Minimal residual disease assessment in transplant-eligible patients with multiple myeloma: real-world applications of multiparametric flow cytometry-DURAClone (CAREMM-2104)

Developing a more efficient method for minimal residual disease (MRD) detection remains challenging in multiple myeloma (MM).[1] Multiparametric flow cytometry (MFC)-based MRD has been recommended because of its high sensitivity and short turn-around-time.[2] This study aimed to develop an MFC-based MRD assessment protocol using the DURAClone and explore whether MRD detection using the DURAClone method can be considered for real-world MM practice.

We prospectively collected bone marrow samples from consecutive patients with MM who underwent autologous stem cell transplantation (ASCT) as their first-line treatment following induction therapy with novel agents (Figure 1A).[3] MFC was performed using a modified DURAClone method following the manufacturer's instructions and principles outlined by the European Myeloma Network.[4] Premixed, dry reagent cocktail (DURAClone RE PC antibody panel) along with CD117-ECD (Beckman Coulter, Marseille, France) used for MRD assessment were obtained from DxFLEX flow cytometers (Beckman Coulter).[5] The DURAClone RE PC antibody panel included CD81-FITC, CD27-PE, CD19-PC5.5, CD200-PC7, CD138-APC, CD56-APC-A750, CD38-PB450, and CD45-KO525. All acquired data files were analyzed using the Kaluza analysis software, version 2.1 (Beckman Coulter), utilizing serial gating according to the consensus guidelines for MRD reporting.[6] The gating strategy employed in our analysis is as Figure 1B. According to the MRD detection guidelines for patients with MM, the estimated limit of detection and lower limit of quantitation were set at 6×10^{-6} and 1×10^{-5}, respectively.[6] The sensitivity was validated as 0.0001% using diluted samples, and excellent linearity was observed up to 10^5 dilutions ($y=1.0741x-0.2879$, $R^2=0.9946$). MRD-positive (MRD^+) and negative (MRD^-) statuses were defined based on the limit of detection threshold. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (IRB no. K21RISI0580) and was conducted in accordance with the principles outlined in the Declaration of Helsinki. All participants provided written informed consent.

A total of 166 patients were included if they had their initial MRD assessment when they achieved a very good partial response (VGPR) or better, as defined by IMWG criteria,[7] at 3±2 months post-ASCT (3 months post-ASCT). Among them, 52 (31.3%) were MRD^+ at 3 months post-ASCT, while 114 (68.7%) were MRD^-. No significant associations were observed between 3 months post-ASCT MRD and known prognostic factors, including International Staging System, lactate dehydrogenase, risks of cytogenetic abnormalities, and presence of plasmacytoma at diagnosis (Table 1).[8] Interestingly, the majority of patients exhibited $CD19^{-/dim+}$ and $CD45^{-/dim+}$ (98.1% and 96.2%, respectively), indicating a consistent absence of their expression in MRD^+ patients. Additionally, MM-associated phenotypes valuable for MRD assessment included $CD27^{-/dim+}$, $CD56^+$, $CD81^{-/dim+}$, $CD200^+$, $CD38^{-/dim+}$, and $CD117^+$ (expressed in 84.6%, 80.8%, 75.0%, 71.2%, 63.5%, and 20.0% of MRD^+ patients with MM, respectively). For the 114 patients who were MRD^-, we followed their MRD status after 12 months (with a permissible variation of ±3 months) from the date of the first MRD assessments (15 months post-ASCT) in 68 patients. Among them, 44 (64.7%) sustained MRD^- status for 1 year, while 24 (35.3%) experienced MRD^+ conversion. Notably, among the 28 patients who achieved VGPR by at 3 months post-ASCT, only nine patients (32.1%) were MRD^-: all nine patients transitioned from VGPR to CR within a median period of 84 (range, 7-428) days from the MRD assessment date (*Online Supplementary Table S1*).

The median follow-up was 24.4 months (95% confidence interval [CI]: 22.8-28.8) from ASCT in the total cohort. The estimated 24-months progression-free survival (PFS) rate and 24-months cumulative incidence of MM progression were 65.4% (95% CI: 56.4-73.0) and 33.1% (95% CI: 25.1-41.4), respectively. The 3-month post-ASCT MRD^+ group exhibited a lower rate of achieving complete remission (CR) compared to the MRD^- group (63.5% *vs*. 92.1%; $P<0.001$). This difference was associated with an adverse impact on the 24-month PFS rates ($P<0.001$; Figure 2A). The 24-month cumulative incidence of MM progression within the MRD^+ group was also significantly higher compared to the MRD^- group ($P=0.001$; Figure 2B). Univariate analysis identified seven variables potentially related to PFS and cumulative incidence of MM progression: 3 months post-ASCT MRD, achieving VGPR at 3 months post-ASCT, immunoglobulin (Ig) D type MM, presence of plasmacytoma, advanced International Staging System, high cytogenetic risk, and high lactate dehydrogenase level at diagnosis (*Online Supplementary Table S2*). Multivariable analysis further demonstrated that 3 months post-ASCT MRD was significantly associated with both PFS (hazard ratio=2.37, 95% CI: 1.25-4.46; $P=0.008$) and the cumulative incidence of MM

progression (hazard ratio=2.45, 95% CI: 1.25-4.82; *P*=0.004), even after adjusting for other potential factors. Patients who experienced MRD$^+$ conversion during follow-up exhibited significantly worse outcomes compared to those who sustained MRD$^-$ status for 1 year. Specifically, the 24-month PFS rate was 69.7% (95% CI: 43.7-85.4) *versus* 92.0% (95% CI: 76.7-97.4); *P*=0.01 (Figure 2C), and the 24-month cumulative incidence of MM progression was 30.3% (95% CI:

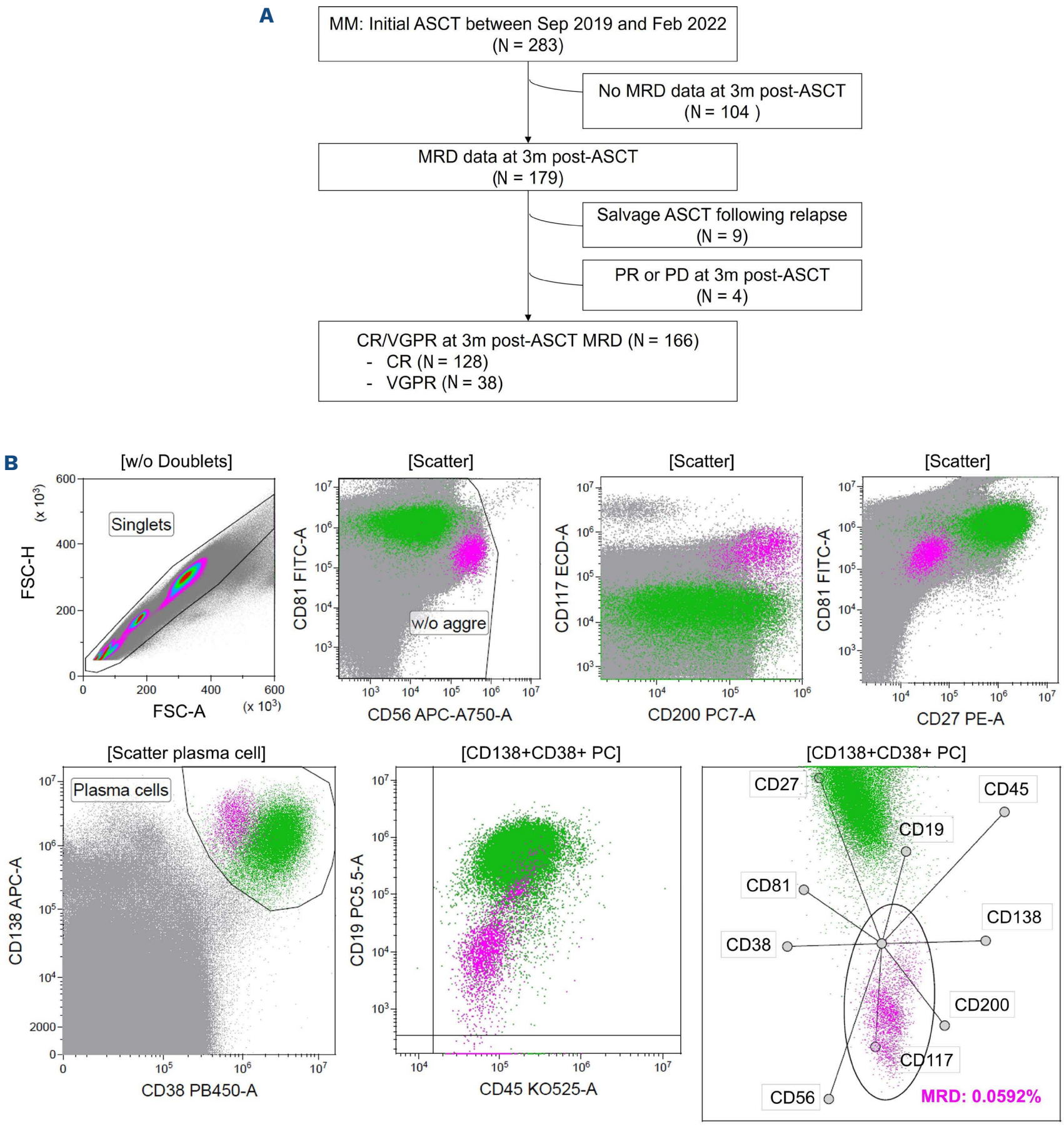

Figure 1. Flow diagram of selection of the study cohort and overview of the gating strategy for minimal residual disease using DURAClone RE PC antibody panel. (A) Flow diagram. (B) Flow cytometry standard data files were processed to exclude double events, debris, and dye aggregation (aggre). Plasma cells were identified through high CD138 and CD38 expression. Clusters of normal (green) and multiple myeloma (MM) (pink) plasma cells were defined using a 2D projection of all 9 fluorescence characteristics (radar plot). An MM-associated phenotype was described as plasma cells with low CD19, CD27, CD38, CD45, and/or CD81 expression, high CD56 expression, and asynchronous CD117 and CD200 expression. Three months (m) post-autologous stem cell transplantation (post-ASCT), 3±2 months post-ASCT. PR: partial response; PD: progressive disease; CR: complete response; VGPR: very good partial response; PC: plasma cell; w/o: without; MRD: minimal residual dsiease.

11.4-51.9) *versus* 5.7% (95% CI: 0.9-17.2), *P*=0.004 (Figure 2D). Furthermore, we observed a significant prognostic impact of subsequent therapy in 3 months post-ASCT MRD$^+$ patients (N=52): 11 patients were not treated further (21.2%), while 41 (78.8%) underwent subsequent therapy such as maintenance, consolidation and/or tandem transplantation. Those without further treatment exhibited a significantly lower 24-month PFS rate compared to those who underwent subsequent therapy (20.5% [95% CI: 3.2-48.2] *vs*. 59.2% [95% CI: 41.2-73.4]; *P*=0.022; Figure 2E), with a trend toward a higher 24-month cumulative incidence of MM progression (69.3% [95% CI: 27.4-90.1] *vs*. 40.8% [95% CI: 24.3-56.7]; *P*=0.07; Figure 2F). Particularly, patients who received consolidation therapy with or without tandem transplant (N=16) had better PFS than those who received maintenance therapy only using the immunomodulatory

Table 1. Comparisons of characteristics between two groups according to minimal residual disease status.

Variables	MRD$^-$ N=114	MRD$^+$ N=52	*P*
Days from ASCT to measurement of MRD, median (Q1-Q3)	88 (78-99)	94 (77-123)	0.185
Age at transplant in years, median (Q1-Q3)	59 (52-63)	58 (53-64)	0.737
Sex, N (%)			0.601
Female	57 (50.0)	23 (44.2)	
Male	57 (50.0)	29 (55.8)	
Type of myeloma, N (%)			0.529
Ig G	46 (40.4)	27 (51.9)	
Ig A	30 (26.3)	11 (21.2)	
Light chain disease	30 (26.3)	12 (23.1)	
Other type	8 (7.0)	2 (3.8)	
International Staging system, N (%), unknown=3			0.311
I	40 (35.1)	14 (26.9)	
II	36 (31.6)	23 (44.2)	
III	35 (30.7)	15 (28.8)	
Lactate dehydrogenase at diagnosis, N (%), unknown=1			0.07
Normal	92 (80.7)	48 (92.3)	
High	21 (18.4)	4 (7.7)	
Cytogenetic abnormality, N (%), unknown=34			
Standard risk	55 (48.2)	22 (42.3)	0.343
High risk	35 (30.7)	20 (38.5)	-
t(4;14)	21 (18.4)	11 (21.2)	0.642
del(17p)	14 (12.2)	10 (19.2)	0.278
t(14;16)	5 (4.4)	3 (5.8)	0.722
Other cytogenetic abnormalities			
1q abnormality	35 (30.7)	19 (36.5)	0.603
del(13q)	42 (36.8)	17 (32.7)	0.395
Presence of plasmacytoma at diagnosis, N (%), unknown=1			0.999
None	82 (72.6)	37 (69.8)	
Present	31 (27.4)	15 (28.3)	
Regimen of induction, N (%)			0.016
VTD	98 (86.0)	42 (80.8)	
VRD	5 (4.4)	9 (17.3)	
VMP	4 (3.5)	0 (0.0)	
DVTD	7 (6.1)	1 (1.9)	
Pretreatment for autologous stem cell infusion, N (%)			0.138
High-dose melphalan	114 (100.0)	51 (98.1)	
Busulfan plus melphalan	0 (0.0)	1 (1.9)	
Infused dose of CD34$^+$ cell, x10^6/kg, median (Q1-Q3), unknown=1	5.6 (4.52-6.93)	5.5 (4.63-6.4)	0.201

MRD: minimal residual disease; ASCT: autologous stem cell transplantation; VTD: bortezomib-thalidomide-dexamethasone; VRD: bortezomib-lenalidomide-dexamethasone; VMP: bortezomib-melphalan-prednisolone; DVTD: daratumumab-bortezomib-thalidomide-dexamethasone.

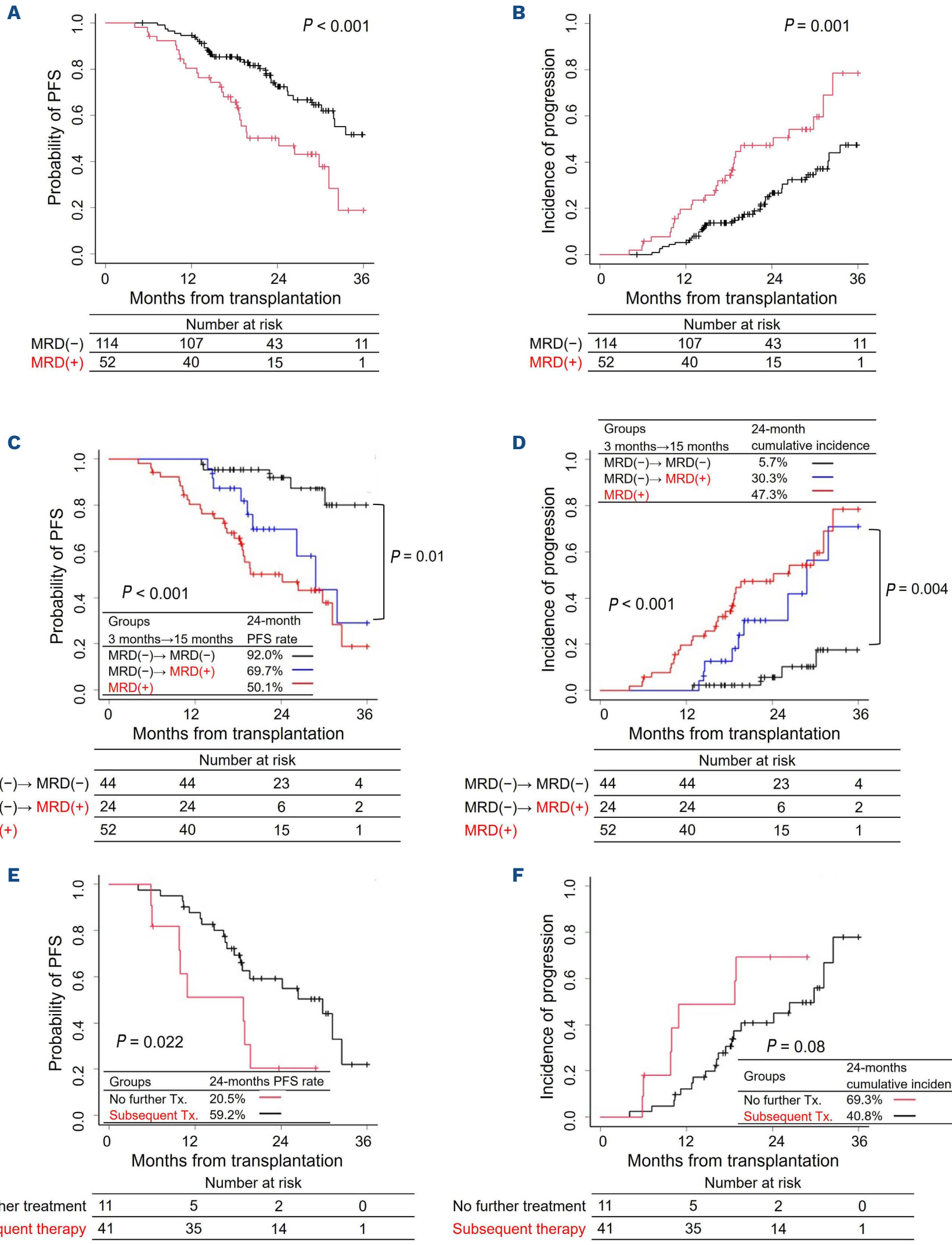

Figure 2. Clinical outcomes according to minimal residual disease status and subgroup analysis. (A) Progression-free survival (PFS), and (B) cumulative incidence of progression according to minimal residual disease (MRD) status. In the MRD⁻ group, (C) higher PFS and (D) lower cumulative incidence in patients who achieved sustained MRD⁻ status for a year compared to patients with MRD⁺ conversion; in the MRD⁺ group, (E) higher PFS and (F) lower cumulative incidence in patients who received subsequent therapy compared to those received no further treatment (Tx).

drug (N=25); however, the difference was not statistically significant (24-month PFS rate, 66.0% [95% CI: 36.5-85.4] *vs*. 55.2% [95% CI: 31.6-73.6]; *P*=0.874).

MRD assessment in MM proves invaluable for tailoring personalized therapy, surpassing traditional response criteria.[9] Currently, two main methods are used to evaluate MRD in MM: immunophenotypic including MFC and molecular techniques such as real-time quantitative-polymerase chain reaction (PCR), digital PCR, or next-generation sequencing.[10] Recently, the International Myeloma Working Group defined MRD negativity as the absence of MM cells with a minimum sensitivity of $<10^{-5}$ either by next-generation sequencing or MFC as the reference method.[7] MFC is a rapid and widely applicable method since the results are not mandatory at diagnosis. It necessitates fresh specimens collected within 48 hours after sampling and a comprehensive understanding of normal antigen expression and maturation patterns of all hematopoietic progenitors.[4,6] Though this study, we demonstrated that the DURAClone method showed high sensitivity (approximately 10^{-5}), cost-effectiveness (<$100/sample), and a quick processing time (approximately 1 hour) for MRD assessment in MM (*Online Supplementary Figure S1*), similar to other MFC techniques such as EuroFlow next-generation flow.[11] Its single customized dried-format tube system facilitates a short turnaround time and minimizes potential sources of human error. Furthermore, the analysis follows a predefined template incorporating dynamic gates and radar plots, ensuring a high level of standardization regardless of the analyzing personnel.[12] The performance of the DURAClone method can be enhanced by its flexibility in incorporating additional MM-associated phenotypes, such as ECD and APC-AF700 coupled fluorochromes.[12] The inclusion of extra antibodies as a drop-in does not compromise assay performance and could prove beneficial for MRD assessment following immunotherapy targeting specific markers.

Consistent with methodologies in previous reports utilizing EuroFlow or next-generation sequencing, our approach to assessing MFC-based MRD using DURAClone at 3 months post-ASCT demonstrated significant prognostic impacts on both PFS and cumulative incidence of MM progression. Importantly, these prognostic impacts were statistically independent of traditional risk factors. Additionally, our observation revealed that patients in VGPR status with negative MRD could transition from VGPR to CR. Conversely, fewer patients in the MRD^+ group achieved CR compared to the MRD^- group. Considering the prolonged half-life (approximately 30 days) of serum Ig and the extended time required for complete M protein clearance, MRD testing is recommended for patients who achieved CR and VGPR.[13] We also emphasized the importance of serial MRD monitoring. Consistent with established guidelines, our study demonstrated that patients sustaining MRD^- status for 1 year exhibit more favorable survival outcomes compared to those experiencing MRD^+ conversion.[14] Furthermore, we elaborated on the clinical implications of the 3 months post-ASCT MRD assessment and its potential to guide further treatment. The Myeloma XI trial demonstrated a significant median PFS benefit with lenalidomide maintenance after ASCT in 3 months post-ASCT MRD^+ patients.[15] Similarly, our real-world cohort-based study revealed that further treatment provides a significant survival benefit in 3 months post-ASCT MRD^+ patients. Our study and previous reports have indicated detectable MRD as an independent risk factor beyond conventional cytogenetic risk, and it may even surpass the risk associated with cytogenetically high-risk MM.[2]

This study has some limitations. They include the relatively short follow-up time and relatively small number of patients, which limits our ability to involve the choice of treatment based on MRD status using the DURAClone method, followed by assessment of clinical outcome. Nevertheless, our study revealed that MRD assessment using the DURAClone method could be feasible, achieving a sensitivity of 10^{-5} and meeting the IMWG recommendation. This enables MRD assessment in patients with MM. Moreover, the DURAClone method-driven MRD negativity at 3 months post-ASCT represents a clinically practical tool for predicting prognosis in MM beyond the conventional risk group, similar to other MFC tools exemplified by the EuroFlow method. The added clinical significance of serial follow-up enhances the reliability of our findings. Further studies to confirm the effect of intensified treatment in MRD^+ patients will potentiate the value of this MRD assessment. Conclusively, this exploratory study provides initial evidence that the faster and more cost-effective MFC-DURAClone method could serve as a viable alternative for clinical MRD assessment in MM.

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Disclosures
No conflicts of interest to disclose.

Contributions
MK and C-KM conceived and designed the study. S-SP, JYL, and C-KM participated in the acquisition of data and clinical review. AA, YK, J-ML, JJ, and MK contributed to sample preparation and performed multiparametric flow cytometry. AA and S-SP wrote the article. The drafted article was reviewed by all authors, including AA, S-SP, YK, JYL, J-ML, JJ, MK, and C-KM.

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Data-sharing statement
The data that support the findings of this study are available from the corresponding author, C-KM, upon reasonable request.

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