

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

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## 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/mm<sup>3</sup> 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

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

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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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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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## 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.<sup>1,2</sup>

107 Although a familial predisposition has been demonstrated,<sup>3</sup> the main risk factor for WM remains a  
108 history of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS),<sup>4</sup> with a  
109 probability of progression to WM or to other lymphoproliferative disorders of 1.5-2% per year.<sup>4</sup>  
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.<sup>5</sup> Several studies using different  
113 techniques, such as Sanger sequencing, polymerase chain reaction (PCR) and allele-specific qPCR  
114 (ASqPCR),<sup>6,7,8</sup> 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<sup>7,8,9,10</sup> and it can also be  
116 identified in peripheral blood (PB),<sup>11</sup> in particular in cell-free DNA (cfDNA) of IgM gammopathy  
117 patients.<sup>12,13</sup>  
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.<sup>14,15,16</sup> However, these patients have a high risk of  
122 transformation from smoldering WM to active WM and should be monitored closely.<sup>17</sup>  
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.<sup>4,18,19,20,21,22,23</sup> 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,<sup>24</sup> 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<sup>25,26</sup>, 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.<sup>27</sup>  
132 Some authors have also tried to assess the impact on patients’ outcome of laboratory prognostic  
133 factors, such as multiparameter flow cytometry (MFC) features<sup>28</sup>, 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,<sup>29,30</sup> being associated with a higher IPSS-WM score and a  
137 shorter time to treatment, PFS and OS.<sup>31,32,33,34</sup>  
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;<sup>9,27,35,36</sup> on the contrary, other studies showed no effect on OS or  
142 time to progression<sup>14</sup> or underlined higher progression rates to symptomatic WM (sWM) and more  
143 aggressive clinical course in *MYD88*<sup>WT</sup> patients.<sup>27,36,37,38</sup> Moreover, *CXCR4*<sup>WHIM</sup> mutations, the  
144 second most frequent genetic alteration in WM, have been proven to impact on response to therapy  
145 and PFS,<sup>39,40</sup> without affecting OS.<sup>33,37</sup> Finally, similar to many other neoplasms, *TP53* alterations  
146 confer worse outcome in terms of OS,<sup>40,41,42</sup> irrespectively of IPSS-WM score; of note, such  
147 alterations were not identified in patients carrying IgM-MGUS.<sup>41,43</sup>

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 sWM<sup>1</sup> 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.<sup>44</sup> Alternatively, *MYD88* mutational status was  
184 determined by droplet digital PCR (ddPCR) on both BM and PB samples, as previously described.<sup>12</sup>

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.<sup>31</sup>

191 Immunophenotypic evaluation was done using conventional methods, panels of monoclonal  
192 antibodies previously described<sup>45</sup> 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

223 IRB Approval: Salamanca University Hospital Ethic Committee, Paseo de San Vicente, 58-182  
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 228 25/11/21).

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*<sup>low</sup> group showed better OS (p=0.005, HR=0.44) and TTFT  
 365 (p=0.024, HR=0.33) compared to *MYD88*<sup>high</sup> 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*<sup>high</sup> 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<sup>low</sup> patients had increased OS (p=0.033, HR=0.65) and TTFT (p=0.008, HR=0.37)  
 381 compared to MFC<sup>high</sup> 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<sup>low</sup>/*MYD88*<sup>low</sup>, IR: either MFC<sup>low</sup>/*MYD88*<sup>high</sup> or  
 391 MFC<sup>high</sup>/*MYD88*<sup>low</sup>; HR: MFC<sup>high</sup>/*MYD88*<sup>high</sup>). 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<sup>L65P</sup> 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;<sup>20,46</sup> 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,<sup>24,25</sup> 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.<sup>27</sup>  
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*<sup>WT</sup> WM patients have inferior survival  
441 outcomes,<sup>36</sup> and show slower and less profound responses with Bruton's tyrosine kinase inhibitor  
442 (BTKi), especially with ibrutinib;<sup>40</sup> 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*<sup>WT</sup> vs *MYD88* L265P WM patients, while  
444 there was an increased occurrence of transformation to diffuse large B-cell lymphoma (DLBCL).<sup>14</sup>  
445 For IgM-MGUS patients, a study by Varettoni et al. showed lower progression rates to overt WM in  
446 *MYD88*<sup>WT</sup> compared to *MYD88* L265P cases.<sup>9</sup> 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.<sup>47</sup> 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 criteria<sup>48</sup>.

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  
504 received research funding from Gilead and Morphosys.  
505

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701 **Tables**  
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<b>Spanish cohort</b>				
	<b>IgM MGUS (n=111)</b>	<b>asymptomatic WM (n=245)</b>	<b>symptomatic WM (n=221)</b>	<b>p-value</b>
<b>Age</b> (Median [IQR])	69.5 [29.0, 87.0]	72.0 [34.0, 93.0]	71.0 [30.0, 94.0]	0.070
<b>Sex</b>				
Female	46 (41.4%)	88 (35.9%)	67 (30.3%)	0.120
Male	65 (58.6%)	157 (64.1%)	154 (69.7%)	
<b>Hb</b> (Median [IQR])	13.9 [8.1, 17.4]	13.2 [6.0, 20.0]	10.4 [3.6, 18.0]	<0.001
<b>ECOG</b>				
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>B symptoms</b>				
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%)	
<b>Platelets</b> (Median [IQR])	227 [73, 1970]	248 [30, 644]	210 [2, 684]	<0.001
<b>B2M</b> ((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%)	
<b>Albumin</b> (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%)	
<b>IgM</b> (Median [IQR])	781 [87, 6130]	1460 [225, 9220]	3300 [19.0, 13000]	<0.001
Missing	9 (8.1%)	23 (9.4%)	22 (10.0%)	
<b>LDH ratio</b> (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%)	
<b>Light Ig type</b>				
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%)	
<b>IPSS-WM</b>				
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%)	
<b>FISH</b>				
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%)	
<b>MYD88 L265P mutation</b>				
Negative	20 (18.0%)	25 (10.2%)	26 (11.8%)	<0.001
Positive	18 (16.2%)	111 (45.3%)	98 (44.3%)	
<b>Not evaluable</b>	73 (65.8%)	109 (44.5%)	97 (43.9%)	
<b>MYD88 L265P mutational ratio</b> (Median [IQR])	0.02 [0.001, 0.0868]	0.0165 [0.00131, 1.76]	0.0362 [0.00158, 1.36]	0.004
<b>Torino cohort</b>				
	<b>IgM MGUS (n=22)</b>	<b>asymptomatic WM (n=71)</b>	<b>symptomatic WM (n=73)</b>	<b>p-value</b>
<b>Age</b> (Median [IQR])	73.0 [48.0, 91.0]	67.0 [24.0, 84.0]	70.0 [43.0, 95.0]	0.091
<b>Sex</b>				
Female	8 (36.4%)	33 (46.5%)	24 (32.9%)	0.237
Male	14 (63.6%)	38 (53.5%)	49 (67.1%)	

<b>Hb</b> (Median [IQR])	10.4 [3.60, 18.0]	14.0 [8.30, 16.0]	13.0 [0, 18.0]	<0.001
<b>ECOG</b>				
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%)	
<b>BMB%</b> (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>B symptoms</b>				
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%)	
<b>Platelets</b> (Median [IQR])	209 [114, 315]	260 [71.0, 632]	203 [29.0, 543]	0.013
<b>B2m</b> ((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%)	
<b>Albumin</b> (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%)	
<b>IgM</b> (Median [IQR])	781 [195, 5360]	1370 [210, 5480]	2960 [205, 9620]	<0.001
Missing	0 (0%)	5 (7.0%)	11 (15.1%)	
<b>LDH</b> (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%)	
<b>Light Ig type</b>				
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%)	
<b>IPSS WM</b>				
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%)	
<b>FISH</b>				
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%)	
<b>MYD88 L265P mutation</b>				
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%)	
<b>MYD88 L265P mutational ratio</b> (Median [IQR])	0.00497 [0.000551, 0.0371]	0.0216 [0.000520, 0.283]	0.0278 [0.000400, 0.713]	0.003

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

	Spanish cohort								
	OS			PFS			TTFT		
	HR	95% IC	p	HR	95% IC	p	HR	95%IC	p
<b>Age</b>									
≤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	<b>0.015</b>
<b>Sex</b>									
Female	-	-		-	-		-	-	

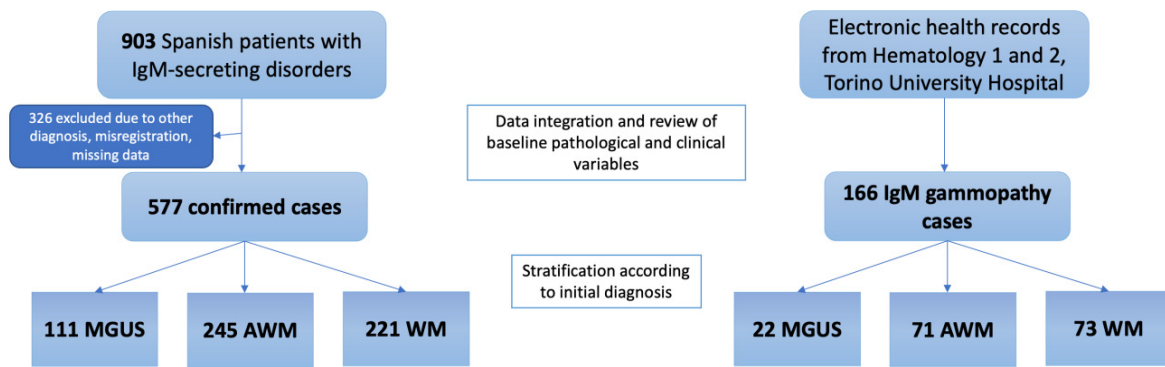
Male	1.651	1.192-2.287	<b>0.003</b>				2.115	0.971-4.606	0.059
<b>Hb</b>									
≤11,5							-	-	
>11,5							0.353	0.136-0.918	<b>0.033</b>
<b>Diagnosis code</b>									
MGUS	-	-							
aWM	1.172	0.722-1.902	0.521						
sWM	2.086	1.277-3.407	<b>0.003</b>						
<b>BMB categ</b>									
Not evaluable				-	-		-	-	
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	<b>0.007</b>
<b>Platelets</b>									
≤100				-	-		-	-	
>100				1.567	0.950-1.869	0.091	0.096	0.027-0.345	<b>&lt;0.001</b>
<b>B2m</b>									
≤3	-	-		-	-				
>3	1.617	1.200-2.180	<b>0.002</b>	1.262	0.905-1.761	0.171			
<b>Albumin</b>									
<3,5							-	-	-
≥3,5							0.449	0.200-1.010	0,053
<b>LDH</b>									
≤1				-	-				
>1				0.533	0.326-0.872	<b>0.012</b>			
<b>Hyperviscosity</b>									
No	-	-							
Yes	1.407	0.943-2.098	0.094						
<b>Torino cohort</b>									
	<b>OS</b>			<b>PFS</b>			<b>TTFT</b>		
	<b>HR</b>	<b>95% IC</b>	<b>p</b>	<b>HR</b>	<b>95% IC</b>	<b>p</b>	<b>HR</b>	<b>95%IC</b>	<b>p</b>
<b>Age</b>									
≤65	-	-		-	-		-	-	
65-75	2.221	1.514-3.260	<b>&lt;0.001</b>	1.389	0.931-2.072	0.107	1.002	0.483-2.083	0.996
≥76	4.136	2.812-6.085	<b>&lt;0.001</b>	2.561	1.675-3.918	<b>&lt;0.001</b>	0.238	0.075-0.757	<b>0.015</b>
<b>Sex</b>									
Female	-	-					-	-	
Male	1.651	1.192-2.287	<b>0.003</b>				2.115	0.971-4.606	0.059
<b>Hb</b>									
≤11,5							-	-	
>11,5							0.353	0.136-0.918	<b>0.033</b>
<b>Diagnosis code</b>									
MGUS	-	-							
aWM	1.172	0.722-1.902	0.521						
sWM	2.086	1.277-3.407	<b>0.003</b>						
<b>BMB categ</b>									
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	<b>0.007</b>
<b>Platelets</b>									
≤100				-	-		-	-	
>100				1.567	0.950-1.869	0.091	0.096	0.027-0.345	<b>&lt;0.001</b>
<b>B2m</b>									
≤3	-	-		-	-				
>3	1.617	1.200-2.180	<b>0.002</b>	1.262	0.905-1.761	0.171			
<b>Albumin</b>									
<3,5							-	-	-
≥3,5							0.449	0.200-1.010	0.053
<b>LDH</b>									
≤1				-	-				
>1				0.533	0.326-0.872	<b>0.012</b>			
<b>Hyperviscosity</b>									
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
<b>MYD88 ratio – cutoff &gt;0,1624318</b>									
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	-	<b>0.049</b>
<b>Flow ratio - cutoff &gt;4,39%</b>									
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
<b>MYD88 ratio – cutoff &gt;0,1624318</b>									
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
<b>Flow ratio - cutoff &gt;4,39%</b>									
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

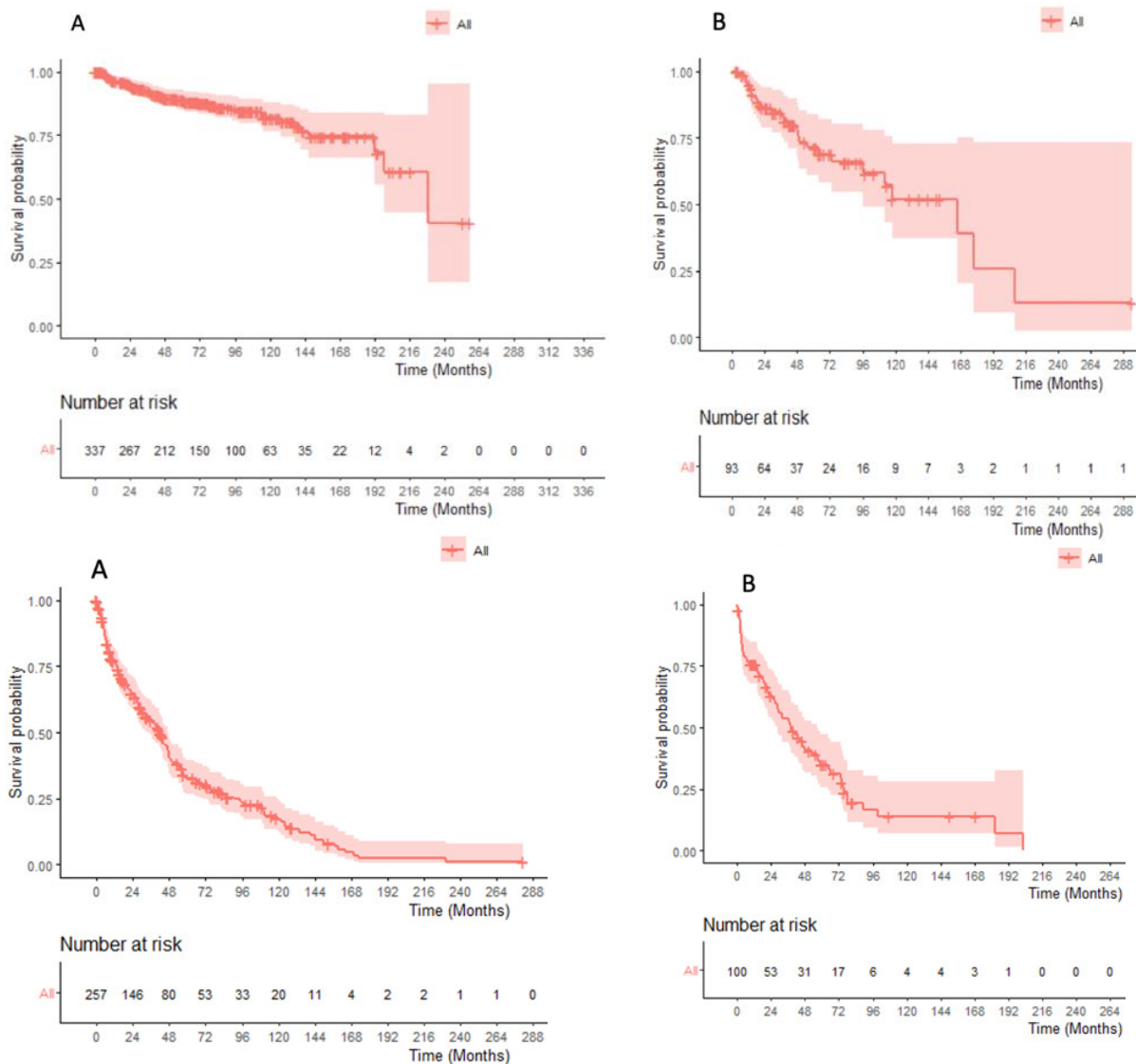
720 **Table 3.** Univariate analysis for OS, TTFT and PFS for molecular and flow cytometry variables in Spanish  
 721 and Torino cohort. Abbreviations: HR, hazard ratio; IC, interval of confidence; p, p-value  
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# Figure 1

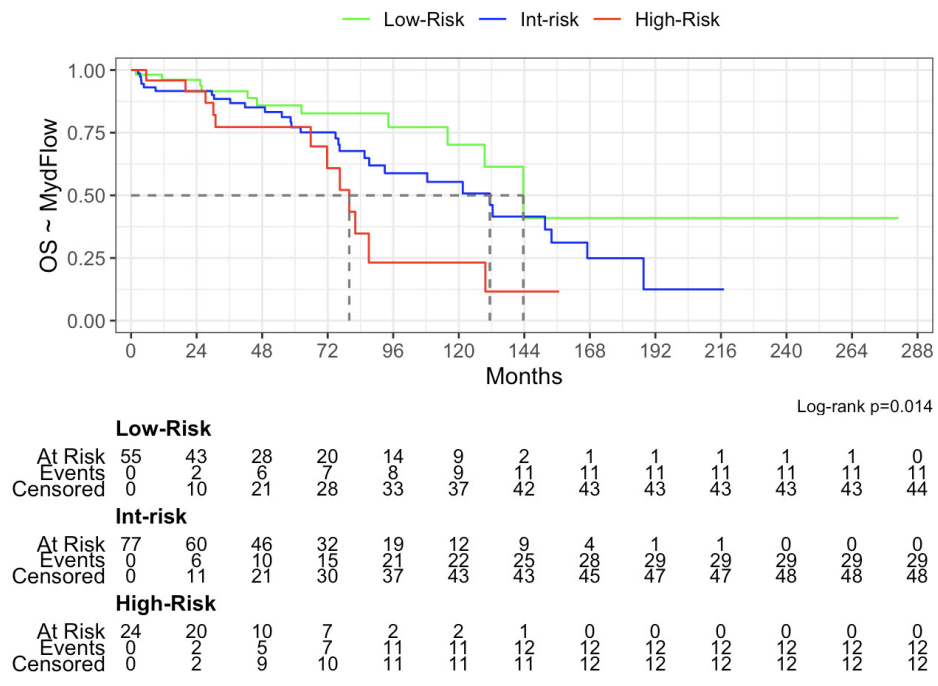


**Figure 1.** Consort diagram of enrolled patients in the two cohorts. Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; aWM, asymptomatic Waldenström Macroglobulinemia; WM, Waldenström Macroglobulinemia; NA, not available; FU, follow-up





**Figure 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<sup>L65P</sup> levels and to BM MFC levels