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ARTICLE

Prognostic value of measurable residual disease in high-risk MDS after intensive chemotherapy in HOVON-SAKK studies

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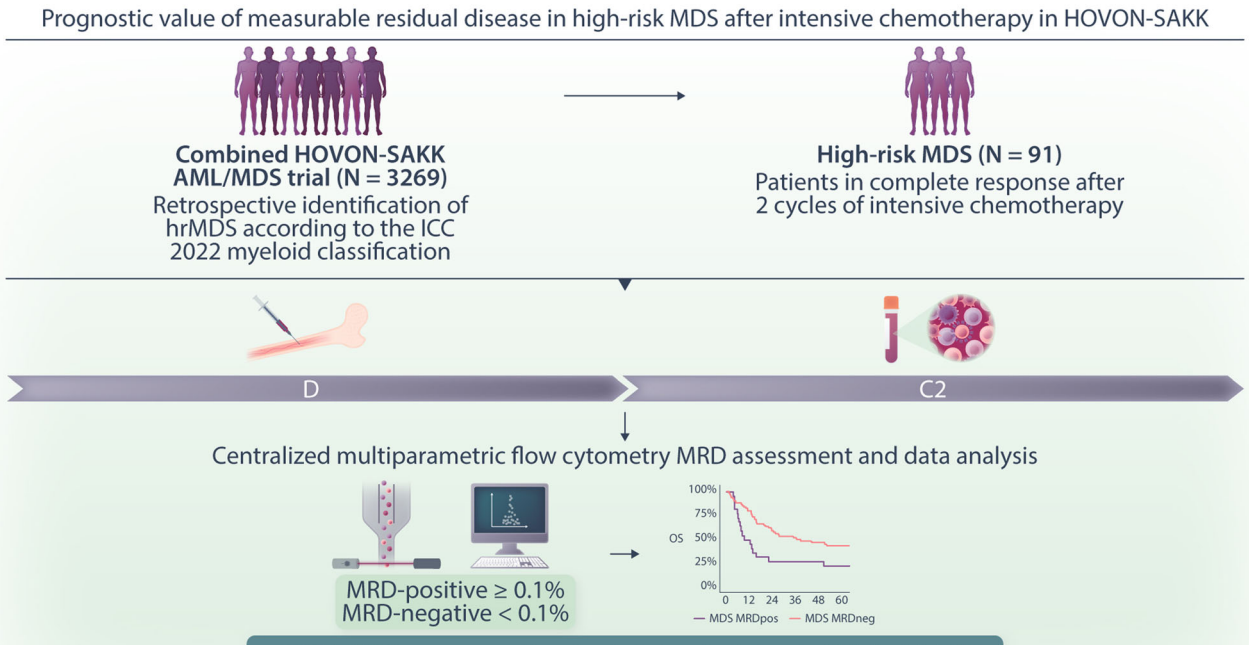
Graphical Abstract



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Abstract

Myelodysplastic syndromes (MDS) are heterogenous disorders in which response assessment remains challenging. In acute myeloid leukemia (AML), measurable residual disease (MRD) by multiparametric flow cytometry (MFC) is prognostic and guides decision-making after two cycles of intensive chemotherapy, but its role in high-risk MDS (hrMDS) is unknown. We aimed to determine the prognostic impact of MFC-MRD (0.1% cutoff) for overall survival (OS) and the cumulative incidence of relapse (CIR) in intensively treated hrMDS. After stringent selection from 3269 patients enrolled in prior HOVON-SAKK MDS/AML trials, we identified 91 ICC 2022-defined hrMDS patients with available MFC-MRD. MFC-MRD positivity was detected in 24% and associated with inferior survival (5-year OS: 22.7% vs. 43.7%; P = 0.010) and higher relapse risk (5-year CIR: 72.7% vs. 47.2%; P = 0.014). In multivariable analyses stratified by trial and adjusted for, among others, the presence of a biallelic *TP53* mutation, MFC-MRD positivity remained associated with poorer OS (hazard ratio (HR) 2.12 [95% CI 1.15–3.90]; P = 0.017) and increased CIR (subdistribution HR 2.15 [95% CI 1.11–4.14]; P = 0.022). To account for the potential confounding effect of allogeneic hematopoietic stem cell transplantation, additional sensitivity analyses were performed and confirmed that MRD positivity remained significantly associated with inferior survival outcomes. Lastly, an exploratory cross-disease comparison showed that MRD-negative (MRDneg) hrMDS patients had similar survival outcomes to MRD-positive (MRDpos) AML patients. These findings demonstrate the prognostic value of MFC-MRD in intensively treated hrMDS and provide rationale for prospective trials of MRD-informed transplant and post-remission strategies.

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INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal hematopoietic stem cell disorders characterized by peripheral blood cytopenia, morphologic dysplasia of hematopoietic cells, and potential to progress to acute myeloid leukemia (AML).[1] The international prognostic scoring system (IPSS) has been developed to classify MDS patients into higher and lower risk categories.[2] The IPSS-molecular (IPSS-M) is the newest edition and refines the IPSS-revised risk classification for MDS patients by incorporating mutation status, next to hematological parameters and cytogenetics.[3] This molecular basis for assessing risk in MDS at diagnosis has led to a more accurate and precise definition of high-risk disease, and it relies less on morphological blast percentages compared to previous risk classifications.

Therapeutic strategies for high-risk MDS (hrMDS) patients consist of hypomethylating agents, intensive chemotherapy for clinically fit patients, and allogeneic stem cell transplantation (allo-HSCT) as the only potentially curative option.[4] Historically, the Dutch–Belgian Cooperative Trial Group for Hematology-Oncology (HOVON-SAKK) consortium has treated hrMDS with intensive chemotherapy, followed by post-remission consolidation, including allo-HSCT.

Recently, the international consortium of MDS described response criteria for hrMDS. These criteria are based on percentages of bone marrow (BM) and peripheral blood (PB) blasts, and other hematologic parameters.[5] The inclusion of MFC and molecular measurable residual disease (MRD) has been debated, but data on MRD in MDS are limited.[6,7] This is in contrast to AML, in which MRD is a well-established indicator for response, showing significant prognostic value and the possibility to guide post-remission treatment.[8,9] For AML, extensive European Leukemia Network (ELN) guidelines on the clinical application of MFC-MRD have been published and were most recently updated in 2025.[10] In MDS, however, only exploratory studies have shown the potential clinical utility of MRD, and extensive high-quality trials are needed before MRD can be implemented as a routine indicator for response in MDS.[6,7]

The HOVON-SAKK MDS/AML trials consist of large cohorts of intensively treated AML and hrMDS patients with long-term follow-up, thereby offering a unique possibility to study the prognostic value of MFC-MRD in hrMDS.

METHODS

Patients and risk stratification

We included patients from six consecutive AML/MDS trials performed by the HOVON-SAKK trial cooperative groups between 2006 and 2019: HO42A, HO81, HO92, HO102, HO103, and HO132.[11–16] In these studies, AML and hrMDS patients were treated identically with two cycles of intensive chemotherapy with or without a novel drug, before receiving post-induction therapy, as previously described.[11–16] HrMDS was defined as IPSS ≥ 1.5 or IPSS-R > 4.5.[2] Following current clinical guidelines, risk was reassessed according to IPSS-M for the purpose of this analysis.[3] Furthermore, patients were reclassified according to the latest ICC2022 (Supporting Information S1: Figure S1).[1]

Sampling and logistics

BM samples were collected across eight European countries between 2006 and 2019. MRD was assessed in patients who achieved complete remission with or without complete count recovery (CR(i)) by cytomorphology after the second cycle of chemotherapy (C2). Criteria for CR, CR with incomplete count recovery (CRi), and relapse were as previously described.[11–16] Details are provided in the supplementary table (Supporting Information S1: Table S1). Standardized karyotyping was performed and scored following ISCN criteria.[17] Mutational analysis results were included for patients in whom next-generation sequencing (NGS) and/or polymerase chain reaction (PCR) targeting common myeloid aberrancies were performed.

MFC-MRD measurements

Details of sample processing and MFC-MRD measurement were as previously described for the HOVON-SAKK AML/MDS trials.[9,11–16,18] In short, MFC-MRD analyses were performed centrally at a single institute, and samples from participating sites were shipped and processed within 72 h after collection. MRD measurement was performed on FACS CANTO-II or FACS Calibur flow cytometers (BD Biosciences). Analysis was performed with Cell QuestPro software (BD) in HO42A and Infinicyt™ software (BD) in the other trials. The leukemia-associated immunophenotype (LAIP) was identified on blast cells defined as CD45-expressing cells combined with a primitive marker (CD34, CD117, or CD133), a myeloid marker (CD13 or CD33), HLA-DR, and aberrant combinations of the LAIP-indicating markers. The patient-specific LAIP was determined at diagnosis and the MRD result was determined after C2, according to ELN 2022 recommendations for MRD measurements in AML.[9] A cutoff of ≥0.1% LAIP of white blood cells (WBC, i.e., CD45-expressing cells) was used to define MRD status. Sample quality was assessed before final inclusion. Adequate samples required the acquisition of at least 100,000 CD45+ cells (preferably >500,000 in recent studies) and sufficient viability.

Statistical analysis

The primary endpoint was overall survival (OS). The secondary endpoint was the cumulative incidence of relapse (CIR), in which death without relapse was considered a competing event. In all analyses, MRD negativity served as the reference group for comparison with MRD positivity. Survival curves for OS (Kaplan–Meier) and CIR (Aalen–Johansen) were created to compare groups using the log-rank test and Gray's test, respectively. Both endpoints were measured from the time of bone marrow collection for MRD assessment after the second induction cycle until death (OS) or until the earliest of relapse or death (CIR).

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Patients without an event were censored at the date of last contact.

To verify the independent prognostic value of MFC-MRD, we performed multivariable Cox regression and competing risk analyses, adjusting for clinically relevant patient-specific variables. The following covariates were evaluated in univariable analyses (Cox regression for OS, Fine–Gray regression for CIR): age, sex, BM blast percentage, prior oncological disease, early remission (i.e., CR after the 1st cycle of induction chemotherapy), cytogenetic risk, presence of a biallelic TP53 mutation as defined by the ICC2022 myeloid classification, and 16 additional main-effect genes from the IPSS-M (*FLT3*-ITD/TKD, *NPM1*, *RUNX1*, *NRAS*, *ETV6*, *IDH2*, *CBL*, *EZH2*, *U2AF1*, *SRSF2*, *DNMT3A*, *ASXL1*, *KRAS*, *SF3B1-a*, and *SF3B1-q*).[3] It is noteworthy that patients with missing molecular data ($n = 20$) were entered into the model as a separate "unknown" category to maximize cohort retention and minimize selection bias. Variables with a $P < 0.2$ in univariable analyses were included in the multivariable models for OS and CIR, provided that categorical variables also had at least five events per group; both models were stratified by trial to adjust for intertrial variability, similar to the approach described by Versluis and colleagues.[19] The proportional hazards assumption was assessed using Schoenfeld residuals for the Cox regression models. Additionally, a sensitivity analysis was performed by including allo-HSCT as a time-dependent variable in the multivariable analyses.

Patient characteristics were compared using the Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Analyses were performed using R statistical software version 4.4.3 (R Foundation for Statistical Computing), SPSS Statistics version 28.0 (IBM Corporation), and Stata (version 18.0; StataCorp.). In all analyses, $P < 0.05$ were considered statistically significant.

RESULTS

Patient characteristics

A total of 3269 patients, of whom 364 were defined as hrMDS at inclusion, were included in the HOVON-SAKK AML trials (Figure 1). Eligibility for analysis was defined according to the ELN recommendations for MFC-MRD assessment in AML: (1) achievement of complete remission after two cycles of intensive chemotherapy; (2) presence of a LAIP at diagnosis; (3) availability of a bone marrow sample obtained after cycle 2; and (4) adequate bone marrow sample quality and data reliability for MRD analysis (e.g., appropriate sample

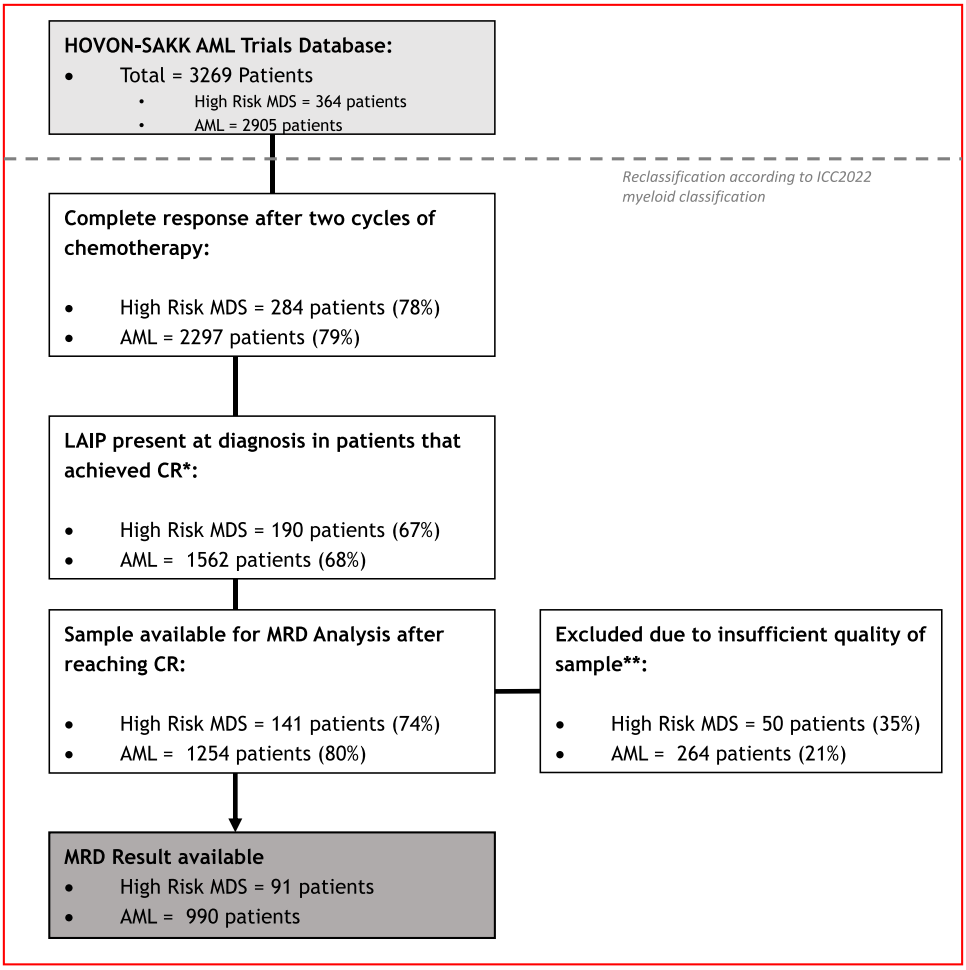

FIGURE 1 **Flow diagram**. *Numbers shown for LAIP at diagnosis refer to patients achieving CR only; across all trials, irrespective of response, a diagnostic LAIP was detected in 77% of MDS and 88% of AML patients. Owing to the retrospective design, it could not always be established with certainty whether cases without a documented diagnostic LAIP were due to missing or inadequate diagnostic samples or due to the absence of a detectable LAIP. **Exclusion was based on either sample quality (e.g., incorrect sample type and poor viability) or data reliability (e.g., insufficient cellular events and laboratory anomalies).

type and viability, sufficient acquired cellular events (preferably >500,000) for MRD evaluability at low levels, absence of laboratory anomalies).[9,10] Across all trials, a LAIP at diagnosis was detected in most patients (77% of MDS and 88% of AML), and in approximately 70% of patients who achieved CR (Figure 1). Applying these stringent criteria resulted in the inclusion of 91 patients with MDS classified according to the ICC 2022 myeloid classification in the final analysis.[1]

To assess potential bias in the exclusion of MDS patients who achieved CR(i) and had a LAIP at diagnosis but were excluded due to inadequate remission sample quality, we compared the survival outcomes between the included MDS patients and those who were excluded due to inadequate remission sample quality. No statistically significant difference in patient characteristics and survival was observed between the two groups, suggesting that the exclusion was random and unlikely to introduce bias into the analysis (Supporting Information S1: Figure S2 and Supporting Information S1: Table S2). By applying these criteria, that is, CR(i) and adequate MRD measurement available after C2, we included 91 MDS patients in our study according to the ICC2022 myeloid classification (Table 1).[1] Determination of IPSS-M scores was possible in 59 of the 91 (65%) MDS patients based on the full availability of the mutational profile, of whom 95% ($n = 56$) were considered high risk (defined as "Moderate high," "High," or "Very High") and 54% ($n = 32$) were classified as "Very High" (Supporting Information S1: Table S3).[3]

Prognostic impact of MRD in MDS

A total of 91 patients with ICC2022 defined MDS with MRD results were included, of whom 24% (22/91) were MRDpos after two cycles of induction chemotherapy. Of the 69 MRDneg hrMDS patients, 14 (20%) had fewer than the preferred 500,000 white blood cell events acquired. High-risk cytogenetics and biallelic *TP53* mutation were more frequently observed in MRDpos patients (Table 1). The median follow-up time of patients who were alive was 84 months (follow-up range: 1–128 months), as estimated using the reverse Kaplan–Meier method. MRDpos MDS patients showed an inferior OS at 5 years when compared with MRDneg patients (22.7% [95% CI 10.5–49.1] vs. 43.7% [95% CI 33.3–57.5]; log-rank P = 0.01) (Figure 2A). Similarly, the CIR was significantly higher in MRDpos MDS patients compared with MRDneg patients (5-year CIR: 72.7% [95% CI 52.9–92.6] vs. 47.2% [95% CI 35.0–59.3]; Gray's test P = 0.01) (Figure 2B). Analyses were repeated using the World Health Organization 2022 classification to confirm the robustness of the findings; the results were consistent and are shown in Supporting Information S1: Figure S3.[20]

Based on univariable analyses, variables associated with OS or CIR at a significance level of P < 0.20 were selected for inclusion in the multivariable models, provided that categorical variables also had at least five events per group. These variables included age (OS only), bone marrow blast percentage (OS only), early remission, and the presence of a biallelic *TP53* mutation (Supporting Information S1: Figure S4). After adjustment for these variables and stratification by trial to account for trial-level heterogeneity, MRD positivity remained an independent prognostic factor associated with inferior OS compared with MRD negativity (adjusted (adj.) HR 2.12 [95% CI 1.15–3.90]; Wald P = 0.02; Figure 3A). In the multivariable competing risk model accounting for nonrelapse mortality as a competing event, MRD positivity remained independently associated with an increased CIR (adj. subdistribution HR 2.15 [95% CI 1.11–4.14]; Wald P = 0.02; Figure 3B). To assess the potential confounding effect of allogeneic hematopoietic stem-cell transplantation (allo-HSCT) on outcomes, we performed sensitivity analyses including allo-HSCT as a time-dependent covariate. In the multivariable model adjusting for allo-HSCT and other relevant covariates, MRD positivity remained an independent predictor for poorer OS (adj. HR 2.19 [95% CI 1.18–4.10]; Wald P = 0.01; Figure 4A). In the corresponding multivariable competing risk model that included allo-HSCT as a time-dependent covariate, MRD positivity was also independently associated with a higher CIR (adj. subdistribution HR 2.80 [95% CI 1.42–5.55]; Wald P = 0.003; Figure 4B). These findings confirm that

TABLE 1 Patient characteristics of hrMDS patients according to the ICC2022 myeloid classification.

Characteristic	Total MDS cohort ($n = 91$[a])	MRD positive ($n = 22$)	MRD negative ($n = 69$)	P
Age, median (range)	59 (27–75)	62 (28–75)	58 (27–73)	0.76
Gender, No. (%)				0.32
Female	33 (36%)	10 (45%)	23 (33%)	
Male	58 (64%)	12 (55%)	46 (67%)	
Blast percentage by morphology, median (range)	12 (1–19)	10 (1–19)	12 (1–19)	0.55
Cytogenetic risk category[b], No. (%)				0.007
Very Good	1 (1%)	1 (5%)	0	
Good	46 (51%)	5 (22%)	41 (59%)	
Intermediate	16 (18%)	3 (14%)	13 (19%)	
Poor	7 (8%)	5 (22%)	2 (3%)	
Very Poor	19 (21%)	8 (36%)	11 (16%)	
Unknown[c]	2 (2%)	0	2 (3%)	
TP53 multi-hit, No. (%)				0.03
Present	12 (13%)	6 (27%)	6 (9%)	
Absent	59 (65%)	11 (50%)	48 (70%)	
Unknown[c]	20 (22%)	5 (23%)	15 (21%)	
Prior oncological disease, No. (%)				0.25
Yes	10 (11%)	4 (18%)	6 (9%)	
No	81 (89%)	18 (82%)	63 (91%)	
IPSS-Molecular[d], No. (%)				1.0
Low risk	3 (3%)	0 (0%)	3 (4%)	
High risk	56 (62%)	12 (55%)	44 (64%)	
NA	32 (35%)	10 (45%)	22 (32%)	
Early remission, No. (%)				0.13
After the 1st Cycle	73 (80%)	15 (68%)	58 (84%)	
After the 2nd Cycle	18 (20%)	7 (32%)	11 (16%)	
Allogeneic HSCT, No. (%)				0.46
Yes	55 (60%)	15 (68%)	40 (58%)	
No	36 (40%)	7 (32%)	29 (42%)	

[a]Three patients included who reached CR with incomplete count recovery.

[b]Cytogenetic Risk Category dichotomized: Low risk defined as "Very Good" and "Good," high risk defined as "Intermediate," "Poor," and "Very Poor."[3,17]

[c]Patients with unknown TP53/cytogenetic risk status were excluded from comparison.

[d]Low risk was defined as "Moderate Low," "Low," or "Very Low," and high risk was defined as "Moderate High," "High," or "Very High" (Supporting Information S1: Table S3).

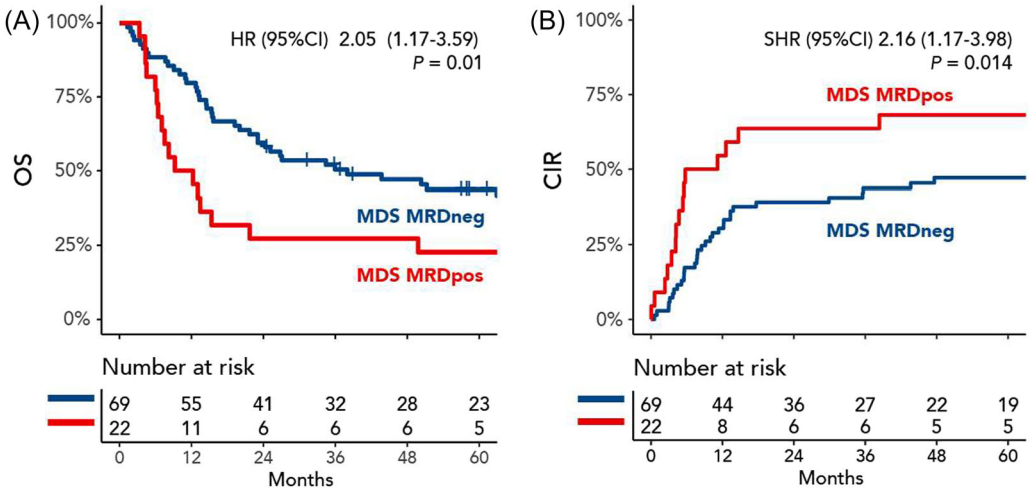

FIGURE 2 **Overall survival (OS) and cumulative incidence of relapse (CIR) based on MFC-MRD results in hrMDS patients according to ICC2022 measured after two induction cycles of intensive chemotherapy**. **(A)** OS (univariable Kaplan–Meier analysis with the log-rank test for comparison). **(B)** CIR (univariable Fine–Gray analysis with Gray's test for comparison). The *X*-axis represents months from MRD sampling, and curves were truncated at 60 months for clarity. Vertical ticks indicate censored observations. See Supporting Information S1: Figure S3 for analysis performed after re-classification of patients according to the WHO 2022 myeloid classification.

the prognostic impact of MFC-MRD remained robust after accounting for allo-HSCT.

MDS Patients with a biallelic TP53 mutation

In MDS, patients with a biallelic *TP53* mutation (MDS-TP53) are considered a distinct entity due to the particularly poor prognosis of this molecular subgroup. Twelve MDS-TP53 patients were included in this study, and as expected, they had worse OS when compared to MDS patients who did not harbor a biallelic TP53 mutation (including four patients with a single hit *TP53* mutation) in univariable analysis (HR 3.38 [95% CI 1.68–6.80]; log-rank P = 0.001) (Supporting Information S1: Figure S4) and multivariable analysis (adj. HR 2.35 [95% CI 1.07–5.17]; Wald P = 0.03) (Figure 3A). Similarly, the presence of a biallelic *TP53* mutational status showed a strong association with an increased CIR in both univariable (subdistribution HR 3.17 [95% CI 1.53–6.56]; Gray's test P = 0.002) and multivariable analyses (adj. subdistribution HR 2.65 [95% CI: 1.14-6.17]; Wald P = 0.02) (Supporting Information S1: Figures S4 and Figure 3B, respectively).

The role of MFC-MRD in this subgroup is unknown. In our cohort, MDS-TP53 patients with an MRDpos result ($n = 6$) had a median survival of 4 months (range: 3–21 months), while MRDneg MDS-TP53 patients ($n = 6$) had a median survival of 14 months (range: 10–73 months). This result should be viewed as merely descriptive, since the small sample size does not allow for a definitive conclusion.

Comparison of AML and MDS

Lastly, an exploratory cross-disease comparison was performed between the MDS cohort ($n = 91$) and the AML cohort ($n = 990$) (Supporting Information S1: Table S4). Remission samples from patients in CR following cycles of intensive chemotherapy were more frequently unassessable in MDS than in AML (35% vs. 21%) due to inadequate quality, but were also deemed unlikely to have introduced bias (Supporting Information S1: Figure S2).

In AML, the ELN 2022 classification is nowadays used to define risk, in which MRD status guides consolidation treatment in the "Intermediate"-risk group within HOVON-SAKK.[8] In univariable analysis, the 5-year OS of MDS patients in CR after two cycles of intensive chemotherapy closely resembled that of adverse-risk AML treated with the same regimen (38.7% [95% CI 29.7–50.3] vs. 40.2% [95% CI 34.6–46.6]; log-rank P = 0.66; Figure 5A). Similarly, the 5-year CIR did not differ significantly between MDS and adverse-risk AML patients (52.3% [95% CI 41.8–62.8] vs. 53.2% [95% CI 47.1–59.2]; Gray's test P = 0.87; Figure 5B). The observed similarities were maintained following MRD stratification (Supporting Information S1: Figure S5) and when analyzed from the date of trial registration in the HOVON-SAKK AML/MDS trials (Supporting Information S1: Figure S6). Furthermore, sensitivity analyses restricted to AML with myelodysplasia-related features (as defined within ELN adverse-risk criteria) showed outcomes comparable to hrMDS (Supporting Information S1: Tables S5 and S6; Supporting Information S1: Figure S7).

MRD positivity in AML was also associated with significantly shorter OS compared with MRD negativity (AML; HR 1.65 [95% CI 1.35-2.03]; P < 0.001) in this cohort. Notably, MRD-negative MDS patients showed a similar 5-year OS to MRDpos AML patients (42.7% [95% CI 36.6–49.8] vs. 43.7% [95% CI 33.3–57.5]; log-rank P = 0.52; Figure 5C). CIR at 5 years was 47.2% [95% CI 35.0–59.3] for MRDneg MDS patients and 56.0% [95% CI 49.3–62.6] for MRDpos AML patients (Gray's test P = 0.08; Figure 5D).

DISCUSSION

Study overview

The prognostic value of MFC-MRD is well established in AML.[8,9] While hrMDS patients have been included in previous AML trials, the role of MRD in MDS has yet to be defined. In this study, we evaluated the prognostic value of MFC-MRD in hrMDS (defined by IPSS-R/M) in CR(i) after two cycles of intensive chemotherapy within HOVON-SAKK trials.[6,7] Our data show that MFC-MRD is a prognostic parameter in

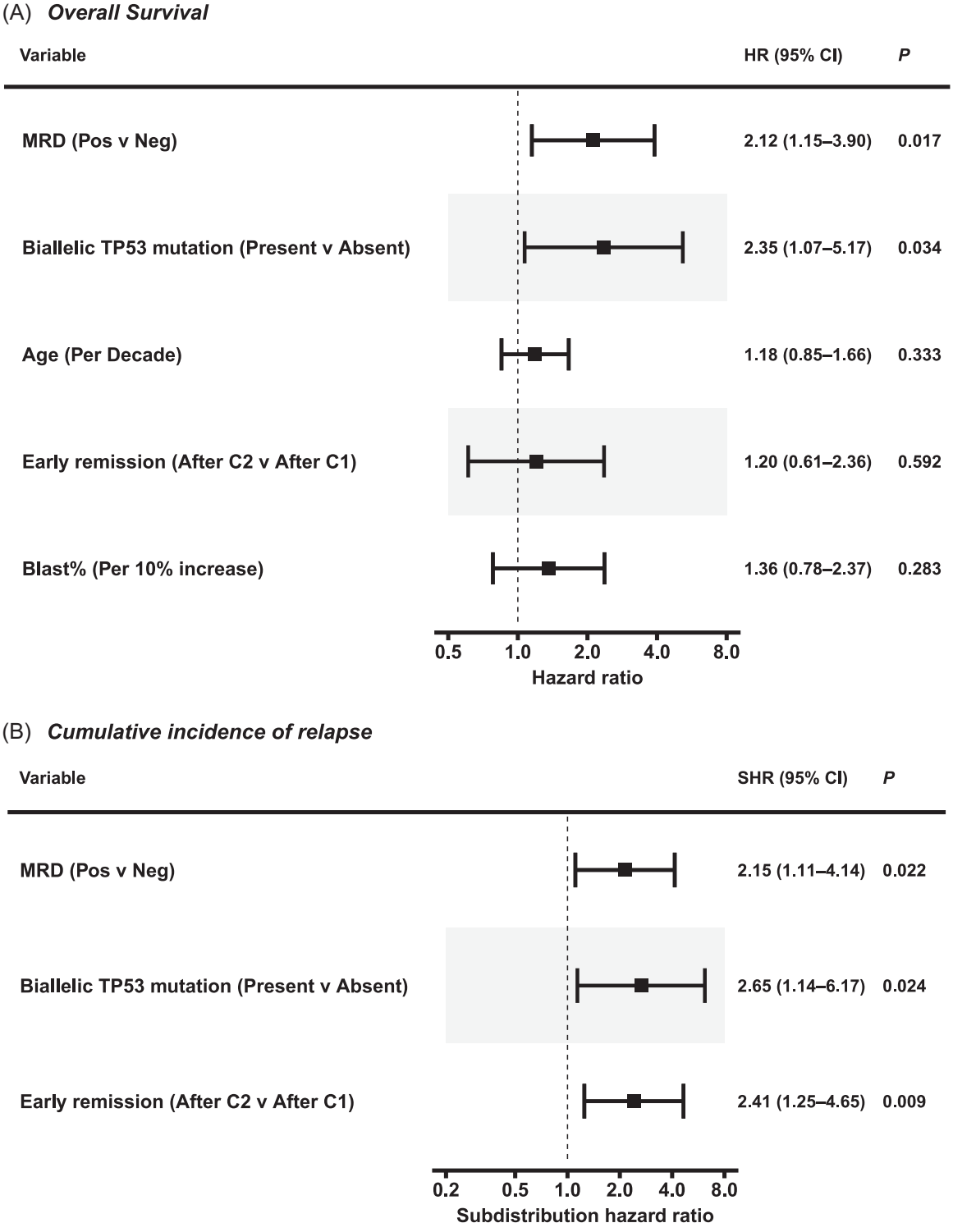

FIGURE 3 **Forest plots. (A)** OS (multivariable Cox regression) and **(B)** CIR (multivariable Fine and Gray regression) in high-risk MDS, showing hazard ratios (adjusted HR/SHR) with 95% confidence intervals for MFC-MRD and other included covariates. P-values from Wald tests. In all comparisons, reference groups are shown second in each variable pair. Patients with unknown biallelic TP53 mutation status ($n = 20$) were classified as a separate group in the model, but excluded from visual display.

patients with hrMDS, that is, positive MRD results after two courses of intensive chemotherapy in hrMDS associated significantly with worse outcomes, which was independent of covariates and similarly observed when adjusted for allo-HSCT and intertrial variability.

A major strength of this study was the stringent application of eligibility criteria. MRD assessment was performed in accordance with ELN recommendations, while MDS diagnoses were established according to the ICC 2022 classification, reflecting contemporary

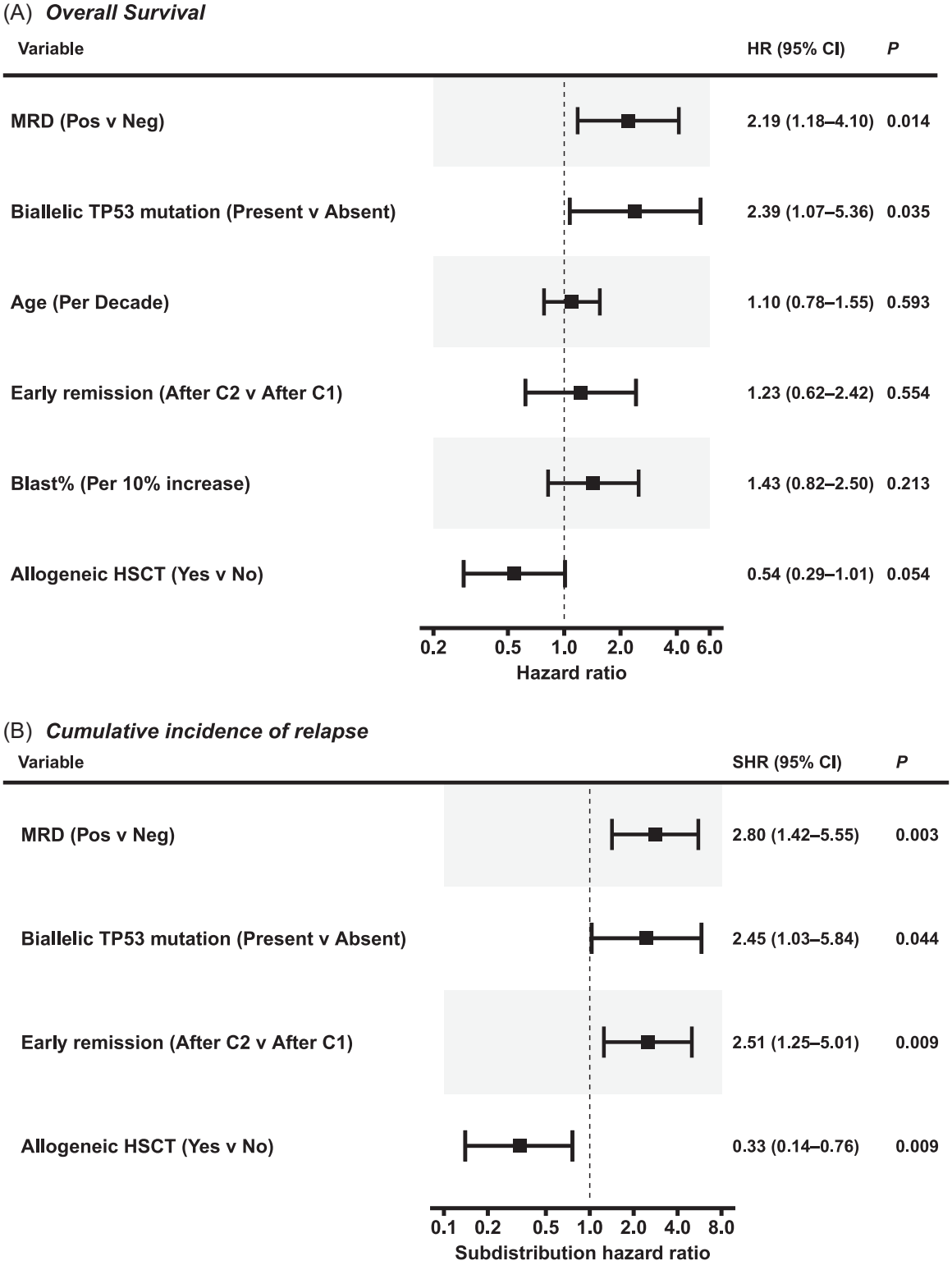

FIGURE 4 **Sensitivity analysis to evaluate the impact of allo-HSCT on prognostic value of MRD. (A)** Multivariable Cox regression analysis for OS. **(B)** Multivariable Fine–Gray analysis for CIR. In all comparisons, reference groups are shown second in each variable pair. Patients with unknown biallelic TP53 mutation status ($n = 20$) were classified as a separate group in the model, but excluded from visual display. HSCT, hematopoietic stem cell transplantation; MRD, measurable residual disease; OS, overall survival.

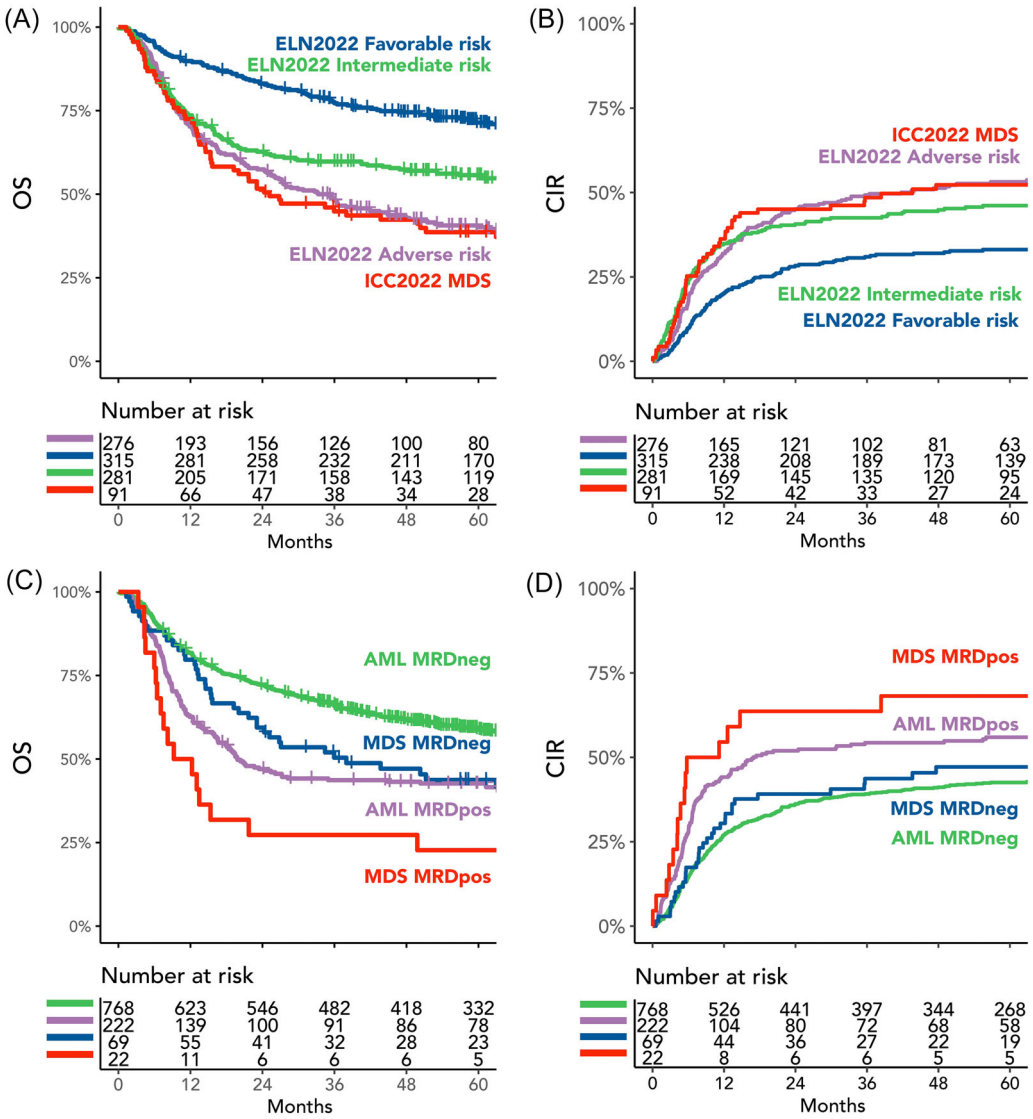

FIGURE 5 **Comparison of hrMDS v. AML.** Overall survival (OS) and cumulative incidence of relapse (CIR) are displayed. **(A)** OS in subgroups (AML Adverse risk v MDS); HR 0.94 [95% CI: 0.70–1.26]; P = 0.66 (univariable Cox regression for HR estimation and log-rank test for comparison). **(B)** CIR in subgroups (AML Adverse risk v MDS); SHR 0.99 [95% CI: 0.89–1.11]; P = 0.87 (univariable Fine & Gray regression for SHR estimation and Gray's test for comparison). **(C)** OS in subgroups (AML MRDpos v MDS MRDneg); HR 0.89 [95% CI: 0.63–1.27]; P = 0.52 (univariable Cox regression for HR estimation and log-rank test for comparison). **(D)** CIR in subgroups (AML MRDpos v MDS MRDneg); SHR 1.41 [95% CI: 0.97–2.05]; P = 0.08 (univariable Fine & Gray regression for SHR estimation and Gray's test for comparison). The *X*-axis represents months from MRD sampling, and curves were truncated at 60 months for clarity. Vertical ticks indicate censored observations. AML Patients with missing ELN risk score were excluded from analysis in panels A & B (*n* = 118). AML, acute myeloid leukemia; CI, confidence interval; HR, hazard ratio; MDS, myelodysplastic syndromes.

advances in diagnostic criteria. Application of the IPSS-M further confirmed that the vast majority of patients belonged to higher-risk disease categories.[1,3,9,10] Together, these criteria resulted in a biologically and clinically well-characterized cohort of 91 patients with MDS (59 patients for whom an updated IPSS-M risk assessment could be performed), providing a robust framework for evaluating the prognostic relevance of MFC-MRD.

The ICC 2022 classification was used as the primary diagnostic framework. Considering the subtle differences between the ICC and WHO classifications, which resulted in minor variations in cohort size, we repeated the analyses using the WHO classification. MFC-MRD remained prognostic in MDS irrespective of the classification system applied, demonstrating that the observed prognostic impact of MRD was not driven by classification-related differences but rather represented a robust biological finding.[1,20]

We observed that MRDpos MDS patients were enriched for genetic aberrations with known poor response to conventional chemotherapy, such as biallelic *TP53* mutations and complex karyotype. In line with the IPSS-M, we show that the presence of a biallelic *TP53* mutation in hrMDS patients is a strong independent predictor of worse survival, as well as MRD positivity.[3]

Given the known adverse prognosis of distinct molecular subgroups in MDS, we hypothesize that MFC-MRD may be influenced by clonal heterogeneity within MDS.[21–24] Thus, an extensive molecular taxonomy, rather than traditional classifications based on blast percentage and specific mutations, may better capture the underlying

biological differences between patients. Such a framework could ultimately enhance the interpretation and clinical utility of MFC-MRD and identify those who would benefit most from MFC-MRD measurements.[25]

Clinical relevance

To accurately interpret the clinical translatability of our findings, several important considerations and limitations must be highlighted. First, our study included high-risk MDS patients deemed fit for intensive AML-like chemotherapy. We acknowledge that such regimens are administered to only a limited group of MDS patients in specialized settings. Therefore, our results primarily reflect the prognostic value of MRD in a fit, intensively treated cohort rather than across the broader MDS population.

Second, achieving CR(i) in MDS is not universally accepted as a surrogate for long-term survival. The International Working Group (IWG) 2023 response criteria note that CR has been linked to improved survival, but caution that relying on CR alone may underestimate a treatment's clinical benefit. The IWG also highlights that the relevance of blast reduction prior to allogeneic HSCT, the only curative therapy for MDS, remains an area of active investigation and continues to evolve.[5]

Our findings directly align with the IWG recommendation for further studies in this context. Specifically, MRD positivity within high-risk MDS patients in morphologic remission was independently associated with inferior OS and higher relapse risk, even after adjusting for transplant as a time-dependent covariate. However, it is important to stress that our study does not advocate for universal intensive chemotherapy in MDS but rather characterizes the prognostic value of MRD among patients already treated with such regimens who achieved morphologic remission.

Third, the data show that the OS of hrMDS patients in this data set equaled that of ELN 2022 adverse-risk AML patients. This might be explained by the overlapping molecular profile between these groups, since myelodysplasia-related genetic abnormalities are part of the ELN AML risk classification and occurred significantly more frequently in adverse-risk AML patients compared to the favorable and intermediate risk.[9] This result supports the current international guidelines recommending allo-HSCT as the primary choice of consolidation for all eligible hrMDS patients.,[19,26,27] in contrast to AML, in which MRD results impact consolidation therapy decisions in intensively treated patients.

MFC-MRD and/or other MRD methodologies such as molecular MRD may also be of value in other clinical situations, especially in the post-allo-HSCT setting to guide immunosuppressive management, as shown recently by Tobiasson and colleagues utilizing patient-specific molecular MRD.[18,28] MRD may guide more or less intensive pre-allo-HSCT conditioning regimens, although no randomized data are available. In addition, given that hypomethylating agents represent a cornerstone of treatment for many patients with hrMDS, investigation of MRD dynamics in hypomethylating agents-treated patients represents an important and clinically relevant area for future studies; however, such analyses were beyond the scope of the present study.[4]

Finally, MFC-MRD could serve as a biomarker in clinical trials evaluating novel agents as an early indicator of efficacy. Our study was not designed to address these questions. Nevertheless, our results show that MRD positivity after intensive therapy is associated with increased relapse rates and worse survival among high-risk MDS patients and provide a rationale for prospective trials to evaluate its integration into MDS management algorithms.

Possibilities for MFC-MRD optimization

An optimized MDS-specific approach may improve the prognostic information of MFC-MRD in MDS, as the current ELN recommendations for MFC-MRD analysis were established for AML.[9,10] The need for refinement of MFC-MRD in MDS is highlighted by the observation of a high relapse rate in MRDneg patients and higher proportion of unassessable remission samples in MDS compared to AML. This may reflect underlying biological differences, as MDS patients often have more hypocellular marrow and may achieve CR after fewer cycles of therapy than AML patients. Differences in marrow cellularity, timing of remission, or other factors may contribute, but detailed data were not consistently available.

Although exclusion due to nonevaluable MRD samples did not introduce detectable selection bias in our study, the proportion of patients without assessable MRD underscores current practical challenges in implementing flow cytometry-based MRD assessment in large, multicenter cohorts. Adequate white blood cell event acquisition is required for accurate interpretation of MRD-negative results. However, in this retrospective pooled cohort, based on clinical trials performed in the past, the currently recommended threshold was not reached in all samples. As a result, low-event MRD-negative samples may have reduced sensitivity and possibly led to false-negative classification, which would be expected to weaken, rather than create, the association between MRD and survival. We re-analyzed the data without these 14 samples and the association was even stronger. Therefore, we retained these patients in the cohort to reflect the real-world setting in which clinical decisions occurred. At present, incorporation of MFC-MRD into clinical trials or routine clinical practice would most reliably be achieved through centralized analysis or through rigorously harmonized protocols across centers and platforms, in line with ongoing international standardization efforts led by the ELN.[10]

In our study, MRD was assessed using the LAIP-based approach with LAIPs optimized for AML.[9] In this approach, LAIPs are determined at diagnosis and followed during therapy. The Different-from-Normal (DfN) approach defines any combination of aberrant cell surface markers as residual disease by comparing them to marker expression in normal BM, an approach routinely used to observe dysplastic features in MDS diagnosis.[29] The DfN approach has a higher risk of false positivity due to marker expression profiles of regenerating bone marrow, which may complicate MRD interpretation in this setting.[18,30,31] Similarly, the role of the DfN approach remains to be defined in the setting of MDS, but may refine MFC-MRD detection. Lastly, another possibility for refinement of MFC-MRD in the hrMDS patients may be the addition of the assessment of immunophenotypic aberrant hematopoietic stem cells and/or the identification of MDS specific LAIPs.[32]

In summary, the current study showed that MFC-MRD measured in hrMDS patients reaching CR after the second cycle of intensive chemotherapy has prognostic value. Despite achieving MRD negativity after intensive chemotherapy, MDS patients show similarly poor survival as MRDpos AML patients. Overall, our findings support the rationale for prospective clinical trials to further investigate the prognostic value of MFC-MRD in MDS to evaluate its potential role in optimizing transplant strategies and guiding post-remission therapeutic decisions, and to underscore that MRD paradigms established in AML should not be automatically extrapolated to hrMDS but instead warrant disease-specific validation and refinement.

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AUTHOR CONTRIBUTIONS

Coen R. Veenstra: Writing—original draft; methodology; writing—review and editing; formal analysis; visualization. **Lok Lam Ngai**: Methodology; visualization; writing—review and editing; writing—original draft; formal analysis. **Patrycja Gradowska**: Writing—review and editing; methodology. **Jurjen Versluis**: Writing—review and editing; methodology. **Tom Reuvekamp**: Writing—review and editing. **Angèle Kelder**: Writing—review and editing; project administration. **Willemijn Scholten**: Writing—review and editing; project administration. **Sander Snel**: Writing—review and editing; project administration. **Jesse M. Tettero**: Writing—review and editing. **Costa Bachas**: Writing—review and editing. **Dimitri A. Breems**: Writing—review and editing. **Thomas Fischer**: Writing—review and editing. **Bjørn T. Gjertsen**: Writing—review and editing. **Laimonas Griskevicius**: Writing—review and editing. **Gunnar Juliusson**: Writing—review and editing. **Johan A. Maertens**: Writing—review and editing. **Markus G. Manz**: Writing—review and editing. **Thomas Pabst**: Writing—review and editing. **Jakob R. Passweg**: Writing—review and editing. **Kimmo Porkka**: Writing—review and editing. **Canan Alhan**: Writing—review and editing. **Theresia M. Westers**: Writing—review and editing. **David C. de Leeuw**: Writing—review and editing. **Bob Löwenberg**: Writing—review and editing. **Gert J. Ossenkoppele**: Writing—review and editing. **Jacqueline Cloos**: Writing—review and editing; supervision. **Arjan A. van de Loosdrecht**: Writing—review and editing; supervision; conceptualization.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data can be made available upon e-mail request with appropriate approval by the investigator team and research administrative offices from HOVON-SAKK.

ETHICS STATEMENT

The clinical trials included in this research were designed by the Acute Myeloid Leukemia working group of the Dutch-Belgian Cooperative Trial Group for Hematology-Oncology (HOVON) Foundation and the Swiss Group for Clinical Cancer Research (SAKK). The study protocols were approved by the ethics committee at each participating center and conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent for enrollment in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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