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

Mattia D'Agostino,[1] Gian Maria Zaccaria,[1] Bachisio Ziccheddu,[2,3] Even H. Rustad,[4] Elisa Genuardi,[1] Andrea Capra,[1] Stefania Oliva,[1] Daniel Auclair,[5] Jennifer Yesil,[5] Paola Colucci,[1] Jonathan J. Keats,[6] Manuela Gambella,[1] Sara Bringhen,[1] Alessandra Larocca,[1] Mario Boccadoro,[1] Niccolò Bolli,[2,7] Francesco Maura,[4] Francesca Gay[1]

1. Myeloma Unit, Division of Hematology, University of Torino, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Torino, Italy
2. Department of Clinical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
3. Department of Molecular Biotechnologies and Health Sciences, University of Turin, Turin, Italy
4. Myeloma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, US-NY
5. Multiple Myeloma Research Foundation (MMRF), Norwalk, US-CT
6. Translational Genomics Research Institute (TGen), US-AZ
7. Department of Oncology and Onco-Hematology, University of Milan, Milan, Italy

Correspondence to: Dr. Francesca Gay, MD, PhD, Myeloma Unit, Division of Hematology, University of Torino, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, via Genova 3 -10126 Torino, Italy. Tel +39 011 6333 4279/4301, Fax: +39 011 63334187, e-mail: fgay@cittadellasalute.to.it.
ORCID ID: 0000-0002-8619-412X

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Resources: all the authors
Data curation: all the authors
Software: MD, AC
Formal analysis: MD, AC
Supervision: MB, NB, FM, FG
Validation: all the authors
Investigation: all the authors
Visualization: MD, GMZ, BZ, AC, NB, FM, and FG
Methodology: MD, GMZ, SO, DA, JY, JJK, MG, MB, NB, FM, and FG
Writing-original draft: MD, GMZ, BZ, AC, NB, FM, and FG
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All the authors approved the final version of the manuscript and 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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Competing interests

MD has served on the advisory board for GSK.
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DA is currently employed by the Multiple Myeloma Research Foundation, Norwalk, US-CT.
JY is currently employed by the Multiple Myeloma Research Foundation, Norwalk, US-CT.
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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.
The other authors declare no competing financial interests.

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 in the United States and 30000 MM patients in Europe die each year, with the main cause of death being the development of refractory disease to the currently available drugs (2–4).

Relapse is caused by MM cell clones with an increasing degree of drug refractoriness and genetic complexity eventually leading to shorter remissions (5). 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 (6).

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) (7). Many efforts aimed at improving the baseline stratification, including the use of gene expression profiles (GEP) and next-generation sequencing (NGS) (8–10). 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% (11,12). 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 therapy [FDT]). FDT was defined as ≤1 year of upfront treatment (13). 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, if 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; the treatment schedule is reported in the Supplementary Appendix).

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 (14–16). 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) (17–19). 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. 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 (19). 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*) (20,21). The cancer cell fraction (CCF) of mutations of interest corrected by tumor purity and MM cell ploidy was estimated using the ABSOLUTE algorithm (22). Moreover, we evaluated the aberrant activity of APOBEC cytidine deaminases (known to be associated with high mutational burden and poor prognosis in MM) (23), using the recently developed fitting algorithm *mmsig* (*Table S1*; https://github.com/evenrus/mmsig) (24). APOBEC activity was defined as *high* or *low* based on its quartile distribution (4th quartile vs others) (23).

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 (25).

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 (25,26). 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 (27), 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. A confirmatory analysis on the same patient population using death within 24 months as an endpoint was conducted (11).

All the analyses were conducted using R version 3.5.1 and bespoke code, which is available upon request.

Data deposition

The access to the Interim Analysis 14 (IA 14) release of CoMMpass was approved by the Data Access Use Committee and downloaded from https://research.themmrf.org/rp/download.

CoMMpass data are deposited in the database of Genotypes and Phenotypes (dbGaP; Study Accession phs000748.v7.p4 - https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000748.v7.p4).

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 (19), 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*).

296 In the Early PD group, 126/191 (66%) patients discontinued the study at the last follow-up:
297 75 (39%) for death due to PD, 26 (14%) for death due to other reasons, 4 (2%) due to
298 withdrawal of consent, 3 (2%) for being lost to follow-up, and 18 (9%) for other reasons.

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

304 Overall response rate (ORR) was significantly lower in Early-PD patients compared to Late-PD
305 patients (80% vs 96%, respectively, p<0.001). Deep responses were also different, with very
306 good partial response (VGPR) rates of 40% vs 57%, complete remission (CR) rates of 2% vs
307 18% and stringent CR rates of 5% vs 8% in Early vs Late PD groups respectively. This
308 translated into a significantly different rate of ≥VGPR in the 2 groups (47% vs 82%, p<0.001;
309 *Table 2*).

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

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

314 Early-PD patients had a significantly higher risk of death compared to the reference
315 population (HR 4.89, 95% CI 3.72-6.43, p<0.001), with 53% of patient deaths at 3 years in the
316 early PD cohort compared with only 12% in the reference cohort. This effect was maintained
317 after adjusting the analysis for age, baseline prognostic factors (ISS, high-risk
318 cytogenetics(27)), treatment and clinical trial enrollment (HR 3.65, 95% CI 2.70-4.93,
319 p<0.001). Of note, 61% of early relapsing patients presented with ISS stage I or II and 74%
320 had conventionally defined standard-risk cytogenetics (27). The median OS of early relapsing

patients was 32.8 months, lower than that of high-risk population defined using baseline ISS III (median OS 54 months) or baseline high-risk cytogenetics (27) (median OS 65 months).

Early-PD patients were defined using a time-dependent endpoint (18 months); consequently, a landmark analysis of OS with a landmark point at 18 months was performed to validate our findings (*Figure 3*). At the landmark timepoint, 121 Early-PD patients and 640 patients in the reference population were evaluable. The main reasons for not being evaluable were death due to PD during the first 18 months in the Early PD population (58/191, 30%) and death due to reasons other than PD during the first 18 months in the reference population (42/735, 6%). The difference in early death rates between the 2 groups led to a possible underestimation of OS differences after the landmark timepoint. Moreover, in this OS comparison we split the reference population in Late PD and No PD patients. The 18-month landmark analysis showed a significantly worse OS in Early-PD patients compared both to Late PD (HR 2.05, 95%, CI 1.25-3.35, p=0.004) and No PD patients (HR 8.05, 95%, CI 4.11-15.74, 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. Only a trend was found for gain(1q) and amp(1q) (*Figure 4*).

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).

To confirm our results, we performed an additional analysis using death within 24 months as an endpoint (11) (*Figure S1*). The adverse effects of TP53 mutation (OR 3.35, p=0.02) and IgL translocation (OR 2.34, p=0.046) were confirmed. Moreover, also 1q abnormalities were significantly correlated with an increased risk of death within 24 months. ASCT (OR 0.44, p=0.02) retained its protective effect.

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 S2A*). 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 S2B.* 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 S2C-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 S3A*). 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 S3B*).

Discussion

MM prognosis is improving and early relapse after upfront treatment is beginning to be recognized as a high-risk feature (28). The same observation had been done for other

hematologic malignancies with an expected indolent course, such as follicular lymphoma and chronic lymphocytic leukemia (29,30).

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 S4*).

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 S5* and by others (17,18).

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. Its adverse effect was confirmed in the risk of death within 24 months from diagnosis. 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 (9), our data further supported the routine testing of TP53 mutation at least in del(17p)-positive patients. Indeed, the presence of del(17p) without TP53 mutation conferred an early PD risk that was similar to that of the overall population.

In our analysis, IgL translocation and IGLL5 mutation also emerged as risk factors of early PD. Both of them have already been associated with poor prognosis (19,31). White et al. showed that mutations in IGLL5 can be associated with translocations juxtaposing IGLL5 (31). In our analysis, IGLL5 mutations and IgL translocations showed a trend toward co-occurrence, though not statistically significant (p=0.06). The higher risk of early relapse observed in IgL-translocated patients, the loss of subclonal IGLL5 mutations at first relapse and the significant effect of IgL translocations but not of IGLL5 mutations in the risk of death within 24 months could suggest that IgL translocations impacted patients' prognosis more than IGLL5 mutations.

Only a trend towards a higher risk of early PD was found for gain(1q) and amp(1q). However, using death within 24 months as an endpoint, the effect of 1q abnormalities was more evident. This was possibly due to the use of a later timepoint allowing more patients to experience an event and to a possible more specific effect of 1q abnormalities on the risk of death.

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

Other factors not included in the current analysis – such as circulating plasma cells (32), high-risk GEP(8,33) and MM cell-extrinsic factors (34) – could also play a role in determining the risk of early PD and should be investigated in future works. Moreover, our analysis focused on MM cells derived from a random bone marrow aspirate, and spatial heterogeneity of high-risk features could also explain some of the early PD cases (35).

ASCT and CT with IMiDs+PIs showed a protective effect against early PD in this patient population. However, the majority of patients in the analyzed cohort were real-world patients and the analysis was consequently performed as per protocol, thus leading to a risk of

443 overestimation of effects of ASCT and CT. With these limitations, our data support the
444 intensification of therapy in patients at risk of early relapse and underline the importance of
445 continuous treatment with combination regimens to optimize long-term disease control (36).

446 Carfilzomib-based induction also showed to reduce the risk of early relapse, although it is
447 difficult to distinguish between treatment and trial effects because the majority of
448 carfilzomib-treated patients were included in a clinical trial, whereas this was not the case for
449 other induction regimens.

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

456 The median age of the analyzed cohort was 63 years, younger than the usual median age of
457 unselected MM patients. Elderly patients were underrepresented and the confirmation of our
458 results in this patient population is warranted. However, other variables that are patient-
459 related but not disease-related (e.g. frailty status) may have a major prognostic role in elderly
460 patients (37).

461 Early-PD patients showed suboptimal responses and, at relapse, were more frequently
462 refractory to PIs and double refractory to IMiDs+PIs, as compared to Late-PD patients. IMiD
463 refractoriness was not different between Early PD and Late PD groups. This was mainly due to
464 the widespread use of PI-containing regimens during the first 18 months of therapy. On the
465 other hand, after the 18-month timepoint, treatment with an IMiD as single agent was widely
466 used in our patient population. Therefore, a high percentage of PI-refractory and IMiD+PI-

refractory cases were observed in the Early PD group, while IMiD-refractory cases were well represented in both the Early PD and Late PD groups.

In conclusion, early PD identifies a high-risk MM population that still represents an unmet clinical need. As compared with FISH, extended genotyping through the routine use of NGS at diagnosis is feasible and may improve the patient stratification and identify patients at risk of early PD (38). Further research is needed to better identify baseline features predicting early 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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635

Figure titles and legends

Figure 1. Study flow

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

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

Abbreviations. PD, progressive disease; OR: odds ratio; IgL: immunoglobulin lambda chain; IGLL5, immunoglobulin lambda like polypeptide 5; LDH: lactate dehydrogenase; V: bortezomib; d: low dose dexamethasone; chemo: conventional chemotherapy; R: lenalidomide; K: carfilzomib; ASCT: autologous stem-cell transplantation; CT: continuous therapy; FDT: fixed-duration therapy; IMiDs: immunomodulatory drugs; PIs: proteasome inhibitors.
Analysis is adjusted for missing values within each variable.

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 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.

690 **Table 2.** Best response to upfront treatment and drug refractoriness after first relapse in
691 Early PD versus Late PD patients
692

	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

693
694 **Abbreviations.** PD, progressive disease; SD stable disease; PR partial response; VGPR very good partial
695 response; CR, complete response; sCR, stringent CR; ORR, overall response rate (≥PR); n, number; IMiDs,
696 immunomodulatory drugs; PIs, proteasome inhibitors**.**
697
698

Figure 1

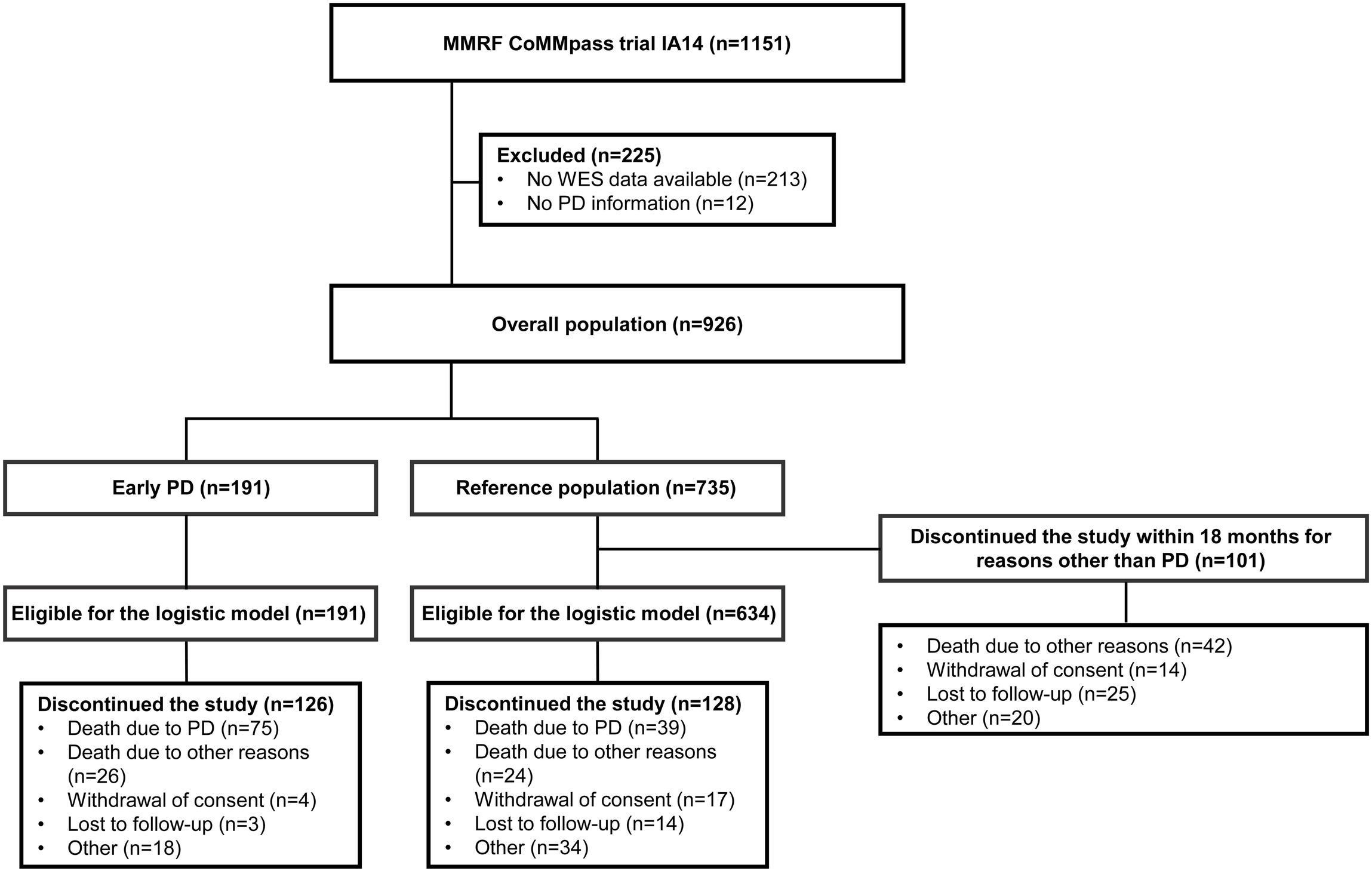

MMRF CoMMpass trial IA14 (n=1151)
Excluded (n=225)
• No WES data available (n=213)
• No PD information (n=12)
Overall population (n=926)
Early PD (n=191)
Reference population (n=735)
Discontinued the study within 18 months for reasons other than PD (n=101)
Eligible for the logistic model (n=191)
Eligible for the logistic model (n=634)
• Death due to other reasons (n=42)
• Withdrawal of consent (n=14)
• Lost to follow-up (n=25)
• Other (n=20)
Discontinued the study (n=126)
• Death due to PD (n=75)
• Death due to other reasons (n=26)
• Withdrawal of consent (n=4)
• Lost to follow-up (n=3)
• Other (n=18)
Discontinued the study (n=128)
• Death due to PD (n=39)
• Death due to other reasons (n=24)
• Withdrawal of consent (n=17)
• Lost to follow-up (n=14)
• Other (n=34)

Figure 2

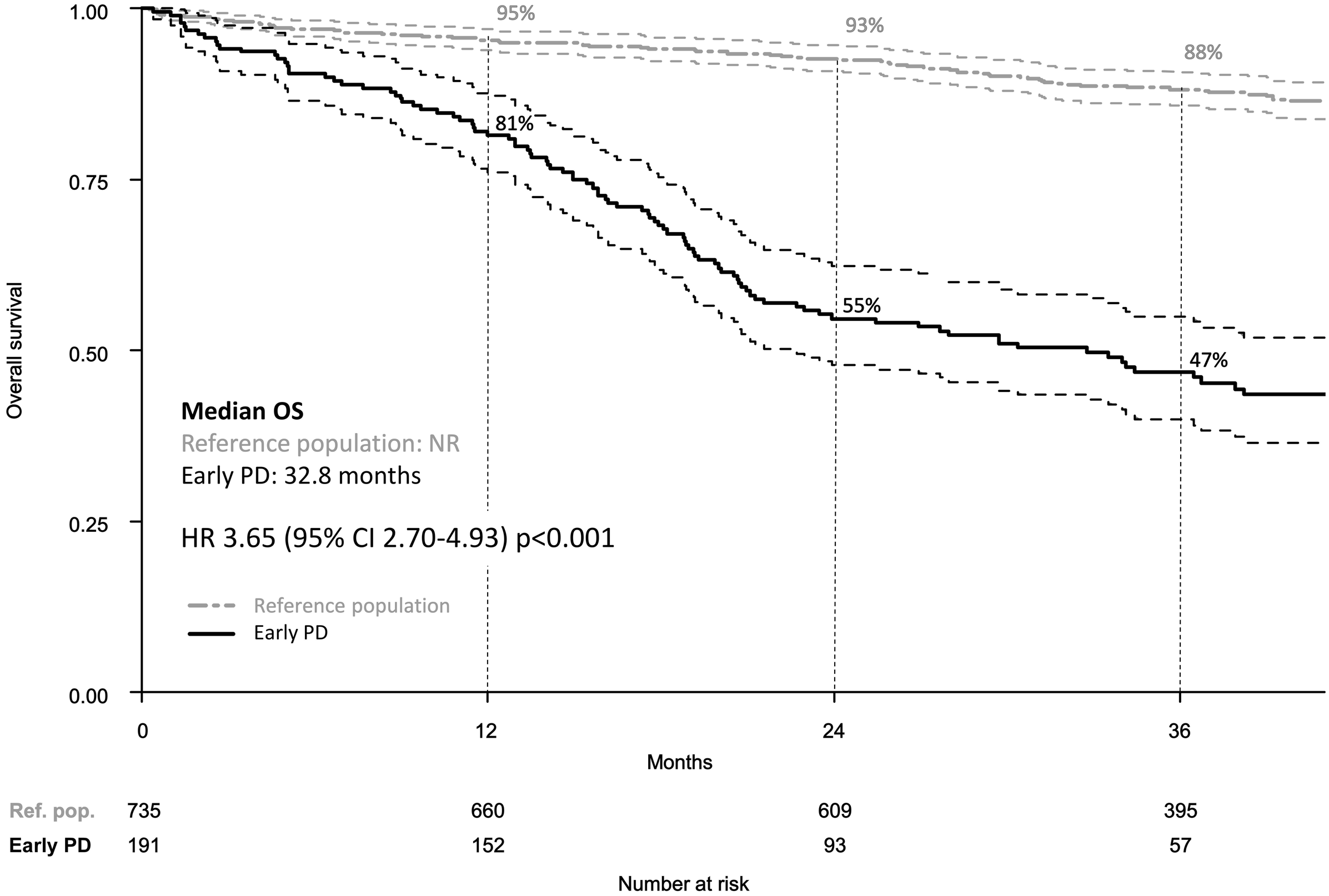

1.00
0.75
0.50
0.25
0.00
Overall survival
95%
93%
88%
81%
55%
47%
Median OS
Reference population: NR
Early PD: 32.8 months
HR 3.65 (95% CI 2.70-4.93) p<0.001
Reference population
Early PD
0
12
24
36
Months
Ref. pop. 735 660 609 395
Early PD 191 152 93 57
Number at risk

Figure 3

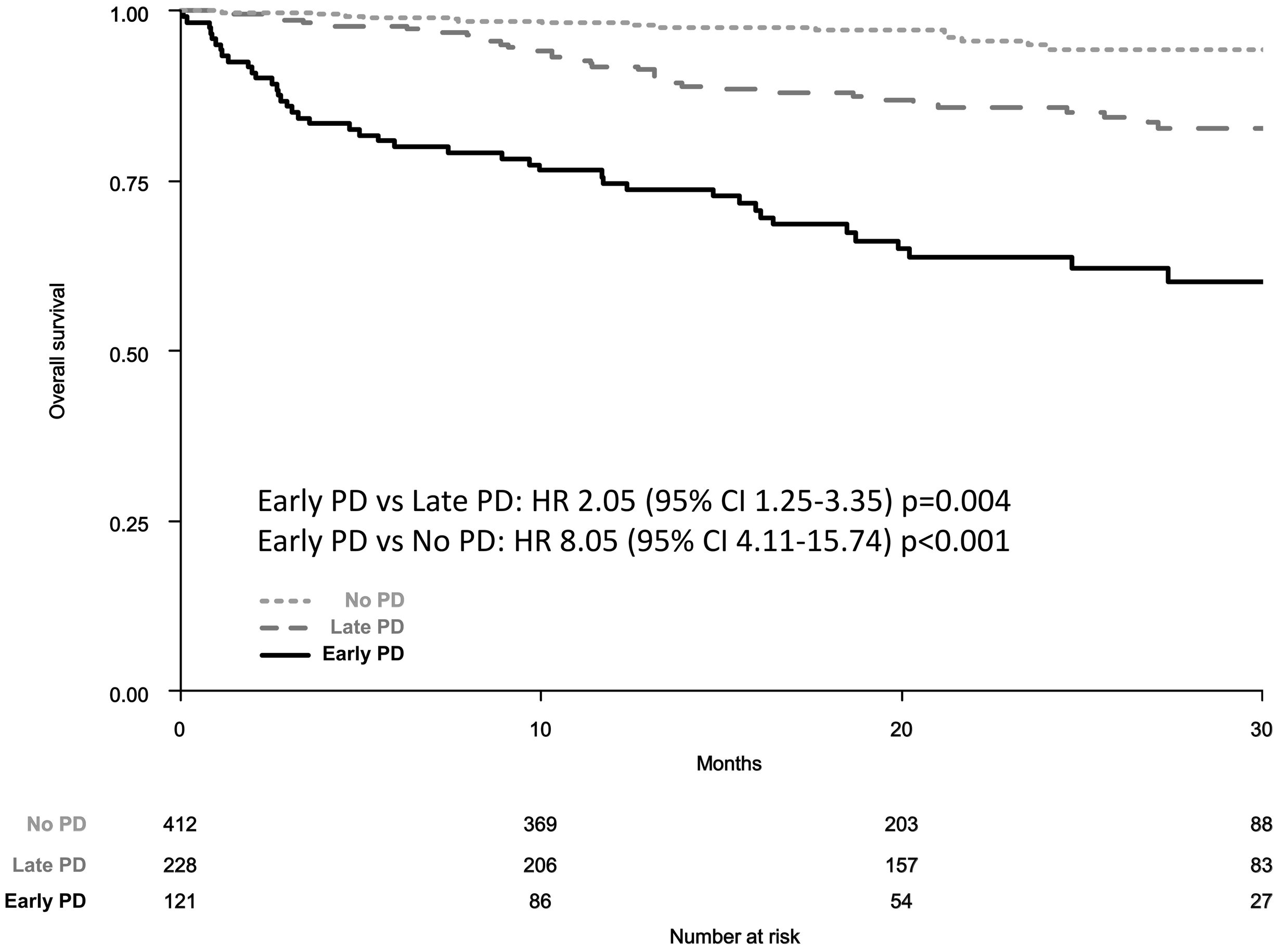
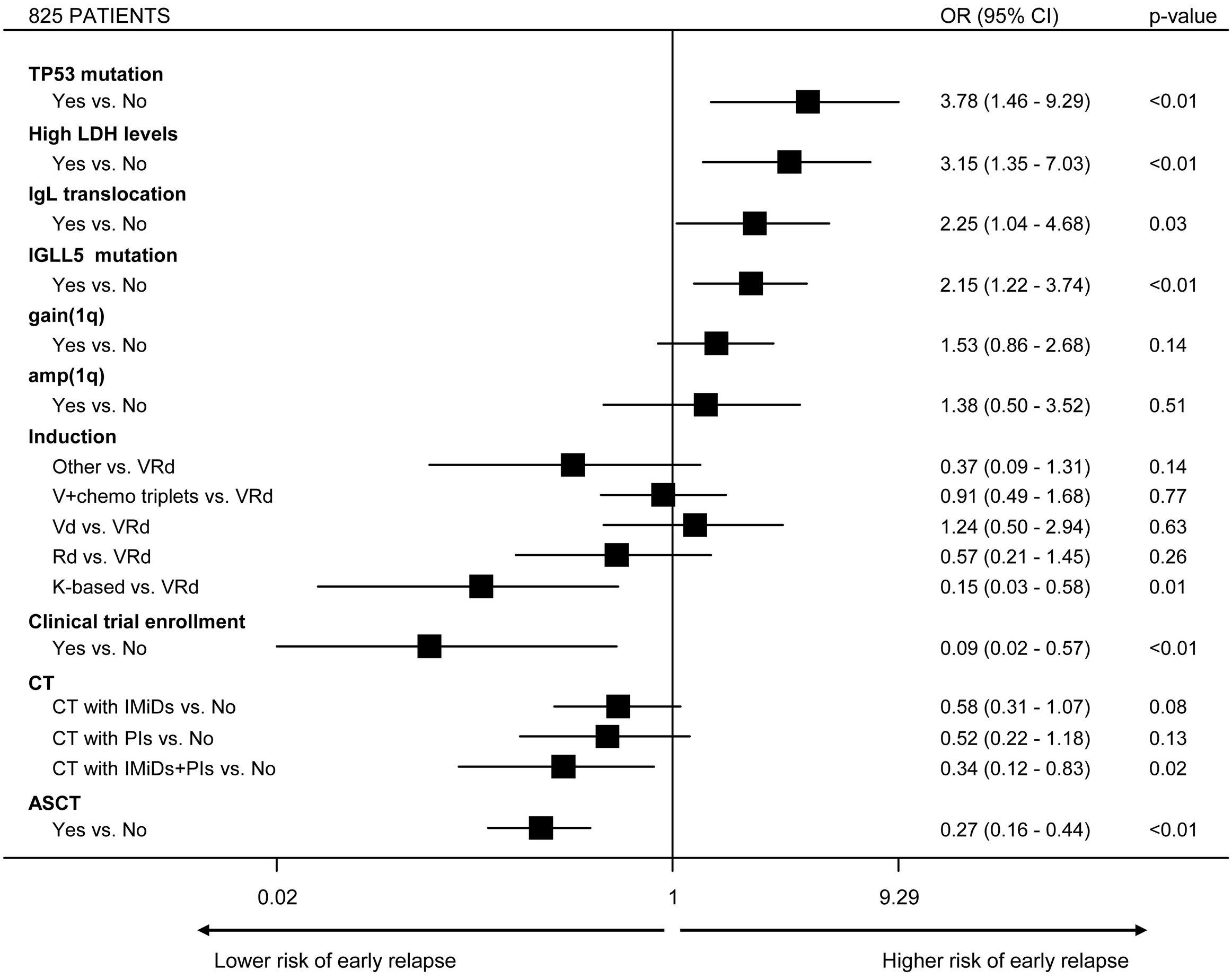

1.00
0.75
0.50
0.25
0.00
Overall survival
Early PD vs Late PD: HR 2.05 (95% CI 1.25-3.35) p=0.004
Early PD vs No PD: HR 8.05 (95% CI 4.11-15.74) p<0.001
No PD
Late PD
Early PD
0
10
20
30
Months
No PD 412 369 203 88
Late PD 228 206 157 83
Early PD 121 86 54 27
Number at risk

Figure 4


825 PATIENTS
OR (95% CI)
p-value
TP53 mutation
Yes vs. No
3.78 (1.46 - 9.29)
<0.01
High LDH levels
Yes vs. No
3.15 (1.35 - 7.03)
<0.01
IgL translocation
Yes vs. No
2.25 (1.04 - 4.68)
0.03
IGLL5 mutation
Yes vs. No
2.15 (1.22 - 3.74)
<0.01
gain(1q)
Yes vs. No
1.53 (0.86 - 2.68)
0.14
amp(1q)
Yes vs. No
1.38 (0.50 - 3.52)
0.51
Induction
Other vs. VRd
0.37 (0.09 - 1.31)
0.14
V+chemo triplets vs. VRd
0.91 (0.49 - 1.68)
0.77
Vd vs. VRd
1.24 (0.50 - 2.94)
0.63
Rd vs. VRd
0.57 (0.21 - 1.45)
0.26
K-based vs. VRd
0.15 (0.03 - 0.58)
0.01
Clinical trial enrollment
Yes vs. No
0.09 (0.02 - 0.57)
<0.01
CT
CT with IMiDs vs. No
0.58 (0.31 - 1.07)
0.08
CT with PIs vs. No
0.52 (0.22 - 1.18)
0.13
CT with IMiDs+PIs vs. No
0.34 (0.12 - 0.83)
0.02
ASCT
Yes vs. No
0.27 (0.16 - 0.44)
<0.01
0.02
1
9.29
Lower risk of early relapse
Higher risk of early relapse