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

Research Article

Early Relapse Risk in Newly Diagnosed Multiple Myeloma Patients Characterized by Next-Generation Sequencing

Running title: Early relapse risk by NGS in NDMM patients

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All the authors collected the data and interpreted the results.
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All the authors approved the final version of the manuscript.
All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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MD has served on the advisory board for GSK.
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FG has received honoraria from Amgen, Celgene, Janssen, Takeda, and Bristol-Myers Squibb; has served on the advisory boards for Amgen, Celgene, Janssen, Takeda, Bristol-Myers Squibb, Roche, AbbVie, Adaptive, and Seattle Genetics.
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Statement of translational relevance

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

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

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

Abstract

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

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

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

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

Introduction

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

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

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

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

Methods

Patients and treatment

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

Main inclusion criteria were: symptomatic NDMM, measurable disease and upfront systemic therapy including an IMiD and/or a PI. CoMMpass data were generated as part of the MMRF Personalized Medicine Initiatives (https://research.themmrf.org and www.themmrf.org).

Data from patients receiving treatment in the context of clinical trials as well as with real word regimens were included. Therapy (source file "mmrf_commpass_IA14_stand_alone_treatment_regimen" available upon request on https://research.themmrf.org) was reviewed and classified according to: type of induction treatment (bortezomib-dexamethasone/bortezomib+chemotherapy triplets/lenalidomide-dexamethasone/bortezomib-lenalidomide-dexamethasone/carfilzomib-based/other), autologous stem-cell transplantation (ASCT; Yes/No), and type of continuous treatment (CT) (IMiDs CT/PIs CT/IMiDs+PIs CT/Fixed duration of therapy [FDT]). FDT was defined as ≤1 year of upfront treatment (12). The definition of variables is detailed in *Tables S1-S2*. Patients were considered evaluable for the ASCT vs no ASCT analysis if they were alive and relapse-free after induction treatment and if the date of ASCT was available. Patients receiving ASCT before PD but after 18 months from the start of treatment (cut-off for the early relapse

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

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

Next-generation sequencing

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

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

Statistical analysis

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

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

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

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

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

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

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

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

Results

Patient characteristics

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

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

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

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

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

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

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

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

Early PD population

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

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

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

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

Risk of early PD

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

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

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

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

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

TP53 mutations

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

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

Longitudinal analysis of mutations associated with early PD

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

Discussion

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

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

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

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

TP53 mutation, which is currently not included in the standard baseline evaluation of MM patients, was the most important factor increasing the risk of early PD emerging from our analysis. TP53 mutation is rare in patients at diagnosis (3.5%), but about 25% of patients 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

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

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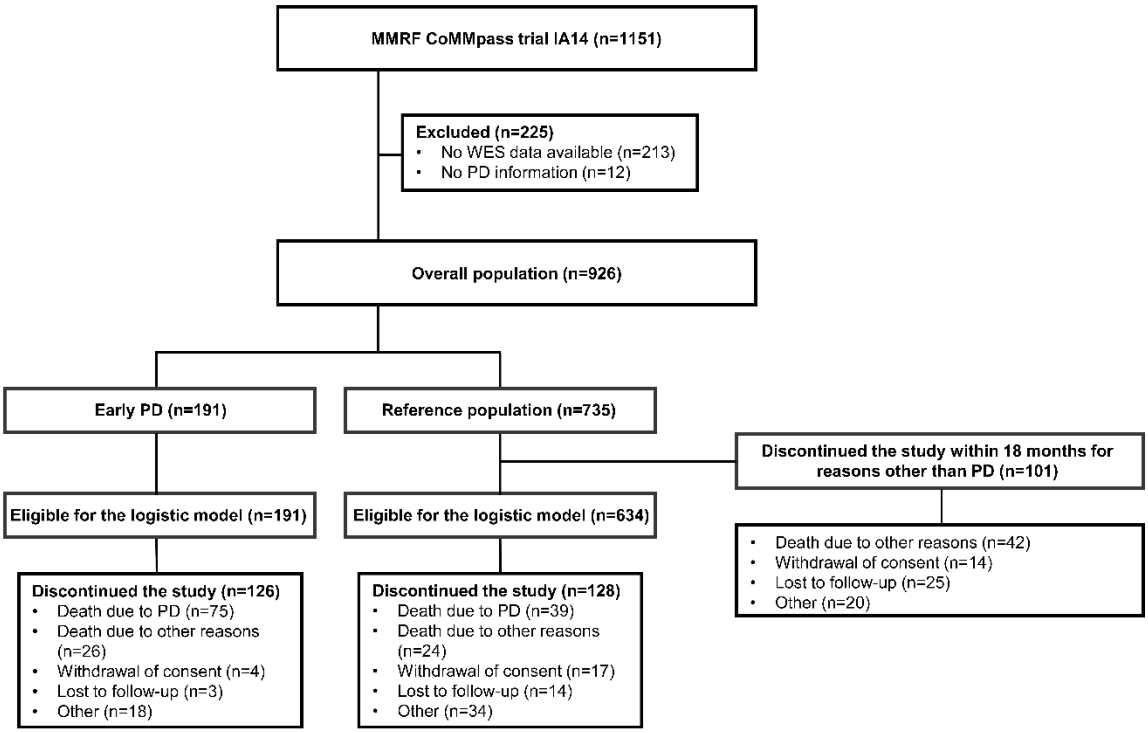
Figures

Figure 1. Study flow

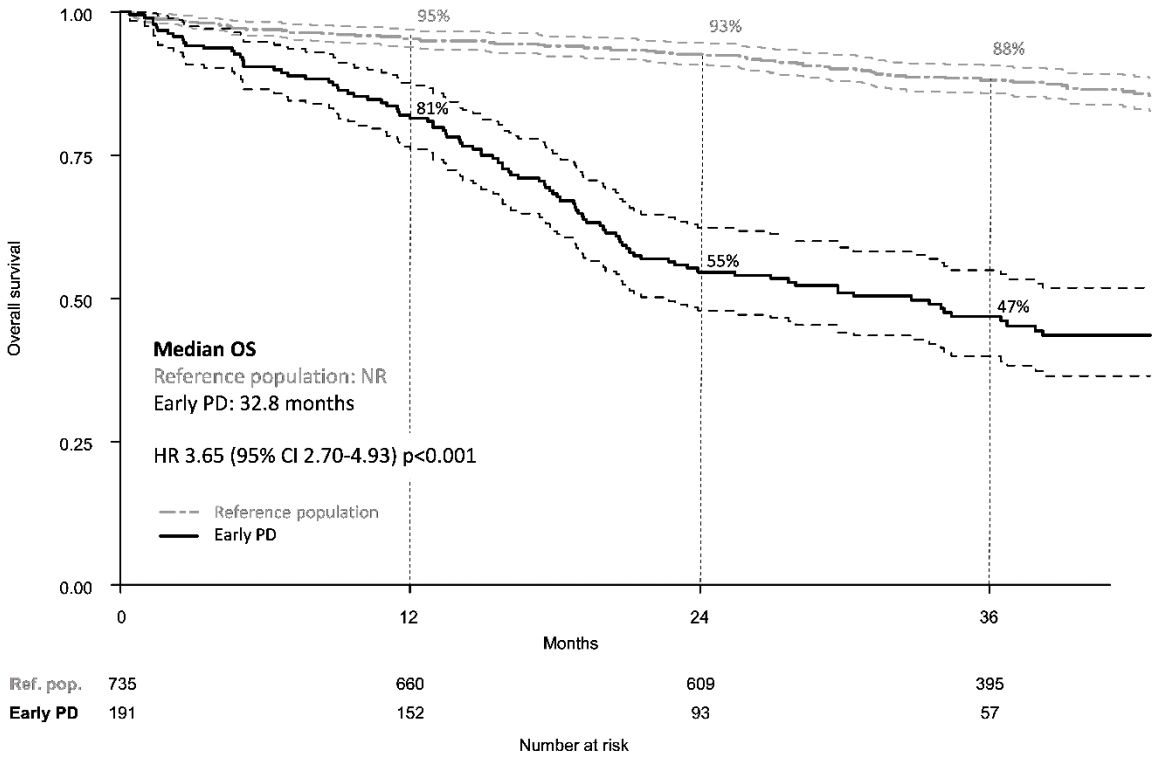
Figure 1



Abbreviations. MMRF: Multiple Myeloma Research Foundation; IA14: Interim analysis 14; WES: whole exome sequencing; PD: progressive disease; n, number.

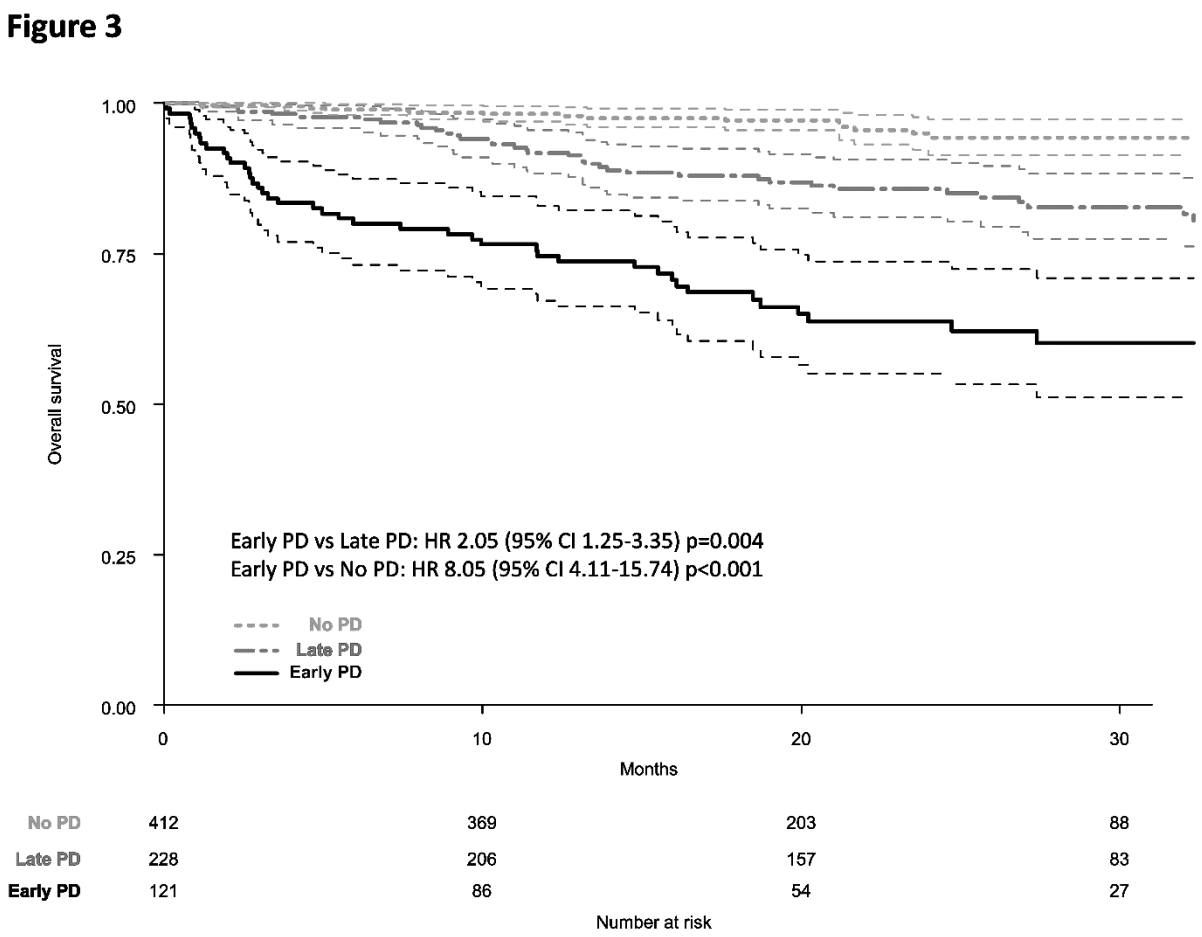
Figure 2. Overall survival for patients with early PD versus reference population

Figure 2



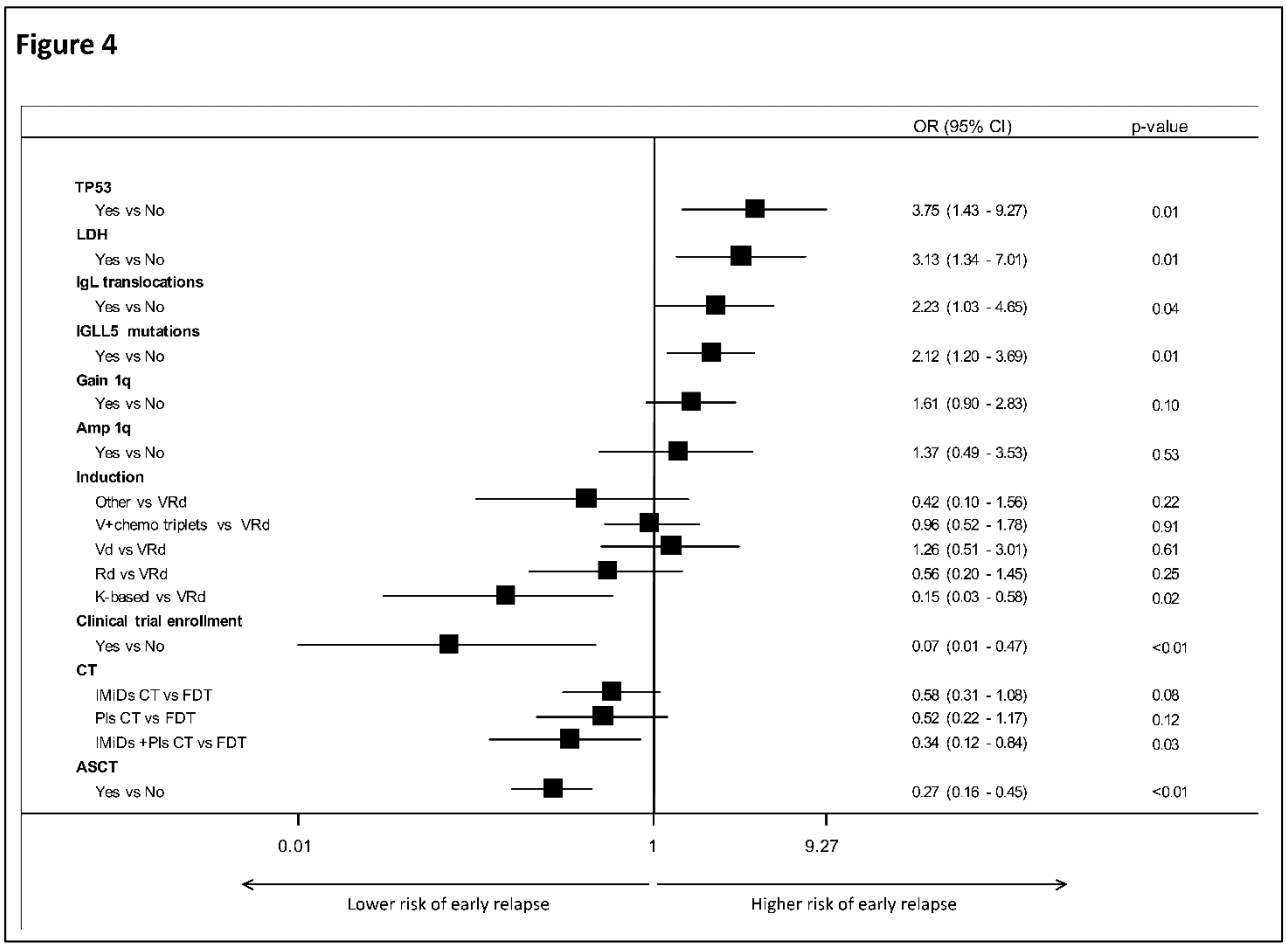
Abbreviations. OS: overall survival; PD: progressive disease; HR: hazard ratio; NR: not reached; ref. pop., reference population.
Dotted lines: 95% confidence intervals. HR adjusted for age, International Staging System (ISS) stage, high-risk cytogenetics [presence of del(17p) and/or t(4;14) and/or t(14;16],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

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



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

631 **Figure 4**. Multivariate logistic regression model evaluating risk factors associated with early
632 PD in the patients actually at risk for the entire 18-month period (n=825)
633



634
635 **Abbreviations**. PD, progressive disease; OR: odds ratio; IgL: immunoglobulin lambda chain; LDH: lactate
636 dehydrogenase; V: bortezomib; d: low dose dexamethasone; chemo: conventional chemotherapy; R:
637 lenalidomide; K: carfilzomib; ASCT: autologous stem-cell transplantation; CT: continuous therapy; FDT: fixed
638 duration of therapy; IMiDs: immunomodulatory drugs; PIs: proteasome inhibitors.
639 Analysis is adjusted for missing values within each variable.
640
641

Tables

Table 1. Patient characteristics

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

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.

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 (n=191)	Late PD (n=228)	P value
Best response to upfront treatment			
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%	$p < 0.001$
Drug refractoriness after first relapse			
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
659 **Abbreviations.** PD, progressive disease; SD stable disease; PR partial response; VGPR very good partial
660 response; CR, complete response; sCR, stringent CR; ORR, overall response rate (≥PR); n, number; IMiDs,
661 immunomodulatory drugs; PIs, proteasome inhibitors**.**
662
663

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

Variable	Categories	Method
ISS	I/II/III	Baseline albumin, β2microglobulin (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 > / ≤ ULN or > / ≤ 90° 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^{th} quartile vs 1^{st}-2^{nd}-3^{rd} quartile)	*mmsig* (https://github.com/evenrus/mmsig)
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 ≤1 year of upfront treatment)
ECOG	0, 1, ≥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) VMp (31) Pad (5) Bendamustine-Vd (1)
K-based	KRd (133) KCd (59) Kd (21) KMp (2)
Vd	Vd (83)
Rd	Rd (61) Claritromycin-Rd (2)
Other	VTd (13)
Other	VRCd (6) Daratumumab-Vd (3) Elotuzumab-Rd (2) Daratumumab-VMp (2) Daratumumab-VTd (1) VCt (1) MpT (1) 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 N=191	Reference population N=634	P value
ISS			**p<0.001**
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)			**p=0.062**
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) Yes No Not evaluable	 4 (2%) 183 (96%) 4 (2%)	 5 (1%) 612 (97%) 17 (3%)	p=0.225
IgL translocations Yes No Not evaluable	 26 (14%) 137 (72%) 28 (15%)	 41 (6%) 479 (76%) 114 (18%)	**p=0.004**
APOBEC signature High Low	 57 (30%) 134 (70%)	 149 (24%) 485 (76%)	**p=0.086**
LDH High Normal Missing	 18 (9%) 113 (59%) 60 (31%)	 34 (5%) 473 (75%) 127 (20%)	**p=0.012**
ECOG 0 1 ≥2 Missing	 51 (27%) 81 (42%) 43 (23%) 16 (8%)	 250 (39%) 253 (40%) 71 (11%) 60 (9%)	**p<0.001**
KRAS mutation Yes No	 60 (31%) 131 (69%)	 152 (24%) 482 (76%)	**p=0.047**
NRAS mutation Yes No	 42 (22%) 149 (78%)	 141 (22%) 493 (78%)	p=1.000
IGLL5 mutation Yes No	 38 (20%) 153 (80%)	 91 (14%) 543 (86%)	**p=0.070**
FAM46C mutation Yes No	 15 (8%) 176 (92%)	 59 (9%) 575 (91%)	p=0.665
DIS3 mutation Yes No	 21 (11%) 170 (89%)	 59 (9%) 575 (91%)	p=0.487
TRAF3 mutation Yes No	 10 (5%) 181 (95%)	 52 (8%) 582 (92%)	p=0.211
BRAF mutation Yes No	 14 (7%) 177 (93%)	 48 (8%) 586 (92%)	p=1.000

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

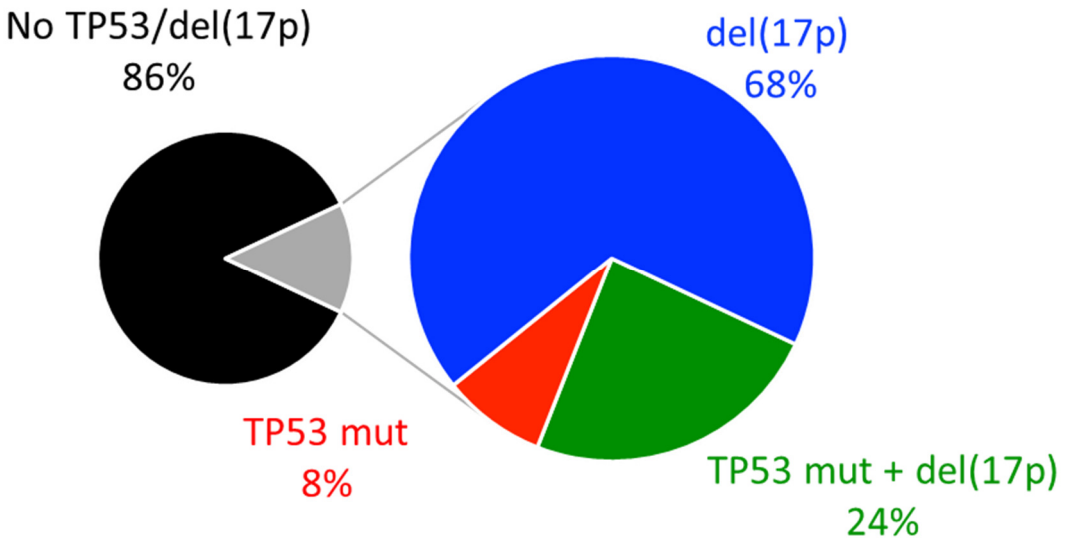
Age median (IQR)	65 (57-72)	62 (55-67)	**p<0.001**
Induction treatment			**p<0.001**
VRd	64 (34%)	227 (36%)	
V+chemo triplets	51 (27%)	140 (22%)	
K-based	24 (13%)	177 (28%)	
Vd	24 (13%)	36 (6%)	
Rd	17 (9%)	36 (6%)	
Other	10 (5%)	18 (3%)	
ASCT			**p<0.001**
Yes	37 (19%)	389 (61%)	
No	114 (60%)	235 (37%)	
Not evaluable	40 (21%)	10 (2%)	
CT			**p=0.018**
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			**p=0.001**
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.

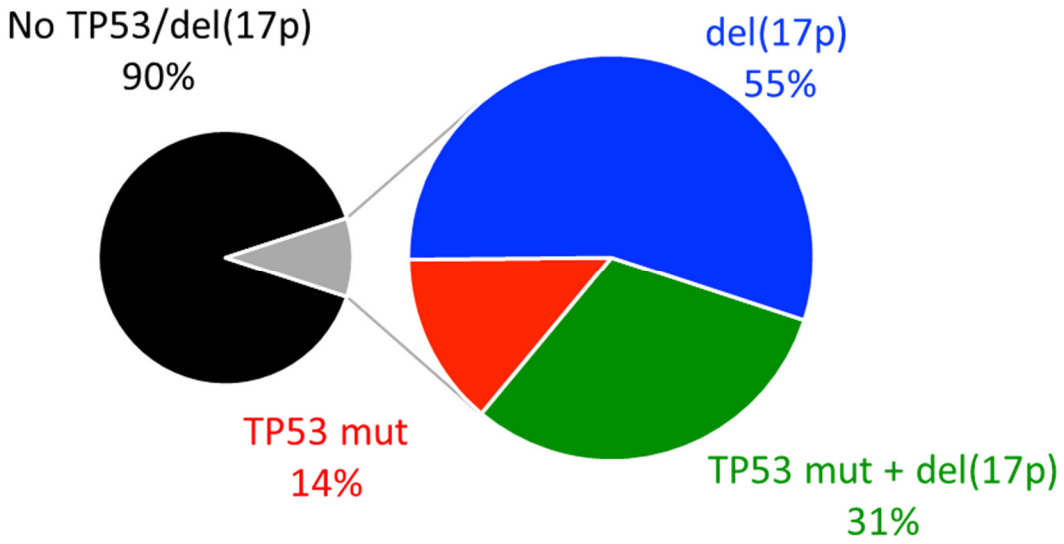
A



B

Subpopulation	Early PD n/evaluable (%)
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



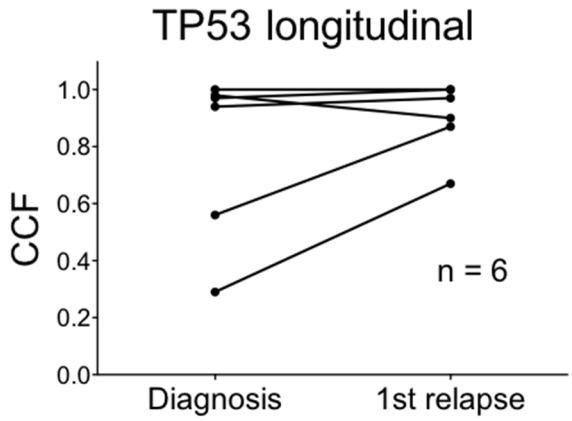
D

Subpopulation	Early PD n/evaluable (%)
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%)

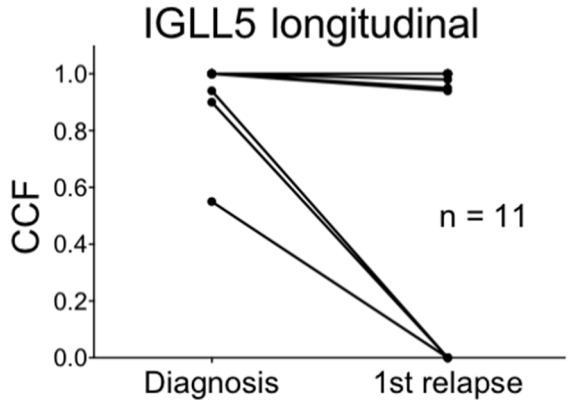
Abbreviations. Del(17p), deletion 17p; mut, mutation; PD, progressive disease; n, number.

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.

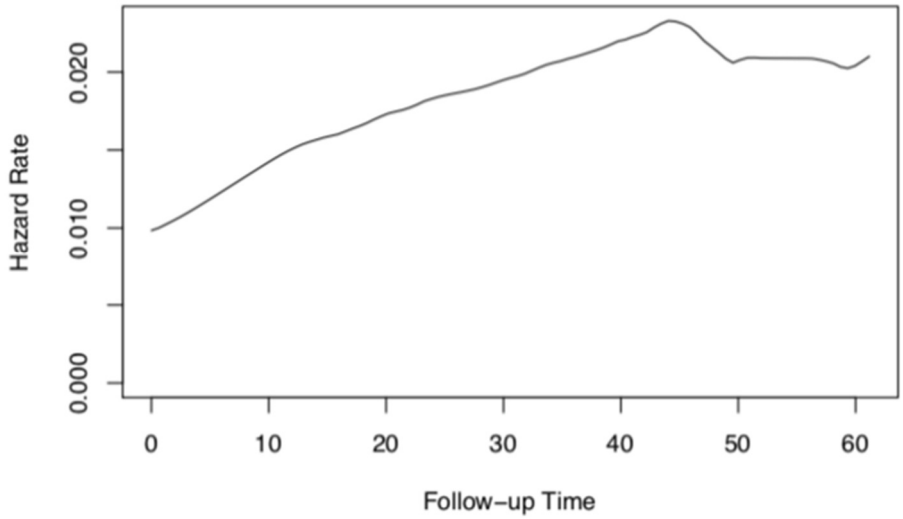


Figure S4. Comparison of Seq-FISH and conventional FISH in a subgroup of patients enrolled in clinical trials and analyzed in the same centralized laboratory (n=166)

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).

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