 1.69 (0.69-4.14)      | 0.25         |
| Albumin <3.5 g/dl         | 2.40 (1.37-4.24)    | <b>0.002</b>     | 1.65 (0.85-3.19)      | 0.14         |
| Serum IgM >4,000 mg/dl    | 1.39 (0.81-2.39)    | 0.23             |                       |              |
| Serum IgM >7,000 mg/dl    | 2.07 (0.74-5.77)    | 0.17             |                       |              |
| Bone marrow >60%          | 0.87 (0.49-1.54)    | 0.63             |                       |              |
| Previously treated        | 1.46 (0.67-3.15)    | 0.34             |                       |              |
| <i>CXCR4</i> mutated      | 1.37 (0.74-2.53)    | 0.31             |                       |              |
| <i>CXCR4</i> WT           | 1.00                |                  |                       |              |
| <i>CXCR4</i> NS           | 1.42 (0.71-2.85)    | 0.32             |                       |              |
| <i>CXCR4</i> FS           | 1.49 (0.61-3.64)    | 0.39             |                       |              |

WT: wild type; NS: nonsense; FS: frameshift

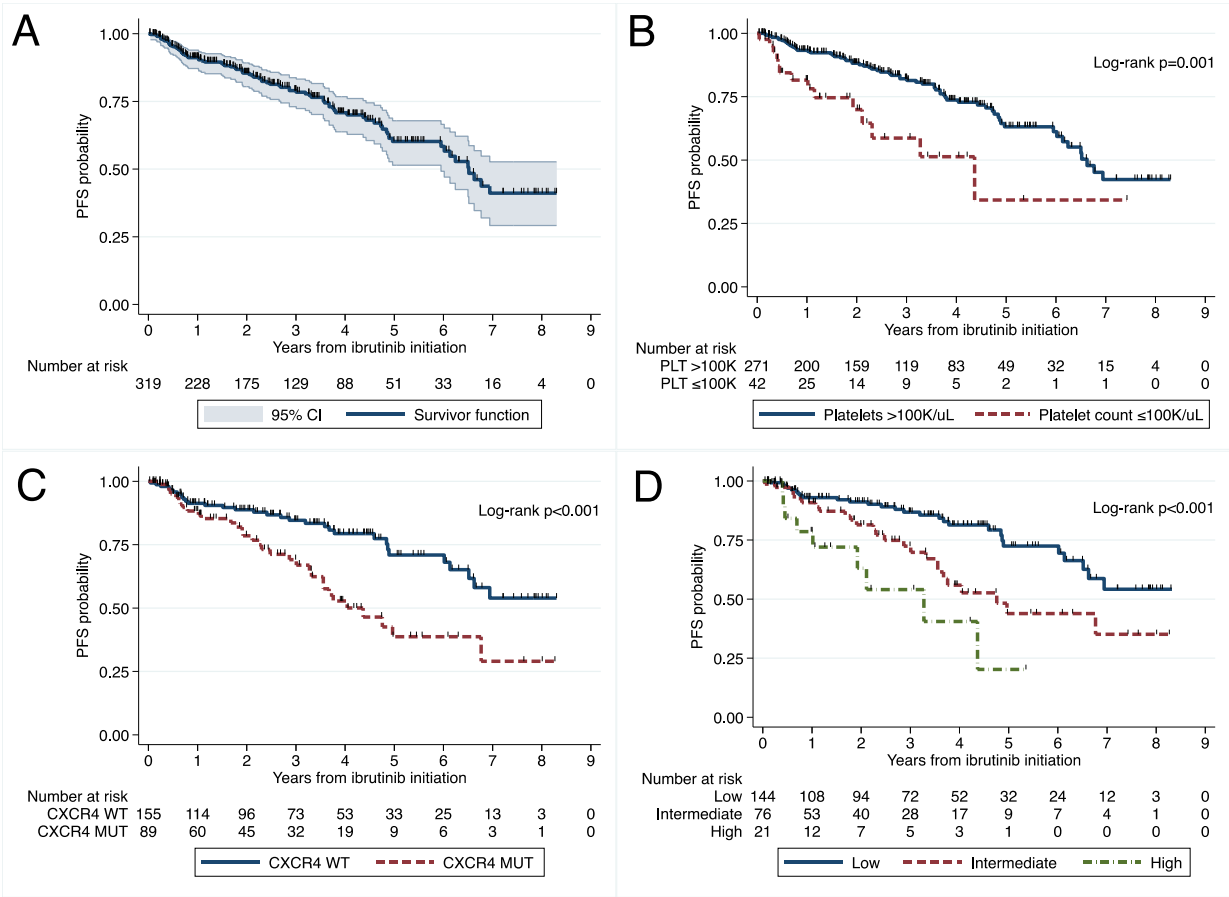
MYD88 mutational status was not included as the sample size for *MYD88* wildtype status was small (n=8)

**Table 4.** Proposed prognostic score for progression-free survival for patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy

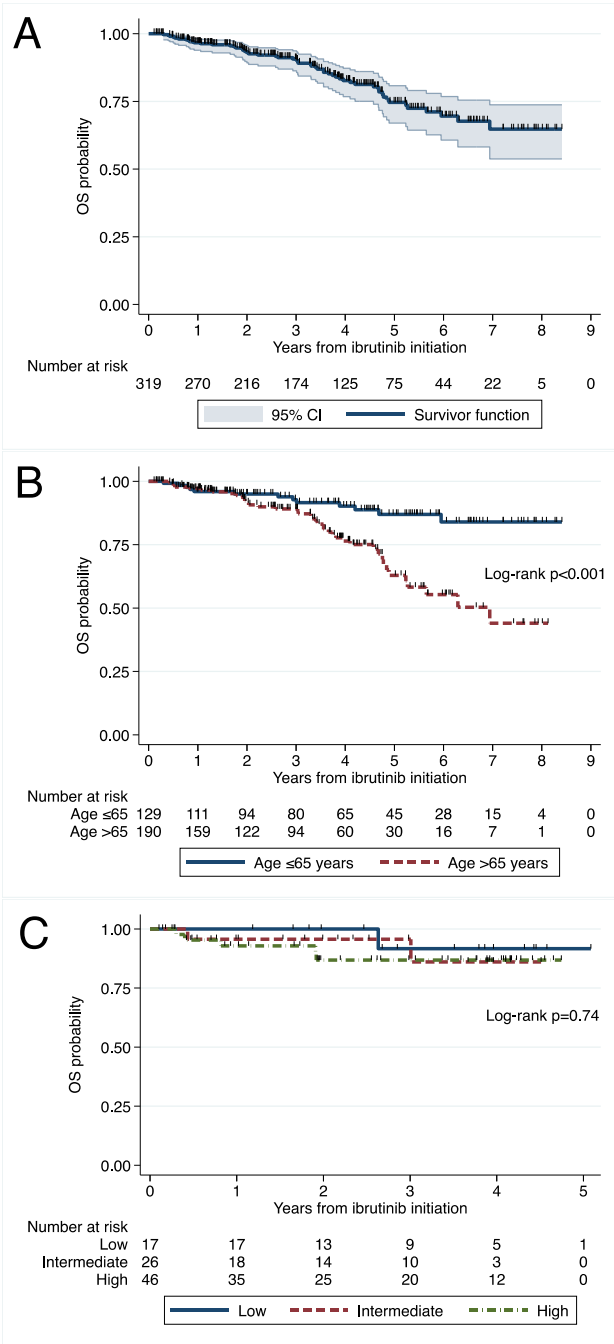
| <b>Variables</b>         |              | <b>HR (95% CI)</b> |               | <b>p</b>                        |                    |
|--------------------------|--------------|--------------------|---------------|---------------------------------|--------------------|
| <i>CXCR4</i> mutations   |              | 2.51 (1.28-4.89)   |               | 0.001                           |                    |
| Platelet count <100 K/ul |              | 1.98 (1.17-3.36)   |               | 0.07                            |                    |
| <b>Stratum</b>           | <b>Score</b> | <b>Number (%)</b>  | <b>Failed</b> | <b>5-year PFS rate (95% CI)</b> | <b>HR (95% CI)</b> |
| Low                      | 0            | 144 (60%)          | 28            | 72% (60-82%)                    | 1.0 (Ref)          |
| Intermediate             | 1            | 76 (32%)           | 25            | 44% (27-60%)                    | 2.2 (1.3-3.8)      |
| High                     | 2            | 21 (9%)            | 9             | 20% (1-55%)                     | 4.7 (2.2-10.1)     |

PFS: progression-free survival; HR: hazard ratio; CI: confidence interval

**Figure 1.** Progression-free survival (PFS) estimates in 319 patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy, for the entire cohort (A), according to platelet count (B), according to *CXCR4* mutational status (C), and according to the proposed PFS scoring system (D)



**Figure 2.** Overall survival estimates in 319 patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy, for the entire cohort (A), according to age (B), and according to the IPSSWM in patients who received ibrutinib as primary therapy (C).



# Figure 1

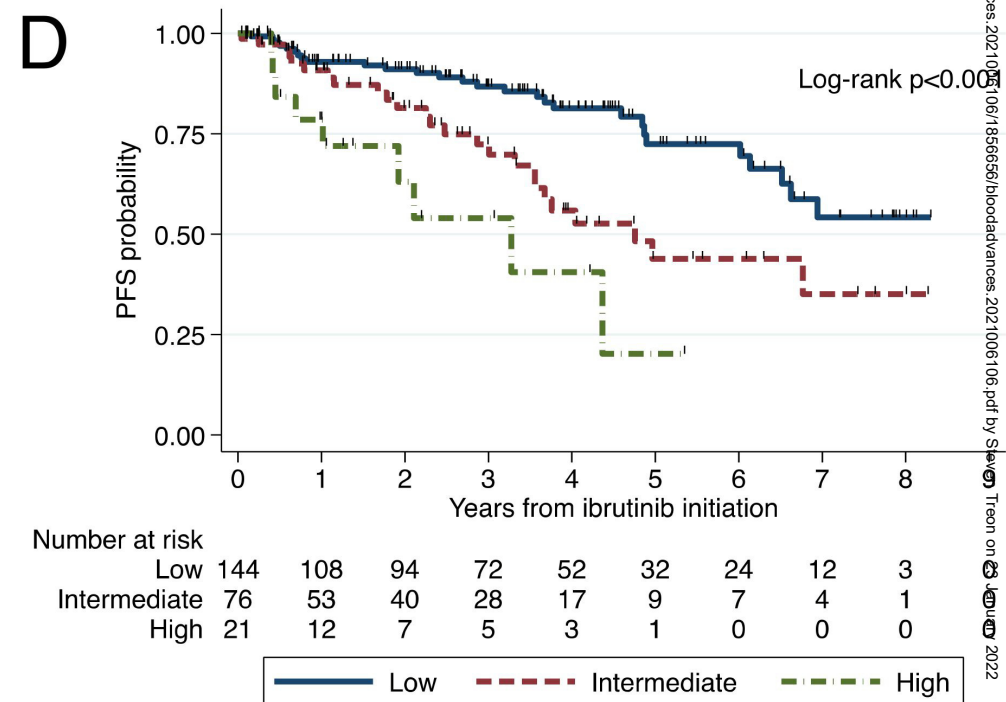
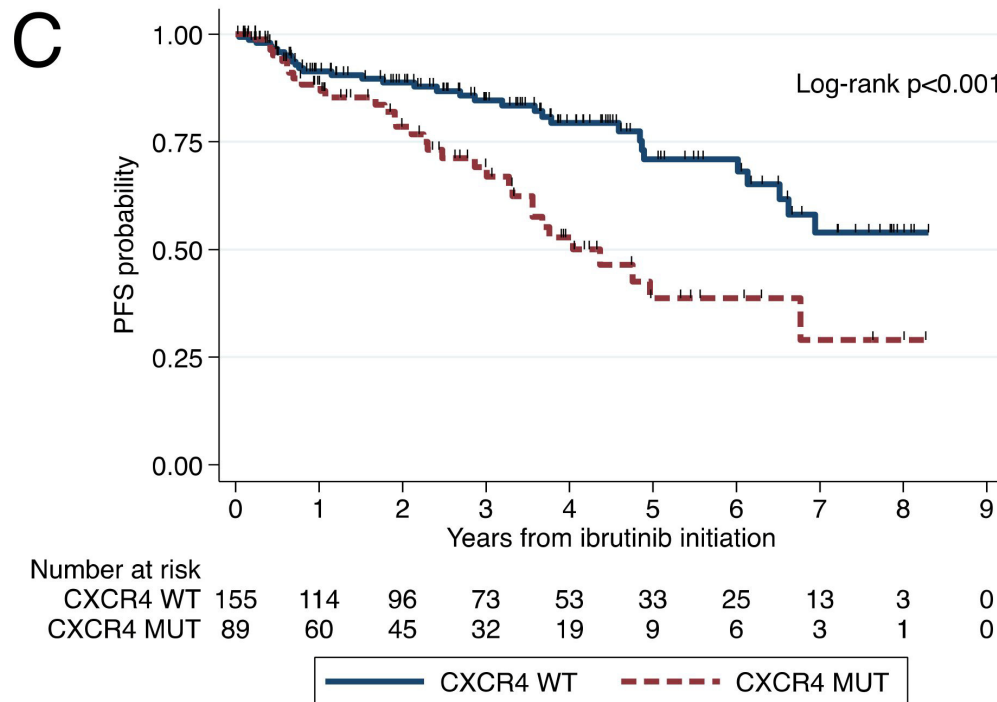
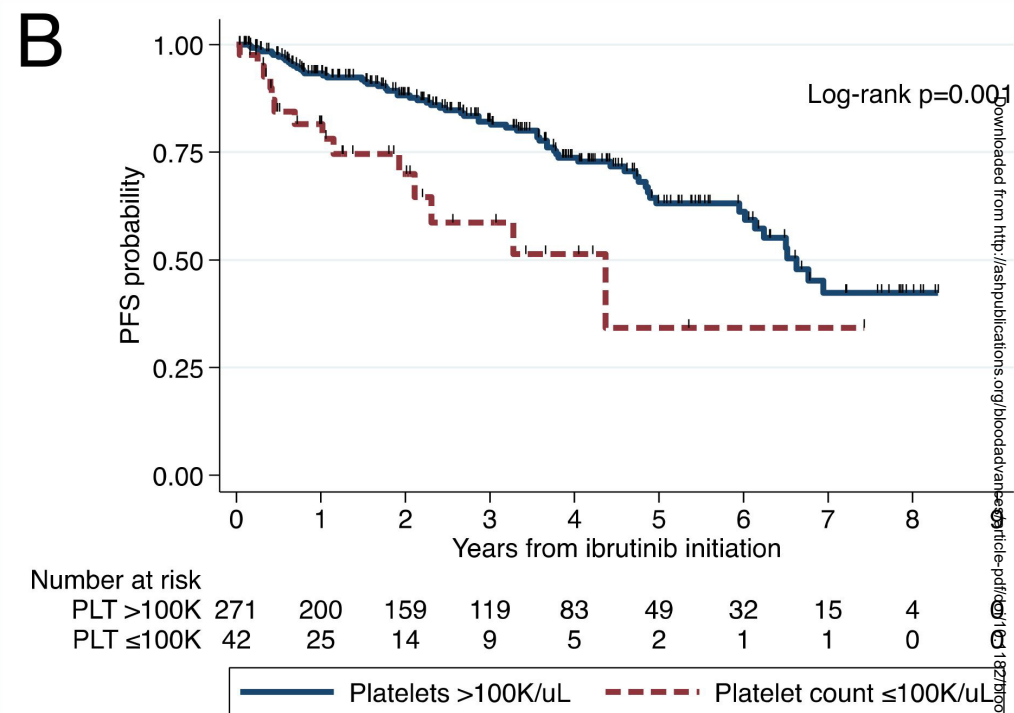
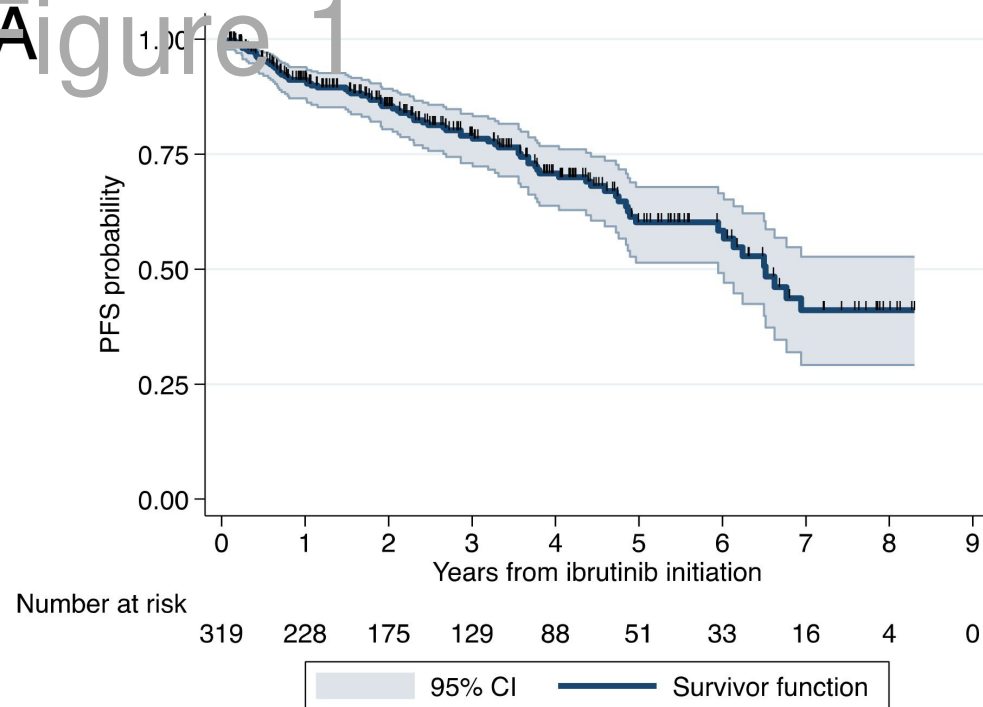
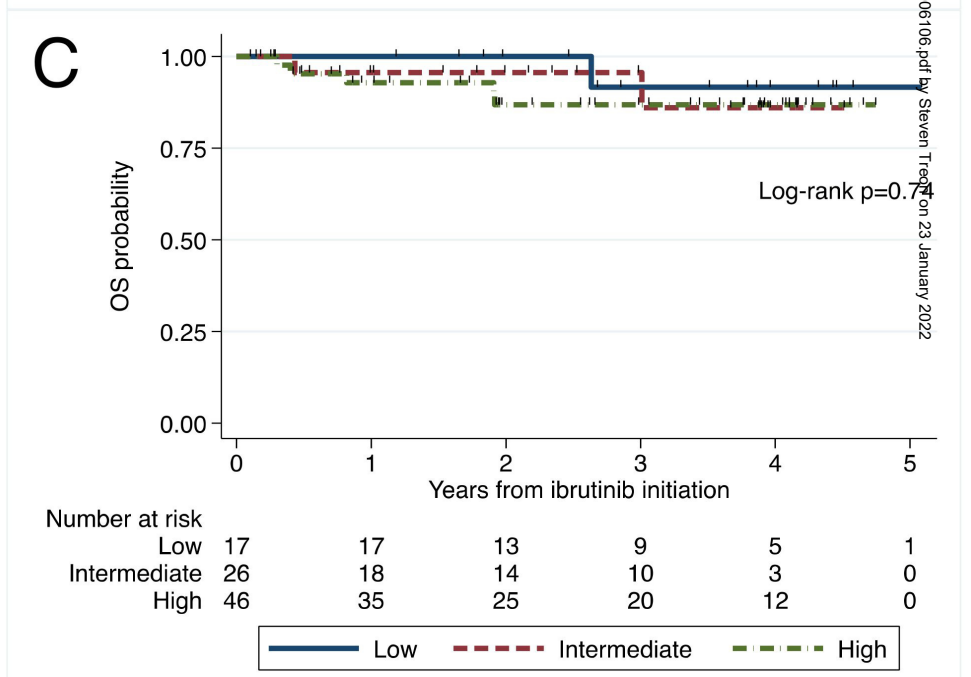
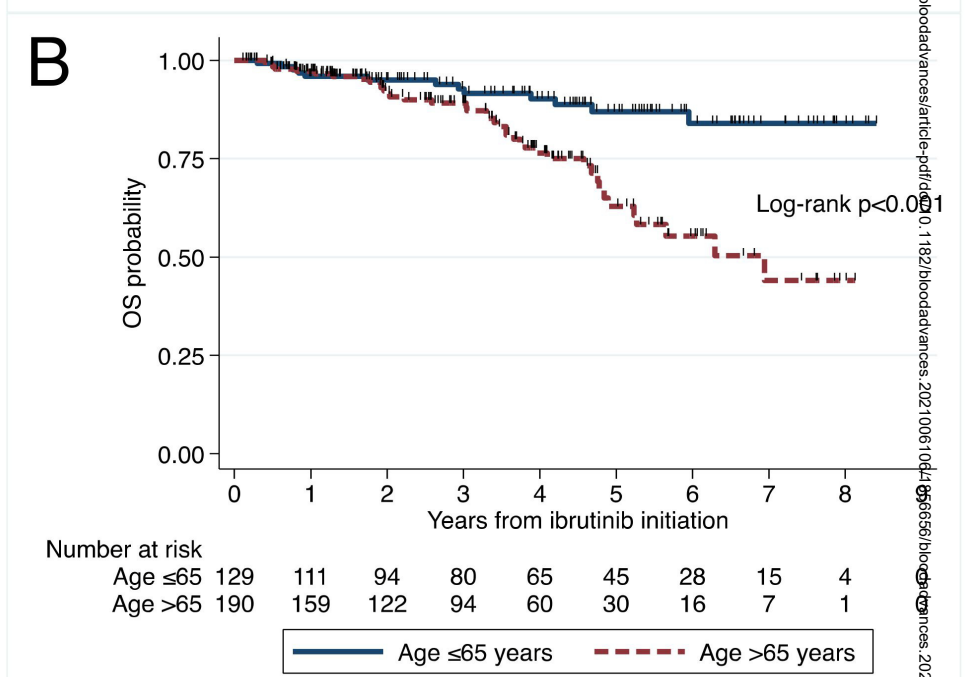
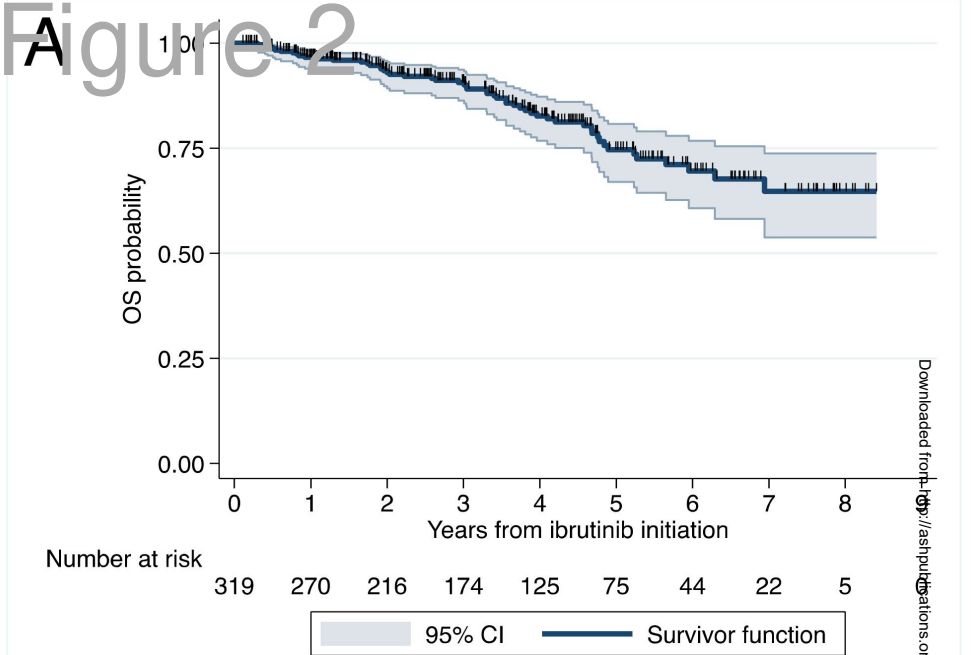


Figure 2



Downloaded from <https://ashpubs.org/bloodadvances/article-pdf/6/1/182/11821006106.pdf> by Steven Trevisan on 23 January 2022