Prognostic value of minimal residual disease detected by EuroFlow next-generation flow cytometry and next-generation sequencing in patients with multiple myeloma achieving complete response and receiving lenalidomide maintenance after autotransplant: a prospective comparison study

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Abstract

Novel agents inducing deeper responses have improved the prognosis of patients with multiple myeloma (MM). To assess minimal residual disease (MRD) and stratify patients achieving complete response (CR), advanced technologies such as EuroFlow next-generation flow cytometry (NGF) and next-generation sequencing (NGS) are increasingly utilized. This prospective study evaluated responses in newly diagnosed MM patients undergoing autologous stem cell transplantation (ASCT) followed by lenalidomide maintenance therapy across multiple Japanese medical centers. Patients achieving CR or stringent CR within 100-365 days post-ASCT were included. MRD levels in the bone marrow were assessed using both NGF and NGS (cutoff: 1×10^{-5}) at three time points: 100-365 days, 1 year, and 2 years post-ASCT. A total of 52 patients were analyzed. MRD levels determined by NGF and NGS showed a strong correlation (r=0.9722; *P*<0.0001). After a median follow-up of 3 years, the 3-year progression-free survival (PFS) and overall survival (OS) rates were 76.5% (95% confidence interval [CI]: 62.3-

85.9%) and 96.2% (95% CI: 85.5-99.0%), respectively. Patients with sustained MRD negativity for >6 months demonstrated superior 3-year PFS compared to those without sustained MRD negativity, as measured by both NGF (100% *vs*. 67.6%; hazard ratio [HR] =0.06; 95% CI: 0.0005-0.50; *P*<0.007) and NGS (90.5% *vs*. 72.2%; HR=0.23; 95% CI: 0.06-0.94; *P*=0.048). These findings highlighted a strong correlation in the MRD levels assessed by NGF and NGS and validated that sustained MRD negativity was significantly associated with prolonged PFS (clinical trial registered at *UMIN 000022238*).

Introduction

The emergence of novel agents, including proteasome inhibitors, immunomodulatory agents, and anti-CD38 monoclonal antibodies, combined with standard-of-care regimens such as autologous stem cell transplantation (ASCT) has significantly improved the outcomes of patients with multiple myeloma (MM).[1-3] These advancements have achieved high rates of complete response (CR) or stringent complete response (sCR), necessitating new techniques to assess responses deeper than CR, particularly minimal residual disease (MRD). To stratify patients with CR based on MRD status, emerging techniques, including next-generation flow cytometry (NGF) and next-generation sequencing (NGS) were utilized. NGF, a two-tube, eight-color assay developed by the EuroFlow consortium, has been approved by the International Myeloma Working Group (IMWG) as a reference method for immunophenotypic MRD assessment.[4] Likewise, LymphoSIGHT®/clonoSEQ® (Adaptive Biotechnologies, Seattle, WA, USA) is the first NGS-based method approved by the IMWG for molecular MRD assessment in the bone marrow (BM).[5] Recently, the IMWG has defined MRD negativity as the absence of phenotypically aberrant clonal plasma cells detected using a method with a minimum sensitivity of 1 in 10^5 nucleated cells or greater. To date, despite the widespread utilization of NGF and NGS, comparative analyses of MRD levels between the two methods remain limited.[6,7]

Recent studies assessing MRD at multiple time points have demonstrated that sustained MRD negativity is associated with improved clinical outcomes.[8-13] Notably, a study employing NGF to assess MRD annually during continuous lenalidomide maintenance demonstrated that patients with sustained MRD negativity for 2 years exhibited no disease progression. Conversely, patients who transitioned from MRD negativity to positivity experienced worse prognoses compared to those with either sustained MRD negativity or persistent MRD positivity.[9] Although therapeutic interventions guided by sequential MRD assessments may improve clinical outcomes, studies on the prognostic effectiveness of sequential MRD assessments remain limited.[14,15] Furthermore, all studies investigating longitudinal MRD status during lenalidomide maintenance have employed NGF methods, leaving the correlation between NGS and NGF results in this context undetermined.[8,9]

Therefore, this study aimed to compare MRD levels assessed by NGF and NGS in a clinical trial investigating the efficacy of continuous lenalidomide maintenance therapy following ASCT. We prospectively monitored MRD levels sequentially using both NGF and NGS in patients who achieved CR or better post-ASCT and analyzed the prognostic effect of sequential MRD status on survival outcomes.

Methods

Study design and procedure

This multicenter, open-label, single-arm prospective study was conducted across 11 Japanese hospitals from September 2016 to July 2021. Transplant-eligible patients aged ≥20 years with newly diagnosed MM received induction regimens comprising bortezomib, lenalidomide, and/or cyclophosphamide, followed by high-dose melphalan (200 mg/m^2) and ASCT, with tandem ASCT permitted. Patients achieving CR or sCR post-ASCT prior to initiating lenalidomide maintenance were eligible for MRD assessment using NGF and NGS. Lenalidomide was administered at 10 mg for 3 weeks with a 1-week rest, with its dosage adjusted appropriately and continued until either progressive disease (PD) or unacceptable toxicity was observed. MRD was assessed at three time points: 100-365 days post-ASCT and before lenalidomide maintenance (PRE), 1 year (± 20 days) post-ASCT (POST1), and 2 years (± 20 days) post-ASCT (POST2). MM was diagnosed per the IMWG criteria,[16] and responses were assessed using the International Uniform Response Criteria.[17] The study received ethical approval from relevant committees, including Kanazawa University's Institutional Review Board (IRB 2016-125), and adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. The trial was registered at the University Hospital Medical Information Network Clinical Trials Registry (*UMIN 000022238*).

Assessments

Disease-related assessments, including imaging and cytogenetics, were performed at diagnosis, with high-risk MM defined by the presence of t(4;14), t(14;16), or del(17p) as detected by fluorescence *in situ* hybridization (FISH).[18] For MRD assessment, BM fluid was collected in aliquots of 2 mL each, totaling 6 mL. The samples were randomly used for EuroFlow-NGF at Kanazawa University, multicolor flow cytometry (MFC) testing at BML, Inc. (Tokyo,

Japan) for a separate project, and NGS at Adaptive Biotechnologies. When bone marrow (BM) puncture fluid was collected sequentially, the later samples collected demonstrated increased dilution due to peripheral blood contamination.[19] To minimize this effect, MRD specimens were collected first; however, the order of collection for NGF at Kanazawa University, MFC at BML, and NGS was not predetermined. The NGF sensitivity threshold was 2×10^{-6} while the NGS-MRD clonoSEQ® assay from Adaptive Biotechnologies achieved a maximum sensitivity of 0.3×10^{-6}. The order of specimen submission for testing was determined independently by each institution. MRD negativity was defined according to the IMWG criteria,[17] with sustained negativity requiring two consecutive negative assessments at least 6 months apart. Additionally, 2-mL aliquots of ASCT autografts were assessed for MRD using clonoSEQ.

Statistical analysis

The concordance of MRD levels between NGF and NGS was analyzed using Pearson's correlation coefficient in log space. Progression-free survival (PFS) and overall survival (OS) were estimated using Kaplan-Meier estimates methods, with group comparisons conducted via the log-rank test. Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI), with Firth's penalized likelihood applied when required. Fisher's exact test was used to analyze categorical variables. All analyses were performed using GraphPad Prism (version 9.3.1; GraphPad Software, Boston, MA, USA) or EZR software (Jichi Medical University Saitama Medical Center, Saitama, Japan), a graphical interface for R (version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria).[20] Statistical significance was set at *P* value <0.05.

Results

Patients

Between December 2016 and November 2018, 63 patients were enrolled in the study, with a data cutoff in October 2021. Of these, 11 patients were excluded from the analysis due to the absence of lenalidomide maintenance therapy. However, patients who did not receive planned maintenance therapy owing to PD and those who died prior to its initiation were included. Ultimately, 52 patients who underwent bortezomib-, lenalidomide-, and/or cyclophosphamide-based induction therapy, followed by ASCT with high-dose melphalan conditioning, consolidation, and lenalidomide maintenance, were eligible for the analysis. The patients' baseline characteristics are summarized in Table 1. The median age at ASCT was 62 years (range, 36-71 years). A median of 30 cycles (range, 0-36 cycles) of lenalidomide maintenance therapy was received. High-risk cytogenetic abnormalities according to the IMWG criteria[18] were observed in 18 patients (35%) and one patient (case 41) exhibiting double-hit disease. Cytogenetic risk could not be assessed in 11 patients (21%) due to the absence of FISH data. Furthermore, five patients exhibited extramedullary disease.

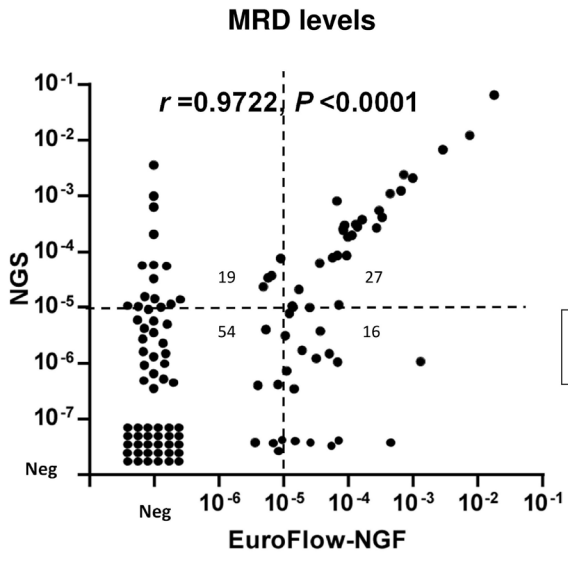
Table 1. Patient baseline characteristics (N=52).

Characteristics	N (%)
Median age, years (range)	62 (36-71)
≥65	20 (38)
Sex: male/female	25/27
PS	
0/1/2/3/4	20/12/10/4/6
MM type	
IgG	30
IgA	8
IgD	1
BJP	13
Light chain	
κ/λ	30/22
Extramedullary disease	5 (10)
ISS	
I/II/III	9/23/20
Induction regimen	
BLD	28
BD	14
VCD	7
others†	3
ASCT	
single	48
tandem	4
Consolidation regimen	
Ld	13
KLD	12
BLD	6
others††	3
none	18
Disease risk	
standard risk	23 (44)
high risk	18 (35)
unknown	11 (21)
Chromosome by G-banding	
normal karyotype	41
hyperdiploid	4
non-hyperdiploid	3
growth failure	4
High-risk cytogenetics	
t(14;16)	2/40 (5)
t(4;14)	8/45 (18)
del(17p)	9/43 (21)
double-hit	1*/52 (2)

†PAD (N=2), CVAD (N=1); ††IRd (N=2), DLd (N=1); *del(17p) and t(4;14). PS: performance status; MM: multiple myeloma; BJP: Bence Jones proteinuria; ISS: International Staging System; ASCT: autologous stem cell transplantation; BLD: bortezomib, lenalidomide, dexamethasone; BD: bortezomib, dexamethasone; VCD: bortezomib, cyclophosphamide, dexamethasone; PAD; bortezomib, doxorubicin, dexamethasone; CVAD: cyclophosphamide, vincristine, doxorubicin, dexamethasone; Ld: lenalidomide, dexamethasone; KLD: carfilzomib, lenalidomide, dexamethasone; IRd: ixazomib, lenalidomide, dexamethasone; DLd: daratumumab, lenalidomide, dexamethasone.

Correlation between next-generation flow cytometry and next-generation sequencing

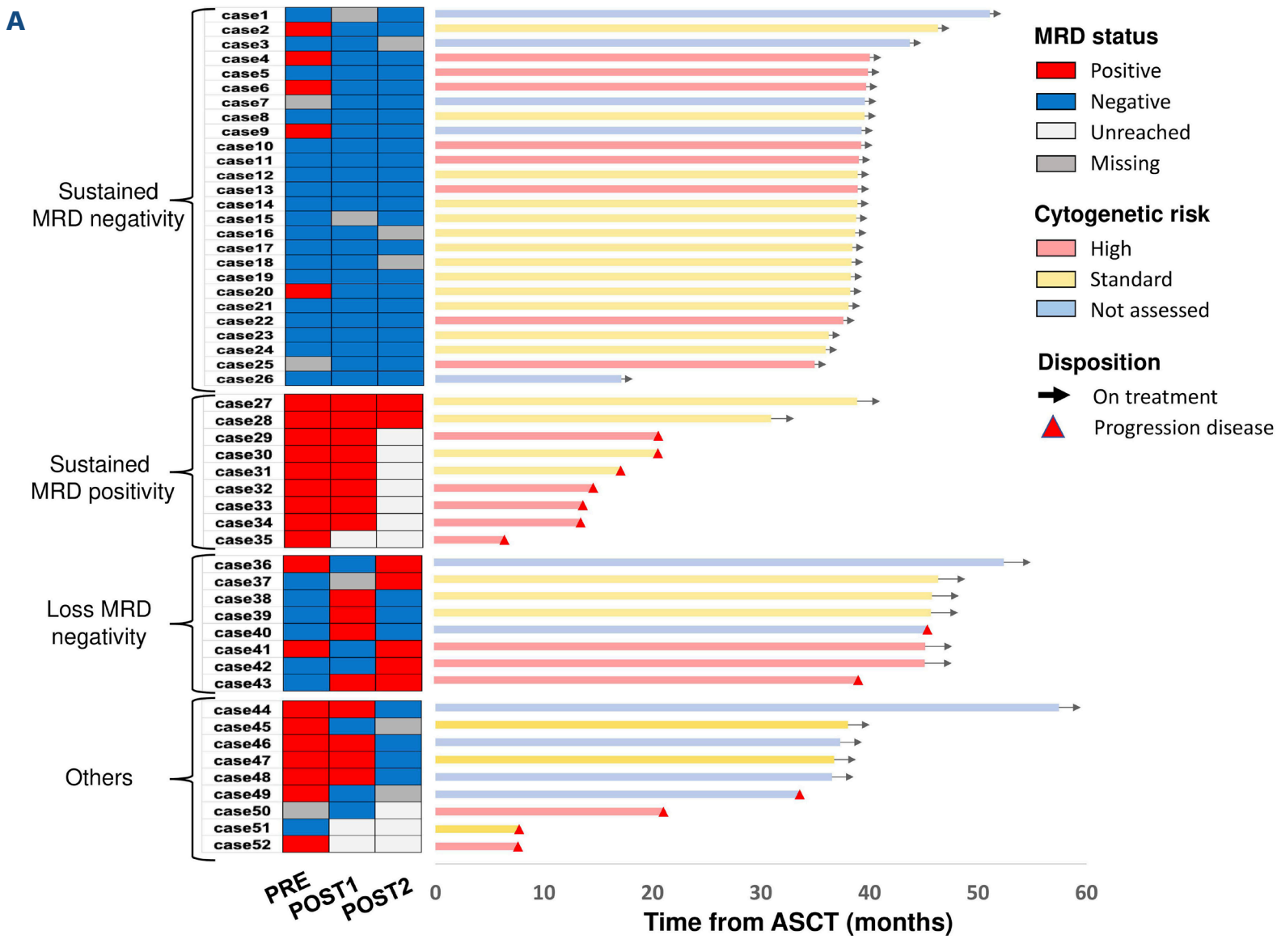
The success rates of MRD assessment using NGF were 94% (49/52), 88% (46/52), and 71% (37/52) at PRE, POST1, and POST2, respectively. MRD was not assessed by NGF in 24 samples due to PD (N=12), BM coagulation at the time of collection (N=9), and shipping errors (N=3). Conversely, the success rates of MRD assessments using NGS were 92% (48/52), 79% (41/52), and 65% (34/52) at PRE, POST1, and POST2, respectively. NGS assessments failed in 33 samples due to the inability to detect amplification in patient-specific regions (i.e., IgH-VJ/DJ and/or IgK/IgL regions) (3 patients, N=9), PD (N=11), shipping errors (N=2), and quality control failures at Adaptive Biotechnologies (N=11). In total, 116 paired samples from 48 patients were available for comparison of MRD levels between the NGS and NGF methods, with a strong correlation observed (r=0.9722; *P*<0.0001) (Figure 1). However, 35 sample pairs (30%) exhibited discordant MRD results; 19 pairs were MRD-positive by NGS and MRD-negative by NGF, while 16 pairs were MRD-negative by NGS and MRD-positive by NGF, with a cutoff value of 1×10^{-5} In the group positive by NGS and negative by NGF, 12 cases were identified, including seven with discordant results at multiple time points. None of these patients experienced PD during the study. Conversely, in the group negative by NGS and positive by NGF, 12 cases were identified, with four showing discordance at different time points. Notably, three patients (cases 32, 43, and 49) subsequently developed PD. Case 32, with a del(17p) chromosomal abnormality, was only NGF positivity at PRE; however, at POST1, both NGS and NGF were positive, followed by progression to PD. Case 43, with no chromo-



		NGF	
		MRD -	MRD +
NGS	MRD -	54	16
	MRD +	19	27

Cutoff: 10^{-5}

Figure 1. Correlation between EuroFlow next-generation flow cytometry and next-generation sequencing. Comparison of minimal residual disease (MRD) levels determined by next-generation flow cytometry (NGF) and next-generation sequencing (NGS). MRD status, using a cutoff value of 1×10^{-5} is shown in the table.



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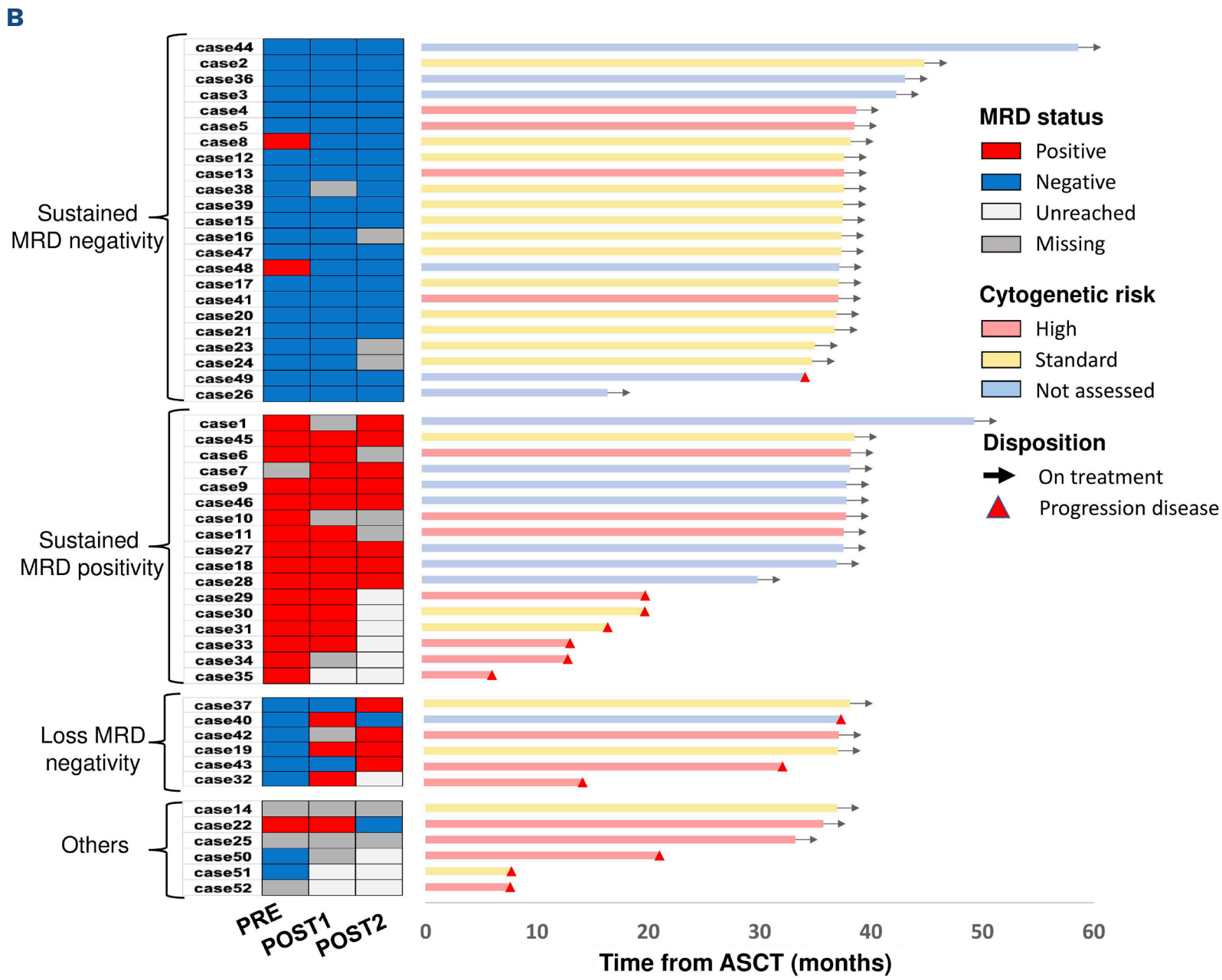

Figure 2. Sequential minimal residual disease assessment and treatment status timelines. Each row represents an individual patient. (A) Patients are divided into 4 groups based on minimal residual disease (MRD) status assessed by next-generation flow cytometry (NGF) at PRE, POST1, and POST2. Cytogenetic risk and patient disposition are annotated. (B) Patients are divided into 4 groups based on MRD status assessed by next-generation sequencing (NGS) at PRE, POST1, and POST2. ASCT: autologous stem cell transplantation; PRE: between days 100 and 365 post-ASCT and before lenalidomide maintenance; POST1: 1 year (± 20 days) post-ASCT; POST2: 2 years (± 20 days) post-ASCT.

somal abnormality, was only NGF positive at POST1, but both NGS and NGF were positive at POST2, and the patient subsequently progressed to PD. Case 49, with chromosomal abnormalities t(4;14) and del(13q), was NGF positive at PRE. At POST1, both NGS and NGF were negative, and the patient subsequently progressed to PD. The proportion of high-risk chromosomal abnormalities was similar between NGS-only and NGF-only MRD-positive cases (4/12 cases and 5/12 cases, respectively) (Figure 2A, B).

Notably, all 13 patients who experienced PD post-ASCT demonstrated concordant results. Among them, seven patients (cases 29, 30, 31, 32, 33, 35, 43) were double-positive and two (cases 40, 51) were double-negative immediately prior to PD. The remaining four (cases 34, 49, 50, 52) were not assessed for MRD using either method (Figure 2A, B).

Outcome

With a median follow-up of 3.0 years, the PFS and OS rates at three years were 76.5% (95% CI: 62.3-85.9%) and 96.2% (95% CI: 85.5-99.0%), respectively (Figure 3A, B). Using NGF, the MRD negativity rates at PRE, POST1, and POST2 were 53% (26/49), 65% (30/46), and 81% (30/37), respectively. Using NGF, the MRD negativity rates at the same time points were 60% (29/48), 59% (24/41), and 65% (22/34), respectively (Figure 2A, B). Patients who were MRD negative by NGF at PRE and POST1 demonstrated significantly higher 3-year PFS rates than those who were MRD positive (92.1% *vs*. 60.6%; HR=0.25; 95% CI: 0.08-0.77; *P*=0.022 and 93.0% *vs*. 55.6%; HR=0.10; 95% CI: 0.03-0.40; *P*=0.0004, respectively). However, at POST2, the difference in PFS rates between MRD-negative and MRD-positive patients was not statistically significant (100% *vs*. 83.3%; HR=0.18; 95% CI: 0.004-8.54; *P*=0.167) (Figure 4A-C). Analysis of OS using NGF revealed no significant differences between MRD-negative and MRD-positive patients at any time point. These findings remained consistent when NGF

assessments were restricted to cases with a sensitivity of 2×10^{-6} (*Online Supplementary Figure S1*).

As assessed by NGS, patients who were MRD negative at POST1 had significantly higher 3-year PFS rates compared to those who were MRD positive (91.3% *vs*. 70.6%; HR=0.20; 95% CI: 0.05-0.83; *P*=0.026). However, no significant differences were observed at PRE (82.4% *vs*. 68.4%; HR=0.58; 95% CI: 0.18-1.86; *P*=0.330) and POST2 (95.2% *vs*. 90.9%; HR=0.99; 95% CI: 0.09-11.0; *P*=1.000) (Figure 4D-F). Similarly, OS analysis using NGS revealed no significant

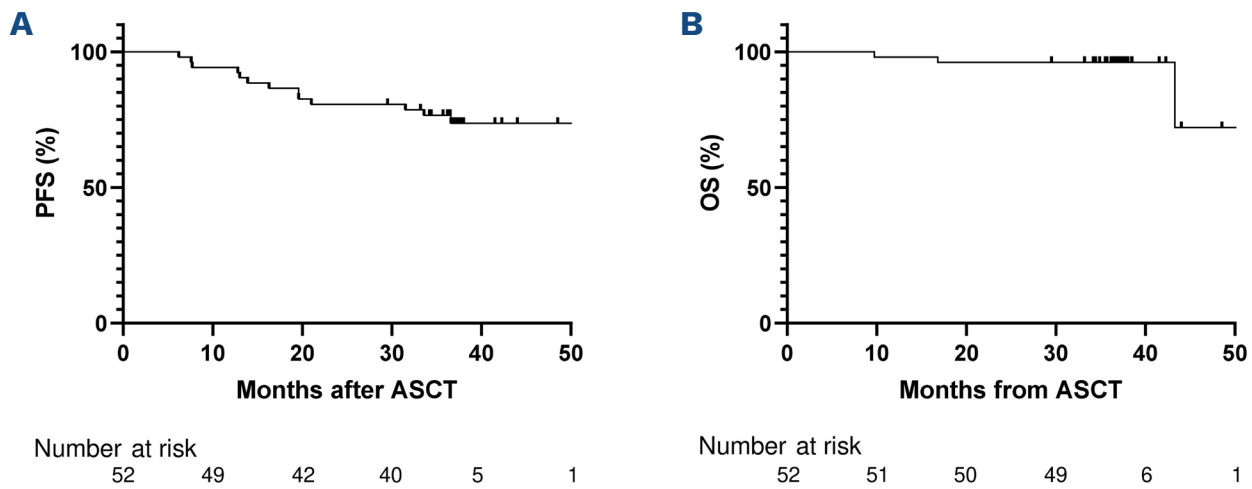

Figure 3. Patient survival outcomes. (A) Progression-free survival (PFS). (B) Overall survival (OS). ASCT: autologous stem cell transplantation.

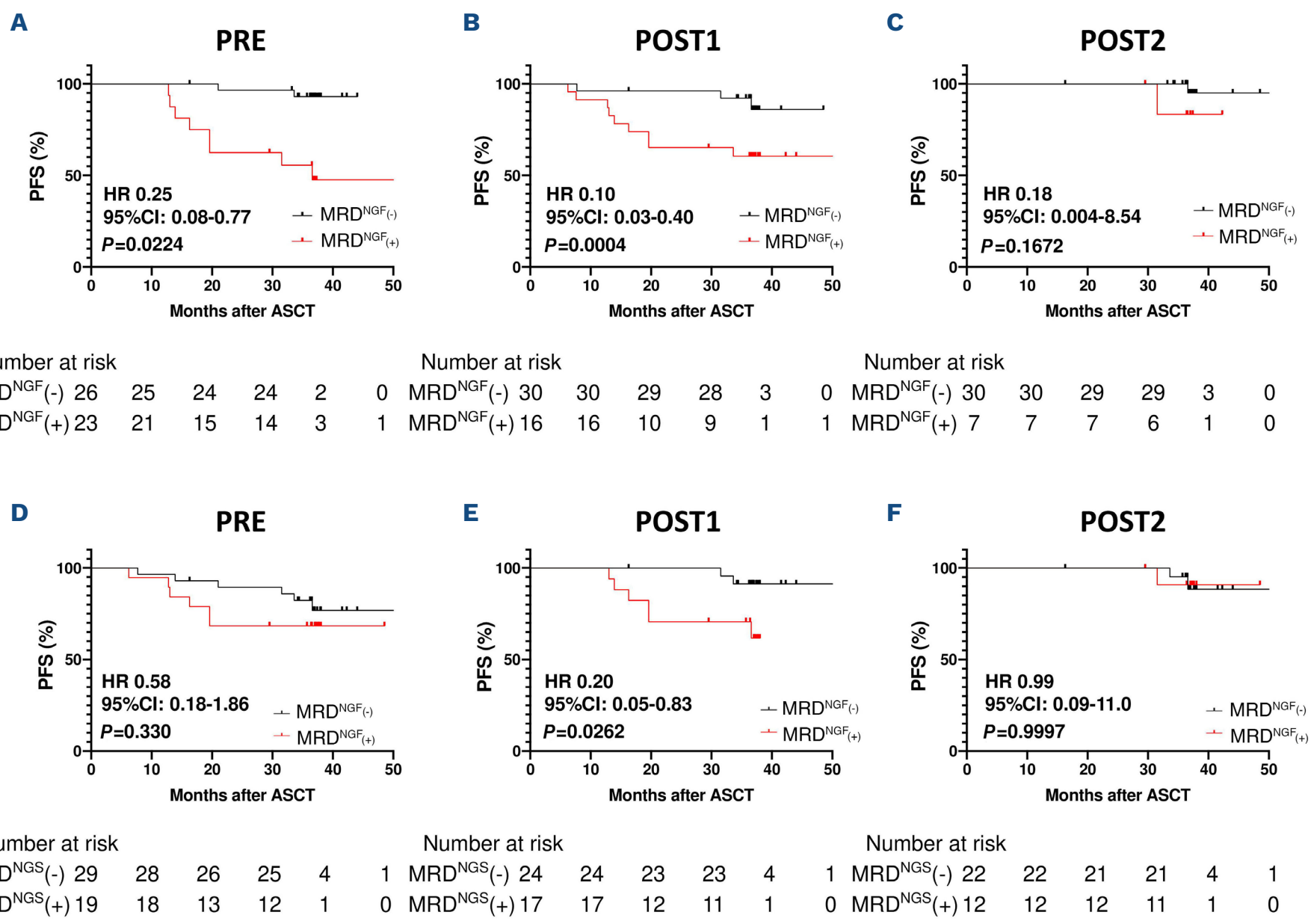

Figure 4. Progression-free survival stratified by minimal residual disease status (cutoff: 10^{-5}). Progression-free survival (PFS) based on minimal residual disease (MRD) negativity by next-generation flow cytometry (NGF) at PRE (A), at POST1 (B), and at POST2 (C). PFS based on MRD status by next-generation sequencing (NGS) at PRE (D), at POST1 (E) and at POST2 (F). ASCT: autologous stem cell transplantation; PRE: between days 100 and 365 post-ASCT and before lenalidomide maintenance; POST1: 1 year (± 20 days) post-ASCT; POST2: 2 years (± 20 days) post-ASCT; HR: hazard ratio; CI: confidence interval.

differences between MRD-positive and MRD-negative patients at any time point (*Online Supplementary Figure S2*). The prognostic impact of sustained MRD negativity, defined as two consecutive MRD-negative assessments at PRE and POST1, was evaluated. Sustained MRD negativity rates were 42% (18/43) and 55% (22/40) by NGF and NGS, respectively, considering non-assessable cases as MRD-positive. Both NGF and NGS demonstrated that patients with sustained MRD negativity had significantly higher 3-year PFS rates compared to those without sustained MRD negativity (100% *vs*. 67.6%; HR=0.06; 95% CI: 0.0005-0.50; *P*=0.007 and 90.5% *vs*. 72.2%; HR=0.23; 95% CI: 0.06-0.94; *P*=0.048, respectively) (Figure 5A, B). To explore the effect of MRD dynamics, comparisons were made between patients with sustained MRD negativity, persistent MRD positivity, and loss of MRD negativity (Figure 5C, D). NGF analysis revealed that patients with sustained MRD negativity had significantly improved PFS compared to both persistent MRD positivity (HR=0.01; 95% CI: 0.0001-0.12; *P*<0.01) and loss of MRD negativity (HR=0.06; 95% CI: 0.0005-0.78; *P*=0.03). Similarly, with NGS, sustained MRD negativity was associated with significantly improved PFS compared to persistent MRD positivity (HR=0.10; 95% CI: 0.01-0.84; *P*=0.01) and loss of MRD negativity (HR=0.07; 95% CI: 0.01-0.63; *P*=0.048).

A PFS comparison was also conducted involving 18 patients with high-risk cytogenetic abnormalities and 23 with standard-risk cytogenetics. High-risk patients had significantly shorter median PFS compared to standard-risk patients (not reached *vs*. not reached; HR=4.04; 95% CI: 1.07-15.3; *P*=0.03) (*Online Supplementary Figure S3A*). However, among MRD-negative patients, no significant

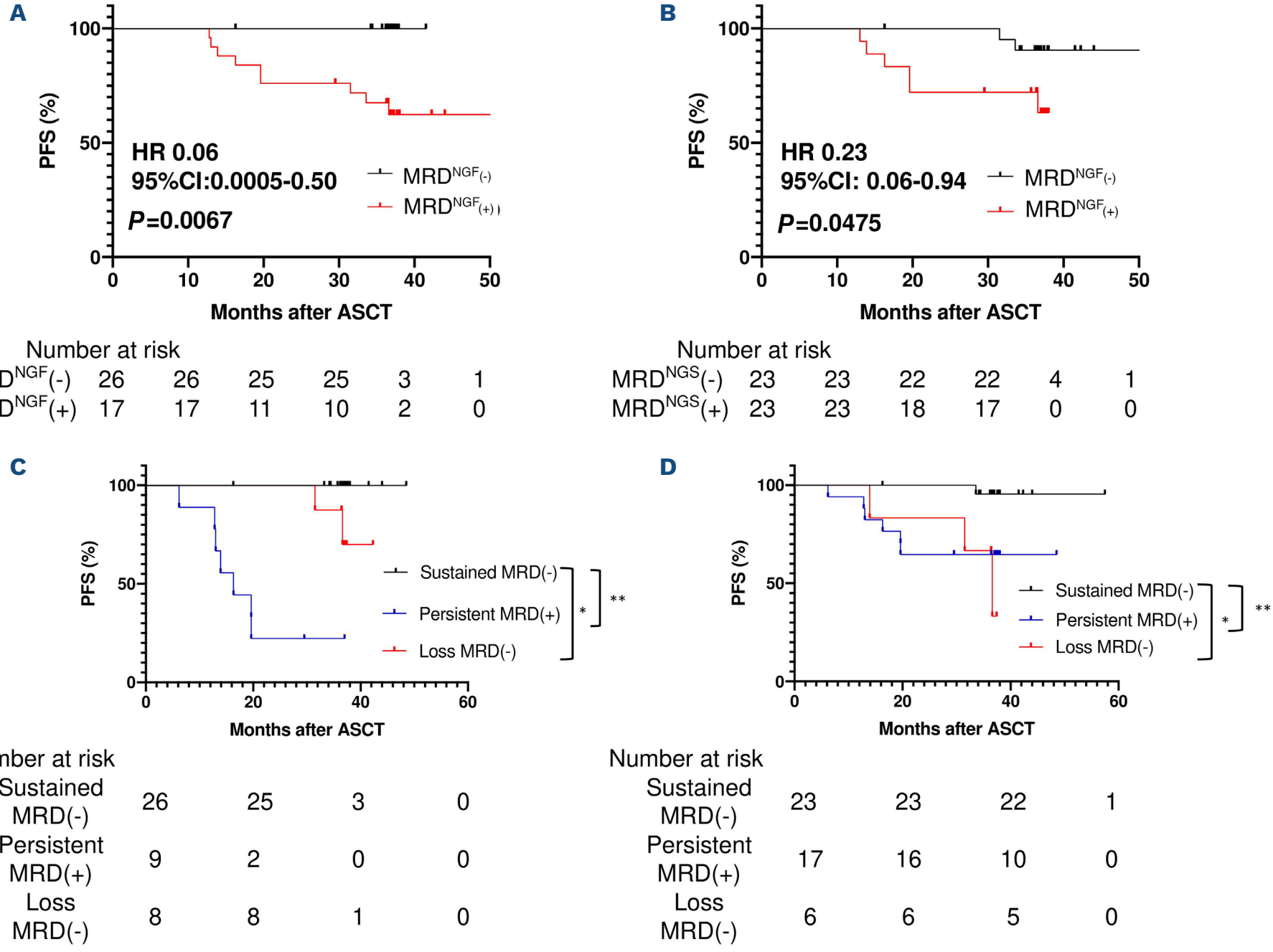

Figure 5. Progression-free survival stratified by sustained minimal residual disease status (10^{-5}; PRE to POST1) and minimal residual disease dynamics. (A) Progression-free survival (PFS) based on sustained minimal residual disease (MRD) status by next-generation flow cytometry (NGF). (B) PFS based on sustained MRD status by next-generation sequencing (NGS). (C) PFS stratified by patients with sustained MRD negative, persistent MRD positive, and loss of MRD negativity using NGF. (D) PFS stratified by patients with sustained MRD negative, persistent MRD positive, and loss of MRD negativity using NGS. PRE: between days 100 and 365 post-ASCT and before lenalidomide maintenance; POST1: 1 year (± 20 days) post-ASCT; POST2: 2 years (± 20 days) post-ASCT; ASCT: autologous stem cell transplantation; HR: hazard ratio; CI: confidence interval. *P<0.05; **P<0.01.

differences in PFS were observed between high-risk and standard-risk patients, suggesting that achieving MRD negativity may mitigate the adverse prognostic impact of high-risk cytogenetics (*Online Supplementary Figure S3B-E*). At PRE, MRD-negative high-risk patients assessed by NGF demonstrated significantly better PFS compared to high-risk MRD-positive patients (not reached *vs*. 13.9 months; HR=0.13; 95% CI: 0.02-1.08; *P*=0.02). However, no significant difference was observed between MRD-negative and MRD-positive standard-risk patients (*Online Supplementary Figure S3B*). At POST1, both high-risk and standard-risk patients who were MRD-negative by NGF had better PFS than MRD-positive patients (not reached *vs*. not reached; HR=0.10; 95% CI: 0.0007-1.22; *P*=0.05 for standard-risk; not reached *vs*. 13.9 months; HR=0.04; 95% CI: 0.004-0.33; *P*<0.01 for high-risk) (*Online Supplementary Figure S3C*). Conversely, MRD assessment by NGS did not reveal significant differences in PFS between MRD-negative and MRD-positive patients at any time point or across risk categories (*Online Supplementary Figure S3D, E*).

Of the 52 cases assessed for MRD levels in autografts, 41 (79%) were analyzed by NGS. Using cutoff values of 1×10^{-5} and 1×10^{-6}, MRD negativity was observed in 24 of 41 cases (59%) and 11 of 41 cases (27%), respectively. The MRD levels in autografts were significantly correlated with those in PRE-BM (r=0.7105; *P*<0.0001). MRD negativity in PRE-BM was observed in six of nine cases (67%) following transplantation of MRD-negative autografts compared to three of 31 cases (10%) with MRD-positive autografts (*P*=0.001) at the cutoff value of $<1\times10^{-6}$ (*Online Supplementary Figure S4A*). No significant differences in PFS or OS were observed between MRD-negative and MRD-positive autografts or between sustained MRD negativity in autografts and PRE-BM (*Online Supplementary Figure S4B-E*). However, no death was recorded among patients who received MRD-negative autografts at the 1×10^{-6} cutoff.

Safety and tolerability

Table 2 summarizes the incidence of grade 3/4 adverse events (AE) during lenalidomide maintenance therapy, occurring in nine patients (16%). Non-hematologic grade 3 AE included infections (6%) and elevated liver function tests (4%), with no grade 4 non-hematologic AE reported. Hematologic grade 3/4 AE consisted of neutropenia (12%), leukopenia (10%), lymphopenia (6%), thrombocytopenia (4%), and anemia (2%). A total of six grade 4 AE were reported in three patients: one experienced neutropenia, lymphopenia, and thrombocytopenia; another experienced neutropenia; and a third had thrombocytopenia. No grade 5 AE or treatment-related deaths occurred throughout the treatment period. Consistent with previous findings, lenalidomide maintenance therapy was well tolerated, with patients completing a median of 30 cycles with appropriate supportive measures.

Table 2. Grade 3/4 adverse events of lenalidomide maintenance (N=52).

AE	N (%)
Non-hematologic	
elevated liver function test	2 (4)
infection	3 (6)
Hematologic AE	
leukopenia	5 (10)
neutropenia	6 (12)
lymphopenia	3 (6)
anemia	1 (2)
thrombocytopenia	2 (4)

AE: adverse event.

Discussion

This study prospectively compared MRD levels assessed by NGF and NGS in lenalidomide maintenance therapy, revealing a strong correlation between the two methods. Notably, MRD negativity by NGF at PRE and POST1, and sustained MRD negativity were significantly associated with prolonged PFS.

Discrepancies in MRD status were observed in 31% of paired samples using a cutoff value of 1×10^{-5}. A previous study comparing MRD levels between NGS and NGF reported a 17% discrepancy.[6] While most discrepant samples in that study were NGS-only MRD-positive, our findings revealed a similar rate of NGS-only and NGF-only MRD-positivity. The cause of this difference remains unclear but may be linked to sample collection methods. In this study, 6 mL of BM were aspirated, and MRD was measured using NGF and NGS at random, without a predetermined selection of method for each sample. One potential explanation for the MRD discrepancies is dilution due to contamination of peripheral blood.[19] As this was a multicenter study, MRD specimens were collected first, but there was no predetermined allocation of specimens for NGS or NGF. The order of specimen collection may vary across centers. Since the first BM aspirate typically contains the highest concentration of tumor cells, different usage across centers could have contributed to the discrepancies. This may have resulted in a positive MRD result for the initial sample and a negative result for subsequent ones. Therefore, clinicians should be cognizant of the BM collection order and the potential dilution effect and consider periodic MRD assessments, for instance, 6 to 12 months interval, to reduce the risk of patchy MM lesions in BM during routine clinical practice. While MRD discrepancies were observed in three patients who progressed to PD (cases 32, 43, and 49), the assessments immediately preceding PD were consistent between the two methods (Figure 2A, B). These findings suggest that simultaneous MRD assessments using both methods may be unnecessary for predicting PD.

Each technique has distinct advantages. NGS requires fewer cells to achieve the necessary sensitivity and permits retrospective analysis of frozen samples. However, it relies on baseline DNA collected at diagnosis. DNA extraction from BM smears or clots can result in fragmentation, preventing MRD measurement. In this study, three of 52 patients (6%) had DNA samples that could not be evaluated using NGS. Conversely, NGF does not require baseline samples but involves rapid BM processing within 48 hours in order to preserve cell quality. It can detect abnormal clones in nearly all patients, achieving sensitivity comparable to NGS.[4] Among the 14 patients who progressed, MRD assessments prior to PD were negative for both methods in four cases (26, 48, 50, and 52). Only case 48 presented with extramedullary disease at baseline. These findings highlight the limitations of BM-based MRD assessment, as the patchy nature of MM in the BM and the presence of extramedullary disease can produce false-negative results.[7,21] To address these limitations, several highly sensitive MRD assessment methods using peripheral blood have emerged. One approach utilizes mass spectrometry to analyze clone-specific sequences, such as CDR1, CDR2, and CDR3, providing sensitivity comparable to NGS in BM, with a detection limit exceeding 1×10^{-6} and potentially mitigating the challenges of patchy BM MRD and extramedullary lesions.[22] Another method involves isolating over 10^6 plasma cells using CD138-magnetic beads, enhancing sensitivity and addressing these same limitations.[23]

Several studies have highlighted the clinical significance of MRD assessment in autografts. Given the large number of cells present in autografts, NGS can achieve a detection sensitivity as high as 1×10^{-7}.[24] In this study, MRD sensitivity was limited to 1×10^{-6} due to the availability of only 2 mL of autograft samples. Achieving true MRD negativity in PRE-BM following autologous transplantation was challenging when MRD-positive autografts were infused, likely reflecting the patchy distribution of myeloma lesions in the BM. Thus, MRD-negative autografts appear to be crucial for achieving MRD-negativity in PRE-BM (*Online Supplementary Figure S4A*). Regarding prognosis, no significant differences in PFS or OS emerged between MRD-negative and MRD-positive autografts, possibly due to the small sample size and the 1×10^{-6} MRD cutoff. However, patients infused with MRD-negative autografts experienced no deaths and exhibited better OS than those receiving MRD-positive autografts (*Online Supplementary Figure S4B-E*).

This study further evaluated the efficacy of continuous lenalidomide maintenance therapy through serial MRD assessments in a relatively homogeneous population. Almost all patients (98%) received induction regimens containing bortezomib and/or lenalidomide, followed by ASCT with melphalan conditioning at 200 mg/m^2 prior to maintenance therapy. MRD negativity at PRE and POST1 and sustained MRD negativity were significantly associated with prolonged PFS when assessed by NGF. Similarly, when assessed by NGS, MRD negativity at POST1 and sustained MRD negativity were significantly correlated with prolonged PFS. Both NGF and NGS are well-validated MRD assessment methods and are strongly associated with prognosis.[21,25] The differences in these findings may be attributed to the study's limited statistical power, given the relatively small sample size. A recent study by Diamond *et al*. reported the prognostic impact of serial MRD assessments by NGF in a single-center phase II study assessing continuous lenalidomide maintenance following unrestricted frontline therapy.[9] The study demonstrated that patients who sustained MRD negativity for 2 years did not experience PD, whereas those who lost MRD negativity were more likely to progress compared to those with sustained MRD negativity or persistent MRD positivity. Consistent with previous studies,[11,12] sustained MRD negativity in this study was associated with excellent PFS. Notably, eight patients lost MRD negativity as assessed by NGF, and six patients lost MRD negativity as assessed by NGS. Those who lost MRD negativity had a significantly poorer prognosis compared to the patients with persistent MRD negativity (Figure 5C, D).

Our cohort comprised a relatively high proportion of patients with high-risk chromosomal abnormalities (HRCA; 38%), although only one case of double-hit disease was observed. We explored the MRD effect on cytogenetic risk and found no significant prognostic differences between MRD-negative high-risk and standard-risk patients. This finding is consistent with previous reports,[26-28] suggesting that high-risk patients who achieve MRD negativity experience PFS similar to that of standard-risk patients. However, it is crucial to note that this study included only one ultra-high-risk patient - that is, with two or more HRCA; who typically struggle to maintain MRD negativity even after initially achieving it.[15,29,30] While some studies support these findings,[26-28] others have reported conflicting results,[8] potentially reflecting the heterogeneity of MM.

Based on our findings and previous studies, clinicians can predict the prognosis of MM patients using MRD status. Sustained MRD-negative patients experience excellent prognosis, and some might discontinue treatments.[14,31] Conversely, patients who shift from MRD negativity to positivity face poor prognosis. Early intervention triggered by MRD positivity in such cases may improve prognosis. To date, the only prospective study we identified, the Remnant study,[32] aims to assess PFS and OS of MM patients with MRD relapse following treatment with daratumumab, carfilzomib and dexamethasone. Further prospective studies are crucial, and treatment adjustments should be based on the outcomes of the "Treatment stop for sustained MRD-negative patients" and "Intervention for patients progressing from MRD negative to positive" studies.

Grade 3/4 AE during lenalidomide maintenance therapy occurred in 16% (9/52) of patients, with primary non-hematological toxicities comprising infections (6%) and elevated liver function tests (4%). Grade 3/4 neutropenia (12%) was

the most commonly observed hematological toxicity. The incidence of grade 3/4 hematological AE was lower than previously reported,[33,34] likely due to the appropriate dose adjustments of lenalidomide implemented in this study. Notably, no cases of second primary malignancies were observed, which may be attributed to the study's relatively short follow-up period.

Our study has some limitations. First, although the pre-planned number of participants were enrolled, the final sample size was smaller than expected, reducing the statistical power of the subgroup analyses. Notably, 17% of the patients were excluded because they received maintenance therapy other than lenalidomide post-ASCT. As a result, the subgroup analysis yielded only preliminary findings. A large-scale clinical trial is crucial to confirm whether achieving MRD negativity in high-risk cases, assessed using both NGS and NGF, confers a prognosis comparable to that of standard-risk patients with MRD negativity. Additionally, further investigation is needed to establish whether MRD progression from negative to positive is associated with poor prognosis as detected by these methods. Second, imaging studies were performed at the discretion of the attending physician, with only 17% (9/52) of patients undergoing baseline positron emission tomography/computed tomography. This may have contributed to false-negative MRD assessments.

In conclusion, this prospective comparative study of MRD assessment in BM cells using EuroFlow-NGF and NGS demonstrated a correlation between MRD levels. Regardless of the assessment method, achieving MRD negativity after ASCT (PRE) and at 1 year of lenalidomide maintenance therapy (POST1) was significantly associated with prolonged PFS in the setting of ASCT. Serial MRD evaluations indicated that sustained MRD negativity is a highly promising prognostic marker. Future investigations should explore whether early intervention can improve prognosis in patients who fail to achieve sustained MRD negativity, and conversely, if with sustained MRD negativity, therapy can be de-escalated or deferred, with attendant avoidance of long-term side effects, including genotoxicity.[35,36]

Disclosures

TYo received honoraria from Takeda, Sanofi, Janssen, Ono, and Bristol-Myers Squibb. HT received honoraria from Janssen, Ono, Sanofi, and Bristol-Myers Squibb; and consultancy from Adaptive Biotechnologies. SY received honoraria from Bristol-Myers Squibb. SF received honoraria from Takeda, Sanofi, Janssen, Ono, and Bristol-Myers Squibb. TI received honoraria fee from Takeda, Sanofi, Janssen, Ono, Bristol-Myers Squibb, Pfizer, and CSL Behring; and research funding from Janssen, Bristol-Myers Squibb, Sanofi, Glaxo Smith Kline, Prothena, Alexionpharma, and Pfizer. KSh has received honoraria from Takeda, ONO, Novartis, Sanofi, Bristol-Myers Squibb, and Janssen; has received advisory from SRL; and has held an endowed chair position with Janssen, Sanofi, Pfizer, and BMS. MM received honoraria fee from Takeda, Sanofi, Janssen, Ono, and Glaxo Smith Kline; has received clinical funding from Janssen, Bristol-Myers Squibb, Glaxo Smith Kline and Pfizer. JS is an employee of Bio Medical Laboratories (BML), INC. All other authors have no conflicts of interest to disclose.

Contributions

TYo, HT, RU, MI, SY, KSa, NT, SO, KaS, KK, TM, MM, YT, TYa, SF, TI, KeS, HM, and KSh collected clinical data and blood samples. TYo, RU, JS, and HT performed flow cytometry. HT designed the research. TYo and HT wrote the manuscript. HT, BGMD and KSh were responsible for critically appraising the article for important intellectual content. All authors critically reviewed the manuscript and checked the final version.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

References

1. Palumbo A, Cavallo F, Gay F, et al. Autologous transplantation and maintenance therapy in multiple myeloma. N Engl J Med. 2014;371(10):895-905.
2. Attal M, Lauwers-Cances V, Hulin C, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. N Engl J Med. 2017;376(14):1311-1320.
3. Voorhees PM, Kaufman JL, Laubach J, et al. Daratumumab, lenalidomide, bortezomib, and dexamethasone for transplant-eligible newly diagnosed multiple myeloma: the GRIFFIN trial. Blood. 2020;136(8):936-945.
4. 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. 2017;31(10):2094-2103.
5. 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. 2016;17(8):e328-e346.
6. Oliva S, Genuardi E, Paris L, et al. Prospective evaluation of minimal residual disease in the phase II FORTE trial: a head-to-

head comparison between multiparameter flow cytometry and next-generation sequencing. eClinicalMedicine. 2023;60:102016.
7. Medina A, Puig N, Flores-Montero J, et al. Comparison of next-generation sequencing (NGS) and next-generation flow (NGF) for minimal residual disease (MRD) assessment in multiple myeloma. Blood Cancer J. 2020;10(10):108.
8. De Tute RM, Pawlyn C, Cairns DA, et al. Minimal residual disease after autologous stem-cell transplant for patients with myeloma: prognostic significance and the impact of lenalidomide maintenance and molecular risk. J Clin Oncol. 2022;40(25):2889-2900.
9. Diamond B, Korde N, Lesokhin AM, et al. Dynamics of minimal residual disease in patients with multiple myeloma on continuous lenalidomide maintenance: a single-arm, single-centre, phase 2 trial. Lancet Haematol. 2021;8(6):e422-e432.
10. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myélo. J Clin Oncol. 2014;32(25):2712-2717.
11. Avet-Loiseau H, San-Miguel J, Casneuf T, et al. Evaluation of sustained minimal residual disease negativity with daratumumab-combination regimens in relapsed and/or refractory multiple myeloma: analysis of POLLUX and CASTOR. J Clin Oncol. 2021;39(10):1139-1149.
12. San-Miguel J, Avet-Loiseau H, Paiva B, et al. Sustained minimal residual disease negativity in newly diagnosed multiple myeloma and the impact of daratumumab in MAIA and ALCYONE. Blood. 2022;139(4):492-501.
13. Schmitz A, Brondum RF, Johnsen HE, et al. Longitudinal minimal residual disease assessment in multiple myeloma patients in complete remission - results from the NMSG flow-MRD substudy within the EMN02/HO95 MM trial. BMC Cancer. 2022;22(1):147.
14. Rosinol L, Oriol A, Rios R, et al. Lenalidomide and dexamethasone maintenance with or without ixazomib, tailored by residual disease status in myeloma. Blood. 2023;142(18):1518-1528.
15. Costa LJ, Chhabra S, Medvedova E, et al. Daratumumab, carfilzomib, lenalidomide, and dexamethasone with minimal residual disease response-adapted therapy in newly diagnosed multiple myeloma. J Clin Oncol. 2022;40(25):2901-2912.
16. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538-548.
17. 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. 2016;17(8):e328-e346.
18. 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. 2015;33(26):2863-2869.
19. Óskarsson JÞ, Rögnvaldsson S, Thorsteinsdottir S, et al. Determining hemodilution in diagnostic bone marrow aspirated samples in plasma cell disorders by next-generation flow cytometry: proposal for a bone marrow quality index. Blood Cancer J. 2023;13(1):177.
20. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
21. Paiva B, Puig N, Cedena M-T, et al. Measurable residual disease by next-generation flow cytometry in multiple myeloma. J Clin Oncol. 2020;38(8):784-792.
22. Noori S, Wijnands C, Langerhorst P, et al. Dynamic monitoring of myeloma minimal residual disease with targeted mass spectrometry. Blood Cancer J. 2023;13(1):30.
23. Notarfranchi L, Zherniakova A, Lasa M, et al. Ultra-sensitive assessment of measurable residual disease (MRD) in peripheral blood (PB) of multiple myeloma (MM) patients using bloodflow. Blood. 2022;140(Suppl 1):2095-2097.
24. 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. 2017;28(10):2503-2510.
25. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood. 2014;123(20):3073-3079.
26. 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. 2017;35(25):2900-2910.
27. 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. 2018;132(23):2456-2464.
28. Goicoechea I, Puig N, Cedena M-T, et al. Deep MRD profiling defines outcome and unveils different modes of treatment resistance in standard and high risk myeloma. Blood. 2021;137(1):49-60.
29. Mina R, Musto P, Rota-Scalabrini D, et al. Carfilzomib induction, consolidation, and maintenance with or without autologous stem-cell transplantation in patients with newly diagnosed multiple myeloma: pre-planned cytogenetic subgroup analysis of the randomised, phase 2 FORTE trial. Lancet Oncol. 2023;24(1):64-76.
30. Tao Y, Jin S, Yang D, et al. Real-world advantage and challenge of post-autologous stem cell transplantation MRD negativity in high-risk patients with double-hit multiple myeloma. BMC Cancer. 2024;24(1):406.
31. Costa LJ, Chhabra S, Medvedova E, et al. Minimal residual disease response-adapted therapy in newly diagnosed multiple myeloma (MASTER): final report of the multicentre, single-arm, phase 2 trial. Lancet Haematol. 2023;10(11):e890-e901.
32. Askeland FB, Rasmussen A, Lysen A, et al. Will survival improve by treating multiple myeloma patients at MRD relapse? The Remnant study. Blood. 2023;142(Suppl 1):4755.
33. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366(19):1782-1791.
34. Jackson GH, Davies FE, Pawlyn C, et al. Lenalidomide maintenance versus observation for patients with newly diagnosed multiple myeloma (Myeloma XI): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2019;20(1):57-73.
35. Richardson PG, Jacobus SJ, Weller EA, et al. Triplet therapy, transplantation, and maintenance until progression in myeloma. N Engl J Med. 2022;387(2):132-147.
36. Samur MK, Roncador M, Aktas Samur A, et al. High-dose melphalan treatment significantly increases mutational burden at relapse in multiple myeloma. Blood. 2023;141(14):1724-1736.