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## Early Relapse Risk in Patients with Newly Diagnosed Multiple Myeloma Characterized by Next-generation Sequencing

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1 **Research Article**

2  
3 **Early Relapse Risk in Newly Diagnosed Multiple Myeloma Patients Characterized by**  
4 **Next-Generation Sequencing**

5  
6 **Running title:** Early relapse risk by NGS in NDMM patients  
7

8  
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36  
37 **Authorship contributions**

38 MD, GMZ, SO, DA, JY, JK, MG, MB, NB, FM and FG conceived and designed the work that led to the submission.

39 All the authors collected the data and interpreted the results.

40 MD, GMZ, BZ, AC, NB, FM, and FG drafted the first version of the manuscript.

41 All the authors revised the manuscript.

42 All the authors approved the final version of the manuscript.

43 All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the  
44 accuracy or integrity of any part of the work are appropriately investigated and resolved.

45  
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49  
50 **Competing interests**

51 MD has served on the advisory board for GSK.

52 SO has received honoraria from Amgen, Celgene, and Janssen; has served on the advisory boards for Adaptive  
53 Biotechnologies, Janssen, Amgen, and Takeda.

54 DA is currently employed by the Multiple Myeloma Research Foundation, Norwalk, US-CT.

55 JY is currently employed by the Multiple Myeloma Research Foundation, Norwalk, US-CT.

56 JK is currently employed by the Translational Genomics Research Institute (TGen), US-AZ.

57 SB has received honoraria from Bristol-Myers Squibb, Celgene, Amgen and Janssen; has served on the advisory  
58 boards for Amgen, Karyopharm, Janssen and Celgene; has received consultancy fees from Takeda and Janssen.  
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63 Mundipharma.  
64 NB has received honoraria from Celgene and Janssen in the last three years, but he has no conflict with regards  
65 to the data presented.  
66 FG has received honoraria from Amgen, Celgene, Janssen, Takeda, and Bristol-Myers Squibb; has served on the  
67 advisory boards for Amgen, Celgene, Janssen, Takeda, Bristol-Myers Squibb, Roche, AbbVie, Adaptive, and Seattle  
68 Genetics.  
69 The other authors declare no competing financial interests.

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77  
78

79 **Statement of translational relevance**

80  
81 Duration of first remission is an important factor for the survival of patients with multiple  
82 myeloma (MM). Conventional baseline risk stratification is not always able to predict a short  
83 duration of first remission and poor survival.

84 In this study, we demonstrated the independent detrimental effect of early relapse (ER)  
85 within 18 months from the start of treatment on the survival of newly-diagnosed MM  
86 patients. Exploiting the molecular characterization through next-generation sequencing (NGS)  
87 of this large cohort of patients, we found additional risk factors increasing the risk of ER,  
88 whereas treatment intensification with carfilzomib-based induction, autologous stem-cell  
89 transplantation and continuous combination therapy may mitigate the risk of ER.

90 We demonstrated that patients relapsing within 18 months from the start of treatment  
91 represent an unmet clinical need and may deserve dedicated trials. NGS may help to better  
92 identify patients at risk. Treatment intensification may reduce early progressive disease in  
93 patients at risk.

94 **Abstract**

95 **Introduction.** Duration of first remission is important for the survival of multiple myeloma  
96 (MM) patients.

97 **Methods.** From the CoMMpass study (NCT01454297), 926 newly-diagnosed MM patients,  
98 characterized by next-generation sequencing, were analyzed to evaluate those who  
99 experienced early progressive disease (PD) (time-to-progression, TTP $\leq$ 18 months).

100 **Results.** After a median follow-up of 39 months, early-PD was detected in 191/926 (20.6%)  
101 patients, 228/926 (24.6%) patients had late-PD (TTP $>$ 18 months), while 507/926 (54.8%)  
102 did not have PD at the current follow-up. Compared to Late-PD patients, Early-PD patients  
103 had a lower at least very good partial response rate (47% vs 82%, p $<$ 0.001) and more  
104 frequently acquired double refractoriness to immunomodulatory drugs (IMiDs) and  
105 proteasome inhibitors (PIs) (21% vs 8%, p $<$ 0.001). Early-PD patients were at higher risk of  
106 death compared to late-PD and no-PD patients (HR 3.65, 95% CI 2.7-4.93, p $<$ 0.001), showing a  
107 dismal median overall survival (32.8 months). In a multivariate logistic regression model,  
108 independent factors increasing the early-PD risk were *TP53* mutation (OR 3.78, p $<$ 0.001),  
109 high LDH levels (OR 3.15, p=0.006),  $\lambda$ -chain translocation (OR 2.25, p=0.033) and *IGLL5*  
110 mutation (OR 2.15, p=0.007). Carfilzomib-based induction (OR 0.15, p=0.014), autologous  
111 stem-cell transplantation (OR 0.27, p $<$ 0.001) and continuous therapy with PIs and IMiDs (OR  
112 0.34, p=0.024) mitigated the risk of early-PD.

113 **Conclusion.** Early PD identifies a high-risk MM population. Further research is needed to  
114 better identify baseline features predicting early PD and the optimal treatment approaches  
115 for patients at risk.

116

117

## 118 **Introduction**

119 The expected survival of newly diagnosed multiple myeloma (NDMM) patients is currently  
120 improving and approaching 8 years, thanks to the use of novel agents and better supportive  
121 care (1). Nevertheless, MM still remains largely incurable and about 12000 MM patients die  
122 each year in the United States, with the main cause of death being the development of  
123 refractory disease to the currently available drugs (2,3).

124 Relapse is caused by MM cell clones with an increasing degree of drug refractoriness and  
125 genetic complexity eventually leading to shorter remissions (4). Since the longest remission  
126 period is usually induced by upfront treatment, the duration of first remission is one of the  
127 most important factors impacting patient prognosis (5).

128 This can become particularly important as a dynamic prognostic marker, if we consider the  
129 complexity associated with the evaluation of baseline prognostic features. The most widely  
130 used staging system is the Revised International Staging System (R-ISS), which is based on  
131 clinical and biological standard features (ISS, chromosomal abnormalities and lactate  
132 dehydrogenase [LDH] levels) (6). Many efforts aimed at improving the baseline stratification,  
133 including the use of gene expression profiles (GEP) and next-generation sequencing (NGS) (7–  
134 9). Of note, according to R-ISS, only 10% of patients are at high risk of progression and/or  
135 death and, according to the NGS-based “double-hit” classification, only 6.1% of patients are at  
136 high risk of progression and/or death, but the overall rate of patients who relapse or die  
137 within two years from diagnosis is about 20% (10,11). This highlights the importance of  
138 dynamic prognostic evaluation and the need for an improved baseline risk stratification. The  
139 identification and treatment of high-risk MM patients currently represent unmet medical  
140 needs. Our aims were (1) to characterize patients with early progressive disease (Early PD;  
141 time-to-progression [TTP]  $\leq 18$  months) after first-line treatment including

142 immunomodulatory drugs (IMiDs) and/or 1st-2nd generation proteasome inhibitors (PIs)  
143 incorporating baseline clinical and next-generation sequencing (NGS) molecular features; (2)  
144 to address the role of different upfront therapies in reducing the risk of Early PD.

145

## 146 **Methods**

### 147 **Patients and treatment**

148 Data from patients enrolled in the prospective observational Multiple Myeloma Research  
149 Foundation (MMRF) CoMMpass study (NCT01454297) were included in this analysis. Ethics  
150 committees or institutional review boards at the study sites approved the study, which was  
151 conducted in accordance with the Declaration of Helsinki. All patients provided written  
152 informed consent.

153 Main inclusion criteria were: symptomatic NDMM, measurable disease and upfront systemic  
154 therapy including an IMiD and/or a PI. CoMMpass data were generated as part of the MMRF  
155 Personalized Medicine Initiatives (<https://research.themmr.org> and [www.themmr.org](http://www.themmr.org)).

156 Data from patients receiving treatment in the context of clinical trials as well as with real  
157 word regimens were included. Therapy (source file  
158 “mmrf\_commpass\_IA14\_stand\_alone\_treatment\_regimen” available upon request on  
159 <https://research.themmr.org>) was reviewed and classified according to: type of induction  
160 treatment (bortezomib-dexamethasone/bortezomib+chemotherapy triplets/lenalidomide-  
161 dexamethasone/bortezomib-lenalidomide-dexamethasone/carfilzomib-based/other),  
162 autologous stem-cell transplantation (ASCT; Yes/No), and type of continuous treatment (CT)  
163 (IMiDs CT/PIs CT/IMiDs+PIs CT/Fixed duration of therapy [FDT]). FDT was defined as  $\leq 1$   
164 year of upfront treatment (12). The definition of variables is detailed in *Tables S1-S2*. Patients  
165 were considered evaluable for the ASCT vs no ASCT analysis if they were alive and relapse-  
166 free after induction treatment and if the date of ASCT was available. Patients receiving ASCT  
167 before PD but after 18 months from the start of treatment (cut-off for the early relapse



168 evaluation) were considered not evaluable. Patients were considered evaluable for the CT  
169 analysis if they were alive and relapse-free after 1 year from the start of treatment, the follow-  
170 up was >1 year and if details of treatment administered after the 1-year timepoint were  
171 available.

172 The Interim Analysis (IA)<sup>14</sup> release of CoMMpass was analyzed. Updated time-to-event  
173 endpoints for CoMMpass patients co-enrolled in the NCT02203643 trial were used (data cut-  
174 off: 30/05/2018).

175

### 176 **Next-generation sequencing**

177 Baseline bone marrow CD138+ cells were obtained before the initiation of systemic therapy  
178 (within 30 days before first-line treatment). Available data on samples at relapse, a pre-  
179 planned objective within the CoMMpass study, were also evaluated. Long-insert whole  
180 genome sequencing (WGS) and whole exome sequencing (WES) were performed by the  
181 Translational Genomics Institute (TGen). Somatic tumor alterations were defined comparing  
182 tumor cells with patient-specific paired normal cells. Details on the definition of the risk  
183 factors explored in this work are provided in previous CoMMpass publications (13–15).  
184 Cytogenetic data reported by single study centers were heterogeneous in terms of  
185 fluorescence in situ hybridization (FISH) probes utilized, number of cells counted and cell  
186 sorting techniques. To uniformly define cytogenetic abnormalities in all patients, copy  
187 number abnormalities (CNAs), immunoglobulin heavy chain (IgH) translocations and  
188 immunoglobulin lambda (IgL) translocations were defined using molecular data (Seq-FISH)  
189 (16–18). The concordance of Seq-FISH and conventional FISH in a subgroup of patients  
190 evaluated in the context of a clinical trial by a centralized laboratory showed a high degree of  
191 concordance (*Figure S1*). The presence or absence of recurrent CNAs [hyperdiploidy,  
192 deletion13q, deletion17p, gain1q (3 CSK1B copies) and amplification(1q) (>3 CSK1B copies)],

193 IgH translocations [t(11;14), t(4;14), t(14;16), t(14;20)] and IgL translocations were  
194 evaluated using calls on WGS long-insert data (18). The threshold for a positive detection of a  
195 CNA by Seq-FISH was 20%. Non-synonymous alterations with an allele ratio of at least 5% in  
196 the tumor sample and less than 2% in the constitutional sample occurring in a customized  
197 panel of 21 genes known to be significantly mutated in MM were also analyzed (*Table S1*)  
198 (19,20). The cancer cell fraction (CCF) of mutations of interest corrected by tumor purity and  
199 MM cell ploidy was estimated using the ABSOLUTE algorithm (21). Moreover, we evaluated  
200 the aberrant activity of APOBEC cytidine deaminases (known to be associated with high  
201 mutational burden and poor prognosis in MM)(22), using the recently developed fitting  
202 algorithm *mmsig* (*Table S1*; <https://github.com/evenrus/mmsig>) (23). APOBEC activity was  
203 defined as *high* or *low* based on its quartile distribution (4th quartile vs others) (22).

204

## 205 **Statistical analysis**

206 Early PD was defined as occurring in the first 18 months from the start of treatment. Patients  
207 not experiencing PD within 18 months from the start of treatment were included in the  
208 reference population. The reference population was further classified in Late PD (occurring  
209 after the first 18 months from the start of treatment) and No PD at the last follow-up. TTP was  
210 defined as the duration from start of treatment to PD; deaths from causes other than  
211 progression were censored (24).

212 Epanechnikov kernel smoothed estimated hazard rates were used to study the risk of PD over  
213 time.

214 Best response to first-line treatment and drug refractoriness after first-line treatment were  
215 evaluated according to the International Myeloma Working Group guidelines (24,25). The  
216 comparison of best response and drug refractoriness in the Early vs Late PD groups was  
217 performed according to two-sided Fisher's exact test.

218 Overall survival (OS) was analyzed as time-to-event data using the Kaplan–Meier method. The  
219 Cox proportional hazards model was used to estimate the hazard ratio (HR) values and the  
220 95% confidence intervals (CIs). In order to account for potential confounders, the comparison  
221 of Early PD vs reference population was adjusted for age, ISS, high-risk cytogenetics (26),  
222 induction treatment, ASCT, CT and clinical trial enrollment. ASCT and CT were considered as  
223 time-dependent variables.

224 An 18-month landmark analysis for OS was also performed, comparing OS in the Early PD vs  
225 Late PD vs No PD groups.

226 To identify risk factors associated with early relapse, patients that were not at risk for  
227 progression for the entire 18-month period after the start of treatment were excluded from  
228 the reference population (n=101, *Figure 1*).

229 Univariate analysis of factors associated with Early PD vs Late/No PD was performed using  
230 Fisher’s exact test, Kruskal-Wallis test or Chi-squared test as appropriate. Starting from the  
231 variables with a p-value <0.15 in univariate analysis, the final logistic model was identified  
232 through a backward selection based on the minimization of the Akaike Information Criterion  
233 (AIC), keeping in the model the therapy-related variables. The final logistic regression model  
234 was used to estimate odds ratio (OR) for Early relapse risk, 95% CIs and p-values.

235 Analysis was conducted using R version 3.5.1 and bespoke code that is available upon request.

236

237

## 238 **Results**

### 239 **Patient characteristics**

240 Data from 1151 patients were available in the CoMMpass IA14. Patients without whole-exome  
241 sequencing (WES) data (n=213) and PD information (n=12) were excluded from the analysis.

242 The remaining 926 patients represented the population analyzed in the current work. Patient  
243 characteristics are shown in *Table 1*.

244 Median age was 63 years and most of the patients had an Eastern Cooperative Oncology  
245 Group (ECOG) performance status of 0 or 1 (39% and 44%, respectively). Baseline prognostic  
246 factors were typical of a NDMM population. 27% of patients presented with ISS stage III and  
247 8% with high LDH levels; 13% of patients presented with del(17p), 14% with t(4;14), 5%  
248 with t(14;16), 1% with t(14;20), 27% with gain(1q) and 7% with amp(1q), while IgL  
249 translocations, a recently described marker of high-risk MM (18), were present in 10% of  
250 evaluable patients.

251 Genes affected by somatic non-synonymous alterations in at least 25 (3%) patients were  
252 analyzed (*Table S3*). Mutational frequency was dominated by alterations in KRAS (25%),  
253 NRAS (21.5%) and IGLL5 (16%) gene.

254 The most frequent induction regimen administered was bortezomib-lenalidomide-  
255 dexamethasone (VRd) (34%), followed by bortezomib+chemotherapy triplets (23%) and  
256 carfilzomib-based treatment (23%).

257 Patients evaluable for the ASCT vs no ASCT comparison were 833. Not evaluable patients  
258 experienced PD during induction (n=40), died for reasons other than PD (n=18), were lost to  
259 follow-up (n=14), withdrew consent (n=5), or discontinued the study for other reasons (n=6).  
260 Ten patients received ASCT after the 18-month endpoint and were considered not evaluable  
261 as well. High-dose chemotherapy followed by ASCT was received by 53% of the evaluable  
262 patients; the median time to ASCT was 169 days (range 78-508).

263 Patients evaluable for CT vs FDT comparison were 609. Not evaluable patients, during the  
264 first year of treatment had PD (n= 112), died for reasons other than PD (n= 32), were lost to  
265 follow-up (n= 21), withdrew consent (n= 16) or discontinued the study for other reasons (n=  
266 15). In 121 patients, information of drugs used during CT was lacking at the current follow-up.

267 74% of evaluable patients received CT (IMiDs 42%, PIs 14% and IMiDs+PIs 18%); 26% of  
268 patients received FDT. The distributions of induction treatment and ASCT in each CT  
269 subgroup are shown in *Table S4*.

270

### 271 **Early PD population**

272 The median follow-up of the entire population was 39 months. 191/926 (20.6%) patients  
273 experienced early PD, while the remaining 735/926 (79.4%) patients were included in the  
274 reference population (*Figure 1*).

275 In the early PD group, 126/191 (66%) patients discontinued the study at the last follow-up:  
276 75 (39%) for death due to PD, 26 (14%) for death due to other reasons, 4 (2%) due to  
277 withdrawal of consent, 3 (2%) for being lost to follow-up, and 18 (9%) for other reasons.

278 In the reference population, 229/735 (31%) patients discontinued the study: 39 (5%) for  
279 death due to PD, 66 (9%) for death due to other reasons, 31 (4%) due to withdrawal of  
280 consent, 39 (5%) for being lost to follow-up, and 54 (7%) for other reasons. In the same  
281 reference population, 228/926 (24.6%) patients experienced a late PD (TTP>18 months),  
282 while 507/926 (54.8%) did not experience PD at the last follow-up.

283 Overall response rate (ORR) was significantly lower in Early-PD patients compared to Late-PD  
284 patients (80% vs 96%, respectively,  $p<0.001$ ). Deep responses were also different, with very  
285 good partial response (VGPR) rates of 40% vs 57%, complete remission (CR) rates of 2% vs  
286 18% and stringent CR rates of 5% vs 8% in Early vs Late PD groups respectively. This  
287 translated into a significantly different rate of  $\geq$ VGPR in the 2 groups (47% vs 82%,  $p<0.001$ ;  
288 *Table 2*).

289 A significantly higher proportion of patients in the Early vs the Late PD group developed a  
290 refractoriness to PIs (50% vs 18%,  $p<0.001$ ) and IMiDs+PIs (21% vs 8%,  $p<0.001$ ), while no  
291 differences were found in terms of IMiD refractoriness (42% vs 38%,  $p=0.541$ ; *Table 2*).

292 OS of Early-PD patients vs the reference population is shown in *Figure 2*.

293 Early-PD patients had a significantly higher risk of death compared to the reference  
294 population (HR 4.89, 95% CI 3.72-6.43,  $p<0.001$ ), with 53% of patient deaths at 3 years in the  
295 early PD cohort compared with only 12% in the reference cohort. This effect was maintained  
296 after adjusting the analysis for age, baseline prognostic factors (ISS, high-risk  
297 cytogenetics(26)), treatment and clinical trial enrollment (HR 3.65, 95% CI 2.70-4.93,  
298  $p<0.001$ ). Of note, 61% of early relapsing patients presented with ISS stage I or II and 74%  
299 had conventionally defined standard-risk cytogenetics (26). The median OS of early relapsing  
300 patients was 32.8 months, lower than that of high-risk population defined using baseline ISS  
301 III (median OS 54 months) or baseline high-risk cytogenetics (26) (median OS 65 months).

302 Early-PD patients were defined using a time-dependent endpoint (18 months); consequently,  
303 a landmark analysis of OS with a landmark point at 18 months was performed to validate our  
304 findings (*Figure 3*). At the landmark timepoint, 121 Early-PD patients and 640 patients in the  
305 reference population were evaluable. The main reasons for not being evaluable were death  
306 due to PD during the first 18 months in the early PD population (58/191, 30%) and death due  
307 to reasons other than PD during the first 18 months in the reference population (42/735,  
308 6%). The difference in early death rates between the 2 groups led to a possible  
309 underestimation of OS differences after the landmark timepoint. Moreover, in this OS  
310 comparison we split the reference population in Late PD and No PD patients. The 18-month  
311 landmark analysis showed a significantly worse OS in Early-PD patients compared both to  
312 Late PD (HR 2.05, 95%, CI 1.25-3.35,  $p=0.004$ ) and No PD patients (HR 8.05, 95%, CI 4.11-  
313 15.74,  $p<0.001$ ).

314

315 **Risk of early PD**

316 We investigated the clinical and prognostic variables impacting the risk of early relapse. In  
317 this analysis, we excluded from the reference population the patients who were not at risk for  
318 the entire 18-month period (101/926, 11%). Excluded patients were those that in the first 18  
319 months died without a PD (n=42), withdrew the consent (n=14), were lost to follow-up  
320 (n=25) or interrupted the protocol for other reasons (n=20).

321 A significantly higher proportion of patients in the early PD group vs the reference population  
322 presented with ISS stage III (39% vs 20%), gain(1q) (26% vs 20%), IgL translocations (14%  
323 vs 6%), high APOBEC signature (30% vs 24%), high LDH (9% vs 5%), ECOG $\geq$ 2 (23% vs 11%),  
324 *KRAS* mutation (31% vs 24%), *IGLL5* mutation (20% vs 14%) and *TP53* mutation (9% vs 3%)  
325 (*Table S5*). These variables were therefore included in multivariate analysis, together with age  
326 and treatment administered.

327 In multivariate analysis (*Figure 4*) *TP53* mutation (OR 3.78, p<0.01), high LDH levels (OR 3.15,  
328 p<0.01), IgL translocation (OR 2.25, p=0.03) and *IGLL5* mutation (OR 2.15, p<0.01) were  
329 significantly correlated with a higher risk of early PD.

330 Receiving ASCT (OR 0.27, p<0.01) and CT with IMiDs+PIs (OR 0.34, p=0.02) were significantly  
331 correlated with a lower risk of early PD. The effect of ASCT was confirmed in age-specific  
332 patient subgroups, showing similar ORs in patients aged  $\leq$ 65 years (n=531, OR 0.27 95% CI  
333 0.13-0.54) and aged 66-75 years (n=222, OR 0.30 95% CI 0.11-0.74).

334 A protective effect of carfilzomib-based induction was also observed (OR 0.15, p=0.01).  
335 Nevertheless, most of carfilzomib-treated patients were enrolled in a clinical trial and the  
336 enrollment effect itself was a protective factor as well (OR 0.09, p<0.01).

337

338 *TP53 mutations*

339 In our analysis, TP53 mutation was the factor with the greatest effect size for early PD. Its  
340 association with MM patients carrying concurrent del(17p) is well known. In this cohort, 865  
341 patients were evaluable for TP53 mutation and del(17p) (*Figure S1A*). One hundred twenty-  
342 one of 865 patients had del(17p) or TP53 mutation. Among them, 82/121 (68%) had del(17p)  
343 only, 10/121 (8%) had TP53 mutation only and 29/121 (24%) had del(17p) and TP53  
344 mutation. Rates of early PD in each patient subgroup are shown in *Figure S1B*. Patients with  
345 del(17p) but not TP53 mutation had an early PD rate of 17.1% (comparable with the general  
346 population), while the bi-allelic group (del(17p)+TP53 mutation) and the TP53-mutation-only  
347 group showed high early PD rates (41.4% and 50%, respectively). Of note, the TP53-  
348 mutation-only group was composed by only 10 patients and the majority of TP53-mutated  
349 patients experiencing early relapse were in the del(17p)+TP53 mutation group.

350 The use of a higher cut-off level to define del(17p) positivity (50% instead of 20%, *Figure S1C-*  
351 *D*) led to a slightly higher early PD rate in del(17p)-only patients (25%). However, the bi-  
352 allelic (del(17p)+TP53 mutation) and the TP53-mutation-only groups still showed the highest  
353 rates of early PD (40.7% and 50%, respectively).

354

355 *Longitudinal analysis of mutations associated with early PD*

356 Considering that TP53 mutation is important to confer early relapse risk, we hypothesized  
357 that TP53-mutated clones needed to be conserved at relapse. Only 6 patients with TP53  
358 mutation at diagnosis had available molecular data at relapse, although in 6/6 cases TP53  
359 mutation was conserved in relapse samples (*Figure S2A*). Moreover, despite the small  
360 numbers, if TP53 mutation was subclonal at diagnosis, a higher cancer cell fraction was found  
361 in paired samples at relapse. This effect was different from the IGLL5 mutations, in which  
362 subclonal cases tended to disappear at relapse (*Figure S2B*).



363

## 364 **Discussion**

365 MM prognosis is improving and early relapse after upfront treatment is beginning to be  
366 recognized as a high-risk feature (27). The same observation had been done for other  
367 hematologic malignancies with an expected indolent course, such as follicular lymphoma and  
368 chronic lymphocytic leukemia (28,29).

369 Here we proposed progression  $\leq 18$  months after the start of first-line treatment as a marker  
370 of high risk and demonstrated its detrimental effect on the OS of NDMM patients.

371 The 18-month cut-off was chosen because our time to ASCT was  $\sim 6$  months and the majority  
372 of published studies on MM patients with early PD defined early PD as a relapse within 12  
373 months from ASCT. Indeed, the hazards of progression in our patient population increased  
374 over time with no identified peak of risk (*Figure S3*).

375 We incorporated in our analysis baseline clinical and biological features to identify risk  
376 factors of early PD. The characterization by NGS of this patient cohort allowed us to  
377 simultaneously study copy number abnormalities (CNAs), translocations and mutations in  
378 genes of interest by using the same platform. This is an advantage of NGS vs conventional  
379 fluorescence in situ hybridization (FISH), which cannot detect mutations and needs specific  
380 probes to detect pre-specified translocations and CNAs. Moreover, NGS and conventional FISH  
381 showed high concordance in detecting the same CNAs and translocations, as shown in *Figure*  
382 *S4* and by others (16,17).

383 TP53 mutation, which is currently not included in the standard baseline evaluation of MM  
384 patients, was the most important factor increasing the risk of early PD emerging from our  
385 analysis. TP53 mutation is rare in patients at diagnosis (3.5%), but about 25% of patients  
386 with del(17p) has also TP53 mutation. As similarly observed by other groups (8), our data

387 further supported the routine testing of TP53 mutation at least in del(17p)-positive patients.  
388 Indeed, the presence of del(17p) without TP53 mutation conferred an early PD risk that was  
389 similar to that of the overall population.

390 In our analysis, IgL translocation and IGLL5 mutation also emerged as risk factors of early PD.  
391 Both of them have already been associated with poor prognosis (18,30). White et al. showed  
392 that mutations in IGLL5 can be associated with translocations juxtaposing IGLL5 (30). In our  
393 analysis, IGLL5 mutations and IgL translocations showed a trend toward co-occurrence,  
394 though not statistically significant ( $p=0.06$ ). The higher OR in IgL-translocated patients and  
395 the loss of subclonal IGLL5 mutations at first relapse could suggest that the Early PD risk was  
396 favored more by IgL translocations than by IGLL5 mutations.

397 In our analysis, the only clinical factor that increased the risk of early PD in multivariate  
398 analysis was baseline LDH, a well-known marker of disease aggressiveness in several  
399 hematologic diseases.

400 Other factors not included in the current analysis – such as circulating plasma cells (31), high-  
401 risk GEP(7,32) and MM cell-extrinsic factors (33) – could also play a role in determining the  
402 risk of early PD and should be investigated in future works. Moreover, our analysis focused on  
403 MM cells derived from a random bone marrow aspirate, and spatial heterogeneity of high-risk  
404 features could also explain some of the early PD cases (34).

405 ASCT and CT with IMiDs+PIs showed a protective effect against early PD in this patient  
406 population. However, the majority of the patients in the analyzed cohort were real-world  
407 patients and the analysis was consequently performed as per protocol, thus leading to a risk  
408 of overestimation of effects of ASCT and CT. With these limitations, our data support the  
409 intensification of therapy in patients at risk of early relapse and underline the importance of  
410 continuous treatment with combination regimens to optimize long-term disease control (35).

411 Carfilzomib-based induction showed to reduce the risk of early relapse as well. However, it is  
412 difficult to distinguish between treatment and trial effects because the majority of  
413 carfilzomib-treated patients were included in a clinical trial, whereas this was not the case for  
414 other induction regimens.

415 Besides clinical trial enrollment, this patient population was heterogeneously treated and our  
416 findings on early PD risk need to be confirmed in homogeneously treated patients. For  
417 instance, among the CT subgroups, heterogeneous upfront treatments before CT were  
418 received (*Table S4*). Nevertheless, the multivariate analysis on the risk of early PD was  
419 adjusted for induction treatment, ASCT, CT and trial enrollment effect, taking into account  
420 these differences.

421 The median age of the analyzed cohort was 63 years, younger than the usual median age of  
422 unselected MM patients. Elderly patients were underrepresented and the confirmation of our  
423 results in this patient population is warranted. However, other variables that are patient-  
424 related but not disease-related (e.g. frailty status) may have a major prognostic role in elderly  
425 patients (36).

426 Early-PD patients showed suboptimal responses and, at relapse, were more frequently  
427 refractory to PIs and double refractory to IMiDs+PIs, as compared to Late-PD patients. IMiD  
428 refractoriness was not different between Early and Late PD groups. This was mainly due to  
429 the widespread use of maintenance therapy with a single-agent IMiD after the 18-month  
430 timepoint inducing a high percentage of IMiD-refractory cases in the Late PD group.

431 In conclusion, early PD identifies a high-risk MM population that still represents an unmet  
432 clinical need. As compared with FISH, extended genotyping through the routine use of NGS at  
433 diagnosis is feasible and may improve the patient stratification and identify patients at risk of  
434 early PD (37). Further research is needed to better identify baseline features predicting early  
435 relapse and the optimal treatment approach. Recently, clinical trials on patients experiencing

436 PD within 18 months from the start of treatment are beginning to emerge (e.g. NCT03601078,  
437 cohorts 2a and 2b), thus suggesting that risk-adapted treatment in this patient population  
438 could soon become a feature of MM clinical management.

439

## 440 **References**

441

- 442 1. Usmani SZ, Hoering A, Cavo M, Miguel JS, Goldschmidt H, Hajek R, et al. Clinical  
443 predictors of long-term survival in newly diagnosed transplant eligible multiple  
444 myeloma — an IMWG Research Project. *Blood Cancer J* [Internet]. 2018 [cited 2019 Oct  
445 7];8:123. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30470751>
- 446 2. Kazandjian D. Multiple myeloma epidemiology and survival: A unique malignancy.  
447 *Semin Oncol* [Internet]. 2016 [cited 2019 Sep 30];43:676–81. Available from:  
448 <http://www.ncbi.nlm.nih.gov/pubmed/28061985>
- 449 3. Mai EK, Haas E-M, Lücke S, Löprrich M, Kunz C, Pritsch M, et al. A systematic  
450 classification of death causes in multiple myeloma. *Blood Cancer J* [Internet]. 2018  
451 [cited 2019 Sep 30];8:30. Available from:  
452 <http://www.ncbi.nlm.nih.gov/pubmed/29520024>
- 453 4. Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, et al. Clonal competition  
454 with alternating dominance in multiple myeloma. *Blood* [Internet]. 2012 [cited 2019  
455 Sep 30];120:1067–76. Available from:  
456 <http://www.ncbi.nlm.nih.gov/pubmed/22498740>
- 457 5. Majithia N, Rajkumar S V, Lacy MQ, Buadi FK, Dispenzieri A, Gertz MA, et al. Early  
458 relapse following initial therapy for multiple myeloma predicts poor outcomes in the  
459 era of novel agents. *Leukemia* [Internet]. 2016 [cited 2019 Sep 30];30:2208–13.  
460 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27211270>
- 461 6. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al.  
462 Revised International Staging System for Multiple Myeloma: A Report From  
463 International Myeloma Working Group. *J Clin Oncol* [Internet]. American Society of  
464 Clinical Oncology; 2015 [cited 2017 Sep 5];33:2863–9. Available from:  
465 <http://ascopubs.org/doi/10.1200/JCO.2015.61.2267>
- 466 7. Kuiper R, Broijl A, van Duin M, van Vliet1 MH, Levin M-D, van Beers1 EH, et al.  
467 PROGNOSTIC AND PREDICTIVE PERFORMANCE OF SKY92 COMBINED WITH R-ISS IN  
468 ELDERLY MULTIPLE MYELOMA PATIENTS IN THE HOVON- 87/NMSG-18 STUDY. 17th  
469 IMW [International Myeloma Workshop] Abstract Book. Boston; 2019. page 15-16  
470 [Abstract #OAB-013]. Available from:  
471 [https://files.aievolution.com/imw1901/docs/17th\\_IMW\\_Abstract\\_Book\\_FINAL\\_V2.pdf](https://files.aievolution.com/imw1901/docs/17th_IMW_Abstract_Book_FINAL_V2.pdf)
- 472 8. Walker BA, Mavrommatis K, Wardell CP, Ashby TC, Bauer M, Davies F, et al. A high-risk,  
473 Double-Hit, group of newly diagnosed myeloma identified by genomic analysis.  
474 *Leukemia* [Internet]. 2019 [cited 2019 Sep 30];33:159–70. Available from:  
475 <http://www.ncbi.nlm.nih.gov/pubmed/29967379>
- 476 9. Bolli N, Biancon G, Moarii M, Gimondi S, Li Y, de Philippis C, et al. Analysis of the  
477 genomic landscape of multiple myeloma highlights novel prognostic markers and  
478 disease subgroups. *Leukemia*. Nature Publishing Group; 2018;32:2604–16.
- 479 10. Avet-Loiseau H. Ultra High-Risk Myeloma. *Hematology* [Internet]. 2010 [cited 2019 Sep  
480 30];2010:489–93. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21239841>
- 481 11. Lonial S, Boise LH, Kaufman J. How I treat high-risk myeloma. *Blood* [Internet]. 2015

- 482 [cited 2019 Sep 30];126:1536–43. Available from:  
483 <http://www.bloodjournal.org/cgi/doi/10.1182/blood-2015-06-653261>
- 484 12. Palumbo A, Gay F, Cavallo F, Di Raimondo F, Larocca A, Hardan I, et al. Continuous  
485 Therapy Versus Fixed Duration of Therapy in Patients With Newly Diagnosed Multiple  
486 Myeloma. *J Clin Oncol* [Internet]. 2015 [cited 2019 Apr 5];33:3459–66. Available from:  
487 <http://ascopubs.org/doi/10.1200/JCO.2014.60.2466>
- 488 13. Keats J, Speyer G, Christofferson A, Stephenson K, Kurdoglu A, Russell M, et al. Interim  
489 Analysis of the MMRF CoMMpass Study: Comprehensive Characterization of Multiple  
490 Myeloma Patients at Diagnosis Reveals Distinct Molecular Subtypes and Clinical  
491 Outcomes. *Clin Lymphoma Myeloma Leuk* [Internet]. Elsevier; 2015 [cited 2019 Sep  
492 30];15:e44–5. Available from:  
493 <https://www.sciencedirect.com/science/article/pii/S2152265015006102>
- 494 14. Miller A, Asmann Y, Cattaneo L, Braggio E, Keats J, Auclair D, et al. High somatic  
495 mutation and neoantigen burden are correlated with decreased progression-free  
496 survival in multiple myeloma. *Blood Cancer J* [Internet]. Nature Publishing Group; 2017  
497 [cited 2019 Sep 30];7:e612–e612. Available from:  
498 <http://www.nature.com/articles/bcj201794>
- 499 15. Maura F, Bolli N, Angelopoulos N, Dawson KJ, Leongamornlert D, Martincorena I, et al.  
500 Genomic landscape and chronological reconstruction of driver events in multiple  
501 myeloma. *Nat Commun*. Springer Science and Business Media LLC; 2019;10.
- 502 16. Miller C, Yesil J, Derome M, Donnelly A, Marrian J, McBride K, et al. A Comparison of  
503 Clinical FISH and Sequencing Based FISH Estimates in Multiple Myeloma: An Mmrf  
504 Commpass Analysis. *Blood* [Internet]. 2016 [cited 2019 Sep 30];128:Abstract #374  
505 [ASH 2016 58th Meeting]. Available from:  
506 <http://www.bloodjournal.org/content/128/22/374>
- 507 17. Goldsmith SR, Fiala MA, Dukeman J, Ghobadi A, Stockerl-Goldstein K, Schroeder MA, et  
508 al. Next Generation Sequencing-based Validation of the Revised International Staging  
509 System for Multiple Myeloma: An Analysis of the MMRF CoMMpass Study. *Clin*  
510 *Lymphoma Myeloma Leuk* [Internet]. 2019 [cited 2019 Sep 30];19:285–9. Available  
511 from: <https://linkinghub.elsevier.com/retrieve/pii/S2152265018315246>
- 512 18. Barwick BG, Neri P, Bahlis NJ, Nooka AK, Dhodapkar M V., Jaye DL, et al. Multiple  
513 myeloma immunoglobulin lambda translocations portend poor prognosis. *Nat Commun*  
514 [Internet]. Nature Publishing Group; 2019 [cited 2019 Sep 30];10:1911. Available from:  
515 <http://www.nature.com/articles/s41467-019-09555-6>
- 516 19. Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I, et al.  
517 Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nat*  
518 *Commun* [Internet]. 2014 [cited 2019 Sep 30];5:2997. Available from:  
519 <http://www.ncbi.nlm.nih.gov/pubmed/24429703>
- 520 20. Kortum KM, Mai EK, Hanafiah NH, Shi C-X, Zhu Y-X, Bruins L, et al. Targeted sequencing  
521 of refractory myeloma reveals a high incidence of mutations in CRBN and Ras pathway  
522 genes. *Blood* [Internet]. 2016 [cited 2019 Sep 30];128:1226–33. Available from:  
523 <http://www.ncbi.nlm.nih.gov/pubmed/27458004>
- 524 21. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute  
525 quantification of somatic DNA alterations in human cancer. *Nat Biotechnol* [Internet].  
526 2012 [cited 2019 Sep 30];30:413–21. Available from:  
527 <http://www.ncbi.nlm.nih.gov/pubmed/22544022>
- 528 22. Maura F, Petljak M, Lionetti M, Cifola I, Liang W, Pinatel E, et al. Biological and  
529 prognostic impact of APOBEC-induced mutations in the spectrum of plasma cell  
530 dyscrasias and multiple myeloma cell lines. *Leukemia* [Internet]. 2018 [cited 2019 Sep  
531 30];32:1044–8. Available from: <http://www.nature.com/articles/leu2017345>

- 532 23. Maura F, Degasperi A, Nadeu F, Leongamornlert D, Davies H, Moore L, et al. A practical  
533 guide for mutational signature analysis in hematological malignancies. *Nat Commun.*  
534 Nature Publishing Group; 2019;10.
- 535 24. Rajkumar SV, Harousseau J-L, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al.  
536 Consensus recommendations for the uniform reporting of clinical trials: report of the  
537 International Myeloma Workshop Consensus Panel 1. *Blood* [Internet]. 2011 [cited  
538 2017 May 9];117:4691–5. Available from:  
539 <http://www.bloodjournal.org/cgi/doi/10.1182/blood-2010-10-299487>
- 540 25. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International  
541 Myeloma Working Group consensus criteria for response and minimal residual disease  
542 assessment in multiple myeloma. *Lancet Oncol* [Internet]. 2016 [cited 2017 Jul  
543 12];17:e328–46. Available from:  
544 <http://linkinghub.elsevier.com/retrieve/pii/S1470204516302066>
- 545 26. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, et al.  
546 International Myeloma Working Group molecular classification of multiple myeloma:  
547 spotlight review. *Leukemia* [Internet]. 2009 [cited 2019 Sep 25];23:2210–21. Available  
548 from: <http://www.nature.com/articles/leu2009174>
- 549 27. Kumar SK, Dispenzieri A, Fraser R, Mingwei F, Akpek G, Cornell R, et al. Early relapse  
550 after autologous hematopoietic cell transplantation remains a poor prognostic factor in  
551 multiple myeloma but outcomes have improved over time. *Leukemia* [Internet]. 2018  
552 [cited 2019 Sep 30];32:986–95. Available from:  
553 <http://www.nature.com/articles/leu2017331>
- 554 28. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early Relapse of  
555 Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin,  
556 Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From  
557 the National LymphoCare Study. *J Clin Oncol* [Internet]. 2015 [cited 2019 Sep  
558 30];33:2516–22. Available from: <http://ascopubs.org/doi/10.1200/JCO.2014.59.7534>
- 559 29. Ahn IE, Farber CM, Davids MS, Grinblatt DL, Kay NE, Lamanna N, et al. Early progression  
560 of disease as a predictor of survival in chronic lymphocytic leukemia. *Blood Adv*  
561 [Internet]. 2017 [cited 2019 Sep 30];1:2433–43. Available from:  
562 <http://www.ncbi.nlm.nih.gov/pubmed/29296893>
- 563 30. White BS, Lanc I, O’Neal J, Gupta H, Fulton RS, Schmidt H, et al. A multiple myeloma-  
564 specific capture sequencing platform discovers novel translocations and frequent, risk-  
565 associated point mutations in IGLL5. *Blood Cancer J* [Internet]. 2018 [cited 2019 Sep  
566 30];8:35. Available from: <http://www.nature.com/articles/s41408-018-0062-y>
- 567 31. Granell M, Calvo X, Garcia-Guiñón A, Escoda L, Abella E, Martínez CM, et al. Prognostic  
568 impact of circulating plasma cells in patients with multiple myeloma: implications for  
569 plasma cell leukemia definition. *Haematologica* [Internet]. 2017 [cited 2019 Oct  
570 7];102:1099–104. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28255016>
- 571 32. Zhou Y, Chen L, Barlogie B, Stephens O, Wu X, Williams DR, et al. High-risk myeloma is  
572 associated with global elevation of miRNAs and overexpression of EIF2C2/AGO2. *Proc*  
573 *Natl Acad Sci* [Internet]. 2010 [cited 2019 Sep 30];107:7904–9. Available from:  
574 <http://www.ncbi.nlm.nih.gov/pubmed/20385818>
- 575 33. Manier S, Sacco A, Leleu X, Ghobrial IM, Roccaro AM. Bone Marrow Microenvironment  
576 in Multiple Myeloma Progression. *J Biomed Biotechnol* [Internet]. 2012 [cited 2019 Sep  
577 30];2012:1–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23093834>
- 578 34. Rasche L, Chavan SS, Stephens OW, Patel PH, Tytarenko R, Ashby C, et al. Spatial  
579 genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. *Nat*  
580 *Commun.* 2017;8:268.
- 581 35. D’Agostino M, De Paoli L, Conticello C, Offidani M, Ria R, Petrucci MT, et al. Continuous

- 582 therapy in standard- and high-risk newly-diagnosed multiple myeloma: A pooled  
583 analysis of 2 phase III trials. *Crit Rev Oncol Hematol* [Internet]. 2018 [cited 2019 Oct  
584 7];132:9–16. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30447931>
- 585 36. Brighen S, D’Agostino M, Paris L, Ballanti S, Pescosta N, Spada S, et al. Lenalidomide-  
586 based induction and maintenance in elderly newly diagnosed multiple myeloma  
587 patients: updated results of the EMN01 randomized trial. *Haematologica* [Internet].  
588 2019 [cited 2019 Oct 17];[Epub ahead of print]. Available from:  
589 <http://www.ncbi.nlm.nih.gov/pubmed/31582542>
- 590 37. Bolli N, Li Y, Sathiaseelan V, Raine K, Jones D, Ganly P, et al. A DNA target-enrichment  
591 approach to detect mutations, copy number changes and immunoglobulin  
592 translocations in multiple myeloma. *Blood Cancer J* [Internet]. 2016 [cited 2019 Nov  
593 11];6:e467. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27588520>
- 594

595 **Figures**

596

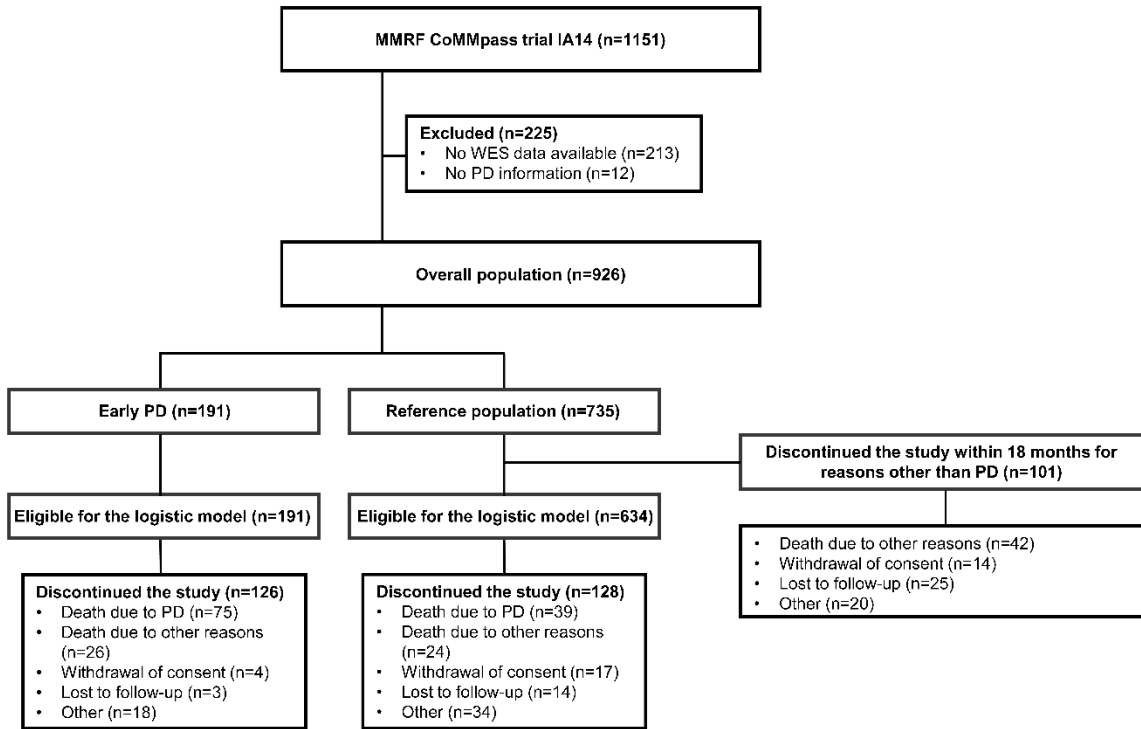
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598 **Figure 1. Study flow**

599

600

**Figure 1**



601

602 **Abbreviations.** MMRF: Multiple Myeloma Research Foundation; IA14: Interim analysis 14; WES: whole exome

603 sequencing; PD: progressive disease; n, number.

604

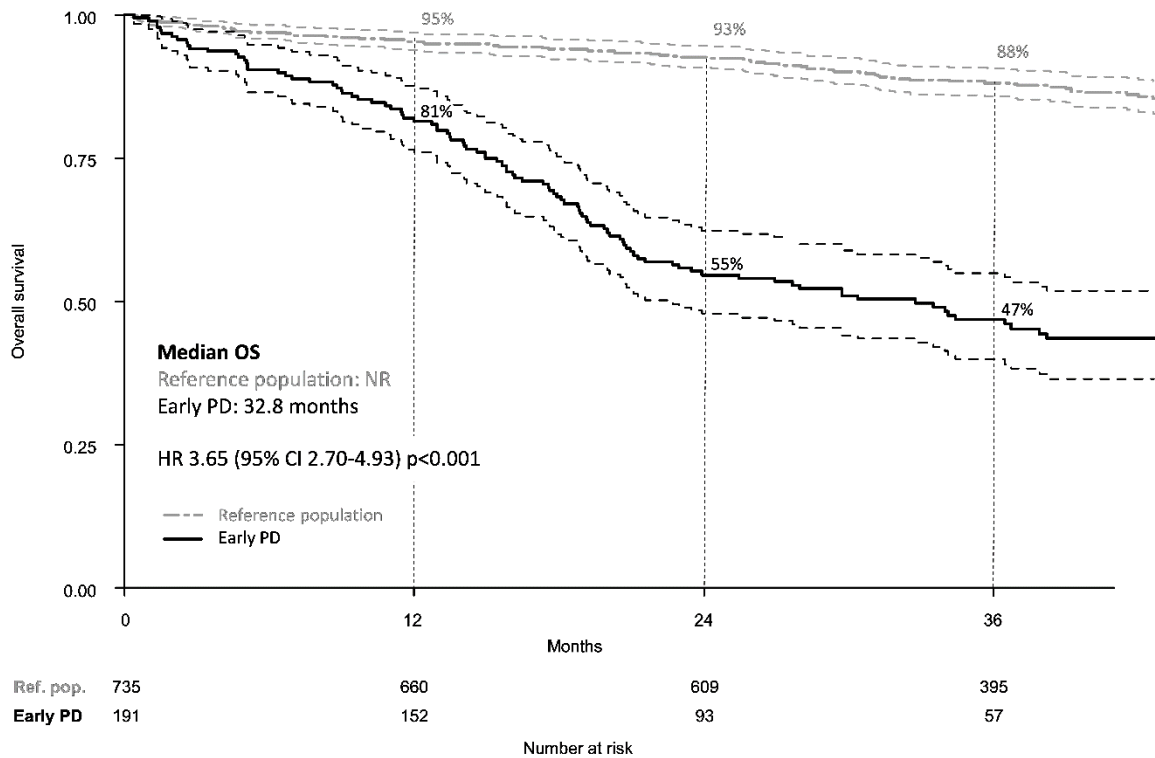
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607 **Figure 2.** Overall survival for patients with early PD versus reference population  
 608

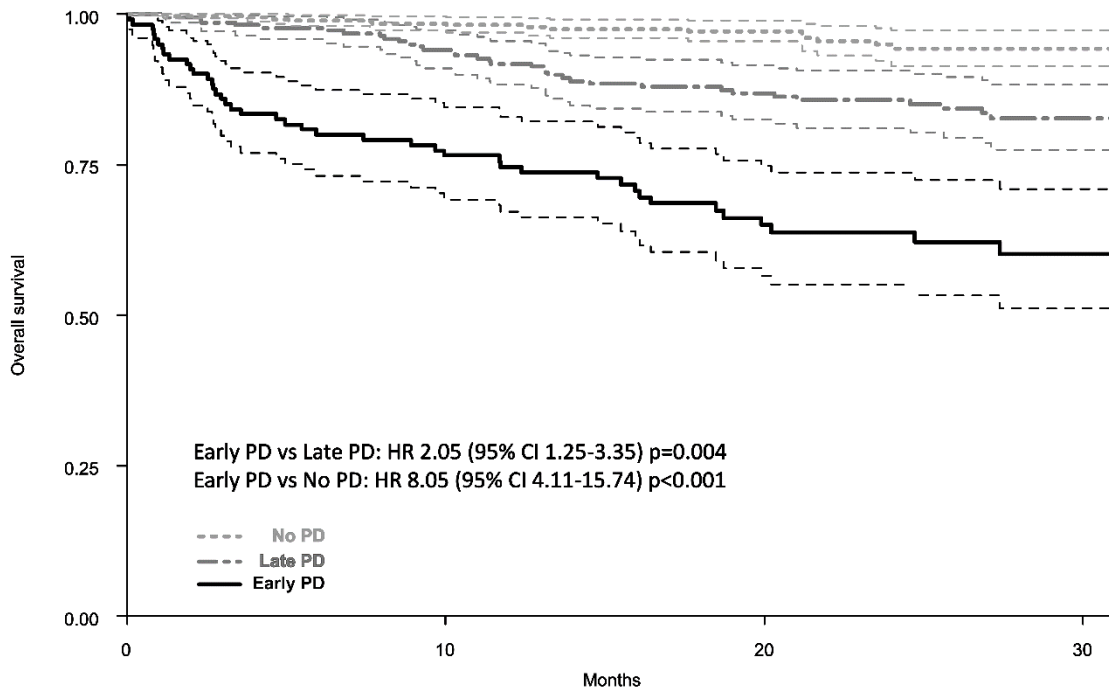
**Figure 2**



609 **Abbreviations.** OS: overall survival; PD: progressive disease; HR: hazard ratio; NR: not reached; ref. pop.,  
 610 reference population.  
 611 Dotted lines: 95% confidence intervals. HR adjusted for age, International Staging System (ISS) stage, high-risk  
 612 cytogenetics [presence of del(17p) and/or t(4;14) and/or t(14;16), induction treatment, autologous stem-cell  
 613 transplantation (ASCT), continuous therapy (CT), and clinical trial enrollment.  
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 615  
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 617  
 618

619 **Figure 3.** 18-month landmark analysis for OS in Early PD versus Late PD versus No PD  
 620 patients  
 621

**Figure 3**



|                 |                |     |     |    |
|-----------------|----------------|-----|-----|----|
| <b>No PD</b>    | 412            | 369 | 203 | 88 |
| <b>Late PD</b>  | 228            | 206 | 157 | 83 |
| <b>Early PD</b> | 121            | 86  | 54  | 27 |
|                 | Number at risk |     |     |    |

622 **Abbreviations.** OS: Overall survival; PD: progressive disease; HR: hazard ratio.  
 623 Dotted lines: 95% confidence intervals. HR adjusted for age, International Staging System (ISS) stage, high-risk  
 624 cytogenetics [presence of del(17p) and/or t(4;14) and/or t(14;16)], induction treatment, autologous stem-cell  
 625 transplantation (ASCT), continuous therapy (CT), and clinical trial enrollment.  
 626  
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642 **Tables**

643 **Table 1.** Patient characteristics

644 *The entire cohort of patients (N=926) is shown.*

| Characteristic            | N (%*)     |
|---------------------------|------------|
| Median follow-up          | 39 months  |
| Median age (IQR)          | 63 (59-69) |
| Induction treatment       |            |
| VRd                       | 319 (34%)  |
| V+chemo triplets          | 216 (23%)  |
| K-based                   | 215 (23%)  |
| Vd                        | 83 (9%)    |
| Rd                        | 63 (7%)    |
| Other                     | 30 (3%)    |
| ASCT                      |            |
| Yes                       | 440 (53%)  |
| No                        | 393 (47%)  |
| Not evaluable             | 93         |
| CT                        |            |
| FDT                       | 159 (26%)  |
| IMiDs                     | 258 (42%)  |
| PIs                       | 83 (14%)   |
| IMiDs+PIs                 | 109 (18%)  |
| Not evaluable             | 317        |
| Clinical trial enrollment |            |
| Yes                       | 166 (18%)  |
| No                        | 760 (82%)  |
| ISS                       |            |
| 1                         | 328 (37%)  |
| 2                         | 325 (36%)  |
| 3                         | 245 (27%)  |
| Missing                   | 28         |
| CNAs                      |            |
| Hyperdiploidy             | 499 (58%)  |
| del(13q)                  | 449 (52%)  |
| del(17p)                  | 111 (13%)  |
| Not evaluable             | 61         |
| gain(1q)                  | 203 (27%)  |
| amp(1q)                   | 53 (7%)    |
| Not evaluable             | 174        |
| IgH translocations        |            |
| t(11;14)                  | 179 (20%)  |
| t(4;14)                   | 123 (14%)  |
| t(14;16)                  | 42 (5%)    |
| t(14;20)                  | 12 (1%)    |
| Not evaluable             | 25         |

|                             |           |
|-----------------------------|-----------|
| IgL translocations          |           |
| Yes                         | 77 (10%)  |
| No                          | 692 (90%) |
| Not evaluable               | 187       |
| APOBEC mutational signature |           |
| High                        | 231 (25%) |
| Low                         | 695 (75%) |
| Not evaluable               | 0         |
| LDH                         |           |
| High                        | 60 (8%)   |
| Normal                      | 657 (92%) |
| Missing                     | 209       |
| ECOG                        |           |
| 0                           | 329 (39%) |
| 1                           | 372 (44%) |
| ≥2                          | 141 (17%) |
| Missing                     | 84        |

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**Abbreviations.** IQR, interquartile range; V, bortezomib; d, low dose dexamethasone; chemo, conventional chemotherapy; R, lenalidomide; K, carfilzomib; IMiDs, immunomodulatory drugs; PIs, proteasome inhibitors; ASCT, autologous stem-cell transplantation; CT, continuous therapy; FDT, fixed duration of therapy; ISS, International Staging System; CNAs, Copy Number Abnormalities; IgH, immunoglobulin heavy chain; IgL, immunoglobulin lambda chain; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group performance status.

\*% calculated on evaluable cases within each variable.

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654

655 **Table 2.** Best response to upfront treatment and drug refractoriness after first relapse in  
 656 Early-PD versus Late-PD patients  
 657

|  | Early PD<br>(n=191) | Late PD (n=228) | P value   |
|--|---------------------|-----------------|-----------|
| <b>Best response to upfront treatment</b>      |                     |                 |           |
| PD   | 9 (6%)              | 0               |           |
| SD   | 22 (14%)            | 8 (4%)          |           |
| PR   | 53 (34%)            | 31 (14%)        |           |
| VGPR   | 63 (40%)            | 129 (57%)       |           |
| CR   | 3 (2%)              | 40 (18%)        |           |
| sCR  | 8 (5%)              | 18 (8%)         |           |
| Not evaluable                                  | 33                  | 2               |           |
| ORR  | 80%                 | 96%             | p < 0.001 |
| ≥VGPR rate                                     | 47%                 | 82%             | p < 0.001 |
| <b>Drug refractoriness after first relapse</b> |                     |                 |           |
| IMiD refractory                                | 80 (42%)            | 86 (38%)        | p = 0.541 |
| PI refractory                                  | 96 (50%)            | 41 (18%)        | p < 0.001 |
| IMiD + PI double refractory                    | 41 (21%)            | 18 (8%)         | p < 0.001 |

658 **Abbreviations.** PD, progressive disease; SD stable disease; PR partial response; VGPR very good partial  
 659 response; CR, complete response; sCR, stringent CR; ORR, overall response rate (≥PR); n, number; IMiDs,  
 660 immunomodulatory drugs; PIs, proteasome inhibitors.  
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# Early Relapse Risk in Newly Diagnosed Multiple Myeloma Patients Characterized by Next-Generation Sequencing

## Supplementary Appendix

- ◆ **Table S1.** List and classification method of the analyzed variables
- ◆ **Table S2.** Induction treatment classification
- ◆ **Table S3.** List of the 21 genes analyzed and mutation frequency
- ◆ **Table S4.** Distribution of upfront treatment and ASCT in CT subgroups
- ◆ **Table S5.** Distribution of variables in Early PD vs reference population
- ◆ **Figure S1.** Sub-analysis on patients with or without baseline del(17p) and/or TP53 mutation
- ◆ **Figure S2.** TP53 and IGLL5 mutations at diagnosis and at first relapse in available longitudinal samples
- ◆ **Figure S3.** Epanechnikov-kernel smoothed estimated hazard rates of progressive disease (PD) over time
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- ◆ **References**

**Table S1.** List and classification method of the analyzed variables

| Variable                                 | Categories  | Method  |
|--|---|---|
| ISS                                      | I/II/III  | Baseline albumin, $\beta$ 2microglobulin (1)  |
| CNAs                                     | Presence/absence of hyperdiploidy, del(13q), del(17p), gain(1q), amp(1q)                          | SeqFISH (2–4)   |
| IgH translocations                       | Presence/absence of t(11;14), t(4;14), t(14;16), t(14;20)   | SeqFISH (2–4)   |
| IgL translocations                       | Presence/absence of lambda chain translocation  | SeqFISH (2–4)   |
| LDH                                      | High/normal   | Baseline value $> / \leq$ ULN or $> / \leq 90^\circ$ percentile if ULN not available                |
| Custom 21-genes panel*                   | Presence/absence of at least 1 nsSNV/INDEL in each gene   | Whole Exome Sequencing  |
| APOBEC mutational signature contribution | High/Low (4 <sup>th</sup> quartile vs 1 <sup>st</sup> -2 <sup>nd</sup> -3 <sup>rd</sup> quartile) | <i>mmsig</i><br>( <a href="https://github.com/evenrus/mmsig">https://github.com/evenrus/mmsig</a> ) |
| Initial induction treatment              | Vd/V+chemo triplets/Rd/VRd/K-based/Other  | Therapy classification (Table S2)   |
| Clinical trial enrollment                | Yes/No  | Treatment in the context of a clinical trial  |
| ASCT                                     | Yes/No  | Therapy classification  |
| CT**                                     | FDT/IMiDs CT/PIs CT/IMiDs+PIs CT  | Therapy classification ( $>$ or $\leq 1$ year of upfront treatment)                                 |
| ECOG                                     | 0, 1, $\geq 2$  | Baseline ECOG   |
| Age                                      | 1-year increase   | Baseline age  |

**Abbreviations.** ISS, International Staging System; CNAs, Copy Number Abnormalities; IgH, immunoglobulin heavy chain; IgL, immunoglobulin lambda chain; LDH, lactate dehydrogenase; ULN, upper limit of normal; nsSNV/INDEL, non-synonymous Single Nucleotide Variants or Insertions-Deletions; APOBEC, Apolipoprotein B mRNA Editing Catalytic Polypeptide-like; V, Bortezomib; d, low dose dexamethasone; chemo, conventional chemotherapy; R, lenalidomide; K, Carfilzomib; ASCT, autologous stem cell transplantation. CT, continuous therapy; FDT, fixed duration of therapy; IMiDs, immunomodulatory drugs; PIs, proteasome inhibitors; ECOG, Eastern Cooperative Oncology Group performance status.

\*Genes analyzed: KRAS, NRAS, IGLL5, FAM46C, DIS3, TRAF3, BRAF, FAT3, DUSP2, HIST1H1E, TP53, EGR1, LTB, ATM, HUWE1, SP140, PRKD2, ACTG1, CYLD, FGFR3, MAX.

\*\*Classification has been made according to the drug classes used after the 1-year timepoint defining the start of CT in our analysis.



**Table S2.** Induction treatment classification

| Induction treatment category | Induction treatment* (n)  |
|------------------------------|---|
| VRd                          | VRd (319)   |
| V+chemo triplets             | VCd (179)<br>VMp (31)<br>Pad (5)<br>Bendamustine-Vd (1)   |
| K-based                      | KRd (133)<br>KCd (59)<br>Kd (21)<br>KMp (2)   |
| Vd                           | Vd (83)   |
| Rd                           | Rd (61)<br>Claritromycin-Rd (2)   |
| Other                        | VTd (13)  |
| Other                        | VRCd (6)<br>Daratumumab-Vd (3)<br>Elotuzumab-Rd (2)<br>Daratumumab-VMp (2)<br>Daratumumab-VTd (1)<br>VCt (1)<br>MpT (1)<br>Td (1) |

**Abbreviations.** V or P, bortezomib; d, low-dose dexamethasone; C, cyclophosphamide; M, melphalan; A, adriamycin; p, prednisone; chemo, conventional chemotherapy; R, lenalidomide; K, carfilzomib; T, thalidomide.

\*The first complete cycle of upfront therapy has been used to classify induction treatment.

**Table S3.** List of the 21 genes analyzed and mutation frequency

*The entire cohort of patients (N=926) is shown.*

| Gene     | % of patients with at least a somatic non-synonymous variant |
|----------|--|
| KRAS     | 25%  |
| NRAS     | 21.5%  |
| IGLL5    | 16%  |
| FAM46C   | 10%  |
| DIS3     | 10%  |
| TRAF3    | 8%   |
| BRAF     | 7%   |
| FAT3     | 5%   |
| DUSP2    | 4.5%   |
| HIST1H1E | 4.5%   |
| TP53     | 3.5%   |
| EGR1     | 3.5%   |
| LTB      | 3.5%   |
| ATM      | 3.5%   |
| HUWE1    | 3%   |
| SP140    | 3%   |
| PRKD2    | 3%   |
| ACTG1    | 3%   |
| CYLD     | 3%   |
| FGFR3    | 3%   |
| MAX      | 3%   |

**Table S4.** Distribution of upfront treatment and ASCT in CT subgroups

|                  | CT Subgroup |           |          |                |
|------------------|-------------|-----------|----------|----------------|
|                  | FDT         | IMiDs CT  | PIs CT   | IMiDs + PIs CT |
| VRd              | 74 (28%)    | 110 (42%) | 20 (8%)  | 58 (22%)       |
| V+chemo triplets | 46 (27%)    | 67 (40%)  | 34 (20%) | 22 (13%)       |
| K-based          | 5 (8%)      | 30 (45%)  | 7 (11%)  | 24 (36%)       |
| Vd               | 19 (39%)    | 16 (33%)  | 13 (27%) | 1 (2%)         |
| Rd               | 8 (18%)     | 28 (62%)  | 6 (13%)  | 3 (7%)         |
| Other            | 6 (33%)     | 7 (39%)   | 4 (22%)  | 1 (6%)         |
| ASCT Yes         | 93 (27%)    | 152 (44%) | 33 (10%) | 69 (20%)       |

**Abbreviations.** V, Bortezomib; d, low-dose dexamethasone; chemo, conventional chemotherapy; R, lenalidomide; K, Carfilzomib; ASCT, autologous stem-cell transplantation. CT, continuous therapy; FDT, fixed duration of therapy; IMiDs, immunomodulatory drugs; PIs, proteasome inhibitors.

**Table S5.** Distribution of variables in Early PD vs reference population

| Variable      | Early PD<br>N=191 | Reference population<br>N=634 | P value           |
|---------------|-------------------|-------------------------------|-------------------|
| ISS           |                   |                               | <b>p&lt;0.001</b> |
| 1             | 45 (24%)          | 258 (41%)                     |                   |
| 2             | 63 (33%)          | 232 (37%)                     |                   |
| 3             | 74 (39%)          | 126 (20%)                     |                   |
| Missing       | 9 (5%)            | 18 (3%)                       |                   |
| Hyperdiploidy |                   |                               | p=0.664           |
| Yes           | 100 (52%)         | 346 (55%)                     |                   |
| No            | 77 (40%)          | 244 (38%)                     |                   |
| Not evaluable | 14 (7%)           | 44 (7%)                       |                   |
| del(13q)      |                   |                               | p=0.732           |
| Yes           | 95 (50%)          | 307 (48%)                     |                   |
| No            | 82 (43%)          | 285 (45%)                     |                   |
| Not evaluable | 14 (7%)           | 47 (7%)                       |                   |
| del(17p)      |                   |                               | p=0.367           |
| Yes           | 26 (14%)          | 71 (11%)                      |                   |
| No            | 151 (79%)         | 521 (82%)                     |                   |
| Not evaluable | 14 (7%)           | 42 (7%)                       |                   |
| gain(1q)      |                   |                               | <b>p=0.062</b>    |
| Yes           | 50(26%)           | 129 (20%)                     |                   |
| No            | 102 (53%)         | 383 (60%)                     |                   |
| Not evaluable | 39 (20%)          | 122 (19%)                     |                   |
| amp(1q)       |                   |                               | p=0.192           |
| Yes           | 14 (7%)           | 30 (5%)                       |                   |
| No            | 138 (72%)         | 482 (76%)                     |                   |
| Not evaluable | 39 (20%)          | 122 (19%)                     |                   |
| t(11;14)      |                   |                               | p=0.250           |
| Yes           | 43 (23%)          | 117 (18%)                     |                   |
| No            | 144 (75%)         | 500 (79%)                     |                   |
| Not evaluable | 4 (2%)            | 17 (3%)                       |                   |
| t(4;14)       |                   |                               | p=1.000           |
| Yes           | 27 (14%)          | 88 (14%)                      |                   |
| No            | 96 (84%)          | 529 (83%)                     |                   |
| Not evaluable | 4 (2%)            | 17 (3%)                       |                   |
| t(14;16)      |                   |                               | p=0.448           |
| Yes           | 7 (4%)            | 34 (5%)                       |                   |
| No            | 180 (94%)         | 583 (92%)                     |                   |
| Not evaluable | 4 (2%)            | 17 (3%)                       |                   |

|                    |           |           |                   |
|--------------------|-----------|-----------|-------------------|
| t(14;20)           |           |           | p=0.225           |
| Yes                | 4 (2%)    | 5 (1%)    |                   |
| No                 | 183 (96%) | 612 (97%) |                   |
| Not evaluable      | 4 (2%)    | 17 (3%)   |                   |
| IgL translocations |           |           | <b>p=0.004</b>    |
| Yes                | 26 (14%)  | 41 (6%)   |                   |
| No                 | 137 (72%) | 479 (76%) |                   |
| Not evaluable      | 28 (15%)  | 114 (18%) |                   |
| APOBEC signature   |           |           | <b>p=0.086</b>    |
| High               | 57 (30%)  | 149 (24%) |                   |
| Low                | 134 (70%) | 485 (76%) |                   |
| LDH                |           |           | <b>p=0.012</b>    |
| High               | 18 (9%)   | 34 (5%)   |                   |
| Normal             | 113 (59%) | 473 (75%) |                   |
| Missing            | 60 (31%)  | 127 (20%) |                   |
| ECOG               |           |           | <b>p&lt;0.001</b> |
| 0                  | 51 (27%)  | 250 (39%) |                   |
| 1                  | 81 (42%)  | 253 (40%) |                   |
| ≥2                 | 43 (23%)  | 71 (11%)  |                   |
| Missing            | 16 (8%)   | 60 (9%)   |                   |
| KRAS mutation      |           |           | <b>p=0.047</b>    |
| Yes                | 60 (31%)  | 152 (24%) |                   |
| No                 | 131 (69%) | 482 (76%) |                   |
| NRAS mutation      |           |           | p=1.000           |
| Yes                | 42 (22%)  | 141 (22%) |                   |
| No                 | 149 (78%) | 493 (78%) |                   |
| IgLL5 mutation     |           |           | <b>p=0.070</b>    |
| Yes                | 38 (20%)  | 91 (14%)  |                   |
| No                 | 153 (80%) | 543 (86%) |                   |
| FAM46C mutation    |           |           | p=0.665           |
| Yes                | 15 (8%)   | 59 (9%)   |                   |
| No                 | 176 (92%) | 575 (91%) |                   |
| DIS3 mutation      |           |           | p=0.487           |
| Yes                | 21 (11%)  | 59 (9%)   |                   |
| No                 | 170 (89%) | 575 (91%) |                   |
| TRAF3 mutation     |           |           | p=0.211           |
| Yes                | 10 (5%)   | 52 (8%)   |                   |
| No                 | 181 (95%) | 582 (92%) |                   |
| BRAF mutation      |           |           | p=1.000           |
| Yes                | 14 (7%)   | 48 (8%)   |                   |
| No                 | 177 (93%) | 586 (92%) |                   |

|                                |                      |                      |                |
|--------------------------------|----------------------|----------------------|----------------|
| FAT3 mutation<br>Yes<br>No     | 12 (6%)<br>179 (94%) | 30 (5%)<br>604 (95%) | p=0.452        |
| DUSP2 mutation<br>Yes<br>No    | 6 (3%)<br>185 (97%)  | 31 (5%)<br>603 (95%) | p=0.424        |
| HIST1H1E mutation<br>Yes<br>No | 7 (4%)<br>184 (96%)  | 27 (4%)<br>607 (96%) | p=0.837        |
| TP53 mutation<br>Yes<br>No     | 17 (9%)<br>174 (91%) | 22 (3%)<br>612 (97%) | <b>p=0.005</b> |
| EGR1 mutation<br>Yes<br>No     | 9 (5%)<br>182 (95%)  | 18 (3%)<br>616 (97%) | p=0.244        |
| LTB mutation<br>Yes<br>No      | 9 (5%)<br>182 (95%)  | 18 (3%)<br>616 (97%) | p=0.244        |
| ATM mutation<br>Yes<br>No      | 5 (3%)<br>186 (97%)  | 24 (4%)<br>610 (96%) | p=0.654        |
| HUWE1 mutation<br>Yes<br>No    | 5 (3%)<br>186 (97%)  | 23 (4%)<br>611 (96%) | p=0.650        |
| SP140 mutation<br>Yes<br>No    | 5 (3%)<br>186 (97%)  | 20 (3%)<br>614 (97%) | p=0.814        |
| PRKD2 mutation<br>Yes<br>No    | 7 (4%)<br>184 (96%)  | 16 (3%)<br>618 (97%) | p=0.451        |
| ACTG1 mutation<br>Yes<br>No    | 5 (3%)<br>186 (97%)  | 21 (3%)<br>613 (97%) | p=1.000        |
| CYLD mutation<br>Yes<br>No     | 8 (4%)<br>183 (96%)  | 16 (3%)<br>618 (97%) | p=0.226        |
| FGFR3 mutation<br>Yes<br>No    | 3 (2%)<br>188 (98%)  | 23 (4%)<br>611 (96%) | p=0.235        |
| MAX mutation<br>Yes<br>No      | 3 (2%)<br>188 (98%)  | 24 (4%)<br>610 (96%) | p=0.166        |

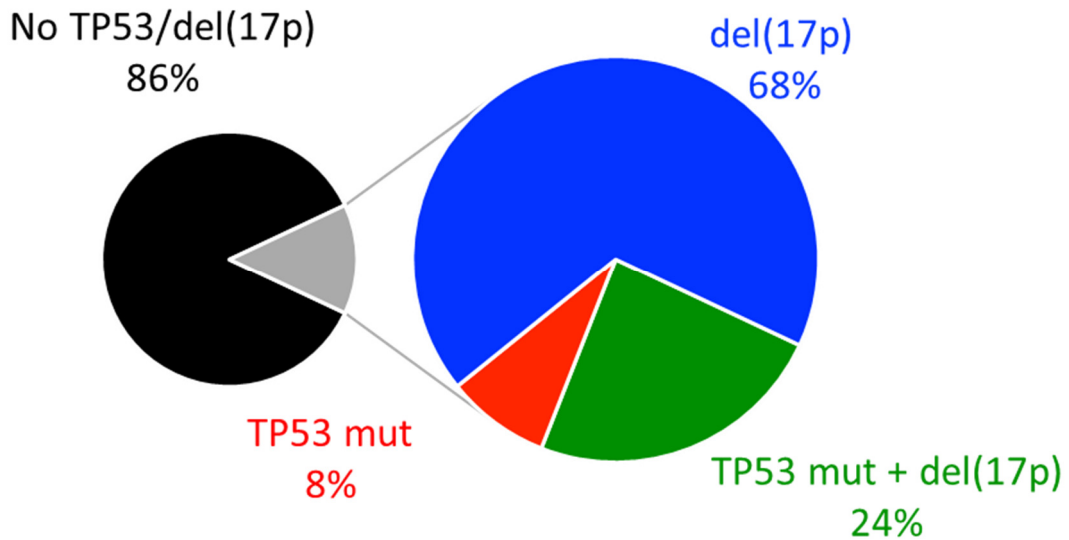
|                           |            |            |                   |
|---------------------------|------------|------------|-------------------|
| Age median (IQR)          | 65 (57-72) | 62 (55-67) | <b>p&lt;0.001</b> |
| Induction treatment       |            |            | <b>p&lt;0.001</b> |
| VRd                       | 64 (34%)   | 227 (36%)  |                   |
| V+chemo triplets          | 51 (27%)   | 140 (22%)  |                   |
| K-based                   | 24 (13%)   | 177 (28%)  |                   |
| Vd                        | 24 (13%)   | 36 (6%)    |                   |
| Rd                        | 17 (9%)    | 36 (6%)    |                   |
| Other                     | 10 (5%)    | 18 (3%)    |                   |
| ASCT                      |            |            | <b>p&lt;0.001</b> |
| Yes                       | 37 (19%)   | 389 (61%)  |                   |
| No                        | 114 (60%)  | 235 (37%)  |                   |
| Not evaluable             | 40 (21%)   | 10 (2%)    |                   |
| CT                        |            |            | <b>p=0.018</b>    |
| FDT                       | 29 (15%)   | 123 (19%)  |                   |
| IMiDs CT                  | 27 (14%)   | 226 (36%)  |                   |
| PIs CT                    | 11 (6%)    | 67 (11%)   |                   |
| IMiDs+PIs CT              | 7 (4%)     | 99 (16%)   |                   |
| Not evaluable             | 117 (61%)  | 119 (19%)  |                   |
| Clinical trial enrollment |            |            | <b>p=0.001</b>    |
| Yes                       | 20 (10%)   | 132 (21%)  |                   |
| No                        | 171 (90%)  | 502 (79%)  |                   |

**Abbreviations.** PD, progressive disease; ISS, International Staging System; IgL, immunoglobulin lambda chain; LDH, lactate dehydrogenase; APOBEC, Apolipoprotein B mRNA Editing Catalytic Polypeptide-like; V, Bortezomib; d, low dose dexamethasone; chemo, conventional chemotherapy; R, lenalidomide; K, Carfilzomib; ASCT, autologous stem-cell transplantation. CT, continuous therapy; FDT, fixed duration of therapy; IMiDs, immunomodulatory drugs; PIs, proteasome inhibitors; ECOG, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; del, deletion, t, translocation; amp, amplification.

**Figure S1.** Sub-analysis on patients with or without baseline del(17p) and/or TP53 mutation

*In panels A-B del(17p) is defined with a 20% cut-off; in panels C-D del(17p) is defined with a 50% cut-off.*

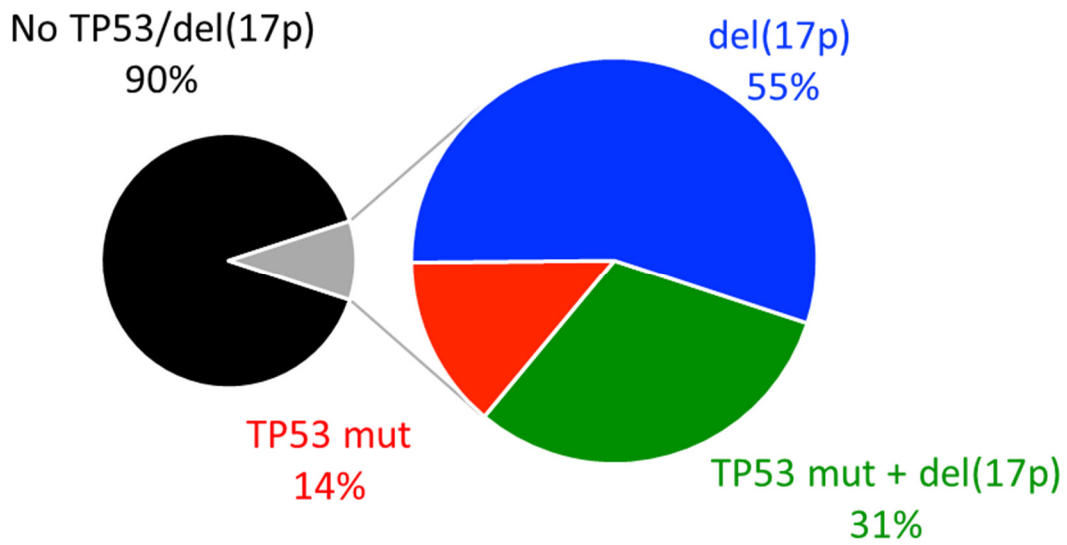
**A**



**B**

| Subpopulation            | Early PD n/evaluatable (%) |
|--------------------------|----------------------------|
| No TP53mut/del(17p)      | 146/744 (19.6%)            |
| del(17p) but not TP53mut | 14/82 (17.1%)              |
| TP53mut but not del(17p) | 5/10 (50%)                 |
| TP53mut + del(17p)       | 12/29 (41.4%)              |



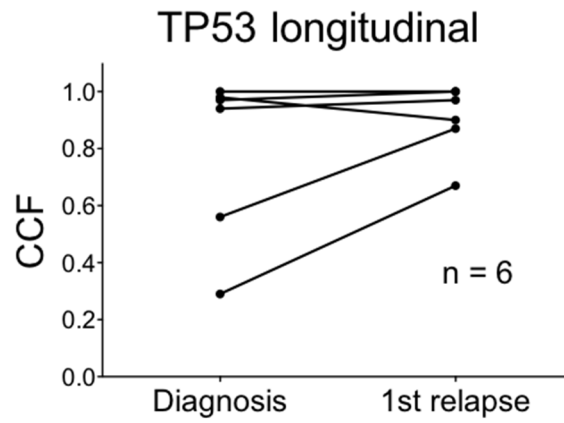
**C****D**

| Subpopulation            | Early PD n/evaluatable (%) |
|--------------------------|----------------------------|
| No TP53mut/del(17p)      | 145/778 (18.6%)            |
| del(17p) but not TP53mut | 12/48 (25%)                |
| TP53mut but not del(17p) | 6/12 (50%)                 |
| TP53mut + del(17p)       | 11/27 (40.7%)              |

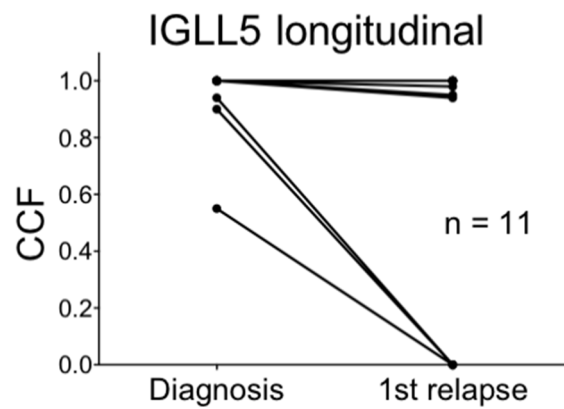
**Abbreviations.** Del(17p), deletion 17p; mut, mutation; PD, progressive disease; n, number.

**Figure S2.** TP53 (Panel A) and IGLL5 (Panel B) mutations at diagnosis and at first relapse in available longitudinal samples

**A**



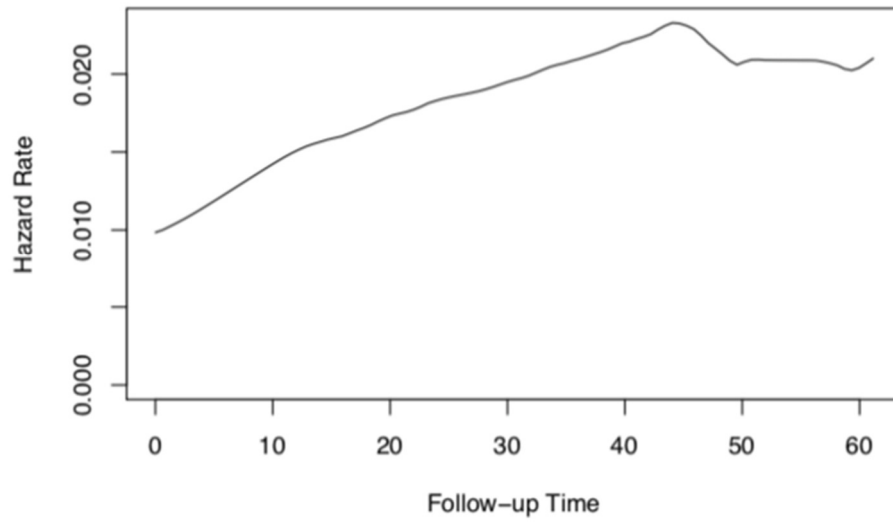
**B**



**Abbreviations.** N, sample size; CCF, cancer cell fraction estimated by ABSOLUTE (5).

**Figure S3.** Epanechnikov-kernel smoothed estimated hazard rates of progressive disease (PD) over time

*The follow-up time is expressed in months.*



**Figure S4.** Comparison of Seq-FISH and conventional FISH in a subgroup of patients enrolled in clinical trials and analyzed in the same centralized laboratory (n=166)

*Representative CNAs [del(13q), Panel A] and IgH t [t (11;14), Panel B] are shown. Overall concordance for del(13q) cases was 96%. Overall concordance for t(11;14) cases was 99%.*

**A**

| del(13q)                   | Seq-FISH negative | Seq-FISH positive |
|----------------------------|-------------------|-------------------|
| Conventional FISH positive | 4 (2%)            | 88 (53%)          |
| Conventional FISH negative | 72 (43%)          | 2 (1%)            |

**B**

| t(11;14)                   | Seq-FISH negative | Seq-FISH positive |
|----------------------------|-------------------|-------------------|
| Conventional FISH positive | 0 (0%)            | 34 (20%)          |
| Conventional FISH negative | 132 (79%)         | 1 (1%)            |

**Abbreviations.** FISH, fluorescence in situ hybridization; Seq-FISH, sequencing-based FISH; CNAs, copy number abnormalities; del(13q), deletion 13q; t(11;14), translocation (11;14).

## References

1. Greipp PR, San Miguel J, Durie BGM, Crowley JJ, Barlogie B, Bladé J, et al. International staging system for multiple myeloma. *J Clin Oncol* [Internet]. American Society of Clinical Oncology; 2005 [cited 2019 Mar 27];23:3412–20. Available from: <http://ascopubs.org/doi/10.1200/JCO.2005.04.242>
2. Miller C, Yesil J, Derome M, Donnelly A, Marrian J, McBride K, et al. A Comparison of Clinical FISH and Sequencing Based FISH Estimates in Multiple Myeloma: An MmrF Compass Analysis. *Blood* [Internet]. 2016 [cited 2019 Sep 30];128:Abstract #374 [ASH 2016 58th Meeting]. Available from: <http://www.bloodjournal.org/content/128/22/374>
3. Goldsmith SR, Fiala MA, Dukeman J, Ghobadi A, Stockerl-Goldstein K, Schroeder MA, et al. Next Generation Sequencing-based Validation of the Revised International Staging System for Multiple Myeloma: An Analysis of the MMRF CoMMpass Study. *Clin Lymphoma Myeloma Leuk* [Internet]. 2019 [cited 2019 Sep 30];19:285–9. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2152265018315246>
4. Barwick BG, Neri P, Bahlis NJ, Nooka AK, Dhodapkar M V., Jaye DL, et al. Multiple myeloma immunoglobulin lambda translocations portend poor prognosis. *Nat Commun* [Internet]. Nature Publishing Group; 2019 [cited 2019 Sep 30];10:1911. Available from: <http://www.nature.com/articles/s41467-019-09555-6>
5. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol* [Internet]. 2012 [cited 2019 Sep 30];30:413–21. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22544022>