

Quantitative MYD88 L265P and flow cytometry levels for outcome determination in IgM gammopathies: the SAL-TO study

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Abstract:

Waldenström macroglobulinemia (WM) is a rare indolent B-cell lymphoproliferative disorder, often preceded by a history of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS). In this retrospective multicentric study, we collected real-life data from 577 IgM gammopathy patients (221 symptomatic WM, sWM, 245 asymptomatic WM, aWM, 111 IgM-MGUS) from 22 Spanish Centers, with a validation cohort of 166 patients (73 sWM, 71 aWM, 22 IgM-MGUS) from University Hospital of Torino, Italy. Median overall survival (OS) was 126.7 months for the Spanish cohort and 202.8 for the Torino cohort. Multivariate analysis identified significant age > 65 years, male gender, diagnosis of sWM and beta-2-microglobulin >3 as significant predictors for shorter OS. Additionally, age > 65 years, bone marrow (BM) biopsy infiltration, haemoglobin <11.5 g/dL and platelets <100.000/mm³ were associated with shorter time to first treatment (TTFT). Pooling data from both cohorts revealed that baseline BM quantitative MYD88 L265P/MYD88 WT ratio > 0.162 (either by ddPCR or quantitative PCR) together with multiparameter flow cytometry (MFC) infiltration >4.39% had a significant impact on OS and TTFT; the combination of MYD88 and MFC levels allowed to stratify patients into high-, intermediate-, and low-risk groups, with high-risk IgM gammopathy patients showing increased disease-related death in competing risk analysis.

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1 TITLE

2

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41 KEY WORDS

42 Waldenström's Macroglobulinemia, IgM-MGUS, prognostic factors, MYD88, molecular medicine,
43 flow cytometry

44

45 KEY POINTS

46

47 Combining MYD88 L265P and flow cytometry allowed stratification into risk groups with distinct
48 survival outcomes

49 RUNNING TITLE

50 Laboratory prognostic tools in WM and IgM-MGUS

51

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62 COMPETING INTEREST

63 GB: speaker's bureau and advisory board: Janssen, BMS, GSK, Novartis.

64 All other authors declare that they have no potential conflicts of interest.

65

66 **Data Sharing**

67 Data are available via email by the corresponding author

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69

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78 **Key Point**

79 Combining MYD88 L265P and flow cytometry allowed stratification into risk groups with distinct
80 survival outcomes

81

82 **Abstract**

83

84 Waldenström macroglobulinemia (WM) is a rare indolent B-cell lymphoproliferative disorder, often
85 preceded by a history of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS).
86 In this retrospective multicentric study, we collected real-life data from 577 IgM gammopathy
87 patients (221 symptomatic WM, sWM, 245 asymptomatic WM, aWM, 111 IgM-MGUS) from 22
88 Spanish Centers, with a validation cohort of 166 patients (73 sWM, 71 aWM, 22 IgM-MGUS) from
89 University Hospital of Torino, Italy. Median overall survival (OS) was 126.7 months for the
90 Spanish cohort and 202.8 for the Torino cohort. Multivariate analysis identified significant age > 65
91 years, male gender, diagnosis of sWM and beta-2-microglobulin >3 as significant predictors for
92 shorter OS. Additionally, age > 65 years, bone marrow (BM) biopsy infiltration, haemoglobin
93 <11.5 g/dL and platelets <100.000/mmc were associated with shorter time to first treatment
94 (TTFT). Pooling data from both cohorts revealed that baseline BM quantitative *MYD88*
95 L265P/*MYD88* WT ratio > 0.162 (either by ddPCR or quantitative PCR) together with
96 multiparameter flow cytometry (MFC) infiltration >4.39% had a significant impact on OS and
97 TTFT; the combination of *MYD88* and MFC levels allowed to stratify patients into high-,
98 intermediate-, and low-risk groups, with high-risk IgM gammopathy patients showing increased
99 disease-related death in competing risk analysis.

100

101

102 **Introduction**

103

104 Waldenström macroglobulinemia (WM) is a rare indolent B-cell lymphoproliferative disorder
105 characterized by bone marrow (BM) involvement by lymphoplasmacytic cells secreting monoclonal
106 IgM protein.^{1,2}

107 Although a familial predisposition has been demonstrated,³ the main risk factor for WM remains a
108 history of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS),⁴ with a
109 probability of progression to WM or to other lymphoproliferative disorders of 1.5-2% per year.⁴
110 Important advances in the understanding of the biology of WM have been made in the last few
111 years. By means of whole genome sequencing (WGS), Treon et al. identified *MYD88* L265P as a
112 highly recurrent somatic mutation in 90% of patients with WM.⁵ Several studies using different
113 techniques, such as Sanger sequencing, polymerase chain reaction (PCR) and allele-specific qPCR
114 (ASqPCR),^{6,7,8} confirmed the presence of *MYD88* L265P mutation in WM; moreover, it was also
115 demonstrated on BM samples in 50-80% of patients with IgM-MGUS^{7,8,9,10} and it can also be
116 identified in peripheral blood (PB),¹¹ in particular in cell-free DNA (cfDNA) of IgM gammopathy
117 patients.^{12,13}
118 From a clinical standpoint, WM typically shows an indolent clinical course, with about 25% of
119 patients being asymptomatic at initial diagnosis, despite a heterogeneous disease behaviour. Indeed,
120 for “smoldering” WM patients there are currently no indications for the initiation of treatment until
121 the development of significant symptoms.^{14,15,16} However, these patients have a high risk of
122 transformation from smoldering WM to active WM and should be monitored closely.¹⁷
123 Over the years, many attempts have been made to predict IgM gammopathy patients’ outcomes
124 based on clinical assessments, although applying different criteria for WM or IgM-MGUS diagnosis
125 and for treatment initiation.^{4,18,19,20,21,22,23} For active WM patients who require treatment, the
126 International Prognostic Scoring System for WM (IPSS-WM) was designed to assess survival after
127 treatment initiation and OS,²⁴ stratifying patients into 3 risk categories with corresponding 5-year
128 survival rates of 87%, 68% and 36%, respectively. Later on, further revisions of the IPSS-WM
129 refined prognostication in active WM^{25,26}, while in the field of asymptomatic WM (aWM) patients,
130 Bustoros et al. developed a prognostic score for disease progression, depending on BMB evaluation
131 and basic clinical findings.²⁷
132 Some authors have also tried to assess the impact on patients’ outcome of laboratory prognostic
133 factors, such as multiparameter flow cytometry (MFC) features²⁸, cytogenetics and molecular
134 biology characterization. Even in absence of disease-defining cytogenetic abnormalities, deletions
135 in the chromosome 6q are present in 40-50% of cases of WM patients, and have been shown to
136 negatively impact on patients’ prognosis,^{29,30} being associated with a higher IPSS-WM score and a
137 shorter time to treatment, PFS and OS.^{31,32,33,34}
138 The impact of molecular factors in determining WM patients’ prognosis is still not entirely
139 elucidated: some studies have suggested that *MYD88* L265P mutation may impact on OS and play a
140 role in disease progression, promoting the transition from IgM-MGUS to WM or to other

141 lymphoproliferative diseases;^{9,27,35,36} on the contrary, other studies showed no effect on OS or
142 time to progression¹⁴ or underlined higher progression rates to symptomatic WM (sWM) and more
143 aggressive clinical course in *MYD88*^{WT} patients.^{27,36,37,38} Moreover, *CXCR4*^{WHIM} mutations, the
144 second most frequent genetic alteration in WM, have been proven to impact on response to therapy
145 and PFS,^{39,40} without affecting OS.^{33,37} Finally, similar to many other neoplasms, *TP53* alterations
146 confer worse outcome in terms of OS,^{40,41,42} irrespectively of IPSS-WM score; of note, such
147 alterations were not identified in patients carrying IgM-MGUS.^{41,43}

148
149

150 **Aims**

151

152 The study aims to: 1) collect a large series of IgM gammopathy patients treated in real life settings;
153 2) determine patients' outcomes in terms of OS, time to first treatment (TTFT) and PFS; 3) identify
154 clinical factors affecting disease progression and survival; 4) evaluate the impact of baseline
155 molecular and flow cytometry analyses impact on survival and need for WM treatment.

156

157

158 **Materials and methods**

159

160 *Patients selection*

161

162 Spanish cohort.

163 The registry of monoclonal gammopathies based in Salamanca and in the region of Castilla y Leon
164 (Spain) was investigated for IgM-secreting disorders (Figure 1); only patients with confirmed IgM-
165 MGUS, aWM or sWM¹ were selected for the present study. All patients provided written informed
166 consent in accordance with Helsinki's declaration.

167

168 Torino cohort.

169 Electronic health records of patients followed up or treated at the Centre of Torino (Division of
170 Haematology 1, Department of Biotechnology and Health Sciences, University Hospital Città della
171 Salute e della Scienza, University of Torino, Italy) were scanned to identify patients with IgM
172 monoclonal gammopathy; patients with confirmed diagnosis of IgM-MGUS, aWM or sWM were
173 selected by the study team, using the same criteria employed for the Spanish cohort (figure 1). All
174 patients provided written informed consent in accordance with Helsinki's declaration.

175

176 *Sample collection and laboratory analysis*

177

178 BM and PB samples were extracted from patients at first diagnosis of IgM gammopathy or at
179 disease progression according to locally established procedures, for both study cohorts; BMB was
180 performed per clinical practice, mainly when criteria for treatment initiation or suspicion of
181 progressive/transformed disease were met.

182 Genomic DNA for molecular studies from evaluated patients was extracted as previously described
183 and analysed for the presence of the *MYD88* L265P.⁴⁴ Alternatively, *MYD88* mutational status was
184 determined by droplet digital PCR (ddPCR) on both BM and PB samples, as previously described.¹²

185 All patients tested in Torino were analysed by ddPCR. Of note, neither cohorts applied CD19+ cells
186 selection prior to molecular study, while it was only performed on selected cases in Spanish cohort
187 prior to fluorescence in situ hybridization (FISH) analysis.

188 Deletions of 6q were assessed in IgM-MGUS and WM by either simple interphase FISH performed
189 on cell nuclei from whole-BM samples or CD19-selected cells using a previously published
190 technique.³¹

191 Immunophenotypic evaluation was done using conventional methods, panels of monoclonal
192 antibodies previously described⁴⁵ and following the general recommendations of the EuroFlow
193 group for the immunophenotypic evaluation of haematological malignancies. The sensitivity of
194 MFC assay was 0,004% for at least 500000 events.

195

196 *Statistical analyses*

197

198 Statistical analyses were carried out using R (v 4.3.1). Survival curves were plotted with Kaplan–
199 Meier method and compared with log-rank test. Medians between groups for continuous variables
200 were compared by the Kruskal-Wallis (for non-normal variables) or the one-way ANOVA test (for
201 normal variables); the chi-squared test or Fisher's exact test for small study samples, were employed
202 for categorical variables. OS was measured from the date of initial diagnosis to the date of death
203 from any cause. For asymptomatic cases, TTFT was defined as the time between diagnosis and
204 progression to sWM requiring active treatment. PFS was defined as the time between first line WM
205 treatment and the date of progression or death from any cause. The Cox proportional hazards model
206 was implemented for the univariate and multivariate survival analyses. In particular, for the
207 multivariable model, an AIC-based backward stepwise algorithm (R function `stats::step`) was used
208 to perform the variable selection (restricting the dataset to have no missing data) in order to
209 determine the most relevant covariates and to analyse potential confounding factors. Once the
210 covariates of interest were obtained from the restricted dataset, they were applied to the full dataset
211 with respect to each endpoint.

212 Given the larger size of the Spanish dataset, the statistical models were constructed using these data.
 213 Subsequently, the Torino dataset was merged to evaluate whether there were differences between
 214 the two populations. An analysis to evaluate the IPSS-WM and the R-IPSS WM scores was also
 215 performed on both cohorts (Spain, Torino). Moreover, the AWM score was tested in aWM patients
 216 from the Torino series.

217 In addition, we worked out a cut-off for quantitative *MYD88* and MFC infiltration in BM; the cut-
 218 off points were identified using the “surv_cutpoint” function in R, on Spanish dataset, and extended
 219 to Torino analysis.

220 Finally, competing risk analysis was performed on OS using “cuminc” function of “tidycmprsk”
 221 package of R.

222

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229

230

231 **Results**

232 *Patients' characteristics*

233

234 Data from 903 patients across 22 Spanish centers were collected between 1976 and 2019 in the
 235 considered registry; 577 cases with local diagnosis of IgM-MGUS, aWM or sWM were selected,
 236 while the remaining cases were excluded due to diagnosis of other lymphoproliferative diseases,
 237 misregistration or missing data (figure 1). Among patients followed in Torino, Italy, 166 IgM
 238 gammopathy patients with available baseline and follow up data were selected according to the
 239 same criteria; of note, these patients were diagnosed from 1988 to 2020, although the vast majority
 240 (161/166) were diagnosed after the year 2000.

241 Demographic and clinical data at initial diagnosis are reported in table 1 (Spanish and Torino
 242 cohort).

243

244 **Table 1.** *Characteristics of IgM gammopathy patients enrolled in the study – Spanish and Torino cohort.*
 245 *Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; WM, Waldenström*
 246 *Macroglobulinemia; Hb, hemoglobin; B2M, beta-2-microglobulin; IgM, immunoglobulin M; LDH, lactate*
 247 *dehydrogenase; Ig, immunoglobulin; k, free light kappa chain; l, free light lambda chain; IPSS-WM,*

248 *International Prognostic Score System for Waldenström Macroglobulinemia; Int, intermediate; FISH,*
 249 *Fluorescence in situ hybridization*

250
 251 When they were first evaluated, 111 Spanish patients were locally diagnosed with IgM-MGUS, 245
 252 with aWM and 221 with sWM. IPSS-WM score for sWM patients at diagnosis was low in 30
 253 (18.2%), intermediate in 68 (41.2%), and high in 67 (40.6%) in the Spanish cohort; low in 10
 254 (20.0%), intermediate in 18 (36.0%), high in 22 (44.0%) in the Torino cohort (p=0.804 for the
 255 distribution in the two cohorts).

256
 257 **Figure 1.** Consort diagram of enrolled patients in the two cohorts. Abbreviations: MGUS, monoclonal
 258 gammopathy of undetermined significance; aWM, asymptomatic Waldenström Macroglobulinemia; WM,
 259 Waldenström Macroglobulinemia; NA, not available; FU, follow-up

260
 261 *Treatments*

262
 263 In the Spanish retrospective series, comprising patients followed from 1976 to 2019, 78/577
 264 (13.5%) patients had missing treatment details in first line and were excluded from analysis of
 265 treatments received. Among 499 patients with available details of treatments received, 321/499
 266 (64.3%) (diagnosed with IgM-MGUS or aWM) were followed-up in a watch and wait (W&W)
 267 approach, while 178/499 (35.7%) WM patients received initial therapy (supplementary table 1). At
 268 last follow-up, 208/321 (64.8%) patients remained asymptomatic, while 41/321 (12.8%) had
 269 progressed to WM and required active treatment; follow-up data were missing about 72 pts.

270 Most employed treatments in first line included chlorambucil-based regimens (n=98/178, 55%),
 271 dexamethasone-rituximab-cyclophosphamide scheme (DRC, n=19/178, 10.7%), fludarabine-
 272 containing regimens (n=6/178, 3.4%). Overall, 51/178 patients (28.6%) received rituximab in first
 273 line (single agent or any combination).

274
 275 In the Torino cohort, 5/166 patients were excluded from analysis of treatment received for missing
 276 information; 60/161 patients (37.3%) remained asymptomatic and did not receive therapy during
 277 follow up. First line treatments used at any time included DRC (n=27/73, 37%), R-Benda (n=8/73,
 278 11%), chlorambucil-based schemes (n=6/73, 8.2%). Rituximab was administered in first line to
 279 69.8% of treated patients.

280
 281 *Survival analyses*

282 **OS**

283 In the Spanish cohort, at last follow up (FU), 305 (52.9%) patients were alive, with a median FU of
 284 100.6 months (IQR 55.5;166.6). Median OS was 126.7 months (IQR: 58.7;193.6); OS at 5 years

285 was 74.0% (95%CI 70.1-78.2), and at 10 years 52.5% (95%CI 47.3-58.2%) (figure 2a). Median OS
 286 for IgM-MGUS vs. aWM vs. sWM patients was 180.6 (IQR 111.3;-) vs. 143.6 (80.9;199.7) vs.
 287 85.0 (38.8;151.6) months, (p<0.001, HR for death in SWM group: 2.481) in univariate analysis
 288 (figure 2c).

289

290 In the Torino cohort, at last FU, 132 (79.5%) patients were alive, with a median FU of 71.2 (IQR
 291 39.6; 115.4) months. Median OS was 202.8 months (IQR 109.0;-); OS at 5 years was 85.7% (95%
 292 CI 80.0-91.8), and 71.6% (61.7-83.1) at 10 years from initial diagnosis (figure 2b). Median OS
 293 stratified for initial diagnosis was 131.0 (131.0;-) months for IgM-MGUS, not-reached (131.4;-)
 294 for aWM, 174.4 (65.9;-) months for sWM (p=0.084) (figure 2).

295

296 **Figure 2.** Overall survival of Spanish cohort (A) and Torino cohort (B); overall survival stratified for initial
 297 diagnosis of MGUS, aWM or sWM (C, D)

298

299 **TTFT**

300 TTFT was evaluated for all asymptomatic patients at baseline, including IgM-MGUS and aWM; for
 301 the Spanish cohort, median TTFT was 228.5 months (IQR 146.9;-), with 89.1% of patients not
 302 requiring treatment after 5 years of FU (95%CI 85.5-92.9). For patients followed up in Torino,
 303 median TTFT was 165.6 months (IQR 49.4;207.9), and probability to remain asymptomatic was
 304 71.4% at 5 years (95%CI 60.9-83.7) (figure 3).

305

306 **Figure 3.** Time to first treatment in asymptomatic patients belonging to Spanish cohort (A) and Torino
 307 cohort (B) (top panels); Progression free survival in patients requiring treatment at any time, belonging to
 308 Spanish cohort (A) and Torino cohort (B) (bottom panels)

309

310 **PFS**

311 PFS was evaluated in patients receiving treatment for symptomatic WM at any time; among
 312 patients with available data, median PFS was 42.4 months (IQR 13.6;91.0) for Spanish cohort, and
 313 38.0 months (IQR 13.9;75.6) for Torino cohort (figure 3, bottom panels). At 2 years, PFS
 314 probability was 64.6% (95%CI 58.8-70.9) and 63.3% (54.2-73.8), respectively. Importantly, there
 315 was no difference in PFS outcome between patients who received active treatment at diagnosis and
 316 patients initially undergoing watch and wait approach and then progressing to WM (p=0.34 and
 317 p=0.24 in the 2 cohorts).

318 Finally, 22/577 (4.8%) and 5/166 (3.1%) patients in the two examined cohorts experienced
 319 transformation from IgM gammopathy to aggressive lymphoma during FU, showing very poor

320 survival (figure S1). No significant difference in the incidence of transformation in the two groups
 321 was highlighted ($p=0.51$).

322
 323 *Clinical prognostic factors*

324
 325 Clinical data at baseline, including age, sex, IgM levels, haemoglobin (Hb), platelets (Plts), ECOG
 326 PS, presence of hyperviscosity, BMB infiltration, albumin, beta2microglobulin (B2M), LDH,
 327 *MYD88 L265P* mutational ratio, MFC infiltration in BM, were tested for prognostic significance in
 328 terms of OS, TTFT (for asymptomatic patients) and PFS (for patients treated at any time);
 329 significant variables for each cohort are resumed in table 3.

330
 331 A multivariable model for OS, PFS, and TTFT (resumed in table 2) was built, as previously
 332 described, for the Spanish cohort. This model was then used to evaluate a merged population (Spain
 333 + Torino) in order to spot eventual differences between the two cohorts. Since a p -value >0.05 was
 334 found, we may conclude that there is no evidence of difference between these groups.

335 For OS prediction, the algorithm identified age > 65 years, male sex, initial diagnosis of SWM and
 336 B2M >3 as statistically significant variables. On the other hand, multivariable analysis for TTFT
 337 was built for asymptomatic patients (IgM-MGUS, aWM), producing a model with age, BMB
 338 infiltration, Hb <11.5 g/dL and Plts $<100.000/mm^3$ as significant variables. Finally, multivariable
 339 analysis for PFS in symptomatic WM patients (SWM at diagnosis + patients progressing from
 340 watch and wait approach to SWM) was performed, and age and LDH ratio resulted statistically
 341 significant.

342
 343 In addition, we evaluated IPSS-WM and R-IPSS-WM scores in Spanish WM patients and found
 344 that they both retained statistical significance ($p <0.001$ for both scores, supplementary figure 2),
 345 confirmed also in multivariable analysis; on the other hand, AWM score could not be tested in
 346 aWM patients from the Torino series due to low number of patients with available complete data
 347 ($n=29$).

348

349 **Table 2.** *Multivariable analysis for OS, TTFT and PFS in Spanish and Torino cohort. Only variables with*
 350 *statistically significant effect for multivariable models are shown. Abbreviations: HR, hazard ratio;*
 351 *IC, interval of confidence; p, p-value; Hb, hemoglobin; BMB, bone marrow biopsy; B2M, beta-2-*
 352 *microglobulin; IgM, immunoglobulin M; LDH, lactate dehydrogenase; IPSSWM, International*
 353 *Prognostic Score System for Waldenström Macroglobulinemia; MGUS, monoclonal gammopathy of*
 354 *undetermined significance; aWM, asymptomatic Waldenström Macroglobulinemia; sWM,*
 355 *symptomatic Waldenström Macroglobulinemia.*

356

357 *Prognostic significance of molecular and flow cytometry factors*

358
359 For patients with available BM *MYD88* L265P evaluation at baseline (n=298, 51.6% for Spanish
360 cohort, n= 142, 85.5% for Torino cohort), we analysed outcome in terms of OS and TTFT
361 according to both quantitative PCR methods results.

362 Patients from Spanish cohort and Torino cohort were pooled and divided into high vs low *MYD88*
363 groups (cutpoint set at 0.162, defined as previously described).

364 In univariate analysis, *MYD88*^{low} group showed better OS (p=0.005, HR=0.44) and TTFT
365 (p=0.024, HR=0.33) compared to *MYD88*^{high} group (table 3). In multivariable analysis, statistical
366 significance was not confirmed (p=0.622) for OS, although there was a trend to significance for
367 TTFT (p=0.06).

368 Regarding the prognostic impact of *MYD88* on OS specifically in IgM-MGUS patients, data were
369 available on 37 patients, classified as *MYD88* low (n=17) or *MYD88*WT (n=20), while no patient
370 scored *MYD88* high; no difference in OS was observed in univariate analysis between these two
371 subgroups (p=0.2, supplementary figure 6).

372 Additionally, molecular burden was compared to the percentage of marrow infiltration by BMB
373 (available only for Torino cohort), showing a moderate positive Pearson's correlation (r=0.653,
374 p<0.0001), as well as to the infiltration reported by BM MFC, showing a strong positive linear
375 correlation (r = 0.73, p < 0.0001). Patients in the *MYD88*^{high} group generally showed higher BMB
376 infiltration (approximately 70 to 90%), though rare outliers with low marrow infiltration (as low as
377 20%) were observed, indicating possible sampling errors as well as biological heterogeneity.

378 The pooled population was also analysed for the prognostic significance of baseline clonal B cell
379 infiltration by MFC in available BM samples, with cut-off point set at 4.39%. In univariable
380 analysis, MFC^{low} patients had increased OS (p=0.033, HR=0.65) and TTFT (p=0.008, HR=0.37)
381 compared to MFC^{high} group (table 3). In multivariable analysis MFC lost statistical significance for
382 OS (p=0.51), while it retained its importance for TTFT (p=0.025, HR=0.37). For IgM-MGUS
383 patients, similarly, MFC burden affected TTFT (p=0.006, supplementary figure 7) but did not show
384 impact on OS (p=0.5).

385

386 **Table 3.** Univariate analysis for OS, TTFT and PFS for molecular and flow cytometry variables in Spanish
387 and Torino cohort. Abbreviations: HR, hazard ratio; IC, interval of confidence; p, p-value
388

389 In addition, we combined both MFC and *MYD88* baseline levels, and patients were stratified into
390 low, intermediate and high risk (LR: MFC^{low}/*MYD88*^{low}, IR: either MFC^{low}/*MYD88*^{high} or
391 MFC^{high}/*MYD88*^{low}; HR: MFC^{high}/*MYD88*^{high}). Based on MFC and molecular results, no IgM-
392 MGUS patient was assigned to the HR group; 16 HR patients had sWM while 8 had aWM;

393 moreover, among sWM patients with high IPSS-WM risk, 7 patients were in the LR group, 18
394 patients in the intermediate group and 6 in the HR group.

395 In univariate analysis, HR group significantly differed from the others in terms of OS (n=156,
396 p=0.005, HR=3.28, figure 4) and TTFT (n=92, p=0.003, HR=9.61); for TTFT, intermediate-risk
397 group (p=0.015, HR=4.76) was also significantly different from reference group (low,
398 supplementary figure 3).

399 Multivariable analysis confirmed these results, highlighting the strong prognostic significance of
400 molecular and MFC evaluations combined (for OS: p=0.038, HR=2.81; for TTFT: p=0.026,
401 HR=7.91 for intermediate vs. low risk and p=0.003, HR=24.68 for high vs. low risk).

402

403 Finally, competing risk analysis was performed; among 545 evaluable cases, at 5 years from
404 diagnosis 12.0% (95%CI:9.6%, 16%) deaths were not related to WM, while 7.1% (95%CI: 5.0%,
405 9.6%) were considered related (supplementary figure 4); among patients in the MYD88/MFC HR
406 group, there was a statistically significant increase in disease-related, but not unrelated deaths at 5
407 years (p=0.002, 2.2%, 95%CI 0.17%-10% vs 19%, 95%CI 5.6%-38% for LR vs HR respectively,
408 supplementary figure 5).

409

410 **Figure 4.** Outcome in terms of OS according to BM MYD88^{L65P} levels and to BM MFC levels

411

412 Discussion

413

414 Previous real life studies reporting on survival outcomes of WM patients, for instance the Rory
415 Morrison Registry in the UK and the Swedish lymphoma registry, showed a five-year OS ranging
416 from 60 to 90%, both with a median follow-up of around 6 years;^{20,46} in our series, including also
417 asymptomatic WM and IgM-MGUS, 5-years OS was comparable (85% for the Torino cohort, 74%
418 for the Spanish cohort), after a median FU of 71 vs 100 months respectively. Of note, the slightly
419 superior OS outcome of the Torino cohort might be explained by the more recent IgM gammopathy
420 diagnosis and the more extensive adoption of rituximab-containing regimens in first and subsequent
421 treatment lines.

422 However, in published registry studies, molecular evaluations and MFC data were limited or not
423 available, so prognostic evaluation relied solely on clinical factors. Indeed, prognostication for
424 symptomatic WM was historically established based on the IPSS-WM score and its revised
425 version,^{24,25} stratifying patients based on age and baseline characteristics.

426 In our study, both IPSS-WM and R-IPSS-WM were confirmed able to correctly identify sWM
427 patients' risk, while for aWM, the score proposed by Bustoros et al was not reproducible, probably

428 due to the low number of patients with data about BMB percentage of invasion available.²⁷
429 Moreover, analysing the whole IgM gammopathy series (IgM-MGUS, aWM and sWM taken
430 together), clinical factors were confirmed important both for OS (age, sex, B2M and diagnosis) and
431 TTFT (age, Hb, Plts and BMB involvement). Indeed, IPSS-WM was initially built at a time (prior
432 to 2002) when nucleoside analogues represented around one third of first line therapies, while
433 rituximab was only administered to 4% of patients; nevertheless, it has been still confirmed a
434 feasible and powerful tool. However, in the rapidly changing field of WM, where both
435 understanding of its biology and treatment were revolutionized by the discovery of *MYD88* L265P
436 mutation, the need to incorporate translational knowledge to prognostic assessment is still unmet.
437 Indeed, while *MYD88* L265P evaluation is already considered as part of standard initial evaluation
438 in suspected WM, particularly helpful in the differential diagnosis with other lymphoproliferative
439 neoplasms, its role in prognostication is still debated.

440 Actually, previous findings have highlighted that *MYD88*^{WT} WM patients have inferior survival
441 outcomes,³⁶ and show slower and less profound responses with Bruton's tyrosine kinase inhibitor
442 (BTKi), especially with ibrutinib;⁴⁰ on the other hand, a large cohort study by Abeykoon et al. did
443 not show inferior OS or time to next treatment in *MYD88*^{WT} vs *MYD88* L265P WM patients, while
444 there was an increased occurrence of transformation to diffuse large B-cell lymphoma (DLBCL).¹⁴
445 For IgM-MGUS patients, a study by Varettoni et al. showed lower progression rates to overt WM in
446 *MYD88*^{WT} compared to *MYD88* L265P cases.⁹ In the same study, *MYD88* L265P allele burden by
447 ASqPCR on BM CD19+ selected mononuclear cells showed a significant correlation with
448 progression from MGUS to WM, albeit with a mild effect; these findings were later confirmed by a
449 recent study by Moreno et al. using ddPCR in IgM-MGUS and aWM.⁴⁷ Of note, for IgM-MGUS
450 patients, reproducibility of prognostic effect of molecular factors might be affected by low disease
451 burden; the use of highly sensitive techniques is therefore key to correctly estimate prognosis.
452 Indeed, past studies have shown heterogeneous results due to different PCR techniques, the variable
453 application of CD19+ sorting and the adoption of different diagnostic criteria⁴⁸.

454 To date, however, there were no published data regarding the effect of high versus low *MYD88*
455 L265P baseline levels on OS and TTFT in WM patients. In our study, highly sensitive techniques
456 for *MYD88* quantitative evaluation, either by ddPCR or ASqPCR, together with MFC analysis,
457 allowed for a precise definition of disease burden at the time of IgM gammopathy diagnosis.
458 Interestingly, the identified MFC cut-off points (4.39% clonal B cell infiltration in BM) to separate
459 high vs low disease burden was actually quite low, close to the median results in aWM subgroup
460 (3.55%). On the contrary, *MYD88* L265P cut-off point (ratio MUT/WT=0.162 (AF 13,4%)) was
461 higher than median *MYD88* L265P identified in sWM group (ratio MUT/WT=0.04 (AF 3.85%)),

462 thus identifying patients with very high molecular disease burden. We also observed a strong
463 correlation of *MYD88* L265P results with BM MFC infiltration, higher than the one between
464 *MYD88* L265P and the percentage of infiltration in BMB by conventional histopathology
465 observation.

466 From a technical standpoint, molecular analysis was not homogeneous between the two cohorts: all
467 Torino samples were analyzed using ddPCR, which offers higher sensitivity, whereas the vast
468 majority (>95%) of Spanish samples were assessed by ASqPCR, reflecting clinical practice at the
469 time of initial diagnosis. Although both techniques correctly identified molecular high-risk patients,
470 this discrepancy represents a limitation of our retrospective, real-world study and highlights the
471 absence of standardized molecular approaches in this disease. This lack of harmonization
472 underscores the need for future efforts aimed at method standardization to ensure comparability and
473 consistency across studies and clinical settings.

474 By multivariable analysis, the combination of MFC and molecular variables identified a high-risk
475 group consisting in around 15% of the evaluable patients. Interestingly, a minority of aWM cases
476 (around 9%), but no IgM-MGUS patients, was classified as HR in terms of the *MYD88*/MFC score:
477 thus, it is conceivable that this kind of evaluation could help represent the risk of progression and
478 disease-related death even in asymptomatic patients. Indeed, a significant fraction (around 22%) of
479 the patients who scored high at IPSS-WM was re-classified as LR by the molecular/MFC score.

480

481

482 **Conclusions**

483 To our knowledge, this is the first time quantitative *MYD88* L265P evaluation and MFC degree of
484 infiltration in BM were proven prognostic of patients' survival and need for treatment in a large
485 IgM gammopathy series; moreover, molecular and flow cytometry results have never been
486 combined before to generate translational data. Validation of these results in prospective studies
487 might add greatly to prognostic evaluation of IgM gammopathy, particularly to improve definition
488 of high-risk WM patients.

489

490 **Author Contribution Statement**

491 D, JC, DD, RGS and SF: Conceptualization

492 ID, VP, MF, DM, SM, SR, GMZ: Data curation

493 ID, JC, VP, EA: Writing - original draft

494 DM, SM, GMZ, MG: Methodology

495 ID, VP, GB, EO, AR, IM, EF CA, AGM, AGC, RH, JD, FC, NP, VGC, SR, MC, CC, EA, MCC,

496 AM, NCG, FS, RGS: Investigation

497 SF, BB, MGD, RGS: Supervision

498 Review and editing: all Authors

499

500 **Conflict of Interest Statement**

501 S.F. is a consultant for Janssen, EUSA Pharma, Abbvie and Sandoz; is on the advisory board of
 502 Janssen, EUSA Pharma, Recordati, Incyte, Roche, Astra Zeneca, Italfarmaco and Behring; received
 503 speaker's honoraria from Janssen, EUSA Pharma, Recordati, Lilly, Beigene, Gilead and Gentili; and
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 505

506 References

- 507
- 508 1. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's
 509 macroglobulinemia: consensus panel recommendations from the Second International
 510 Workshop on Waldenstrom's Macroglobulinemia. *Semin Oncol.* 2003 Apr;30(2):110-5. doi:
 511 10.1053/sonc.2003.50082. PMID: 12720118.
- 512 2. Campo E, Jaffe ES, Cook JR, et al. The International Consensus Classification of Mature
 513 Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Blood.* 2022 Sep
 514 15;140(11):1229-1253. doi: 10.1182/blood.2022015851. Erratum in: *Blood.* 2023 Jan
 515 26;141(4):437. doi: 10.1182/blood.2022019016. PMID: 35653592; PMCID: PMC9479027.
- 516 3. Kristinsson SY, Björkholm M, Goldin LR, McMaster ML, Turesson I, Landgren O. Risk of
 517 lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic
 518 lymphoma/Waldenstrom macroglobulinemia patients: a population-based study in Sweden.
 519 *Blood.* 2008 Oct 15;112(8):3052-6. doi: 10.1182/blood-2008-06-162768. Epub 2008 Aug
 520 13. PMID: 18703425; PMCID: PMC2569164.
- 521 4. Kyle RA, Larson DR, Therneau TM, et al. Long-Term Follow-up of Monoclonal
 522 Gammopathy of Undetermined Significance. *N Engl J Med.* 2018 Jan 18;378(3):241-249.
 523 doi: 10.1056/NEJMoa1709974. PMID: 29342381; PMCID: PMC5852672.
- 524 5. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's
 525 macroglobulinemia. *N Engl J Med.* 2012 Aug 30;367(9):826-33. doi:
 526 10.1056/NEJMoa1200710. PMID: 22931316.
- 527 6. Jiménez C, Sebastián E, Chillón MC, et al. MYD88 L265P is a marker highly characteristic
 528 of, but not restricted to, Waldenström's macroglobulinemia. *Leukemia.* 2013
 529 Aug;27(8):1722-8. doi: 10.1038/leu.2013.62. Epub 2013 Feb 28. PMID: 23446312.
- 530 7. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the
 531 MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related
 532 lymphoid neoplasms. *Blood.* 2013 Mar 28;121(13):2522-8. doi: 10.1182/blood-2012-09-
 533 457101. Epub 2013 Jan 25. PMID: 23355535.
- 534 8. Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenström macroglobulinemia,
 535 immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative
 536 disorders using conventional and quantitative allele-specific polymerase chain reaction.
 537 *Blood.* 2013 Mar 14;121(11):2051-8. doi: 10.1182/blood-2012-09-454355. Epub 2013 Jan
 538 15. Erratum in: *Blood.* 2013 Jun 27;121(26):5259. Varettoni, Maria [corrected to Varettoni,
 539 Marzia]. PMID: 23321251; PMCID: PMC3596964.
- 540 9. Varettoni M, Zibellini S, Boveri E, et al. A risk-stratification model based on the initial
 541 concentration of the serum monoclonal protein and MYD88 mutation status identifies a
 542 subset of patients with IgM monoclonal gammopathy of undetermined significance at high
 543 risk of progression to Waldenström macroglobulinaemia or other lymphoproliferative
 544 disorders. *Br J Haematol.* 2019 Nov;187(4):441-446. doi: 10.1111/bjh.16086. Epub 2019 Jul
 545 5. PMID: 31276195.
- 546 10. Landgren O, Staudt L. MYD88 L265P somatic mutation in IgM MGUS. *N Engl J Med.*
 547 2012 Dec 6;367(23):2255-6; author reply 2256-7. doi: 10.1056/NEJMc1211959. PMID:
 548 23215570.
- 549 11. Xu L, Hunter ZR, Yang G, et al. Detection of MYD88 L265P in peripheral blood of patients
 550 with Waldenström's Macroglobulinemia and IgM monoclonal gammopathy of undetermined

- 551 significance. *Leukemia*. 2014 Aug;28(8):1698-704. doi: 10.1038/leu.2014.65. Epub 2014
552 Feb 10. PMID: 24509637.
- 553 12. Drandi D, Genuardi E, Dogliotti I, et al. Highly sensitive *MYD88*^{L265P} mutation detection by
554 droplet digital polymerase chain reaction in Waldenström macroglobulinemia.
555 *Haematologica*. 2018 Jun;103(6):1029-1037. doi: 10.3324/haematol.2017.186528. Epub
556 2018 Mar 22. PMID: 29567768; PMCID: PMC6058774.
- 557 13. Dogliotti I, Jiménez C, Varettoni M, et al. Diagnostics in Waldenström's
558 macroglobulinemia: a consensus statement of the European Consortium for Waldenström's
559 Macroglobulinemia. *Leukemia*. 2023 Feb;37(2):388-395. doi: 10.1038/s41375-022-01762-
560 3. Epub 2022 Nov 26. PMID: 36435884; PMCID: PMC9898035.
- 561 14. Abeykoon JP, Yanamandra U, Kapoor P. New developments in the management of
562 Waldenström macroglobulinemia. *Cancer Manag Res*. 2017 Mar 10;9:73-83. doi:
563 10.2147/CMAR.S94059. PMID: 28331368; PMCID: PMC5354523.
- 564 15. Kapoor P, Ansell SM, Fonseca R, et al. Diagnosis and Management of Waldenström
565 Macroglobulinemia: Mayo Stratification of Macroglobulinemia and Risk-Adapted Therapy
566 (mSMART) Guidelines 2016. *JAMA Oncol*. 2017 Sep 1;3(9):1257-1265. doi:
567 10.1001/jamaoncol.2016.5763. PMID: 28056114; PMCID: PMC5556979.
- 568 16. Pophali PA, Bartley A, Kapoor P, et al. Prevalence and survival of smouldering
569 Waldenström macroglobulinaemia in the United States. *Br J Haematol*. 2019
570 Mar;184(6):1014-1017. doi: 10.1111/bjh.15201. Epub 2018 Mar 13. PMID: 29532912.
- 571 17. Kyle RA, Benson JT, Larson DR, et al. Progression in smoldering Waldenström
572 macroglobulinemia: long-term results. *Blood*. 2012 May 10;119(19):4462-6. doi:
573 10.1182/blood-2011-10-384768. Epub 2012 Mar 26. PMID: 22451426; PMCID:
574 PMC3362362.
- 575 18. Facon T, Brouillard M, Duhamel A, et al. Prognostic factors in Waldenström's
576 macroglobulinemia: a report of 167 cases. *J Clin Oncol*. 1993 Aug;11(8):1553-8. doi:
577 10.1200/JCO.1993.11.8.1553. PMID: 8336194.
- 578 19. Andrade-Campos M, Murillo-Flórez I, García-Sanz R, Giraldo P. Immunoparesis in IgM
579 gammopathies as a useful biomarker to predict disease progression. *Clin Chem Lab Med*.
580 2017 Aug 28;55(10):1598-1604. doi: 10.1515/cclm-2016-0748. PMID: 28284031.
- 581 20. Brandefors L, Melin B, Lindh J, Lundqvist K, Kimby E. Prognostic factors and primary
582 treatment for Waldenström macroglobulinemia - a Swedish Lymphoma Registry study. *Br J*
583 *Haematol*. 2018 Nov;183(4):564-577. doi: 10.1111/bjh.15558. Epub 2018 Sep 10. PMID:
584 30198549.
- 585 21. García-Sanz R, Montoto S, Torrequebrada A, et al. Waldenström macroglobulinaemia:
586 presenting features and outcome in a series with 217 cases. *Br J Haematol*. 2001
587 Dec;115(3):575-82. doi: 10.1046/j.1365-2141.2001.03144.x. PMID: 11736938.
- 588 22. Kyle RA, Ansell SM, Kapoor P. Prognostic factors and indications for treatment of
589 Waldenström's Macroglobulinemia. *Best Pract Res Clin Haematol*. 2016 Jun;29(2):179-186.
590 doi: 10.1016/j.beha.2016.08.014. Epub 2016 Aug 23. PMID: 27825464; PMCID:
591 PMC5117990.
- 592 23. Dhodapkar MV, Hoering A, Gertz MA, et al. Long-term survival in Waldenström
593 macroglobulinemia: 10-year follow-up of Southwest Oncology Group-directed intergroup
594 trial S9003. *Blood*. 2009 Jan 22;113(4):793-6. doi: 10.1182/blood-2008-07-172080. Epub
595 2008 Oct 17. PMID: 18931340; PMCID: PMC2630265.
- 596 24. Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for
597 Waldenström macroglobulinemia. *Blood*. 2009 Apr 30;113(18):4163-70. doi:
598 10.1182/blood-2008-08-174961. Epub 2009 Feb 5. PMID: 19196866.
- 599 25. Kastritis E, Kyrtsolis MC, Hadjiharissi E, et al. Validation of the International Prognostic
600 Scoring System (IPSS) for Waldenström's macroglobulinemia (WM) and the importance of

- 601 serum lactate dehydrogenase (LDH). *Leuk Res.* 2010 Oct;34(10):1340-3. doi:
602 10.1016/j.leukres.2010.04.005. Epub 2010 May 5. PMID: 20447689.
- 603 26. Kastiris E, Morel P, Duhamel A, et al. A revised international prognostic score system for
604 Waldenström's macroglobulinemia. *Leukemia.* 2019 Nov;33(11):2654-2661. doi:
605 10.1038/s41375-019-0431-y. Epub 2019 May 22. PMID: 31118465.
- 606 27. Bustoros M, Sklavenitis-Pistofidis R, Kapoor P, et al. Progression Risk Stratification of
607 Asymptomatic Waldenström Macroglobulinemia. *J Clin Oncol.* 2019 Jun 1;37(16):1403-
608 1411. doi: 10.1200/JCO.19.00394. Epub 2019 Apr 16. PMID: 30990729; PMCID:
609 PMC6544461.
- 610 28. Paiva B, Montes MC, García-Sanz R, Ocio EM, Alonso J, de Las Heras N, Escalante F,
611 Cuello R, de Coca AG, Galende J, Hernández J, Sierra M, Martín A, Pardal E, Báñez A,
612 Alonso J, Suarez L, González-López TJ, Perez JJ, Orfao A, Vidriales MB, San Miguel JF.
613 Multiparameter flow cytometry for the identification of the Waldenström's clone in IgM-
614 MGUS and Waldenström's Macroglobulinemia: new criteria for differential diagnosis and
615 risk stratification. *Leukemia.* 2014 Jan;28(1):166-73. doi: 10.1038/leu.2013.124. Epub 2013
616 Apr 22. PMID: 23604227.
- 617 29. Guerrero ML, Tsakmaklis N, Xu L, et al. *MYD88* mutated and wild-type Waldenström's
618 Macroglobulinemia: characterization of chromosome 6q gene losses and their mutual
619 exclusivity with mutations in *CXCR4*. *Haematologica.* 2018 Sep;103(9):e408-e411. doi:
620 10.3324/haematol.2018.190181. Epub 2018 Mar 29. PMID: 29599202; PMCID:
621 PMC6119142.
- 622 30. Sekiguchi N, Nomoto J, Nagata A, et al. Gene Expression Profile Signature of Aggressive
623 Waldenström Macroglobulinemia with Chromosome 6q Deletion. *Biomed Res Int.* 2018 Oct
624 4;2018:6728128. doi: 10.1155/2018/6728128. PMID: 30402490; PMCID: PMC6193339.
- 625 31. García-Sanz R, Dogliotti I, Zaccaria GM, et al. 6q deletion in Waldenström
626 macroglobulinaemia negatively affects time to transformation and survival. *Br J Haematol.*
627 2021 Mar;192(5):843-852. doi: 10.1111/bjh.17028. Epub 2020 Aug 11. PMID: 32780894.
- 628 32. Hunter ZR, Xu L, Tsakmaklis N. Insights into the genomic landscape of *MYD88* wild-type
629 Waldenström macroglobulinemia. *Blood Adv.* 2018 Nov 13;2(21):2937-2946. doi:
630 10.1182/bloodadvances.2018022962. PMID: 30401751; PMCID: PMC6234368.
- 631 33. Varettoni M, Zibellini S, Defrancesco I, et al. Pattern of somatic mutations in patients with
632 Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined
633 significance. *Haematologica.* 2017 Dec;102(12):2077-2085. doi:
634 10.3324/haematol.2017.172718. Epub 2017 Oct 5. PMID: 28983055; PMCID:
635 PMC5709107.
- 636 34. García-Sanz R, García-Álvarez M, Medina A, Askari E, González-Calle V, Casanova M, de
637 la Torre-Loizaga I, Escalante-Barrigón F, Bastos-Boente M, Báñez A, Vidaña-Bedera N,
638 Alonso JM, Sarasquete ME, González M, Chillón MC, Alcoceba M, Jiménez C. Clonal
639 architecture and evolutionary history of Waldenström's macroglobulinemia at the single-cell
640 level. *Dis Model Mech.* 2023 Aug 1;16(8):dmm050227. doi: 10.1242/dmm.050227. Epub
641 2023 Aug 23. PMID: 37493341; PMCID: PMC10461465.
- 642 35. Varettoni M, Zibellini S, Rizzo E. Targeted Next Generation Sequencing Identifies Novel
643 Genetic Mutations in Patients with Waldenström's Macroglobulinemia/Lymphoplasmacytic
644 Lymphoma or IgM-Monoclonal Gammopathies of Undetermined Significance. *Blood* 2016;
645 128 (22): 2928. doi: <https://doi.org/10.1182/blood.V128.22.2928.2928>
- 646 36. Treon SP, Gustine J, Xu L. *MYD88* wild-type Waldenström Macroglobulinaemia:
647 differential diagnosis, risk of histological transformation, and overall survival. *Br J*
648 *Haematol.* 2018 Feb;180(3):374-380. doi: 10.1111/bjh.15049. Epub 2017 Nov 27. PMID:
649 29181840.
- 650 37. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in *MYD88* and
651 *CXCR4* are determinants of clinical presentation and overall survival in Waldenström

- 652 macroglobulinemia. *Blood*. 2014 May 1;123(18):2791-6. doi: 10.1182/blood-2014-01-
653 550905. Epub 2014 Feb 19. PMID: 24553177.
- 654 38. Hunter ZR, Xu L, Tsakmaklis N, et al. Insights into the genomic landscape of MYD88 wild-
655 type Waldenström macroglobulinemia. *Blood Adv*. 2018 Nov 13;2(21):2937-2946. doi:
656 10.1182/bloodadvances.2018022962. PMID: 30401751; PMCID: PMC6234368.
- 657 39. Castillo JJ, Moreno DF, Arbelaez MI, Hunter ZR, Treon SP. CXCR4 mutations affect
658 presentation and outcomes in patients with Waldenström macroglobulinemia: A systematic
659 review. *Expert Rev Hematol*. 2019 Oct;12(10):873-881. doi:
660 10.1080/17474086.2019.1649132. Epub 2019 Jul 30. PMID: 31343930.
- 661 40. Treon SP, Sarosiek S, Castillo JJ. How I use genomics and BTK inhibitors in the treatment
662 of Waldenström macroglobulinemia. *Blood*. 2024 Apr 25;143(17):1702-1712. doi:
663 10.1182/blood.2022017235. PMID: 38211337; PMCID: PMC11103089.
- 664 41. Poulain S, Roumier C, Bertrand E, Renneville A, Caillault-Venet A, Doye E, Geffroy S,
665 Sebda S, Nibourel O, Nudel M, Herbaux C, Renaud L, Tomowiak C, Guidez S, Tricot S,
666 Roche-Lestienne C, Quesnel B, Preudhomme C, Leleu X. *TP53 Mutation and Its Prognostic*
667 *Significance in Waldenstrom's Macroglobulinemia*. *Clin Cancer Res*. 2017 Oct
668 15;23(20):6325-6335. doi: 10.1158/1078-0432.CCR-17-0007. Epub 2017 Jul 28. PMID:
669 28754818.
- 670 42. Varettoni M, Zibellini S, Drandi D. TP53 mutations in paired bone marrow and cell-free
671 DNA samples of patients with Waldenström macroglobulinemia or IgM monoclonal
672 gammopathy of undetermined significance prospectively enrolled in the FIL_biowm study
673 of fondazione italiana linfomi. *Blood* 2025; 146 (Supplement 1): 5318.
674 doi: <https://doi.org/10.1182/blood-2025-5318>
- 675 43. Gustine JN, Tsakmaklis N, Demos MG, et al. TP53 mutations are associated with mutated
676 MYD88 and CXCR4, and confer an adverse outcome in Waldenström macroglobulinaemia.
677 *Br J Haematol*. 2019 Jan;184(2):242-245. doi: 10.1111/bjh.15560. Epub 2018 Sep 5. PMID:
678 30183082.
- 679 44. Jiménez C, Chillón Mdel C, Balanzategui A. Detection of MYD88 L265P mutation by real-
680 time allele-specific oligonucleotide polymerase chain reaction. *Appl Immunohistochem Mol*
681 *Morphol*. 2014 Nov-Dec;22(10):768-73. doi: 10.1097/PAI.000000000000020. PMID:
682 24992174.
- 683 45. van Dongen JJ, Lhermitte L, Böttcher S et al. EuroFlow antibody panels for standardized n-
684 dimensional flow cytometric immunophenotyping of normal, reactive and malignant
685 leukocytes. *Leukemia*. 2012 Sep;26(9):1908-75. doi: 10.1038/leu.2012.120. Epub 2012 May
686 3. PMID: 22552007; PMCID: PMC3437410.
- 687 46. Uppal E, Khwaja J, Bomszyk J, et al. The Rory Morrison WMUK Registry for
688 Waldenström macroglobulinaemia: The growth of a national registry for a rare disorder. *Br*
689 *J Haematol*. 2023 Jun;201(5):905-912. doi: 10.1111/bjh.18680. Epub 2023 Jan 25. PMID:
690 36698318.
- 691 47. Moreno DF, López-Guerra M, Paz S, et al. Prognostic impact of MYD88 and CXCR4
692 mutations assessed by droplet digital polymerase chain reaction in IgM monoclonal
693 gammopathy of undetermined significance and smouldering Waldenström
694 macroglobulinaemia. *Br J Haematol*. 2023 Jan;200(2):187-196. doi: 10.1111/bjh.18502.
695 Epub 2022 Oct 9. PMID: 36210485; PMCID: PMC10092069.
- 696 48. Drandi D, Decruyenaere P, Ferrante M. Nucleic Acid Biomarkers in Waldenström
697 Macroglobulinemia and IgM-MGUS: Current Insights and Clinical Relevance. *Diagnostics*
698 (Basel). 2022 Apr 12;12(4):969. doi: 10.3390/diagnostics12040969. PMID: 35454017;
699 PMCID: PMC9028641.

701 **Tables**

702

Spanish cohort				
	IgM MGUS (n=111)	asymptomatic WM (n=245)	symptomatic WM (n=221)	p-value
Age (Median [IQR])	69.5 [29.0, 87.0]	72.0 [34.0, 93.0]	71.0 [30.0, 94.0]	0.070
Sex				
Female	46 (41.4%)	88 (35.9%)	67 (30.3%)	0.120
Male	65 (58.6%)	157 (64.1%)	154 (69.7%)	
Hb (Median [IQR])	13.9 [8.1, 17.4]	13.2 [6.0, 20.0]	10.4 [3.6, 18.0]	<0.001
ECOG				
0	68 (61.3%)	136 (55.5%)	46 (20.8%)	<0.001
1	19 (17.1%)	55 (22.4%)	74 (33.5%)	
2	3 (2.7%)	12 (4.9%)	48 (21.7%)	
3	0 (0%)	3 (1.2%)	10 (4.5%)	
4	1 (0.9%)	2 (0.8%)	2 (0.9%)	
Missing	20 (18.0%)	37 (15.1%)	41 (18.6%)	
B symptoms				
No	92 (82.9%)	203 (82.9%)	117 (52.9%)	<0.001
Yes	1 (0.9%)	7 (2.9%)	73 (33.0%)	
Missing	18 (16.2%)	35 (14.3%)	31 (14.0%)	
Platelets (Median [IQR])	227 [73, 1970]	248 [30, 644]	210 [2, 684]	<0.001
B2M ((Median [IQR])	2.00 [0.5, 6.4]	2.46 [0.15, 18.0]	3.26 [0.4, 18.6]	<0.001
Missing	21 (18.9%)	55 (22.4%)	45 (20.4%)	
Albumin (Median [IQR])	4.10 [2.9, 5.0]	3.90 [2.3, 5.2]	3.60 [1.6, 4.9]	<0.001
Missing	20 (18.0%)	38 (15.5%)	32 (14.5%)	
IgM (Median [IQR])	781 [87, 6130]	1460 [225, 9220]	3300 [19.0, 13000]	<0.001
Missing	9 (8.1%)	23 (9.4%)	22 (10.0%)	
LDH ratio (Median [IQR])	0.73 [0.27, 1.8]	0.65 [0.28, 1.76]	0.65 [0.25, 4.0]	0.043
Missing	18 (16.2%)	35 (14.3%)	35 (15.8%)	
Light Ig type				
K	69 (62.2%)	160 (65.3%)	157 (71.0%)	0.039
L	37 (33.3%)	62 (25.3%)	38 (17.2%)	
Negative	0 (0%)	2 (0.8%)	1 (0.5%)	
Missing	5 (4.5%)	21 (8.6%)	25 (11.3%)	
IPSS-WM				
High	0 (0%)	13 (5.3%)	67 (30.3%)	<0.001
Int	45 (40.5%)	126 (51.4%)	68 (30.8%)	
Low	30 (27.0%)	45 (18.4%)	30 (13.6%)	
Missing	36 (32.4%)	61 (24.9%)	56 (25.3%)	
FISH				
Abnormal	4 (3.6%)	20 (8.1%)	34 (15.5%)	0.001
Normal	26 (23.4%)	93 (38.0%)	65 (29.5%)	
Not evaluable	81 (73%)	132 (53.9%)	121 (54.1%)	
MYD88 L265P mutation				
Negative	20 (18.0%)	25 (10.2%)	26 (11.8%)	<0.001
Positive	18 (16.2%)	111 (45.3%)	98 (44.3%)	
Not evaluable	73 (65.8%)	109 (44.5%)	97 (43.9%)	
MYD88 L265P mutational ratio (Median [IQR])	0.02 [0.001, 0.0868]	0.0165 [0.00131, 1.76]	0.0362 [0.00158, 1.36]	0.004
Torino cohort				
	IgM MGUS (n=22)	asymptomatic WM (n=71)	symptomatic WM (n=73)	p-value
Age (Median [IQR])	73.0 [48.0, 91.0]	67.0 [24.0, 84.0]	70.0 [43.0, 95.0]	0.091
Sex				
Female	8 (36.4%)	33 (46.5%)	24 (32.9%)	0.237
Male	14 (63.6%)	38 (53.5%)	49 (67.1%)	

Hb (Median [IQR])	10.4 [3.60, 18.0]	14.0 [8.30, 16.0]	13.0 [0, 18.0]	<0.001
ECOG				
0	16 (72.7%)	59 (83.1%)	30 (41.1%)	<0.001
1	4 (18.2%)	8 (11.3%)	15 (20.5%)	
2	1 (4.5%)	1 (1.4%)	10 (13.7%)	
3	0 (0%)	0 (0%)	7 (9.6%)	
4	0 (0%)	0 (0%)	1 (1.4%)	
Missing	1 (4.5%)	3 (4.2%)	10 (13.7%)	
BMB% (Median [IQR])	0 [0, 0]	30.0 [10.0, 90.0]	70.0 [10.0, 95.0]	<0.001
Missing	15 (68.2%)	8 (11.3%)	10 (13.7%)	
B symptoms				
No	20 (90.9%)	69 (97.2%)	53 (72.6%)	0.002
Yes	1 (4.5%)	0 (0%)	10 (13.7%)	
	1 (4.5%)	2 (2.8%)	10 (13.7%)	
Platelets (Median [IQR])	209 [114, 315]	260 [71.0, 632]	203 [29.0, 543]	0.013
B2m ((Median [IQR])	2.52 [1.4, 6.6]	1.95 [1.1, 13.2]	3.10 [1.5, 12.6]	0.002
Missing	10 (45.5%)	23 (32.4%)	42 (57.5%)	
Albumin (Median [IQR])	3.80 [3.0, 5.1]	4.00 [3.0, 5.3]	3.75 [2.5, 4.8]	0.012
Missing	11 (50.0%)	34 (47.9%)	35 (47.9%)	
IgM (Median [IQR])	781 [195, 5360]	1370 [210, 5480]	2960 [205, 9620]	<0.001
Missing	0 (0%)	5 (7.0%)	11 (15.1%)	
LDH (Median [IQR])	0.8 [0.3, 1.8]	0.8 [0.3, 1.4]	0.7 [0.4, 2.4]	0.554
	8 (36.4%)	25 (35.2%)	22 (30.1%)	
Light Ig type				
K	15 (68.2%)	50 (70.4%)	52 (71.2%)	0.982
L	4 (18.2%)	15 (21.1%)	15 (20.5%)	
Missing	3 (13.6%)	6 (8.5%)	6 (8.2%)	
IPSS WM				
High	0 (0%)	4 (5.6%)	22 (30.1%)	<0.001
Int	15 (68.2%)	30 (42.3%)	18 (24.7%)	
Low	6 (27.3%)	23 (32.4%)	10 (13.7%)	
Missing	1 (4.5%)	14 (19.7%)	23 (31.5%)	
FISH				
Abnormal	0 (0%)	2 (2.8%)	2 (2.9%)	0.193
Normal	0 (0%)	2 (2.8%)	0 (0%)	
Not evaluable	22 (100%)	67 (94.4%)	68 (97.1%)	
MYD88 L265P mutation				
Negative	3 (13.6%)	5 (7.0%)	10 (13.7%)	0.376
Positive	11 (50.0%)	50 (70.4%)	53 (72.6%)	
Missing	8 (36.4%)	16 (22.5%)	10 (13.7%)	
MYD88 L265P mutational ratio (Median [IQR])	0.00497 [0.000551, 0.0371]	0.0216 [0.000520, 0.283]	0.0278 [0.000400, 0.713]	0.003

703
704 **Table 1.** Characteristics of IgM gammopathy patients enrolled in the study – Spanish and Torino cohort.
705 Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; WM, Waldenström
706 Macroglobulinemia; Hb, hemoglobin; B2M, beta-2-microglobulin; IgM, immunoglobulin M; LDH, lactate
707 dehydrogenase; Ig, immunoglobulin; k, free light kappa chain; l, free light lambda chain; IPSS-WM,
708 International Prognostic Score System for Waldenström Macroglobulinemia; Int, intermediate; FISH,
709 Fluorescence in situ hybridization

710

	Spanish cohort								
	OS			PFS			TTFT		
	HR	95% IC	p	HR	95% IC	p	HR	95%IC	p
Age									
≤65	-	-		-	-		-	-	
65-75	2.221	1.514-3.260	<0.001	1.389	0.931-2.072	0.107	1.002	0.483-2.083	0.996
≥76	4.136	2.812-6.085	<0.001	2.561	1.675-3.918	<0.001	0.238	0.075-0.757	0.015
Sex									
Female	-	-		-	-		-	-	

Male	1.651	1.192-2.287	0.003				2.115	0.971-4.606	0.059
Hb									
≤11,5							-	-	
>11,5							0.353	0.136-0.918	0.033
Diagnosis code									
MGUS	-	-							
aWM	1.172	0.722-1.902	0.521						
sWM	2.086	1.277-3.407	0.003						
BMB categ									
Not evaluable				-	-		-	-	
Negative				0.937	0.423-2.074	0.873	1.498	0.523-4.291	0.452
Positive				1.332	0.950-1.869	0.096	2.902	1.337-6.298	0.007
Platelets									
≤100				-	-		-	-	
>100				1.567	0.950-1.869	0.091	0.096	0.027-0.345	<0.001
B2m									
≤3	-	-		-	-				
>3	1.617	1.200-2.180	0.002	1.262	0.905-1.761	0.171			
Albumin									
<3,5							-	-	-
≥3,5							0.449	0.200-1.010	0,053
LDH									
≤1				-	-				
>1				0.533	0.326-0.872	0.012			
Hyperviscosity									
No	-	-							
Yes	1.407	0.943-2.098	0.094						
Torino cohort									
	OS			PFS			TTFT		
	HR	95% IC	p	HR	95% IC	p	HR	95%IC	p
Age									
≤65	-	-		-	-		-	-	
65-75	2.221	1.514-3.260	<0.001	1.389	0.931-2.072	0.107	1.002	0.483-2.083	0.996
≥76	4.136	2.812-6.085	<0.001	2.561	1.675-3.918	<0.001	0.238	0.075-0.757	0.015
Sex									
Female	-	-					-	-	
Male	1.651	1.192-2.287	0.003				2.115	0.971-4.606	0.059
Hb									
≤11,5							-	-	
>11,5							0.353	0.136-0.918	0.033
Diagnosis code									
MGUS	-	-							
aWM	1.172	0.722-1.902	0.521						
sWM	2.086	1.277-3.407	0.003						
BMB categ									
Not evaluable				-	-		-	-	
Negative				0.937	0.423-2.074	0.873	1.498	0.523-4.291	0.452
Positive				1.332	0.950-1.869	0.096	2.902	1.337-6.298	0.007
Platelets									
≤100				-	-		-	-	
>100				1.567	0.950-1.869	0.091	0.096	0.027-0.345	<0.001
B2m									
≤3	-	-		-	-				
>3	1.617	1.200-2.180	0.002	1.262	0.905-1.761	0.171			
Albumin									
<3,5							-	-	-
≥3,5							0.449	0.200-1.010	0.053
LDH									
≤1				-	-				
>1				0.533	0.326-0.872	0.012			
Hyperviscosity									
No	-	-							
Yes	1.407	0.943-2.098	0.094						

712 **Table 2.** Multivariable analysis for OS, TTFT and PFS in Spanish and Torino cohort. Only variables with
 713 statistically significant effect for multivariable models are shown. Abbreviations: HR, hazard ratio;
 714 IC, interval of confidence; p, p-value; Hb, hemoglobin; BMB, bone marrow biopsy; B2M, beta-2-
 715 microglobulin; IgM, immunoglobulin M; LDH, lactate dehydrogenase; IPSSWM, International
 716 Prognostic Score System for Waldenström Macroglobulinemia; MGUS, monoclonal gammopathy of
 717 undetermined significance; aWM, asymptomatic Waldenström Macroglobulinemia; sWM,
 718 symptomatic Waldenström Macroglobulinemia.
 719

Spanish cohort									
	OS			PFS			TTFT		
	HR	95% IC	p	HR	95% IC	p	HR	95% IC	p
MYD88 ratio – cutoff >0,1624318									
Neg	-	-	-	-	-	-	-	-	-
Low	0.995	-	0.982	0.789	-	0.333	0.864	-	0.754
High	1.823	-	0.061	1.013	-	0.969	3.478	-	0.049
Flow ratio - cutoff >4,39%									
Neg	-	-	-	-	-	-	-	-	-
Low	0.912	-	0.792	<0.001	-	0.995	3.215	-	0.268
High	1.421	-	0.279	<0.001	-	0.995	7.196	-	0.056
Torino cohort									
	OS			PFS			TTFT		
	HR	95% IC	p	HR	95% IC	p	HR	95% IC	p
MYD88 ratio – cutoff >0,1624318									
Neg	-	-	-	-	-	-	-	-	-
Low	1.153	-	0.822	0.811	-	0.575	0.724	-	0.670
High	3.063	-	0.16	1.430	-	0.434	2.874	-	0.395
Flow ratio - cutoff >4,39%									
Neg	-	-	-	-	-	-	-	-	-
Low	<0.001	-	0.998	<0.001	-	0.997	0.35	-	0.396
High	<0.001	-	0.998	<0.001	-	0.997	1.48	-	0.621

720
 721 **Table 3.** Univariate analysis for OS, TTFT and PFS for molecular and flow cytometry variables in Spanish
 722 and Torino cohort. Abbreviations: HR, hazard ratio; IC, interval of confidence; p, p-value
 723
 724

Figure 1

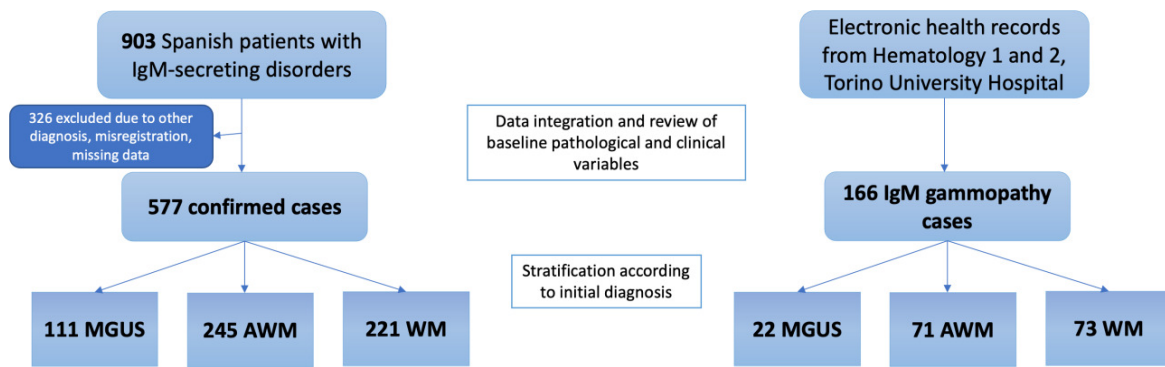


Figure 1. Consort diagram of enrolled patients in the two cohorts. Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; aWM, asymptomatic Waldenström Macroglobulinemia; WM, Waldenström Macroglobulinemia; NA, not available; FU, follow-up

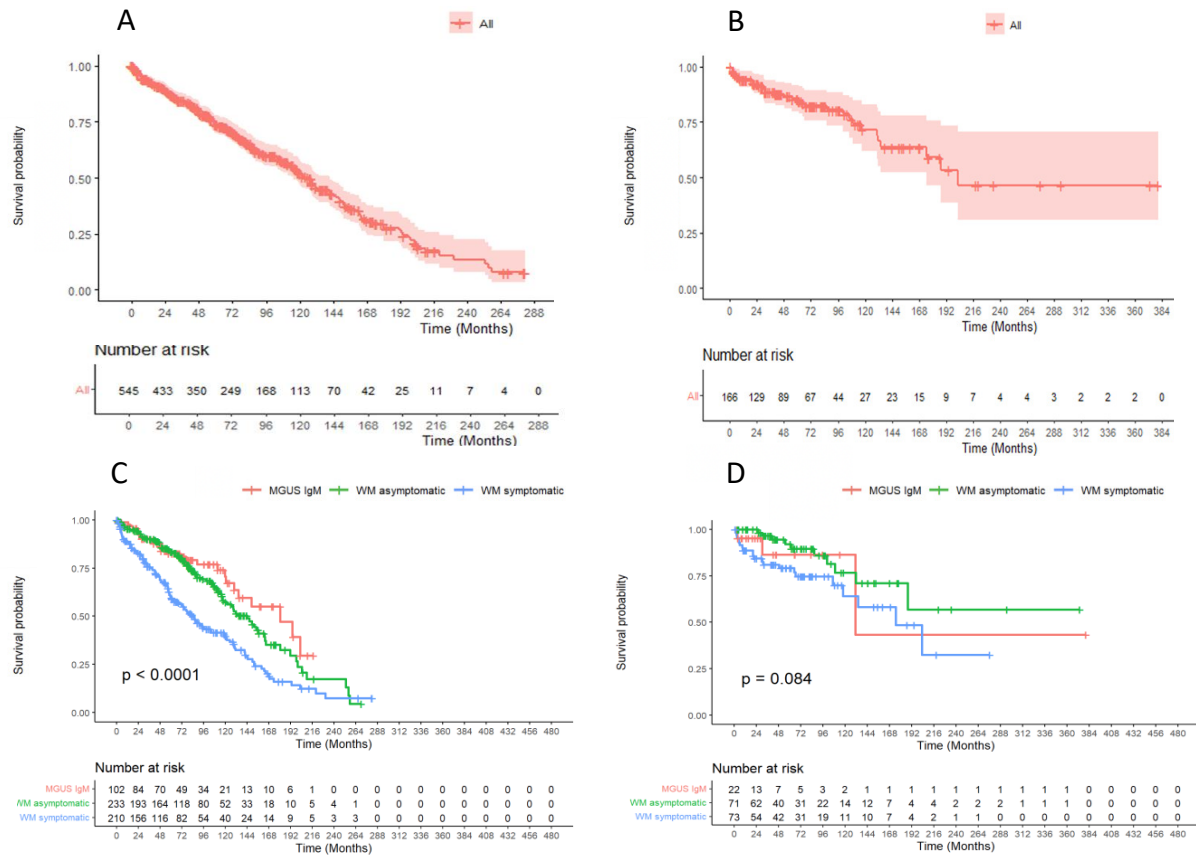


Figure 2. Overall survival of Spanish cohort (A) and Torino cohort (B); overall survival stratified for initial diagnosis of MGUS, aWM or sWM (C, D)

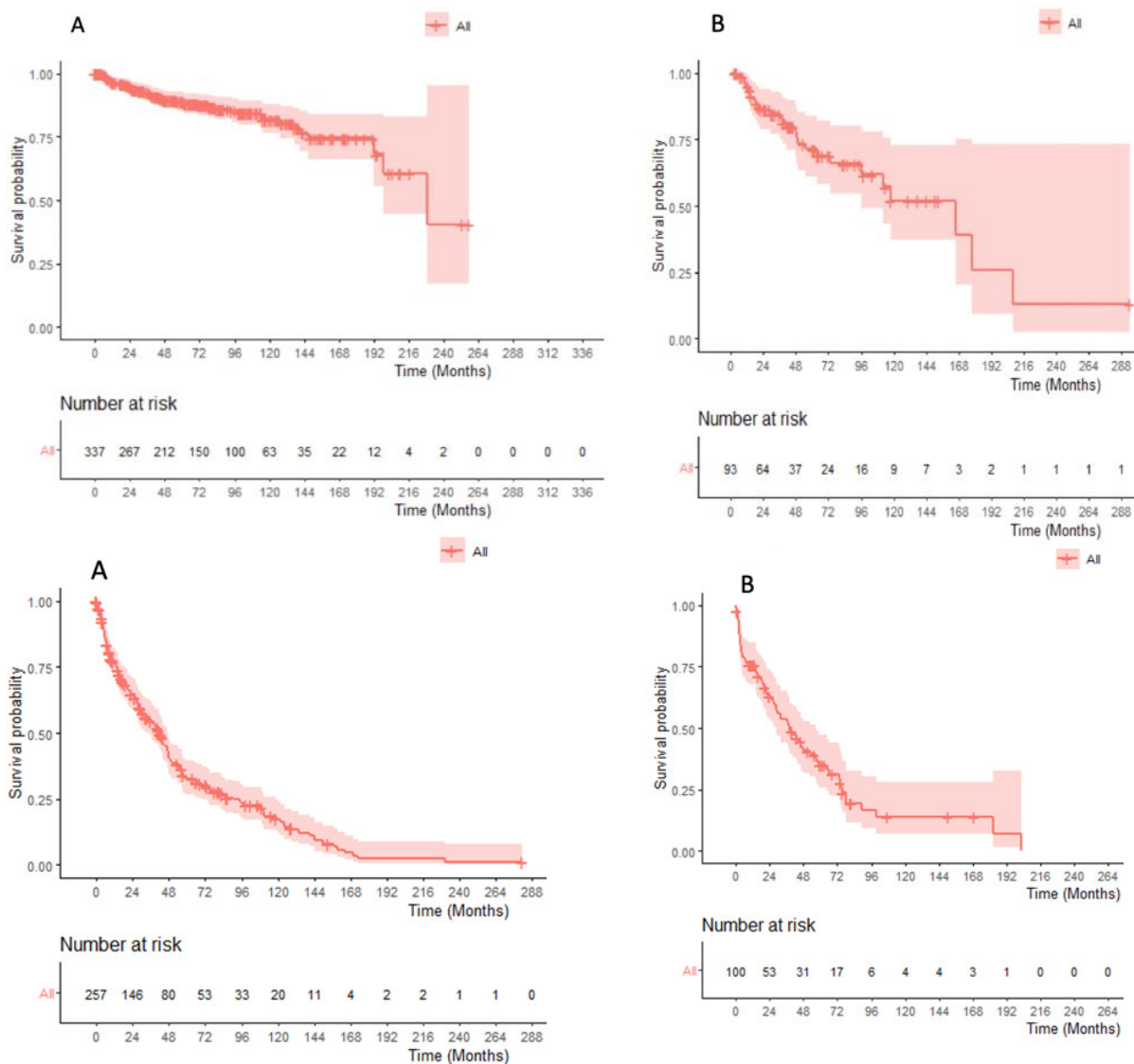


Figure 3. Time to first treatment in asymptomatic patients belonging to Spanish cohort (A) and Torino cohort (B) (top panels); Progression free survival in patients requiring treatment at any time, belonging to Spanish cohort (A) and Torino cohort (B) (bottom panels)

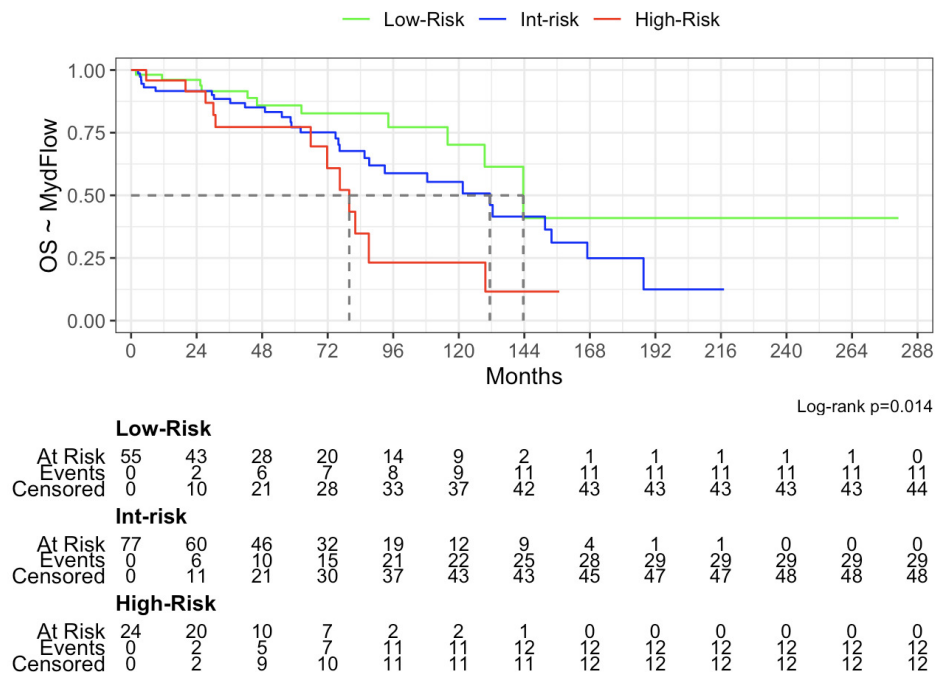


Figure 4. Outcome in terms of OS according to BM MYD88^{L65P} levels and to BM MFC levels