Measurable residual disease by next-generation flow cytometry in multiple myeloma

Paiva, B.; Puig, N.; Cedena, M.T.; Rosinol, L.; Cordon, L.; Vidriales, M.B.; ... ; PETHEMA Programa Estudio Terapeuti

original reports

Measurable Residual Disease by Next-Generation Flow Cytometry in Multiple Myeloma

Bruno Paiva, PhD[1]; Noemi Puig, MD, PhD[2]; Maria-Teresa Cedena, MD[3]; Laura Rosiñol, MD, PhD[4]; Lourdes Cordón, PhD[5]; María-Belén Vidriales, MD, PhD[2]; Leire Burgos, PhD[1]; Juan Flores-Montero, MD, PhD[6,7]; Luzalba Sanoja-Flores, MSc[6,7]; Lucia Lopez-Anglada, MD, PhD[3]; Roberto Maldonado, MSc[3]; Javier de la Cruz, MD[3]; Norma C. Gutierrez, MD, PhD[2]; Maria-Jose Calasanz, PhD[1]; Maria-Luisa Martin-Ramos, PhD[3]; Ramón Garcia-Sanz, MD, PhD[2]; Joaquin Martinez-Lopez, MD[3]; Albert Oriol, MD[8]; María-Jesús Blanchard, MD[9]; Rafael Rios, MD[10]; Jesus Martin, MD[11]; Rafael Martinez-Martinez, PhD[12]; Anna Sureda, MD, PhD[13]; Miguel-Teodoro Hernandez, MD, PhD[14]; Javier de la Rubia, MD[5,15]; Isabel Krsnik, MD, PhD[16]; Jose-Maria Moraleda, MD[17]; Luis Palomera, MD, PhD[18]; Joan Bargay, MD, PhD[19]; Jacques J.M. Van Dongen, MD, PhD[20]; Alberto Orfao, MD, PhD[6,7]; Maria-Victoria Mateos, MD, PhD[2]; Joan Blade, MD, PhD[4]; Jesús F. San-Miguel, MD, PhD[1]; and Juan-José Lahuerta, MD[3]; on behalf of the GEM (Grupo Español de Mieloma)/PETHEMA (Programa Para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Group

abstract

PURPOSE Assessing measurable residual disease (MRD) has become standard with many tumors, but the clinical meaning of MRD in multiple myeloma (MM) remains uncertain, particularly when assessed by next-generation flow (NGF) cytometry. Thus, we aimed to determine the applicability and sensitivity of the flow MRD-negative criterion defined by the International Myeloma Working Group (IMWG).

PATIENTS AND METHODS In the PETHEMA/GEM2012MENOS65 trial, 458 patients with newly diagnosed MM had longitudinal assessment of MRD after six induction cycles with bortezomib, lenalidomide, and dexamethasone (VRD), autologous transplantation, and two consolidation courses with VRD. MRD was assessed in 1,100 bone marrow samples from 397 patients; the 61 patients without MRD data discontinued treatment during induction and were considered MRD positive for intent-to-treat analysis. The median limit of detection achieved by NGF was 2.9×10^{-6}. Patients received maintenance (lenalidomide ± ixazomib) according to the companion PETHEMA/GEM2014MAIN trial.

RESULTS Overall, 205 (45%) of 458 patients had undetectable MRD after consolidation, and only 14 of them (7%) have experienced progression thus far; seven of these 14 displayed extraosseous plasmacytomas at diagnosis and/or relapse. Using time-dependent analysis, patients with undetectable MRD had an 82% reduction in the risk of progression or death (hazard ratio, 0.18; 95% CI, 0.11 to 0.30; $P < .001$) and an 88% reduction in the risk of death (hazard ratio, 0.12; 95% CI, 0.05 to 0.29; $P < .001$). Timing of undetectable MRD (after induction *v* intensification) had no impact on patient survival. Attaining undetectable MRD overcame poor prognostic features at diagnosis, including high-risk cytogenetics. By contrast, patients with Revised International Staging System III status and positive MRD had dismal progression-free and overall survivals (median, 14 and 17 months, respectively). Maintenance increased the rate of undetectable MRD by 17%.

CONCLUSION The IMWG flow MRD-negative response criterion is highly applicable and sensitive to evaluate treatment efficacy in MM.

J Clin Oncol 38:784-792. © 2019 by American Society of Clinical Oncology

ASSOCIATED CONTENT

Appendix

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on September 17, 2019 and published at ascopubs.org/journal/jco on November 26, 2019: DOI https://doi.org/10.1200/JCO.19.01231

INTRODUCTION

Assessing measurable residual disease (MRD) has become a standard procedure in many hematologic malignancies,[1-4] but the lack of effective therapies for multiple myeloma (MM) delayed the interest to perform MRD studies in this disease until the past decade. Although initial data were obtained using low-sensitive and nonstandardized methods[5-19] or with next-generation sequencing (NGS) applied in retrospective series of patients treated with older regimens,[20,21] depth of response based on MRD emerged as one of the most relevant prognostic factors in MM.[15,22,23] Thus, the International Myeloma Working Group (IMWG) updated the response criteria in 2016 to foster standardized assessment of MRD in prospective trials, which should incorporate MRD-negative definitions based on NGS, next-generation flow cytometry (NGF), and positron-emission tomography/computerized tomography (PET/CT).[24] Thus far, a few studies have investigated the utility of PET/CT[25-30] and NGS[27,31-34] in patients treated with novel regimens, and the impressive results obtained with NGS in five trials[31-35] validated the new IMWG sequencing MRD-negative response criterion. By contrast, no prospective studies

using NGF in trials have validated the encouraging preliminary results reported by EuroFlow,[36] and many questions about the utility of MRD in patients with MM remain unanswered.[37]

PATIENTS AND METHODS

Study Design

Assessment of MRD was a secondary end point in the PETHEMA/GEM2012MENOS65 clinical trial (Appendix, online only). This open-label, phase III study included 458 patients receiving six induction cycles of bortezomib, lenalidomide, and dexamethasone (VRD), autologous stem-cell transplantation (ASCT) conditioned with busulfan and melphalan (ie, Bu-Mel) or melphalan (ie, Mel-200) high-dose therapy (HDT), and two consolidation cycles of VRD (Appendix Fig A1, online only).[38] The primary end point was progression-free survival (PFS) after Bu-Mel versus Mel-200, and it has not been met yet. Afterward, patients were enrolled in the PETHEMA/GEM2014MAIN clinical trial that randomly assigned them to maintenance with lenalidomide and low-dose dexamethasone (Rd) or Rd plus ixazomib for 2 years, after which patients continued with Rd for an additional 3 years if MRD positive or stopped therapy if MRD negative. Each study site's independent ethics committee approved the protocol, and informed consent forms were required prior to patient enrollment. Studies were registered at www.clinicaltrials.gov (ClinicalTrials.gov identifiers: NCT01916252, NCT02406144) and EudraCT (EudraCT identifiers: 2012-005683-10, 2014-000554-10), and the studies were conducted per the ethical principles of the Declaration of Helsinki.

MRD Assessment

MRD was predefined to be assessed at the time of suspected complete remission (CR), after induction, at day 100 after HDT/ASCT, and after consolidation. Of the 458 patients enrolled, 397 had at least one assessment of MRD performed after one or more of these defined treatment phases. The 61 patients without assessment of MRD discontinued therapy during induction. Another 26 patients discontinued therapy during intensification. Other reasons for missing MRD data (n = 52 [4.5%] of 1,152) are described in Figure 1. Overall, 377, 352, and 357 patients had MRD evaluable after induction, HDT/ASCT, and consolidation, respectively. MRD continued to be assessed every year during maintenance (PETHEMA/GEM2014MAIN), and data from the first 2 years were analyzed.

MRD was evaluated using NGF developed by EuroFlow (Appendix, online only).[36] The number of viable nucleated cells was systematically registered, and the limit of detection (LOD) achieved by NGF was determined in each sample according to the following formula: (20/number of viable nucleated cells) $\times$ 100. Patients had detectable MRD whenever the percentage of phenotypically aberrant clonal plasma cells (PCs) was equal to or greater than the LOD achieved in the corresponding sample. To determine the impact of detectable MRD levels on survival, patients were grouped according to the following MRD logarithmic levels: $\geq 2 \times 10^{-6}$ to $< 10^{-5}$, $\geq 10^{-5}$ to $< 10^{-4}$, and $\geq 10^{-4}$. Conversely, patients had undetectable MRD when phenotypically aberrant clonal PCs were either absent or present at percentages lower than the LOD achieved in the corresponding sample.

Statistical Analyses

To avoid narrowing the study by considering only those patients who experienced a response to treatment (ie, favorable disease course), MRD results were analyzed on the intent-to-treat population (n = 458) unless otherwise specified. Thus, patients with active disease who discontinued treatment were considered to have detectable MRD at that moment and thereafter. As expected, these patients had poor PFS (median, 13 months). Patients without an assessment of MRD at a specific timepoint for reasons other than discontinuing treatment also were considered to have detectable MRD at that timepoint.

The effect of an MRD response was evaluated using Cox proportional hazards models that considered MRD as a time-dependent covariable; all patients had disease at diagnosis (time = 0), and their MRD statuses were updated after induction (time = 1), HDT/ASCT (time = 2), and consolidation (time = 3). The effect of MRD conversions during maintenance was not included because of short follow-up. Cox regression models for PFS and overall survival (OS) were adjusted for sex, age, International Staging System (ISS), serum lactate dehydrogenase (LDH) levels, and fluorescence in situ hybridization cytogenetics; high-risk cytogenetics was defined by the presence of t(4;14), t(14;16) and/or del(17p) alterations. The regression models were performed by entering an interaction term between patient subgroup and MRD status.

Survival probabilities according to persistent versus undetectable MRD after consolidation were estimated using the Kaplan-Meier method. Briefly, differences were tested for statistical significance with the (two-sided) log-rank test, and hazard ratios (HRs; with two-sided 95% CIs) were estimated with a Cox regression model. PFS was defined as the time from MRD assessment until disease progression or death of any cause, and OS was defined as the time from MRD assessment until death. To estimate survival probabilities in all patients stratified at diagnosis by the Revised ISS (R-ISS; n = 404), PFS was defined as the time from study entrance until disease progression or death of any cause, and OS was defined as the time from study entrance until death. Multivariable Cox regression models with forward selection were performed to evaluate, at each step, the prognostic value of risk stratification at diagnosis based on the R-ISS and after treatment based on MRD status. Variables were introduced in the models if P was $< .05$. Statistical analyses were conducted using SAS (SAS

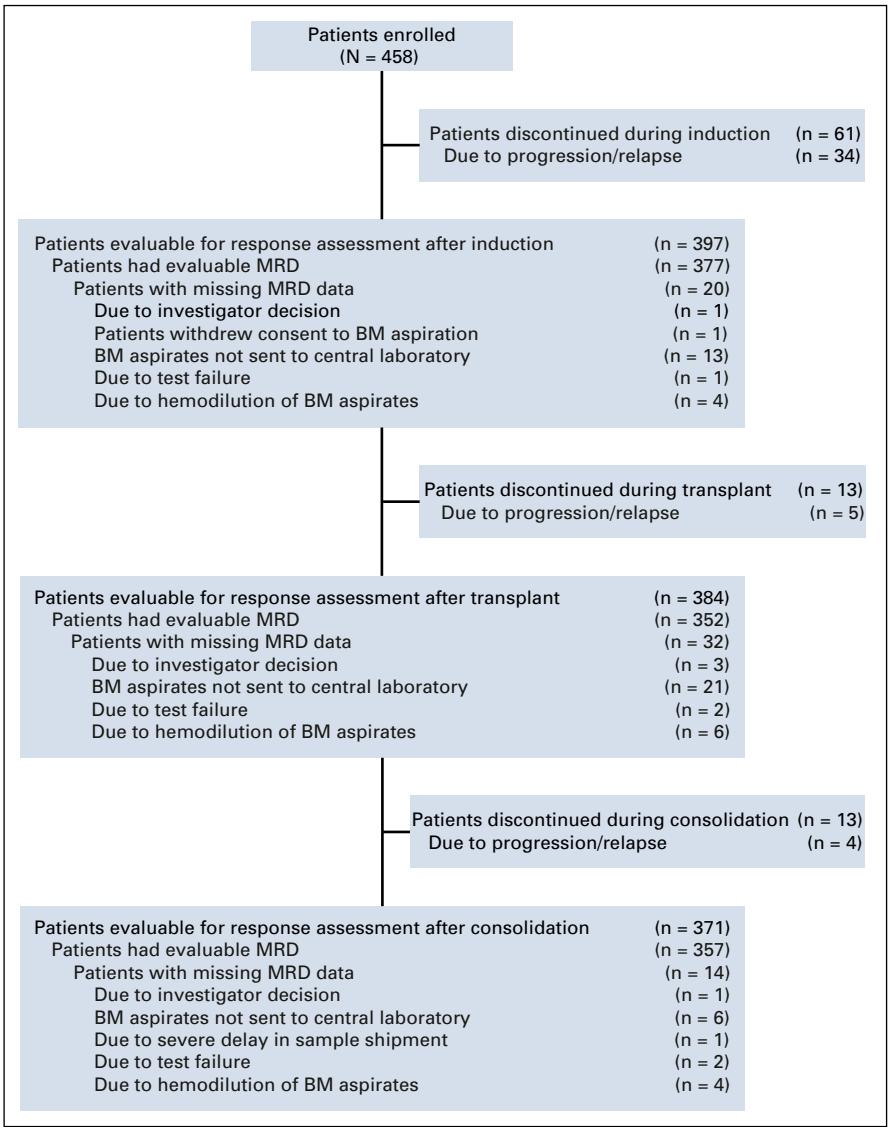

FIG 1. Patient disposition and measurable residual disease (MRD) assessments in the GEM2012MENOS65 clinical trial. BM, bone marrow.

Institute, Cary, NC), STATA (version 15.0; StataCorp, College Station, TX), and Statistical Package for Social Sciences (version 20.0; SPSS, Chicago, IL).

RESULTS

Applicability and Sensitivity of NGF

A total of 1,119 bone marrow (BM) aspirates were tested after induction, HDT/ASCT, or consolidation, and evaluable data were obtained from 1,114 aspirates (99.6%). NGF was unsuccessful in five samples (0.4%) because of inadequate specimen processing and/or instrument setup. The percentages of B-cell precursors, nucleated red blood cells, and mast cells were evaluated in each sample to determine the extent of hemodilution. Samples with $< 0.01\%$ BM PCs, B-cell precursors, nucleated red blood cells, and mast cells (n = 14 of 1,114) were considered severely hemodiluted and inadequate for MRD assessment. The median LOD achieved by NGF in the 1,100 BM aspirates evaluable for MRD was 2.9×10^{-6} (range, 1×10^{-6} to 1.14×10^{-4}). The logarithmic ranges of $< 2 \times 10^{-6}$, 2×10^{-6} to $< 10^{-5}$, $\geq 10^{-5}$ to $< 10^{-4}$, and $\geq 10^{-4}$ were achieved in 11 (1%), 965 (88%), 1,099 (99.9%), and 1,100 samples (100%), respectively.

Rates of Undetectable MRD With VRD Induction, HDT/ASCT, and VRD Consolidation

In the intent-to-treat population (n = 458), 129 (28%), 194 (42%), and 208 (45%) patients had undetectable MRD after induction, HDT/ASCT, and consolidation, respectively. After consolidation, 34% of patients had MRD levels $\geq 10^{-4}$, 10% had levels of $\geq 10^{-5}$ to $< 10^{-4}$, and 11% had levels of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$.

Despite similar rates of undetectable MRD after HDT/ASCT and consolidation, a detailed analysis of MRD log levels among patients with persistent MRD showed that 8% had MRD levels in the logarithmic range of 2×10^{-6} to $< 10^{-5}$ after HDT/ASCT, but subsequent consolidation reduced

MRD levels to a range of 2×10^{-6} to $< 10^{-5}$ in 19% of MRD-positive patient cases (Appendix Table A1, online only).

Patient MRD statuses according to conventional response criteria are listed in Appendix Table A2 (online only). Of note, all patients in partial response or less and undetectable MRD after induction remained MRD negative in subsequent timepoints, and all but two reached CR. Similarly, 32 of the 33 patients with very good partial response and undetectable MRD after induction remained MRD negative, and 18 reached CR.

Characteristics of Patients Who Experienced Disease Progression Despite Undetectable MRD

With a median follow-up of 40 months, disease progression occurred in 14 patients (7%) with undetectable MRD versus 101 patients (40%) with persistent MRD after consolidation ($P < .001$). Appendix Table A3 (online only) lists the characteristics of patients who experienced progression despite an undetectable MRD: five of 14 displayed ISS-III status, four of 14 had high LDH levels, and only two of 14 had high-risk cytogenetic abnormalities. More than half of the patients were early responders with negative MRD after induction (eight of 14). Interestingly, many patients experienced relapse without detectable M-protein (nine of 14) or BM infiltration (seven of 14) and with extraosseous plasmacytomas (six of 14); in fact, these traits were already observed in five of these patients at diagnosis.

Risk of Disease Progression in Patients With Persistent Versus Undetectable MRD

Using a Cox proportional hazards model in which the patient's MRD status was treated as a continuous time-dependent variable, we found that achieving undetectable MRD before maintenance was associated with an 82% reduction in the risk of progression or death (HR, 0.18; 95% CI, 0.11 to 0.30; $P < .001$) and an 88% reduction in the risk of death (HR, 0.12; 95% CI, 0.05 to 0.29; $P < .001$).

Using MRD as a fixed covariable, the Kaplan-Meier estimate of the 36-month PFS rate was 87% versus 50% in patients with undetectable versus persistent MRD after consolidation (Fig 2A). The 36-month OS rate was 96% versus 88% in patients with undetectable versus persistent MRD (Fig 2B). Data on time to progression and cumulative incidence of relapse are shown in Appendix Fig A2 (online only). The HRs for PFS and OS according to patient MRD statuses after consolidation were 0.21 (95% CI, 0.12 to 0.36; $P < .001$) and 0.26 (95% CI, 0.10 to 0.67; $P = .005$), respectively. The effect of MRD in reducing the risk of progression or death was independent of treatment arm (Appendix Tables A4 and A5, online only) and of whether patients were in CR (Appendix Fig A3, online only) or in less than CR (HR, 0.10; 95% CI, 0.01 to 0.72; $P = .02$). By contrast, there were no significant differences in PFS ($P = .21$) and OS ($P = .60$) in patients with positive MRD in CR versus less than CR after consolidation.

Positive MRD in the Logarithmic Range of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$ Is Clinically Relevant

Among patients with persistent MRD, there were no significant differences in PFS according to the logarithmic range of MRD levels (ie, $\geq 2 \times 10^{-6}$ to $< 10^{-5}$, $\geq 10^{-5}$ to $< 10^{-4}$, and $\geq 10^{-4}$). Thus, even patients who had very low but positive MRD levels in the logarithmic range of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$ displayed significantly inferior PFS ($P < .001$) and a trend for inferior OS ($P = .07$) compared with patients who had undetectable MRD (Appendix Fig A4, online only).

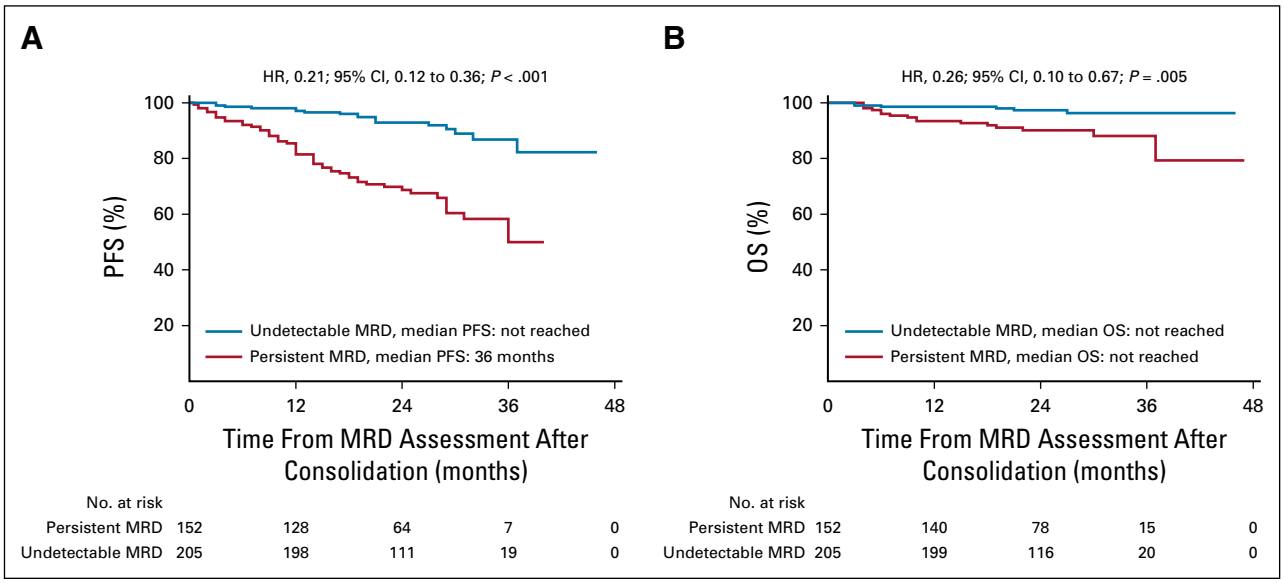

FIG 2. Survival according to undetectable *v* persistent measurable residual disease (MRD). The Kaplan-Meier estimates of (A) progression-free survival (PFS) and (B) overall survival (OS) after MRD assessment after consolidation (n = 357). HR, hazard ratio.

Undetectable MRD Is of Similar Significance at Distinct Timepoints

There were no significant differences in survival between patients with undetectable MRD achieved after induction or after treatment intensification (ie, HDT/ASCT and/or consolidation). The 36-month rates of PFS were 88% and 85% ($P = .38$), respectively, whereas the 36-month rates of OS were 94% and 99% ($P = .17$), respectively (Appendix Fig A5, online only).

MRD Responses Modulate Patients' Risks at Diagnosis

The observed reduction in the risk of progression or death observed in the intent-to-treat population with undetectable MRD was consistent across all patients, including those with high-risk cytogenetics (Fig 3; Appendix Table A6, online only). The reduction in the risk of progression or death also was evident in patients with elevated LDH levels; however, probably because of its association with extramedullary disease, the HR was higher when compared with patients who had normal LDH (0.47 *v* 0.18, respectively). Similar results were observed with regard to OS (Appendix Fig A6, online only).

When combining all risk parameters for the R-ISS according to the IMWG guidelines (prognostic value of the R-ISS is listed in Appendix Table A7, online only), there were no significant differences in the 36-month PFS rate for patients with R-ISS-I, R-ISS-II, or R-ISS-III statuses if MRD was undetectable after treatment (95%, 94%, and 88%, respectively; Fig 4A). Thus, patients with R-ISS-III status had their poor prognoses overcome through the achievement of undetectable MRD. By contrast, outcomes were progressively poor for patients with R-ISS-I, R-ISS-II, and R-ISS-III statuses when MRD remained positive (36-month PFS rates of 62%, 53%, and 28%, respectively; Fig 4B). Similar results were observed when OS was considered (Appendix Fig A7, online only). In Cox regression models with forward selection, undetectable MRD was selected in the first regression as the variable with the highest predictive value for PFS (HR, 0.12; 95% CI, 0.07 to 0.21; $P < .001$) and OS (HR, 0.09; 95% CI, 0.04 to 0.23; $P < .001$). In a second regression, both the R-ISS and MRD statuses showed significant predictive values (Table 1).

Impact of Maintenance Therapy on Patients' MRD Statuses

Overall, patient MRD status after consolidation remained stable during the first 2 years of maintenance: approximately half of patients (103 [45%] of 190) had sustained undetectable MRD, and one fifth (40 [21%] of 190) had persistent MRD. Conversions from positive to negative MRD during maintenance were observed in 33 (17%) of 190 patients, whereas the remaining 14 (7%) of 190 patients lost the negative MRD status (Appendix Table A8, online only). Longitudinal analysis of patients with paired MRD assessment from consolidation into the first and second years of maintenance denoted that most conversions from MRD positive to negative status were achieved during the first year.

DISCUSSION

The triple combination of proteasome inhibitors, immunomodulatory agents, and corticosteroids is emerging as

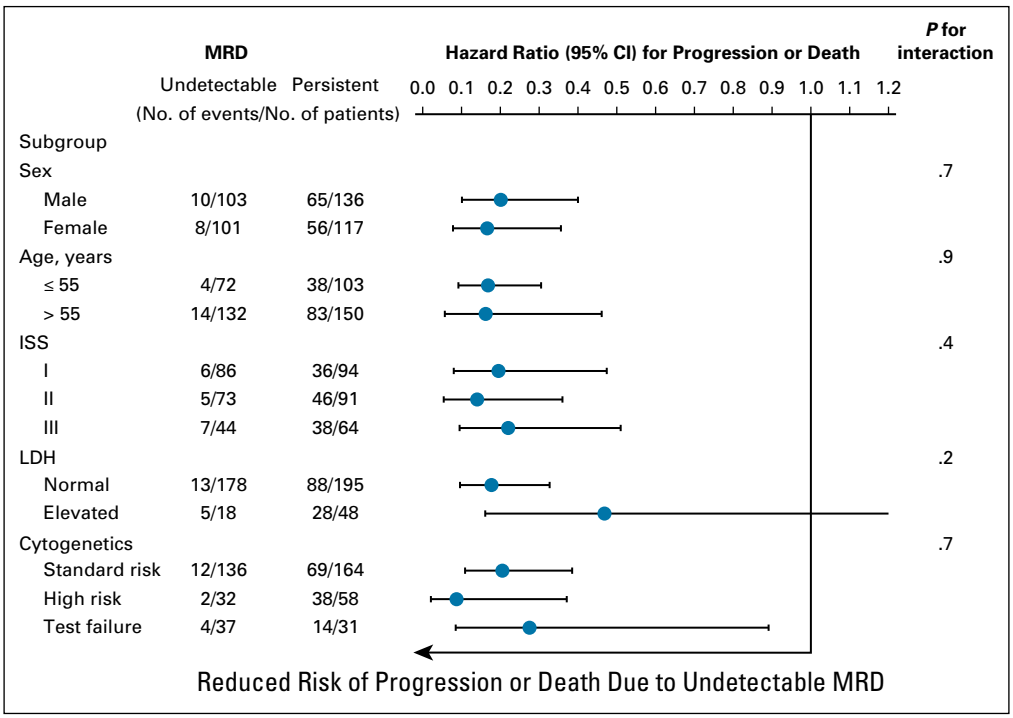

FIG 3. Subgroup analysis of progression-free survival according to patients' time-dependent measurable residual disease status. The intent-to-treat patient population was sub grouped according to sex, age, International Staging System (ISS), lactate dehydrogenase (LDH) levels, and cytogenetic abnormalities.

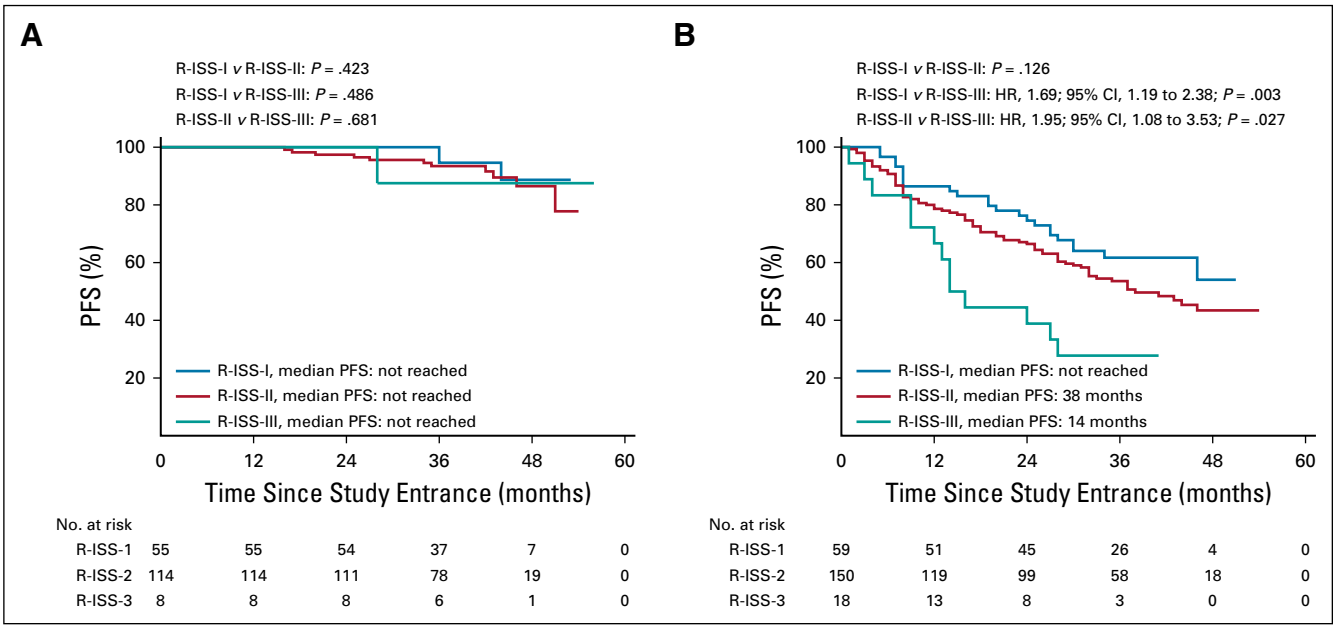

FIG 4. Modulating patients' risk at diagnosis according to depth of response after treatment, defined by measurable residual disease (MRD) status. Impact on progression-free survival of the Revised International Staging System (R-ISS) in patients with (A) undetectable *v* (B) persistent MRD. Of note, ISS was unavailable in six of 458 patient cases, lactate dehydrogenase in 19 of 458, and fluorescence in situ hybridization in 68 of 458. HR, hazard ratio.

a standard of care for patients with MM.[39-41] Based on Cassiopeia,[42] the efficacy of this triplet can be increased by adding anti-CD38 monoclonal antibodies. Here, in a prospective study with limited missing MRD data, we report that a VRD/ASCT/VRD treatment scheme provides almost 50% MRD-negative rates; with a median follow-up of 40 months, patients with undetectable MRD after consolidation showed very low risk of disease progression (7%), with 3-year survival rates reaching 90%. These are unprecedented results that identify new outcomes for transplant-eligible patients and establish undetectable MRD as the new treatment end point for MM.

Despite the positive results accumulated in the past decade,[22,23] MRD assessment has been considered in MM an exploratory test without clinical implications. Thus, MRD has been valuable to identify a false CR (ie, patients in CR with similar outcome to those in partial response because of persistent MRD),[15] but the achievement of undetectable MRD using low-sensitive methods was associated with a reduction in the risk of progression or death of only 60%.[15] Here, we show that an MRD-negative response defined by NGF identifies a group of patients with significantly lower risk of progression when compared with previous studies using flow cytometry.[5,6,12,13,15,16,18,40] Thus, our prospective analysis conducted in a large series of homogeneously treated patients validates the IMWG flow MRD-negative response criterion and supports its translation from trials into clinical practice. Of note, MRD assessment in trials typically is performed at predefined timepoints and irrespective of depth of response to prevent missing data. Accordingly, some patients in less than CR had undetectable MRD at a given timepoint, but most achieved CR later. This result reinforces that, in clinical

TABLE 1. Multivariable Analyses of PFS and OS, Incorporating Risk Stratification at Baseline According to the Revised International Staging System and Response Assessment After Treatment According to MRD status

	PFS			OS		
Model	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
First regression						
Undetectable *v* persistent MRD	0.12	0.07 to 0.21	< .001	0.09	0.04 to 0.23	< .001
Second regression						
Undetectable *v* persistent MRD	0.12	0.07 to 0.21	< .001	0.09	0.04 to 0.23	< .001
R-ISS I/II *v* III	0.46	0.26 to 0.80	.006	0.29	0.15 to 0.55	< .001

Abbreviations: HR, hazard ratio; MRD, measurable residual disease; OS, overall survival; PFS, progression-free survival; R-ISS, Revised International Staging System.

practice, MRD should be performed whenever patients achieve CR.

Despite the increased sensitivity of next-generation techniques, some patients with undetectable MRD develop early progression.[20,27,34,36] Here, we show that approximately half of these patients, some of them with extraosseous plasmacytomas at diagnosis, presented new plasmacytomas as an isolated criterion of disease progression without detectable M-protein or BM infiltration. Thus, it appears that these were true false-negative MRD results, reinforcing the need to combine NGF or NGS with PET/CT to monitor treatment efficacy,[25,30] particularly in patients presenting with extramedullary or macrofocal disease as well as elevated LDH levels.

After the promising results reported by Martinez-Lopez et al,[20] subsequent studies[21,27,31-34] confirmed the prognostic value of NGS-based MRD assessment in MM and established it as the gold standard among molecular methods to evaluate treatment efficacy in this disease. Because the clinical meaning of persistent MRD $< 10^{-5}$ remained uncertain, some studies used an LOD of 10^{-5} to define negative MRD,[20,31-33] while others adopted an LOD of 10^{-6}.[21,34] Although, for prognostic purposes, the IMWG threshold of $< 10^{-5}$ is adequate, our study extends the findings by Perrot et al[34] and Flores-Montero et al[36] and supports a negative MRD defined with an LOD of 10^{-6}.

Based on low-sensitive flow cytometry, we and others reported that patients who achieved MRD negativity after induction had superior outcomes compared with patients who achieved this response after HDT/ASCT,[5,16] probably because of the inability of these methods to detect MRD $< 0.01\%$.[20,34,36] Here, we showed that, using sensitive NGF, patients with undetectable MRD before or after HDT/ASCT had virtually identical survival. These results, obtained with a sequential scheme, suggest that persistent MRD after induction may be used as an indication for early intensification. Whether patients with undetectable MRD after induction are candidates to harvest stem cells for a late ASCT after disease progression (or, eg, MRD reappearance) should be investigated in randomized clinical trials. In addition, 17% of patients converted from detectable to undetectable MRD during maintenance, most of them in the first year. This rate is lower than in other trials (eg, Myeloma XI, EMN02, BMT CTN 0702), which could be related to exposure to lenalidomide during induction/consolidation, which would render patients less sensitive during maintenance, and/or to the higher sensitivity of NGF versus less-sensitive flow cytometry assays used in those studies. Of note, none of the patients converting from detectable to undetectable MRD during maintenance have experienced progression thus far, which strengthens the clinical value of maintenance therapy.

In 2015, the IMWG developed the R-ISS to effectively risk stratify patients on the basis of three diagnostic parameters.[43] Here, we show that achieving undetectable MRD by NGF overcame the poor prognosis of adverse factors identified at diagnosis, including high-risk cytogenetics. This unveils that risk is dynamic, because patients with adverse prognoses may shift into a favorable one upon achieving deep responses to treatment. The opposite also holds true; patients with R-ISS-I or R-ISS-II statuses and detectable MRD have a PFS closer to that of patients with R-ISS-III status and persistent MRD, rather than the other patients with R-ISS-I or R-ISS-II statuses with undetectable MRD. These results underline that MRD assessment helps resolve the variability in patient survival predicted by the R-ISS and highlight the value of the R-ISS to predict early versus late disease progression in patients with detectable MRD. Accordingly, patients with R-ISS-III status and persistent MRD showed median PFS and OS times of only 14 and 17 months, respectively. This observation is clinically meaningful, because these patients should be offered alternative treatment strategies before insurmountable disease progression occurs. In conclusion, the IMWG flow MRD-negative response criterion assessed in the BM is highly applicable and sensitive to evaluate treatment efficacy in MM.

AFFILIATIONS

[1]Clinica Universidad de Navarra, Centro de Investigacion Medica Aplicada Instituto de Investigacion Sanitaria de Navarra, Pamplona, Spain
[2]Hospital Universitario de Salamanca, Instituto de Investigacion Biomedica de Salamanca, Salamanca, Spain
[3]Hospital 12 de Octubre, Madrid, Spain
[4]Hospital Clínic Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[5]Hospital Universitario y Politécnico La Fe, Valencia, Spain
[6]Centro de Investigación Biomédica en Red de Cáncer, Instituto Carlos III, Madrid, Spain
[7]University of Salamanca, Salamanca, Spain
[8]Institut Català d'Oncologia i Institut Josep Carreras, Badalona, Spain
[9]Hospital Ramón y Cajal, Madrid, Spain
[10]Hospital Virgen de las Nieves, Granada, Spain
[11]Hospital Universitario Virgen del Rocío, Instituto de Biomedicina de Sevilla, Sevilla, Spain
[12]Hospital Universitario San Carlos, Madrid, Spain
[13]Institut Català d'Oncologia L'Hospitalet, Barcelona, Spain
[14]Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain
[15]Catholic University of Valencia, Valencia, Spain
[16]Hospital Puerta de Hierro, Madrid, Spain
[17]Hospital Virgen de la Arrixaca, Murcia, Spain
[18]Hospital Clínico Lozano Blesa, Zaragoza, Spain
[19]Hospital Son Llatzer, Palma de Mallorca, Spain
[20]Leiden University Medical Center, Leiden, the Netherlands

CORRESPONDING AUTHOR

Jesús F. San-Miguel, MD, PhD, Clinica Universidad de Navarra; Centro de Investigacion Medica Aplicada, Av Pio XII 36, 31008 Pamplona, Spain; e-mail: sanmiguel@unav.es.

EQUAL CONTRIBUTION

B.P., N.P., M.-T.C., and L.R. contributed equally to this study as first authors. J.F.S.-M. and J.-J.L. contributed equally to this study as last authors.

PRIOR PRESENTATION

Presented as an oral abstract at the 59th American Society of Hematology Annual Meeting and Exposition, Atlanta, GA, December 11, 2017.

SUPPORT

Supported by grants from the Centro de Investigación Biomédica en Red—Área de Oncología—del Instituto de Salud Carlos III (CIBERONC; grants No. CB16/12/00369, CB16/12/00400, and CB16/12/00284), Instituto de Salud Carlos III/Subdirección General de Investigación Sanitaria (FIS Nos. PI15/01956, PI15/02049, PI15/02062, and PI18/01709), Asociación Española Contra el Cáncer (No. GCB120981SAN and Accelerator Award), Beca Leonardo a Investigadores y Creadores Culturales 2017, Fundación BBVA (IN[17]_BBM_TRA_0236), the Black Swan Research Initiative of the International Myeloma Foundation, and the European Research Council (ERC) 2015 Starting Grant (MYELOMANEXT).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.19.01231.

AUTHOR CONTRIBUTIONS

Conception and design: Bruno Paiva, Juan Flores-Montero, Luzalba Sanoja-Flores, Jesus Martin, Rafael Martinez-Martinez, Joan Bargay, Jacques J.M. Van Dongen, Alberto Orfao, Joan Blade, Jesús F. San-Miguel, Juan-José Lahuerta
Financial support: Bruno Paiva, Jacques J.M. Van Dongen, Jesús F. San-Miguel
Administrative support: Roberto Maldonado, Jesus Martin, Jacques J.M. Van Dongen, Jesús F. San-Miguel, Juan-José Lahuerta
Provision of study material or patients: Bruno Paiva, Laura Rosiñol, Lourdes Cordón, María-Belén Vidriales, Maria-Jose Calasanz, Ramón Garcia-Sanz, Joaquin Martinez-Lopez, Albert Oriol, Rafael Rios, Jesus Martin, Rafael Martinez-Martinez, Anna Sureda, Javier de la Rubia, Luis Palomera, Jacques J.M. Van Dongen, Maria-Victoria Mateos, Joan Blade, Jesús F. San-Miguel, Juan-José Lahuerta
Collection and assembly of data: Bruno Paiva, Noemi Puig, Maria-Teresa Cedena, Lourdes Cordón, María-Belén Vidriales, Lucia Lopez-Anglada, Roberto Maldonado, Norma C. Gutierrez, Maria-Jose Calasanz, Maria-Luisa Martin-Ramos, Ramón Garcia-Sanz, Joaquin Martinez-Lopez, Albert Oriol, María-Jesús Blanchard, Rafael Rios, Jesus Martin, Rafael Martinez-Martinez, Miguel-Teodoro Hernandez, Isabel Krsnik, Jose-Maria Moraleda, Luis Palomera, Joan Bargay, Jacques J.M. Van Dongen, Maria-Victoria Mateos, Jesús F. San-Miguel, Juan-José Lahuerta
Data analysis and interpretation: Bruno Paiva, Noemi Puig, Maria-Teresa Cedena, Laura Rosiñol, María-Belén Vidriales, Leire Burgos, Javier de la Cruz, Ramón Garcia-Sanz, Albert Oriol, Rafael Rios, Jesus Martin, Rafael Martinez-Martinez, Anna Sureda, Javier de la Rubia, Jose-Maria Moraleda, Joan Bargay, Jacques J.M. Van Dongen, Joan Blade, Jesús F. San-Miguel, Juan-José Lahuerta
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

ACKNOWLEDGMENT

We thank all investigators and participating centers of the GEM (Grupo Español de Mieloma)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Group (a list of investigators is in the Appendix). The authors also thank Alfonso Santiago and Carmen Carrero for the administrative support by PETHEMA and Arturo Touchard for data management.

REFERENCES

1. Jongen-Lavrencic M, Grob T, Hanekamp D, et al: Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med 378:1189-1199, 2018
2. Dimier N, Delmar P, Ward C, et al: A model for predicting effect of treatment on progression-free survival using MRD as a surrogate end point in CLL. Blood 131: 955-962, 2018
3. Etienne G, Guilhot J, Rea D, et al: Long-term follow-up of the French stop imatinib (STIM1) study in patients with chronic myeloid leukemia. J Clin Oncol 35: 298-305, 2017
4. Jen EY, Xu Q, Schetter A, et al: FDA approval: Blinatumomab for patients with B-cell precursor acute lymphoblastic leukemia in morphologic remission with minimal residual disease. Clin Cancer Res 25:473-477, 2019
5. Paiva B, Vidriales MB, Cerveró J, et al: Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. Blood 112:4017-4023, 2008
6. Paiva B, Martinez-Lopez J, Vidriales MB, et al: Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol 29:1627-1633, 2011
7. Puig N, Sarasquete ME, Balanzategui A, et al: Critical evaluation of ASO RQ-PCR for minimal residual disease evaluation in multiple myeloma: A comparative analysis with flow cytometry. Leukemia 28:391-397, 2014
8. Ferrero S, Ladetto M, Drandi D, et al: Long-term results of the GIMEMA VEL-03-096 trial in MM patients receiving VTD consolidation after ASCT: MRD kinetics' impact on survival. Leukemia 29:689-695, 2015
9. Oliva S, Gambella M, Gilestro M, et al: Minimal residual disease after transplantation or lenalidomide-based consolidation in myeloma patients: A prospective analysis. Oncotarget 8:5924-5935, 2017
10. Oliva S, Hofste op Bruinink D, Říhová L, et al: Minimal residual disease (MRD) monitoring by multiparameter flow cytometry (MFC) in newly diagnosed transplant eligible multiple myeloma (MM) patients: Results from the EMN02/HO95 phase 3 trial. J Clin Oncol. 35: 2017 (suppl; abstr 8011).
11. Hahn TE, Wallace PK, Fraser R, et al: Minimal residual disease (MRD) assessment before and after autologous hematopoietic cell transplantation (AutoHCT) and maintenance for multiple myeloma (MM): Results of the prognostic immunophenotyping for myeloma response (PRIMeR) study. Biol Blood Marrow Transplant 25:S4-S6, 2019

12. Paiva B, Gutiérrez NC, Rosiñol L, et al: High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. Blood 119:687-691, 2012
13. Paiva B, Chandia M, Puig N, et al: The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapsed multiple myeloma. Haematologica 100:e53-e55, 2015
14. Paiva B, Cedena MT, Puig N, et al: Minimal residual disease monitoring and immune profiling in multiple myeloma in elderly patients. Blood 127:3165-3174, 2016
15. Lahuerta J-J, Paiva B, Vidriales M-B, et al: Depth of response in multiple myeloma: A pooled analysis of three PETHEMA/GEM clinical trials. J Clin Oncol 35: 2900-2910, 2017
16. Rawstron AC, Child JA, de Tute RM, et al: Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: Impact on outcome in the Medical Research Council myeloma IX study. J Clin Oncol 31:2540-2547, 2013
17. de Tute RM, Rawstron AC, Gregory WM, et al: Minimal residual disease following autologous stem cell transplant in myeloma: Impact on outcome is independent of induction regimen. Haematologica 101:e69-e71, 2016
18. Rawstron AC, Gregory WM, de Tute RM, et al: Minimal residual disease in myeloma by flow cytometry: Independent prediction of survival benefit per log reduction. Blood 125:1932-1935, 2015
19. Ladetto M, Pagliano G, Ferrero S, et al: Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol 28:2077-2084, 2010
20. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al: Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood 123:3073-3079, 2014
21. Takamatsu H, Takezako N, Zheng J, et al: Prognostic value of sequencing-based minimal residual disease detection in patients with multiple myeloma who underwent autologous stem-cell transplantation. Ann Oncol 28:2503-2510, 2017
22. Munshi NC, Avet-Loiseau H, Rawstron AC, et al: Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: A meta-analysis. JAMA Oncol 3:28-35, 2017
23. Landgren O, Devlin S, Boulad M, et al: Role of MRD status in relation to clinical outcomes in newly diagnosed multiple myeloma patients: A meta-analysis. Bone Marrow Transplant 51:1565-1568, 2016
24. Kumar S, Paiva B, Anderson KC, et al: International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 17:e328-e346, 2016
25. Moreau P, Attal M, Caillot D, et al: Prospective evaluation of magnetic resonance imaging and [^{18}F]fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial: Results of the IMAJEM study. J Clin Oncol 35:2911-2918, 2017
26. Zamagni E, Nanni C, Dozza L, et al. Standardization of 18F-FDG PET/CT according to deauville criteria for MRD evaluation in newly diagnosed transplant eligible multiple myeloma patients: Joined analysis of two prospective randomized phase III trials. Blood.132: 2018 (suppl; abstr 257).
27. Korde N, Roschewski M, Zingone A, et al: Treatment with carfilzomib-lenalidomide-dexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. JAMA Oncol 1:746-754, 2015
28. Usmani SZ, Sawyer J, Rosenthal A, et al: Risk factors for MDS and acute leukemia following total therapy 2 and 3 for multiple myeloma. Blood 121:4753-4757, 2013
29. Zamagni E, Nanni C, Mancuso K, et al: PET/CT Improves the definition of complete response and allows to detect otherwise unidentifiable skeletal progression in multiple myeloma. Clin Cancer Res 21:4384-4390, 2015
30. Rasche L, Alapat D, Kumar M, et al: Combination of flow cytometry and functional imaging for monitoring of residual disease in myeloma. Leukemia 33: 1713-1722, 2019
31. Mateos M-V, Dimopoulos MA, Cavo M, et al: Daratumumab plus bortezomib, melphalan, and prednisone for untreated myeloma. N Engl J Med 378:518-528, 2018
32. Dimopoulos MA, Oriol A, Nahi H, et al: Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med 375:1319-1331, 2016
33. Palumbo A, Chanan-Khan A, Weisel K, et al: Daratumumab, bortezomib, and dexamethasone for multiple myeloma. N Engl J Med 375:754-766, 2016
34. Perrot A, Lauwers-Cances V, Corre J, et al: Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood 132:2456-2464, 2018
35. Facon T, Kumar SK, Plesner T, et al. Phase 3 randomized study of daratumumab plus lenalidomide and dexamethasone (D-Rd) versus lenalidomide and dexamethasone (Rd) in patients with newly diagnosed multiple myeloma (NDMM) ineligible for transplant (MAIA). Blood.132: 2018 (suppl; abstr LBA-2).
36. Flores-Montero J, Sanoja-Flores L, Paiva B, et al: Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia 31:2094-2103, 2017
37. Moreau P, Zamagni E: MRD in multiple myeloma: More questions than answers? Blood Cancer J 7:639, 2017
38. Rosinol L, Oriol A, Rios R, et al: Bortezomib, lenalidomide and dexamethasone (VRD-GEM) as induction therapy prior autologous stem cell transplantation (ASCT) in multiple myeloma (MM): Results of a prospective phase III Pethema/GEM trial. Blood. 130:2017 (suppl; abstr).
39. Rosiñol L, Oriol A, Teruel AI, et al: Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: A randomized phase 3 PETHEMA/GEM study. Blood 120:1589-1596, 2012
40. Attal M, Lauwers-Cances V, Hulin C, et al: Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. N Engl J Med 376:1311-1320, 2017
41. Durie BGM, Hoering A, Abidi MH, et al: Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): A randomised, open-label, phase 3 trial. Lancet 389: 519-527, 2017
42. Moreau P, Attal M, Hulin C, et al: Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): A randomised, open-label, phase 3 study. Lancet 394:29-38, 2019
43. Palumbo A, Avet-Loiseau H, Oliva S, et al: Revised international staging system for multiple myeloma: A report from International Myeloma Working Group. J Clin Oncol 33:2863-2869, 2015

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Measurable Residual Disease by Next-Generation Flow Cytometry in Multiple Myeloma

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/journal/jco/site/ifc.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Bruno Paiva
Honoraria: Amgen, Bristol-Myers Squibb, Celgene, Janssen-Cilag, Takeda
Consulting or Advisory Role: Celgene, Janssen-Cilag, Sanofi, Takeda
Speakers' Bureau: Celgene
Research Funding: Celgene, Janssen-Cilag, Sanofi, Takeda

Noemi Puig
Honoraria: Amgen, Celgene, Janssen, Takeda, The Binding Site
Consulting or Advisory Role: Amgen, Celgene, Janssen, Takeda
Speakers' Bureau: Celgene
Research Funding: Celgene, Janssen, Amgen, Takeda
Travel, Accommodations, Expenses: Amgen, Celgene, Janssen, Takeda

Laura Rosiñol
Honoraria: Janssen-Cilag, Celgene, Amgen, Takeda
Consulting or Advisory Role: Janssen-Cilag, Celgene, Amgen

Juan Flores-Montero
Patents, Royalties, Other Intellectual Property: Methods, reagents and kits for detecting minimal residual disease: Patent number: PCT/NL/2013/050420US 62/072.498 (Inst); methods, reagents and kits for flow cytometry immunophenotyping: Patent number: PCT/NL2010/050332US2012/0165213 (Inst)
Travel, Accommodations, Expenses: Janssen Scientific Affairs

Norma C. Gutiérrez
Honoraria: Janssen-Cilag
Travel, Accommodations, Expenses: Kite/Gilead

Ramón Garcia-Sanz
Honoraria: Janssen, Takeda, Amgen
Consulting or Advisory Role: Janssen
Research Funding: Gilead (Inst), Incyte (Inst)
Patents, Royalties, Other Intellectual Property: BIOMED 2 primers (Inst)
Travel, Accommodations, Expenses: Jsnssen, Takeda (I)

Joaquin Martinez-Lopez
Speakers' Bureau: Novartis, Janssen-Cilag, Celgene, Bristol-Myers Squibb, Incyte
Research Funding: Bristol-Myers Squibb

Albert Oriol
Consulting or Advisory Role: Celgene, Janssen, Amgen
Speakers' Bureau: Amgen, Celgene

María-Jesús Blanchard
Consulting or Advisory Role: Janssen-Cilag, Amgen
Speakers' Bureau: Celgene

Rafael Rios
Consulting or Advisory Role: Janssen, Celgene

Jesus Martin
Travel, Accommodations, Expenses: Amgen

Rafael Martinez-Martinez
Travel, Accommodations, Expenses: Celgene

Anna Sureda
Honoraria: Takeda, Bristol-Myers Squibb, Merck Sharp & Dohme, Celgene, Janssen, Sanofi, Roche, Novartis, Gilead Sciences
Consulting or Advisory Role: Takeda, Bristol-Myers Squibb, Gilead Sciences, Celgene, Janssen, Novartis
Speakers' Bureau: Takeda
Other Relationship: Sanofi, Takeda, Roche, Celgene, Gilead Sciences

Miguel-Teodoro Hernandez
Consulting or Advisory Role: Celgene, Janssen, Amgen, Takeda
Speakers' Bureau: Janssen, Celgene, Amgen, Roche Pharma AG, Novartis
Research Funding: Celgene (Inst)

Javier de la Rubia
Expert Testimony: Amgen, Celgene, Janssen
Travel, Accommodations, Expenses: Amgen, Janssen

Isabel Krsnik
Honoraria: Celgene, Janssen, Takeda, Amgen
Consulting or Advisory Role: Celgene
Research Funding: Janssen, Prothena

Jose-Maria Moraleda
Consulting or Advisory Role: Gilead Sciences, Celgene
Research Funding: Pfizer (Inst)

Luis Palomera
Honoraria: Janssen-Cilag
Consulting or Advisory Role: Celgene, Janssen-Cilag, Amgen

Jacques J.M. Van Dongen
Honoraria: BD Biosciences (Inst)
Consulting or Advisory Role: Cytognos (Inst)
Research Funding: GlaxoSmithKline (Inst)
Patents, Royalties, Other Intellectual Property: Patents concerning methods for diagnosis of lymphoproliferative disorders (Inst)

Alberto Orfao
Consulting or Advisory Role: Cytognos
Speakers' Bureau: Novartis, Pfizer, AbbVie, BD Biosciences, Alexion Pharmaceuticals

Maria-Victoria Mateos
Honoraria: Janssen-Cilag, Celgene, Amgen, Takeda
Consulting or Advisory Role: Takeda, Janssen-Cilag, Celgene, Amgen, AbbVie, GlaxoSmithKline, Pharmamar-zeltia

Joan Blade
Honoraria: Janssen, Celgene, Amgen, Takeda

Jesús F. San-Miguel
Consulting or Advisory Role: Amgen (Inst), Celgene (Inst), Takeda (Inst), Bristol-Myers Squibb (Inst), MSD (Inst), Novartis (Inst), Sanofi (Inst), Janssen (Inst), Roche (Inst), AbbVie (Inst)

Juan-José Lahuerta
Consulting or Advisory Role: Celgene, Takeda, Amgen, Janssen-Cilag
Travel, Accommodations, Expenses: Celgene

No other potential conflicts of interest were reported.

APPENDIX

List of Investigators in the GEM (Grupo Español de Mieloma)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Group

The following are study group investigators and locations: Dr. María Casanova Espinosa, Complejo Hospitalario Costa del Sol; Dr. José Luís Guzman Zamudio, H. Especialidades de Jerez de la Frontera; Dr. Eduardo Ríos Herranz, H. Nuestra Señora de Valme; Dr. Rafael Rios Tamayo, H. Universitario Virgen de las Nieves; Dr. Jesús Martín Sánchez, Complejo Hospitalario Regional Virgen del Rocío; Dr. Luís Palomera Bernal, H. Clínico Universitario Lozano Blesa; Dr. Ana Pilar González Rodríguez, H. Universitario Central de Asturias; Dr. María Esther González García, H. Cabueñes; Dr. Antonia Sampol Mayol, Complejo Asistencial Son Espases; Dr. Joan Bargay Lleonart, H. Son Llátzer; Dr. Alexia Suárez, H. de Gran Canaria Dr. Negrín; Dr. Miguel Teodoro Hernández García, H. Universitario de Canarias; Dr. Carmen Montes Gaisán, H. Universitario Marqués de Valdecilla; Dr. Belén Hernández Ruiz, H. General de Ciudad Real; Dr. Felipe Casado Montero, Complejo Hospitalario de Toledo; Dr. Dunia de Miguel Llorente, H. Universitario de Guadalajara; Dr. Fernando Solano Ramos, H. Nuestra Señora del Prado (Talavera); Dr. Ángela Ibañez Garcia, H. General de Albacete; Dr. Mariví Mateos Manteca, H. Clínico de Salamanca; Dr. José Mariano Hernández Martín, Complejo Hospitalario H. General de Segovia; Dr. Fernando Escalante Barrigón, H. de León; Dr. Javier García Frade, H. Universitario Rio Hortega; Dr. Alfonso García de Coca, H. Clínico Universitario de Valladolid; Dr. Carlos Aguilar Franco, H. Santa Bárbara; Dr. Jorge Labrador Gómez, Hospital Universitario de Burgos; Dr. Elena Cabezudo Pérez, H. Althaia, Xarxa Asistencial de Manresa (Sant Joan de Deu); Dr. Joan Bladé Creixentí, H CLINIC; Dr. Ana Ma Sureda Balari, H. Durán i Reynals—ICO LHospitalet; Dr. Yolanda González Montes, ICO Girona, H. Universitario de Girona Dr. Josep Trueta; Dr. Lourdes Escoda Teigell, H. Universitario Joan XXII de Tarragona; Dr. Antonio García Guiñón, Hospital Universitari Arnau de Vilanova de Lleida; Dr. Eugenia Abella Monreal, H. del Mar; Dr. Juan Alfonso Soler Campos, H. de Sabadell (Parc Taulí); Dr. Josep Ma Martí Tutusaus, Hospital Universitario Mútua de Terrassa; Dr. Albert Oriol Rocafiguera, H. Germans Trias i Pujol; Dr. Miquel Granell Gorrochategui, H. de la Santa Creu i Sant Pau; Dr. Mercedes Gironella Mesa, H. Vall d'Hebrón; Dr. Carmen Cabrera Silva, H. San Pedro de Alcántara (Complejo Hospitalario de Cáceres); Dr. Marta Sonia González Pérez, Complejo Hospitalario Universitario de Santiago; Dr. Ana Dios Loureiro, Complejo Hospitalario de Pontevedra; José Angel Méndez Sánchez, Complejo Hospitalario de Ourense; Dr. María Josefa Nájera Irazu, H. San Pedro; Dr. Francisco Javier Peñalver Párraga, H. Universitario Fundación de Alcorcón; Dr. Juan José Lahuerta Palacios, H. Universitario 12 de Octubre; Dr. Pilar Bravo Barahona, H. de Fuenlabrada; Dr. Cristina Encinas Rodríguez, H. General Universitario Gregorio Marañón; Dr. José Ángel Hernández Rivas, H. Infanta Leonor; Dr. Jaime Pérez de Oteyza, H. Universitario Madrid—Sanchinarro; Dr. Rebeca Iglesias del Barrio, Centro Oncológico MD Anderson; Dr. Ana López de la Guia, H. Universitario La Paz; Dr. Adrián Alegre Amor, H. Universitario de la Princesa; Dr. Elena Prieto Pareja, Fundación Jiménez Díaz—UTE; Dr. Isabel Krsnik Castelló, Hospital Universitario Puerta de Hierro—Majadahonda; Dr. Ma Jesús Blanchard Rodríguez, H. Ramón y Cajal; Dr. Rafael Martínez, H. Universitario de San Carlos; Dr. Rosalía Riaza Grau, H. Severo Ochoa; Dr. Eugenio Giménez Mesa, H. Infanta Sofía; Dr. Elena Ruiz Sainz, Hospital del Tajo; Dr. Felipe de Arriba, H. Morales Meseguer; Dr. Jose María Moraleda Jiménez, H. Universitario Virgen de la Arrixaca; Dr. Marta Romera, H. General Universitario Santa Lucia; Dr. Felipe Prósper Cardoso, Clínica Universidad de Navarra; Dr. José Ma Arguiñano Pérez, Complejo Hospitalario de Navarra; Dr. María Puente Pomposo, H. de Cruces; Dr. Ernesto Pérez Persona, H. de Txagorritxu; Dr. Ana Isabel Teruel Casasús, H. Clínico Universitario de Valencia; Dr. Paz Ribas García, H. Universitario Dr. Peset; Dr. Isidro Jarque Ramos, H. Universitario La Fe; Dr. María Blanca Villarrubia Lor, H. General Universitario de Alicante; Dr. Pedro Luis Fernández García, H. Torrevieja Salud UTE; and Dr. Pedro Luis Fernández García, H. del Vinalopo.

Supplementary Methods

Secondary end points of the PETHEMA/GEM2012MENOS65 clinical trial. The following were secondary end points:

- Complete response (CR) rates with negative immunofixation after each phase of treatment (induction, autologous stem-cell transplantation [ASCT] and consolidation);
- Measurable residual disease (MRD) in patients with immunofixation-negative CR and after each phase of treatment (induction, autologous stem-cell transplantation, and consolidation);
- Overall survival after ASCT with busulfan + melphalan versus melphalan; and
- Safety and tolerability of induction and consolidation treatments.

Next-generation flow. MRD was assessed using the next-generation flow (NGF) method developed by EuroFlow for highly sensitive and standardized MRD detection in multiple myeloma (Sanoja-Flores L, et al: Blood Cancer J 8:117, 2018).[36] In brief, the method is based on a (standardized) lyse-wash-and-stain protocol and an optimized eight-color, two-tube antibody panel for accurate identification of phenotypically aberrant, clonal plasma cells (PCs): Tube 1 includes CD138-BV421, CD27-BV510, CD38-FITC, CD56-PE, CD45-PerCPCy5.5, CD19-PECy7, CD117-APC, and CD81-APCH7; tube 2 includes CD138-BV421, CD27-BV510, CD38-FITC, CD56-PE, CD45-PerCPCy5.5, CD19-PECy7, cyKAPPA-APC, and cyLAMBDA-APCH7 (Arana P, et al: Leukemia 32:971-978, 2018).[36] The two-tube strategy allows detection of MRD with specific confirmation of light-chain (mono)clonality on phenotypically aberrant PCs, identified by antigen underexpression (CD19, CD27, CD38, CD45, CD81) or overexpression (CD56, CD117, CD138) compared with normal PCs (Arana P, et al: Leukemia 32:971-978, 2018).[36] Data acquisition was performed in a FACSCanto II flow cytometer (BD, San Jose, CA) using the FACSDiva 6.1 software (BD). Data analysis was performed by experienced operators using the Infinicyt software (Cytognos SL, Salamanca, Spain). MRD assessments were performed blinded for clinical outcomes in three PETHEMA/GEM laboratories and data were centralized for MRD analyses.

Cytogenetic characterization. Fluorescence in-situ hybridization was performed at diagnosis in the same three PETHEMA/GEM laboratories that perform MRD monitoring. Immunomagnetically enriched PCs from 390 of 458 patients were tested for chromosome 1 alterations, *IGH* translocations and del(17p13). Patients with t(4;14), t(14;16), and/or del(17p13) were classified as high risk (n = 90); others were classified as standard risk (n = 300).

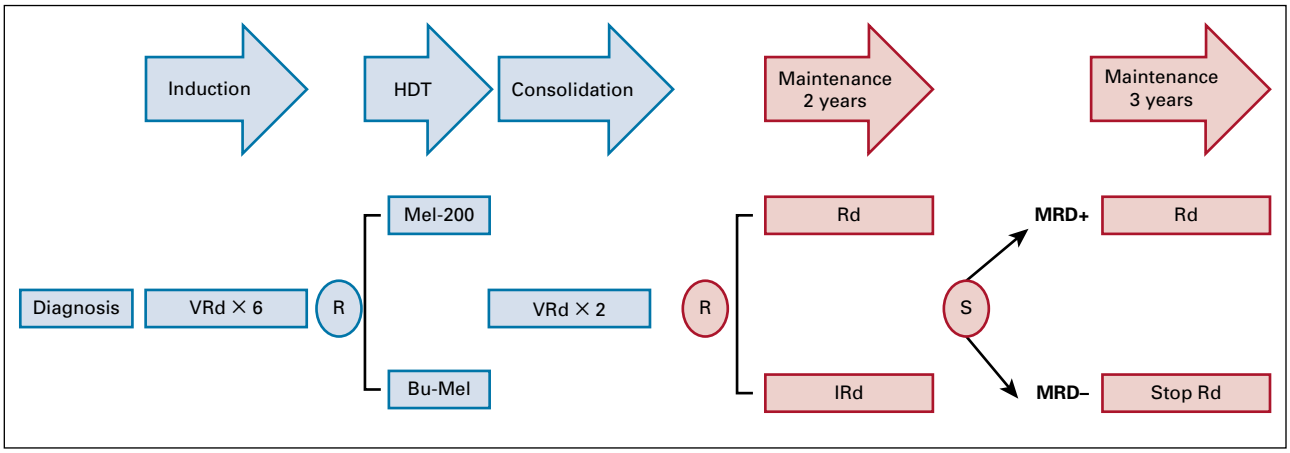

FIG A1. Schema of the PETHEMA GEM2012MENOS65 (in light blue) and GEM2014MAIN trials (in light red). Bu-Mel, busulfan + melphalan; d, low-dose dexamethasone; HDT, high-dose therapy; I, ixazomib; Mel, melphalan; MRD, measurable residual disease; R, lenalidomide; V, bortezomib.

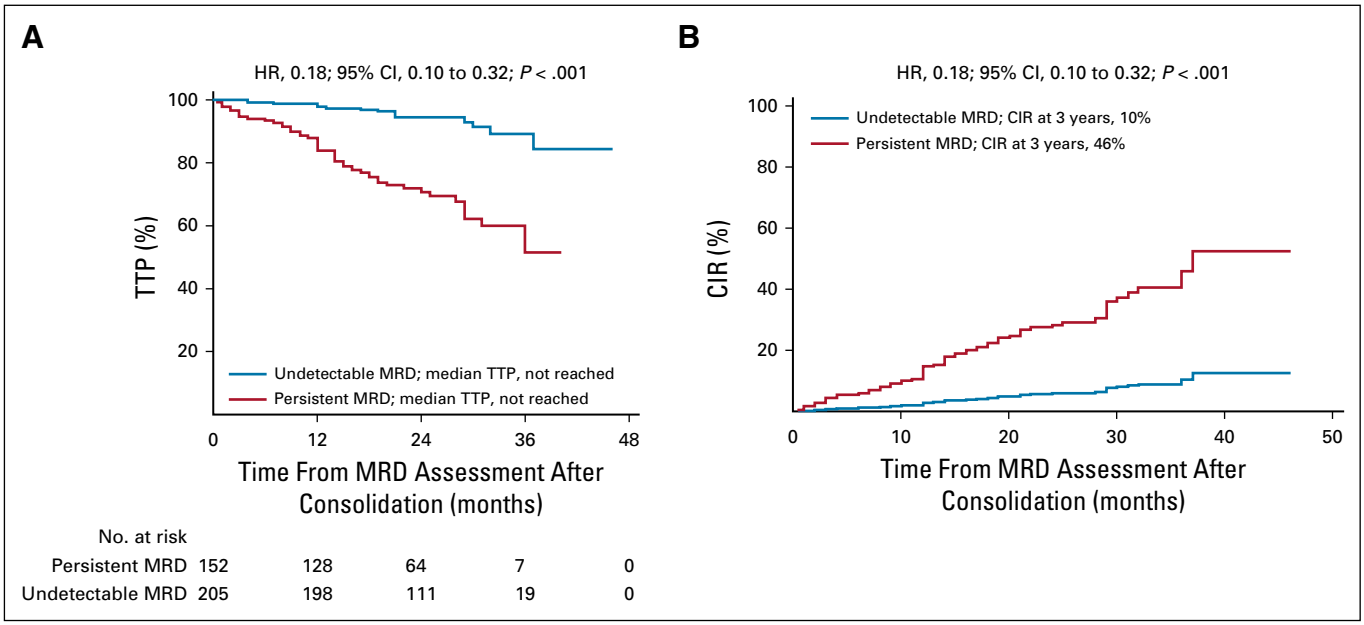

FIG A2. (A) Kaplan-Meier estimates of time-to progression (TTP), defined as the time from measurable residual disease (MRD) assessment until disease progression, according to patients' MRD statuses after consolidation (n = 357). Data from patients who died in the absence of progression were thus censored. The 3-year TTP rates were 89% *v* 51% for patients with undetectable *v* persistent MRD, respectively. (B) The cumulative incidence of relapse (CIR) was calculated from the time from MRD assessment to the date of disease progression, considering death without disease progression as a competing event. HR, hazard ratio.

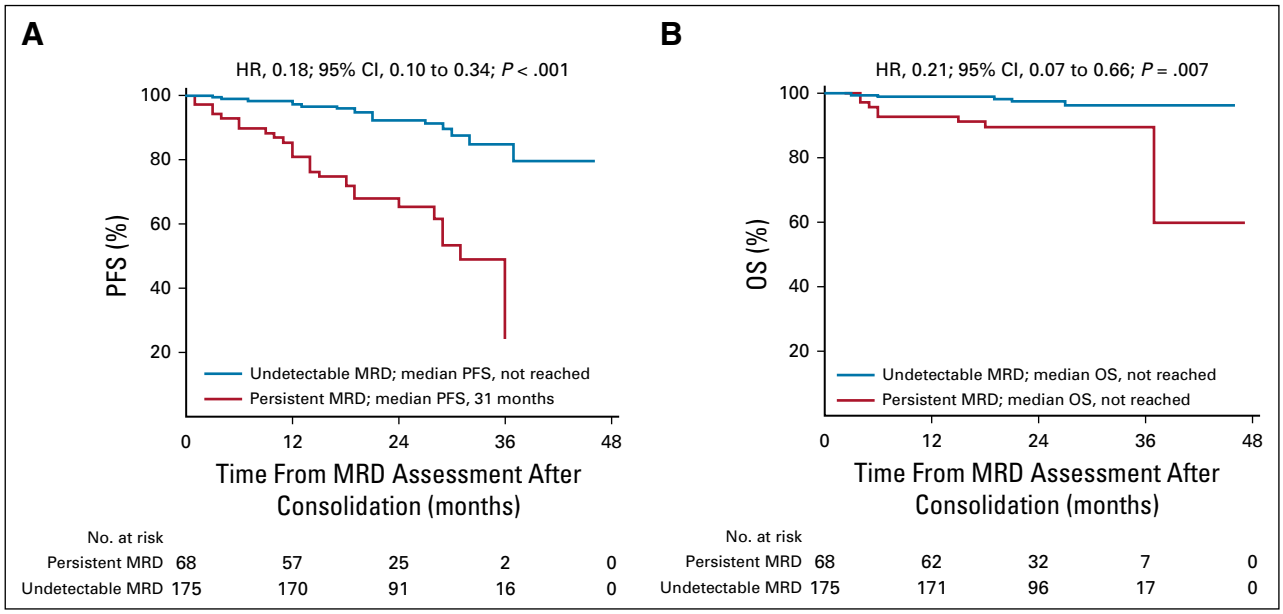

FIG A3. Survival according to undetectable *v* persistent measurable residual disease (MRD) in patients in complete remission (CR) after consolidation. (A) The Kaplan-Meier estimate of the 36-month progression-free survival (PFS) rate was 85% *v* 24% in patients with undetectable *v* persistent MRD. (B) The 36-month overall survival (OS) rate was 96% *v* 89% in patients with undetectable *v* persistent MRD. HR, hazard ratio.

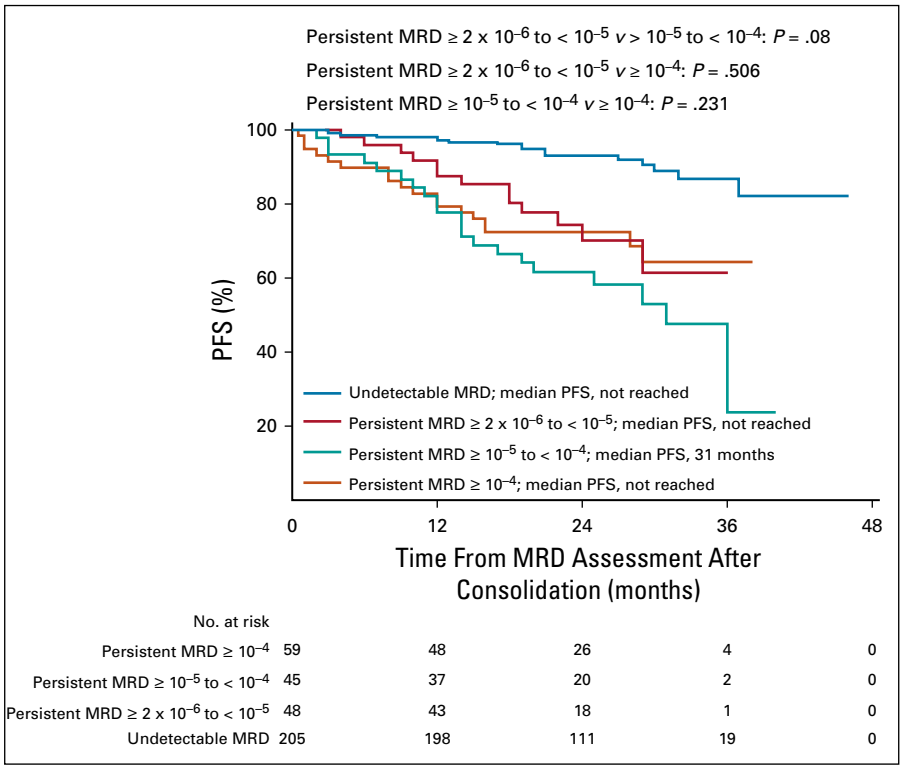

FIG A4. Progression-free survival (PFS) according to the presence of undetectable *v* persistent measurable residual disease (MRD) in the logarithmic ranges of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$, $\geq 10^{-5}$ to $< 10^{-4}$ and $\geq 10^{-4}$ after consolidation (n = 357). All pairwise comparisons between patients with negative *v* positive MRD in in the logarithmic ranges of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$, $\geq 10^{-5}$ to $< 10^{-4}$ and $\geq 10^{-4}$ were statistically significant ($P < .001$). By contrast, there were no statistically significant differences in PFS of patients with positive MRD in the logarithmic range of $\geq 2 \times 10^{-6}$ to $< 10^{-5}$ *v* those with MRD levels of $\geq 10^{-5}$ to $< 10^{-4}$ or *v* those with MRD $\geq 10^{-4}$. There were also no statistically significant differences when comparing the PFS of patients with MRD in the logarithmic range of $\geq 10^{-5}$ to $< 10^{-4}$ *v* those with MRD $\geq 10^{-4}$.

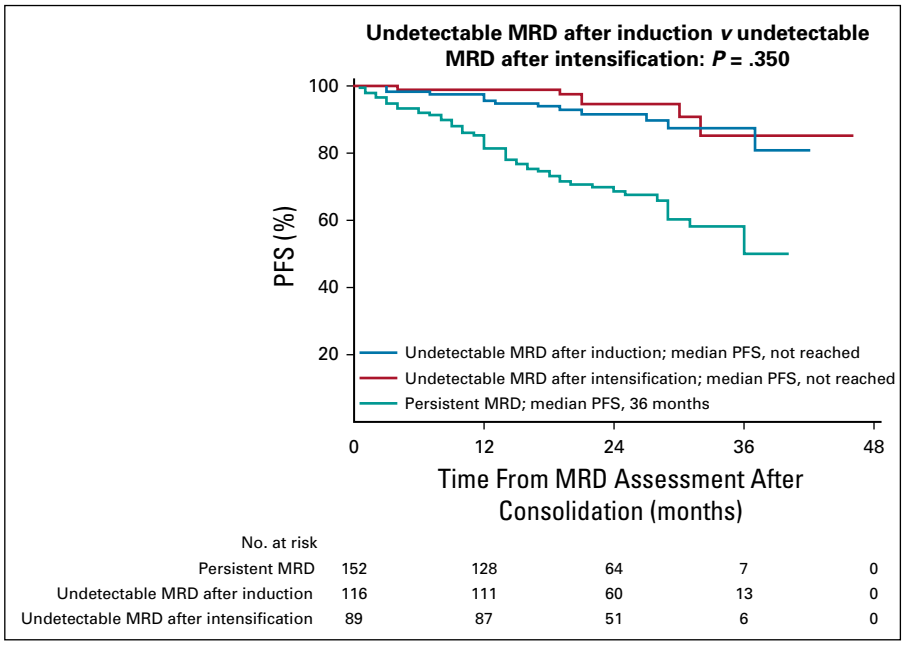

FIG A5. Progression-free survival (PFS) after consolidation (n = 357) according to the moment at which measurable residual disease (MRD) is undetectable: after induction *v* after intensification (either after high-dose therapy or consolidation). Data from patients with persistent MRD across the entire duration of the study were also plotted. Of note, 16 patients achieved negative MRD after induction (n = 1) or after high-dose therapy/autologous stem-cell transplantation (n = 15) but lost their MRD-negative status at the end of consolidation; five of the 16 experienced disease progression.

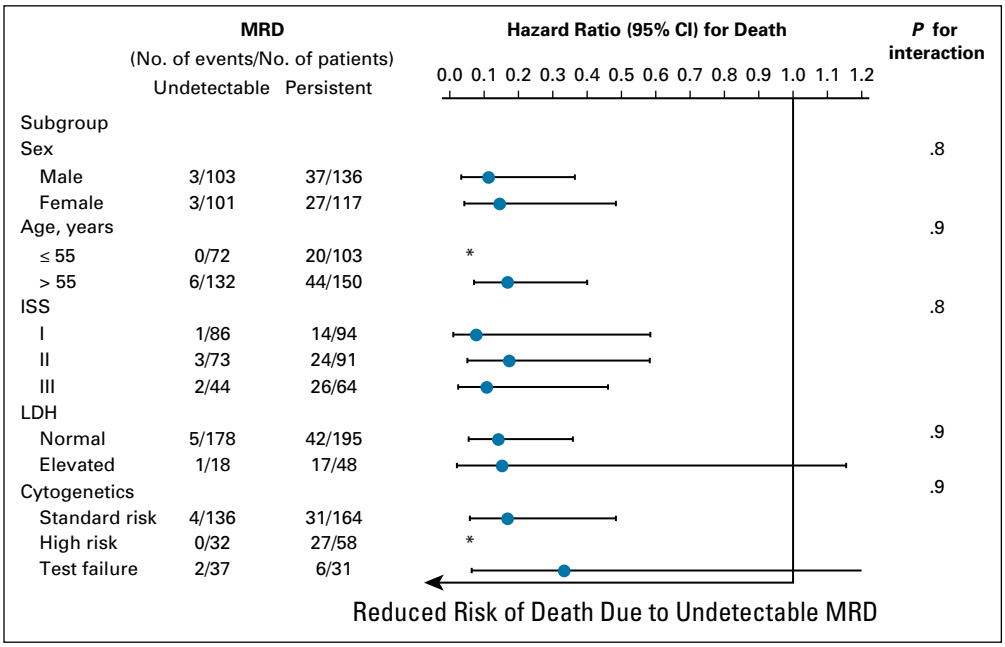

FIG A6. Subgroup analysis of overall survival according to patients' time-dependent measurable residual disease status. The intent-to-treat patient population was subgrouped according to sex, age, International Staging System (ISS), lactate dehydrogenase (LDH) levels, and cytogenetic alterations. (*) No events in this subgroup category.

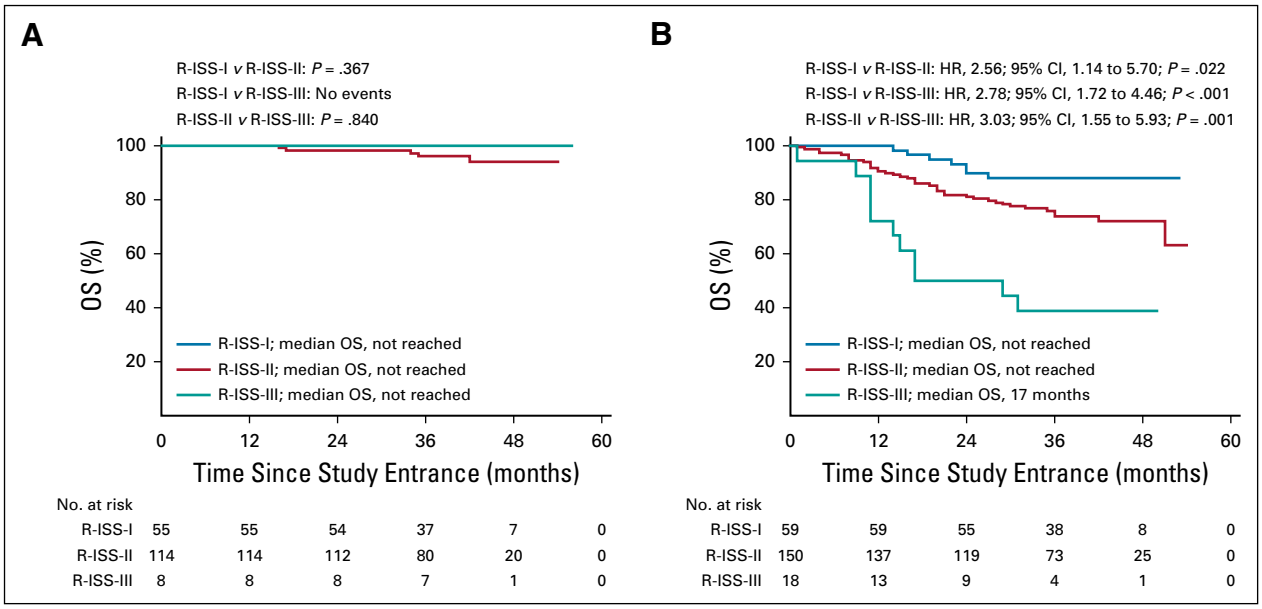

FIG A7. Modulating patients' risks at diagnosis according to depth of response after treatment, defined by measurable residual disease (MRD) status. Impact on overall survival (OS) of the Revised International Staging System (R-ISS) in patients with (A) undetectable *v* (B) persistent measurable residual disease. The Kaplan-Meyer OS curves of patients with R-ISS-I and -III are superimposed, because no events were observed in either patient subgroup. HR, hazard ratio.

TABLE A1. Longitudinal MRD Response Rates After Induction, HDT/ASCT, and Consolidation in the Intent-to-Treat Patient Population Enrolled in the GEM2012MENOS65 Clinical Trial

	No. (%) of Occurrences by Phase of Therapy (N = 458)		
MRD Status	**VRD × 6**	**HDT/ASCT**	**VRD × 2**
On intent-to-treat protocol (n = 458)			
Undetectable MRD	129 (28)	194 (42)	208 (45)
Persistent MRD	329 (72)	264 (58)	250 (55)
MRD log levels $\geq 10^{-4}$	244 (74)	170 (64)	156 (63)
MRD log levels $\geq 10^{-5}$ and $< 10^{-4}$	66 (20)	73 (28)	46 (18)
MRD log levels $\geq 2 \times 10^{-6}$ and 10^{-5}	19 (6)	21 (8)	48 (19)
Longitudinally (n = 317)			
Undetectable MRD	110 (35)	173 (55)	185 (58)
Persistent MRD	207 (65)	144 (45)	132 (42)
MRD log levels $\geq 10^{-4}$	139 (67)	61 (42)	47 (36)
MRD log levels $\geq 10^{-5}$ and $< 10^{-4}$	52 (25)	65 (45)	42 (32)
MRD log levels $\geq 2 \times 10^{-6}$ and 10^{-5}	16 (8)	18 (13)	43 (32)

NOTE. Three hundred seventeen patients had MRD assessed at all three timepoints, and results for this cohort are shown in the lower part of the Table. Patients with MRD-positive statuses were subgrouped according to detectable MRD log levels to evaluate the impact of different treatment stages in persistent MRD.

Abbreviations: HDT/ASCT, high-dose therapy followed by autologous stem-cell transplantation; MRD, measurable residual disease; VRD × 2, consolidation therapy; VRD × 6, induction therapy.

TABLE A2. MRD Status Assessed After Induction, HDT/ASCT, and Consolidation in the GEM2012MENOS65 Clinical Trial According to Conventional Response Criteria

	No. (%) of Occurrences by Treatment Stage								
	Induction (n = 377)			HDT/ASCT (n = 352)			Consolidation (n = 357)		
MRD Status	CR (n = 160)	VGPR (n = 130)	≤ PR (n = 87)	CR (n = 200)	VGPR (n = 121)	≤ PR (n = 31)	CR (n = 243)	VGPR (n = 89)	≤ PR (n = 19)
Undetectable MRD	87 (54)	33 (25)	9 (10)	143 (71.5)	43 (35.5)	4 (13)	175 (72)	28 (31.5)	0 (0)
Persistent MRD	73 (46)	97 (75)	78 (90)	57 (28.5)	78 (64.5)	27 (87)	68 (28)	61 (68.5)	19 (100)

NOTE. Conventional response criteria used: CR, VGPR, and ≤ PR. The numbers of patients in CR, VGPR, and ≤ PR after induction, HDT/ASCT, and consolidation are reported for the cohort of patients with MRD assessment at that specific treatment stage.

Abbreviations: CR, complete remission; HDT/ASCT, high-dose therapy followed by autologous stem-cell transplantation; MRD, measurable residual disease; ≤ PR, partial response or less; VGPR, very good partial response.

TABLE A3. Characteristics of Patients Who Experienced Progression Despite Undetectable MRD

	Diagnosis				Response			Relapse				
Patient	ISS	LDH	FISH	Bone-Related Plasmacytomas (imaging)	Depth	Moment of Undetectable MRD	LOD	Time Since Diagnosis (months)	M-Protein	BM PCs (%)	Clonal PCs (%)	Extraosseous Plasmacytomas (imaging)
1	III	Normal	SR	No (PET/CT)	sCR	IND	10^{-6}	51	Yes	40	100	No (PET/CT)
2	I	Elevated	HR	No (CT, MRI)	sCR	CONS	10^{-6}	46	Yes	2	0	No (CT, MRI)
3	II	Normal	SR	Yes (PET/CT)	VGPR	IND	10^{-6}	20	No	4	0	Yes (PET/CT)
4	I	Normal	SR	No (WB-MRI)	sCR	HDT	10^{-6}	44	Yes	36	28	No (WB-MRI)
5	III	Elevated	HR	Yes (PET/CT)	sCR	IND	10^{-6}	28	No	3	0	Yes (PET/CT)
6	II	Normal	SR	No (WB-MRI)	sCR	IND	10^{-6}	43	No	0.2	0	Yes (PET/CT)
7	I	Normal	SR	Yes (PET/CT)	sCR	IND	10^{-5}	36	No	2	0	Yes (PET/CT)
8	II	Normal	SR	No (PET/CT)	CR	CONS	10^{-6}	34	Yes	NE	NE	NE
9	III	Normal	TF	No (x-rays, CT)	sCR	HDT	10^{-6}	35	Yes	17	100	NE
10	I	Normal	SR	No (CT)	sCR	CONS	10^{-6}	36	No	2	86	Yes (CT)
11	III	Elevated	SR	Yes (PET/CT)	sCR	IND	10^{-6}	25	No	1	0	Yes (PET/CT)
12	I	Normal	TF	No (PET/CT)	sCR	IND	10^{-6}	33	No	5	50	No (PET/CT)
13	I	Normal	TF	NE (x-rays)	CR	CONS	10^{-6}	20	No	58	100	NE
14	III	Elevated	SR	Yes (PET/CT)	sCR	IND	10^{-6}	27	No	1	0	No* (PET/CT)

NOTE. The incidence of +1q (7/14) was higher than that typically observed in newly-diagnosed multiple myeloma (approximately 30%).

Abbreviations: BM PCs, bone marrow plasma cells; CONS, consolidation; CR, complete response; CT, computed tomography; FISH, fluorescence in situ hybridization; HDT, high-dose therapy; HR, high risk [t(4;14), t(14;16) and/or del(17p)]; IND, induction; ISS, International Staging System; LDH, lactate dehydrogenase; LOD, limit of detection achieved at the time of the latest MRD assessment; MRD, measurable residual disease; MRI, magnetic resonance imaging; NE, not evaluated; PCs, plasma cells; PET, positron emission tomography; sCR, stringent CR; SR, standard risk; TF, test failure; VGPR, very good partial response; WB-MRI, whole-body MRI.

*Multifocal disease.

TABLE A4. PFS and OS of Patients With Undetectable MRD Conditioned With Bu-Mel or Mel-200 High-Dose Therapy

	Survival Rate (%) by Conditioning Regimen		
Survival Type	**Bu-Mel (n = 180)**	**Mel-200 (n = 177)**	***P***
36-month PFS	88	86	.61
36-month OS	98	95	.38

NOTE. Achieving undetectable MRD before maintenance was associated with a 77% reduction in the risk of progression or death (HR, 0.23; $P < .001$) in patients treated with Bu-Mel and an 81% reduction (HR, 0.19; $P < .001$) in patients treated with Mel-200 high-dose therapy.

Abbreviations: Bu-Mel, busulfan + melphalan; HR, hazard ratio; Mel, melphalan; MRD, measurable residual disease; OS, overall survival; PFS, progression-free survival.

TABLE A5. PFS and OS of Patients With Undetectable MRD Receiving Maintenance With RD or IRD

	Survival Rate (%) by Maintenance Regimen		
Survival Type	**IRD (n = 163)**	**RD (n = 150)**	***P***
36-month PFS	86	90	.50
36-month OS	98	94	.18

NOTE. Achieving undetectable MRD before maintenance was associated with a 77% reduction in the risk of progression or death (HR, 0.23; $P < .001$) in patients treated with IRD and an 80% reduction (HR, 0.20; $P = .001$) in patients treated with RD.

Abbreviations: HR, hazard ratio; IRD, RD + ixazomib; MRD, measurable residual disease; OS, overall survival; PFS, progression-free survival; RD, lenalidomide + dexamethasone.

TABLE A6. Subgroup Analysis of Disease Progression Rates According to Patients' Time-Dependent MRD Statuses

Subgroup	No. of Patients	No. (%) of Progressions/No. of Patients by MRD Status	
		Undetectable	Persistent
Sex			
Male	239	8 (8)/103	55 (40)/136
Female	218	6 (6)/101	46 (39)/117
P		.78	.90
Age, years			
≤ 55	175	4 (6)/72	35 (35)/103
> 55	282	10 (8)132	66 (44)/150
P		.77	.12
ISS			
I	179	6 (7)/85	29 (31)94
II	164	2 (3)/73	41 (45)91
III	108	6 (14)/44	30 (47)/64
P		.08	.06
LDH			
Normal	373	10 (6)/178	75 (39)/195
Elevated	70	4 (22)/18	23 (48)/48
P		.03	.25
Cytogenetics			
Standard risk	300	9 (7)/136	56 (34)/164
High risk	90	2 (6)/32	34 (59)/58
Test failure	69	3 (8)/37	11 (36)/31
P		.94	.004

NOTE. The intent-to-treat patient population was subgrouped according to sex, age, ISS, LDH levels, and cytogenetic abnormalities.

Abbreviations: ISS, International Staging System; LDH, lactate dehydrogenase; MRD, measurable residual disease.

TABLE A7. Multivariable Analyses of PFS and OS According to the R-ISS

R-ISS Subgroup	PFS			OS		
	Median (months)	36-Month Rate (%)	P	Median (months)	36-Month Rate (%)	*P*
I	NR	77		NR	94	
II	NR	70	.002	NR	83	< .001
III	28	46		NR	58	

Abbreviations: NR, not reached; OS, overall survival; PFS, progression-free survival; R-ISS, Revised International Staging System.

TABLE A8. Impact of Maintenance Therapy on Patients' MRD Statuses

MRD Status	No. (%) of Patients		
	CONS→M1 (n = 190)	M1→M2 (n = 190)	CONS→M1→M2 (n = 190)
MRD− → MRD−	107 (56.3)	123 (64.7)	103 (54.2)
MRD+ → MRD−	33 (17.4)	13 (6.8)	33 (17.4)
MRD− → MRD+	10 (5.3)	17 (8.9)	14 (7.4)
MRD+ → MRD+	40 (21.1)	37 (19.5)	40 (21.1)

NOTE. According to the GEM2014MAIN study design, MRD was assessed every year during maintenance. The longitudinal comparison from CONS into M1 and M2 was performed in the 190 patients with MRD assessment at all three timepoints. Results also are reported for the paired comparison between CONS and M1 (CONS→M1) and the paired comparison between M1 and M2 (M1→M2). Patients were categorized into sustained MRD negativity from consolidation to maintenance (MRD− → MRD−), conversion from MRD+ into MRD− during maintenance (MRD+ → MRD−), loss of MRD negativity during maintenance (MRD− → MRD+), and persistent MRD from consolidation to maintenance (MRD+ → MRD+). Of note, none of the patients who converted from MRD+ into MRD− during maintenance or who lost their MRD negativity during maintenance have experienced disease progression thus far.

Abbreviations: +, positive; −, negative; CONS, consolidation; M1, first year of maintenance; M2, second year of maintenance; MRD, measurable residual disease.