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ORIGINAL ARTICLE

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Prognostic impact of pretransplant measurable residual disease assessed by peripheral blood *WT1*-mRNA expression in patients with AML and MDS

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Abstract

Objective: As peripheral blood (PB) *Wilm's Tumor 1* (*WT1*)-mRNA expression is established as MRD-marker during conventional AML chemotherapy, impact of pretransplant *WT1* expression remains unclear. Therefore, we aimed to assess prognostic impact of pretransplant *WT1* expression on post-transplant outcome in patients with AML/MDS.

Methods: In 64 AML/MDS patients, pretransplant *WT1* expression was retrospectively analyzed using a standardized assay offering high sensitivity, specificity, and a validated cut-off. Patients were divided into three groups determined by pretransplant remission and *WT1* expression. Post-transplant outcome of these groups was compared regarding cumulative incidence of relapse (CIR), relapse-free (RFS), and overall survival (OS).

Results: Pretransplant forty-six patients (72%) showed hematologic remission, including 21 (46%) MRD-negative and 25 (54%) MRD-positive patients indicated by *WT1* expression, while 18 refractory patients (28%) showed active disease. Two-year estimates of post-transplant CIR, RFS, and OS were similar in MRD-positive (61%, 37%, 54%) and refractory patients (70%, 26%, 56%), but significantly inferior compared with MRD-negative patients (10%, 89%, 90%). After multivariable adjustment, pretransplant MRD negativity measured by *WT1* expression retained its prognostic impact on CIR ($P = .008$), RFS ($P = .005$), and OS ($P = .049$).

Conclusions: PB *WT1* expression represents a useful method to estimate pretransplant MRD, which is highly predictable for post-transplant outcome and may help improving peri-transplant management in AML/MDS patients.

Novelty Statement: Measurement of peripheral blood (PB) *Wilm's Tumor 1 (WT1)*-mRNA expression using a standardized assay represents a practicable approach to sensitively assess pretransplant minimal residual disease (MRD) and is applicable in the majority of patients with AML/MDS independent from disease-specific molecular features. Pretransplant PB *WT1* expression enables refined estimation of post-transplant relapse risk and overall survival in patients with AML and MDS. In patients with pretransplant MRD positivity assessed by *WT1* expression, an optimization of peri-/post-transplant management (eg, pretransplant salvage therapy, an intensification of conditioning regimen or prophylactic/pre-emptive treatment strategies post-transplant) appears reasonable to overcome the negative prognostic impact of pretransplant MRD.

KEYWORDS
acute myeloid leukemia, myelodysplastic syndrome, transplantation, measurable residual disease, WT1, relapse

1 | INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment approach for many patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). In patients with AML, allo-HSCT is most effective when performed in complete remission (CR), which is usually achieved after intensive chemotherapy (CTX).[1,2] The role of pretransplant cytoreduction in advanced MDS or AML derived from MDS (sAML) is still a matter of debate,[3-6] but those 20% to 50% of patients, who achieve remission either after CTX or hypomethylating agents (HMA), have a good chance to achieve long-term survival. Still, even in the absence of morphologically detectable disease at the time of transplant, relapse remains the main cause of treatment failure, suggesting that conventional morphology is incapable to detect clinically relevant amounts of malignant cells, which subsequently drive post-transplant relapse.[1,7] In accordance with this, several groups have shown in general that the presence of submicroscopic levels of leukemia (ie, measurable residual disease, MRD) detected by multiparametric flow cytometry (MFC), PCR-, or NGS-based techniques at the time of transplantation offers prognostic information in patients undergoing allo-HSCT in hematologic remission.[8-19] However, the following methodological and biological aspects limit their use in clinical routine: (1) molecular aberrations suitable for sensitive MRD monitoring are only present in subgroups of patients and/or instable during the course of disease; (2) lack of reproducible standardized assays and/or high technical and resource-consuming efforts; (3) the need for bone marrow (BM) as optimal sample source. In contrast, we and other have demonstrated that measurement of *Wilms Tumor 1 (WT1)*-mRNA expression offers several of these advantageous properties, as it is present in about 80 to 90% of patients with AML and advanced MDS and can be quantitatively measured in peripheral blood (PB) using a standardized assay with high sensitivity and specificity.[20-24] In the current retrospective analysis, we aimed to determine the prognostic impact of pretransplant MRD detected by PB *WT1*-mRNA expression on post-transplant outcome in patients with AML or MDS undergoing allo-HSCT in hematologic remission.

2 | METHODS

2.1 | Study Design

This retrospective analysis included 64 patients with AML (n = 50) without recurrent genetic abnormalities, advanced MDS, or chronic myelomonocytic leukemia (CMML) (n = 14), who were allografted at our center between March 2013 and January 2020 either in hematologic CR (n = 46, 72%) or with active disease after being refractory to chemotherapy (n = 18, 28%). Further inclusion criteria were PB *WT1*-mRNA overexpression at diagnosis and available information about PB *WT1*-mRNA expression level before transplantation. Post-transplant outcome in terms of overall (OS) and relapse-free survival (RFS) as well as relapse incidence (CIR) and non-relapse mortality (NRM) were compared between patients with MRD-positive (CR_{MRD+}) and MRD-negative (CR_{MRD-}) CR prior transplant assessed by PB *WT1*-mRNA expression status as well as 18 refractory patients with morphological evidence of disease at transplantation. All patients gave written informed consent according to the Declaration of Helsinki, and the analysis was approved of the institutional review board (approval numbers: 3973, 3768 and 3541).

2.2 | Quantitative assessment of peripheral blood cell WT1-mRNA expression

Quantitative assessment of *WT1*-mRNA expression in PB mononuclear cells was performed using the Ipsogen® WT1 ProfilQuant® Kit according to the manufacturers' instructions with technical and quality settings as previously described.[23] This plasmid-based, standardized, ELN-certified assay offers a validated cut-off level of 50 *WT1* copies/10^4 *ABL* copies in PB to distinguish between normal and overexpression of *WT1*-mRNA.[20] This cut-off was established and validated using 620 diagnostic and 129 follow-up samples from 504 AML patients and 118 peripheral blood samples from healthy volunteers in a systemic evaluation of nine published and in-house PCR-based assays in a network of 11 laboratories. As another advantage, this assay includes specific plasmids, primers, and probes, which enables stable and checkable performance of the assay and exact quantification of *WT1*-mRNA expression within each sample via standard curve. These properties make results and the specific cut-off reproducible and comparable between different laboratories when using this assay. All patients with CR prior transplant were categorized for post-transplant outcome analyses based on this validated cut-off level into those with normalized (defined as <50 *WT1* copies/10^4 ABL copies) PB *WT1*-mRNA expression representing CR_{MRD-} and those with *WT1*-mRNA overexpression (defined as >50 *WT1* copies/10^4 ABL copies) reflecting CR_{MRD+}. The results from MRD analyses were available to the transplant team.

2.3 | Definitions and Response Criteria

Remission status prior transplant and conditioning intensity was defined as previously reported.[2,25-28] Furthermore, depth of remission regarding MRD was defined as described above.[29] Post-transplant hematologic relapse was defined as ≥5% BM blasts, detection of

blasts in PB, and/or extramedullary disease. Molecular relapse after allo-HSCT was defined as presence of disease-specific cytogenetic aberrations, reoccurrence of known mutations in at least 1% of reads, a decrease in donor chimerism <95%, evidence of a mixed XY-FISH of >4% residual recipient cells, or an increase of PB *WT1*-mRNA expression above the cut-off of 50 copies per 10^4 *ABL* copies detected at two separate time points during an interval of 14 days and in absence of any criterion defining hematologic relapse. Both hematologic and molecular relapse were appraised as event for CIR and RFS.

2.4 | Statistical analyses

Data lock for this analysis was June 1, 2020. For categorical variables, frequencies were given and differences were estimated applying cross tabulation and Fisher's exact test. For continuous variables, medians (ranges) were given and the Mann-Whitney test was used to detect differences. OS was calculated as date from allo-HSCT to death from any cause or last follow-up in survivors. RFS was defined as time from allo-HSCT until (i) molecular or hematologic relapse or (ii) death with those censored at last contact who were alive and had not experienced relapse until then. Both OS and RFS were estimated using Kaplan-Meier method, and log-rank test was used for univariate comparisons. Relapse incidence and non-relapse mortality (NRM) were considered as competing risks and calculated using cumulative incidence (CI) estimates employing Gray test for univariate comparisons. Multivariate analysis was performed using a multiple Cox regression model with a step-wise backward procedure and included only variables influencing outcome in univariate analysis with a *P*-value <.1. For relapse incidence and NRM variables, multivariate analyses have to be interpreted as a cause-specific hazards model. In all analyses, a *P*-value <.05 was considered to be statistically significant. Statistical analyses were performed using GraphPad Prism® 5.01 (GraphPad Software Inc, La Jolla, USA), SPSS Statistic for Windows (SPSS Inc Chicago, IL), and R 4.0.0.

3 | RESULTS

3.1 | Patient Characteristics

For this analysis, we identified 46 patients with MDS/MPN (n = 8, 17%), sAML (n = 14, 30%), therapy-related (n = 3, 7%), or de novo AML (n = 21, 46%) with PB *WT1*-mRNA overexpression (median 2187.6, range 76 to 26 433.1 *WT1* copies per 10^4 ABL copies) at diagnosis, who underwent first allo-SCT in hematologic CR at our institution between March 2013 and January 2020. Besides *WT1*-mRNA overexpression at diagnosis, these patients had no molecular marker such as NPM1 or other recurrent genetic abnormalities suitable for sensitive PCR-based MRD monitoring. As indicated in Table S1, individual patients exhibited mutations in genes such as FLT3, DNMT3A, or ASXL1. However, these molecular markers are not optimal for MRD monitoring due to instability during course of disease, lack of sensitive assays in clinical routine, and/or association with clonal hematopoiesis. Detailed information regarding patients and transplant characteristics is summarized in Tables 1 and 2. Patients had achieved first (n = 44, 96%) or second CR (n = 2, 4%) after treatment with a median of 2 (range: 1-5) cycles of intensive CTX (n = 39, 85%) or after a median of 2 (range: 1-7) cycles of HMA (n = 7, 15%). Of these 46 patients, 21 (46%) were MRD-negative indicated by normalized PB *WT1*-mRNA values (<50 *WT1* copies per 10^4 ABL copies; median 7.3, range 0 to 42.6 *WT1* copies per 10^4 ABL copies), while 25 patients (54%) still showed *WT1*-mRNA overexpression in PB (≥50 *WT1* copies per 10^4 ABL copies; median 149, range 52 to 4 002.9 *WT1* copies per 10^4 ABL copies) thus being MRD-positive. The majority of patients received peripheral blood stem cells (n = 43, 93%) from a HLA-matched unrelated donor (n = 27, 59%) after reduced intensity conditioning (RIC, n = 30, 65%), whereas 16 patients (35%) underwent allo-HSCT after myeloablative conditioning according to the definitions determined by Bacigalpuo and colleagues.[27] Further details regarding conditioning regimen of the entire study population including refractory patients are displayed in Table S2. Median time between diagnosis and allo-HSCT was 3.7 months (range, 0.7 to 37.8) and was similar between CR_{MRD+} patients (median 3.6 months, range 0.7 to 18.6 months) and CR_{MRD-} patients (median 3.7 months, range 2.2 to 37.8 months, *P* =.772). Median time between MRD assessment and allo-HSCT was 21 days (range, 4 to 70) and also comparable between CR_{MRD+} patients (median 21 days, range 7-69) and CR_{MRD-} patients (median 20 days, range 4 to 70, *P* =.674). Furthermore, with the exception of a significantly higher frequency of an abnormal karyotype, there were no differences between patients with CR_{MRD+} and CR_{MRD-} regarding common patient-, disease-, or transplant-related factors (Table 2). Eighteen additional patients with AML, MDS, or CMML were primary refractory to induction chemotherapy and underwent allo-SCT during the same time-period with active hematologic disease. Baseline characteristics of these 18 patients were comparable to the 46 patients transplanted in CR (Table 2) including the fact that with except for *WT1*-mRNA overexpression they neither exhibited a molecular marker suitable for sensitive PCR-based MRD monitoring.

3.2 | Outcome after allo-HSCT

A total of 14 deaths, 21 relapses, and 2 NRM events contributed to the probability estimates for OS, RFS, relapse, and NRM stratified by MRD status in the 46 patients with CR at transplant. After a median post-transplant follow-up period of 33.7 months (range: 2.8-67.3 months), the 2-year OS, RFS, CIR, and NRM rate of these 46 patients with hematologic CR was 68% [95% CI: 51%-80%], 58% [95% CI: 41%-72%], 41% [95% CI: 27%-58%], and 4% [95% CI: 1%-17%], respectively (Figure 1). As indicated in Figure 2, in univariate analysis, the estimates for OS (two year probability: 54% vs 90%, log-rank *P* =.03), RFS (two year probability: 37% vs 89%, log-rank *P* <.01), and CIR (two year probability: 61% vs 10%, Gray test *P* <.01) significantly

TABLE 1 Patients demographics and transplant characteristics

Remission status prior allo-HSCT	CR (n = 46)		refractory (n = 18)		
Characteristic	**No.**	**%**	**No.**	**%**	***P***
Age, median (range), y	58 (21-73)		59 (27-69)		.61
Gender					.78
Male	25	54	11	61	
Female	21	46	7	49	
WHO 2016 diagnosis[a]					
AML					
AML-MRC	14	30	7	39	.56
t-MN	3	7	0	0	.55
AML NOS	20	43	5	28	.27
Myeloid sarcoma	1	2	0		>.99
MDS/MPN					
MDS-EB2	6	13	5	28	.27
CMML	2	4	1	6	>.99
Karyotype					
Normal	22	48	7	39	.58
Aberrant	24	52	11	61	
Complex	10	22	7	39	.21
ELN cytogenetic/molecular genetic risk[b]					
Favorable	0	0	0	0	>.99*
Intermediate	15	39	5	41	
Adverse	22	58	7	58	
Missing	1	3	0	0	
IPSS-R cytogenetic Risk[c]					
Very good/good	0	0	2	40	.08[#]
Intermediate	1	17	2	40	
Poor/very poor	5	83	1	20	
Disease Status at allo-HSCT[b/d]					
CR1	44	96	-		
CR2	2	4	-		
MRD Status prior transplant[e]					
CR_{MRD+}	25	54	18	100	
CR_{MRD-}	21	46	-	-	
Conditioning[f]					
Myeloablative	16	35	7	39	.78
Dose-reduced	30	65	11	61	
HCT-CI					
Low	27	59	11	61	>.99
Int/high	19	41	7	39	
Donor Type					
Matched related	9	20	5	28	.51
Matched unrelated	27	58	9	50	.58
Mismatched related	1	2	0	0	
Mismatched unrelated	9	20	4	22	>.99
Haploidentical	0	0	0	0	
Immunosuppression					
MMF + CSA	10	22	5	28	.74
MMF + FK506	36	78	13	72	
In vivo T-cell depletion					
Yes	36	78	13	72	.74
No	10	22	5	28	
Graft source					
PBSC	43	93	18	100	.55
BM	3	7	0	0	

Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia related changes; AML NOS, acute myeloid leukemia not otherwise specified; BM, bone marrow; CMML, chronic myelomonocytic leukemia; CR, complete remission; CSA, ciclosporin A; EB2, excess blasts 2; ELN, european leukemia net; FK506, Tacrolimus; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IPSS-R, revised international prognostic scoring system; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MPN, myeloproliferative neoplasia; MRD, minimal residual disease; no., number; *P*, *P*-value; PBSC, peripheral blood stem cells; t-MN, therapy-related myeloid neoplasm; WHO, world health organization; WT1, Wilm's Tumor 1; y, years.

[a]according to Arber et al Blood 2016.[25]

[b]according to ELN-criteria, Döhner et al Blood.[2]

[c]acoording to IPSS-R, Greenberg et al Blood 2012.[28]

[d]according to Cheson et al Blood 2006.[26]

[e]regarding WT1-mRNA expression (CR_{MRD+} = ≥ 50 WT1 copies/10^4ABL copies; CR_{MRD-} = <50 WT1 copies/10^4ABL copies).

[f]according to Bacigalupo et al BBMT 2009.[27]

*No significant differences regarding frequency of favorable and intermediate compared to adverse ELN risk categories among patients with hematologic CR prior transplant and refractory patients.

[#]No significant differences regarding frequency of very good/good and intermediate compared to poor and very poor IPSS-R cytogenetic risk categories among patients with hematologic CR prior transplant and refractory patients.

differed between patients with CR_{MRD+} and CR_{MRD-} suggesting that pretransplant MRD status assessed by *WT1*-mRNA expression enables risk stratification of patients undergoing allo-HSCT in hematologic CR. We then compared the outcome of patients receiving allo-HSCT in hematologic CR with the cohort of 18 patients with active disease at allo-HSCT. Of note, the outcome of CR_{MRD+} patients in terms of OS, RFS, and CIR did not differ with the outcome of refractory patients (OS (two year probability: 54% vs 56%, log-rank *P* =.94), RFS (two year probability: 37% vs 26%, log-rank *P* =.27), and CIR (two year probability: 61% vs 70%, Gray test *P* =.32), while CR_{MRD-} patients had a significantly better outcome (Figure 2).

Focusing on other patient-, disease-, or transplant-related parameters, we found that in addition to the MRD status, the presence of a complex karyotype, high-risk genetics, and the use of a related donor were associated with a shorter OS in univariate analysis. A complex KT was also the only factor besides MRD status, which showed an impact on RFS and relapse incidence in univariate analysis (Table S3). Due to the low number of events (n = 4), no parameter associated with NRM could be ascertained.

In multivariate analyses, pretransplant MRD negativity measured by *WT1*-mRNA expression was associated with longer RFS, lower relapse incidence, and longer OS than MRD positivity. While no other parameter besides MRD status influenced RFS and relapse

TABLE 2 Comparison of patient- and transplant-related characteristics according to MRD status prior transplant assessed by WT1-mRNA expression in patients with hematologic complete remission

Characteristic	All	CR_{MRD+} #	CR_{MRD-} #	*P*
No. of patients	46 (100%)	25	21	
Follow-up, median (range), mo	33.7 (2.8-67.3)	37.4 (2.8-59.4)	26.3 (2.9-67.3)	.77
Age, median (range), y	58 (21-73)	61 (24-69)	56 (21-52)	.14
Gender				>.99
Male	22 (48%)	12	10	
Female	24 (52%)	13	11	
WHO 2016 diagnosis[a]				
AML				
AML-MRC	14 (30%)	10	4	*, &, §
De novo AML (therapy rel AML, AML NOS, Myeloid sarcoma)	24 (52%)	10	14	
MDS/MPN				
MDS-EB2	6 (13%)	4	2	
CMML	2 (4%)	1	1	
Karyotype				
Normal	22 (48%)	8	14	.04
Aberrant	24 (52%)	17	7	.31
Complex	10 (22%)	7	3	
Cytogenetic risk category[b]				
Low/int	16 (35%)	7	9	.36
High	27 (59%)	16	11	
Missing	3 (7%)	2	1	
WT1-mRNA expression at diagnosis, median (range)	2188 (74-26433)	2140 (94-19547)	2397 (74-26433)	.76
FLT3-ITD mutation status				
mut and high ratio	8 (17%)	3	5	.45
wt or mut and low ratio	31 (67%)	17	14	
Missing	7 (15%)	5	2	
Time to between diagnosis to allo-HSCT, months, median (range)	3.7 (0.7-37.8)	3.6 (0.7-18.8)	3.7 (2.2-37.8)	.74
HCT-CI				
Low	27 (59%)	12	15	.14
Int/high	19 (41%)	13	6	
Conditioning[c]				
Myeloablative	16 (35%)	7	9	.36
Dose-reduced	30 (65%)	18	12	
Donor Type				
Related	10 (22%)	5	5	>.99
Unrelated	36 (78%)	20	16	

(Continues)

Characteristic	All	CR_{MRD+} #	CR_{MRD-} #	*P*
HLA matching				
Matched	36 (78%)	20	16	>.99
Mismatched	10 (22%)	5	5	

Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; AML NOS, acute myeloid leukemia not otherwise specified; AML-MRC, acute myeloid leukemia with myelodysplasia related changes; BM, bone marrow; CMML, chronic myelomonocytic leukemia; CR; complete remission; EB2, excess blasts 2; ELN, european leukemia net; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IPSS-R, revised international prognostic scoring system; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasia; MRD, minimal residual disease; mut, mutation; no., number; PBSC, peripheral blood stem cells; rel, related; WHO, world health organization; wt, wildtype; WT1, Wilm's Tumor 1; y, years.

[a]according to Arber et al Blood 2016[25]

[b]for MDS IPSS-R risk cytogenetics were applied (Greenberg et al Blood 2012,[28]) and very good, good, and intermediate risk categories were assigned to low/intermediate group, whereas poor and very poor risk categories were included into high-risk group; for AML ELN risk cytogenetics were used (Döhner et al Blood 2017, (2)) and favorable and intermediate risk categories were assigned to low/intermediate risk group, whereas adverse risk category was included into high-risk group.

[c]according to Bacigalupo et al BBMT 2009.[27]

#regarding WT1-mRNA expression (CR_{MRD+} = ≥ 50 WT1 copies/10^4ABL copies; CR_{MRD-} = < 50 WT1 copies/10^4ABL copies).

*No significant differences regarding the frequency of AML diagnosis compared to MDS/MPN diagnosis among the CR_{MRD+} and CR_{MRD-} group (*P* =.71).

&No significant differences regarding the frequency of MDS/MPN/sAML diagnosis compared to diagnosis of de novo AML among the CR_{MRD+} and CR_{MRD-} group (*P* =.09).

§No significant differences regarding the frequency of MDS diagnosis compared to diagnosis of sAML among the CR_{MRD+} and CR_{MRD-} group (*P* >.99).

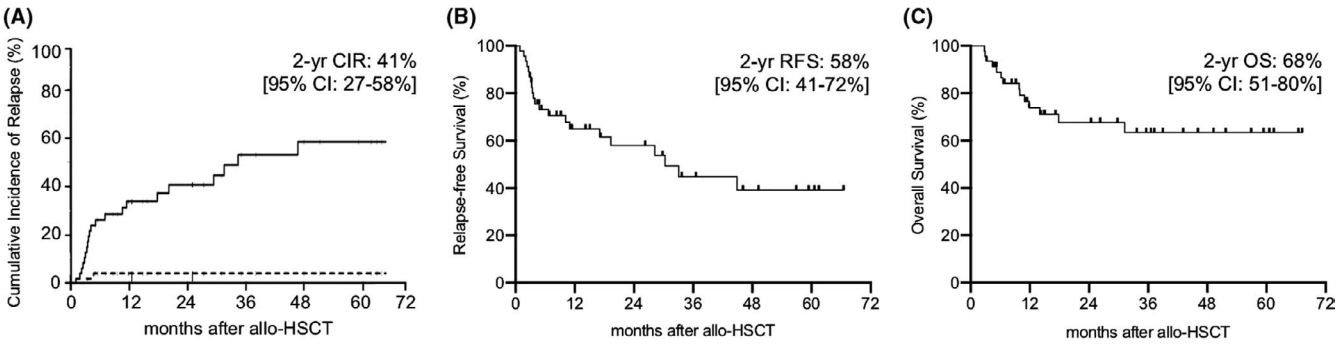

FIGURE 1 Post-transplant outcome of 46 patients receiving allogeneic transplantation in hematologic complete remission. Figure 1 represents outcome in terms of (A) cumulative incidence of relapse and non-relapse mortality (dotted line), (B) relapse-free, and (C) overall survival for patients with hematologic complete remission at time of transplantation

incidence, the use of a related donor was confirmed as predictor for shorter OS (Table 3).

3.3 | Relapse pattern and salvage therapy

Of the 46 patients with hematologic remission at allo-SCT, twenty-one (46%) experienced either molecular (n = 8, 38%) or hematologic (n = 13, 62%) relapse after a median of 11 months (range, 1-66 months). This included 18 (86%) of CR_{MRD+} patients and 3 (14%) of CR_{MRD-} patients (Table S4). Of the 25 CR_{MRD+} patients, 24 achieved MRD negativity after transplant, while one patient did not achieve MRD negativity and immediately showed disease progression. Of the remaining 17 relapses within the CR_{MRD+} cohort, 14 showed rise of *WT1*-mRNA expression at the time of relapse, while 3 patients remained below the validated cut-off of 50 *WT1*-mRNA copies, thus being in excellent agreement with our previous results.[23] All 3 patients in the CR_{MRD-} cohort, who relapsed after allo-SCT, showed rise of *WT1*-mRNA expression at relapse (Figure S1). Of interest, the time to relapse did not differ between CR_{MRD+} patients and those relapsing patients, who had undergone allo-HSCT with active hematologic disease (median time 3.8 months vs 4.3 months, *P* =.55). Nineteen of the 21 relapsing patients (90%) received a salvage therapy consisting of HMA and donor lymphocyte infusions (DLI), which induced CR in 9 of them (47%), while the 2 other patients received radiotherapy and DLI or best supportive care, respectively. This also includes all 8 patients with molecular relapse. All of them were treated with HMA at the stage of molecular relapse, which led to CR in 5 (63%) of them.

4 | DISCUSSION

The results from this analysis show that measurement of PB *WT1*-mRNA expression using an ELN-certified assay enables determination of MRD in patients with hematologic remission prior transplant,

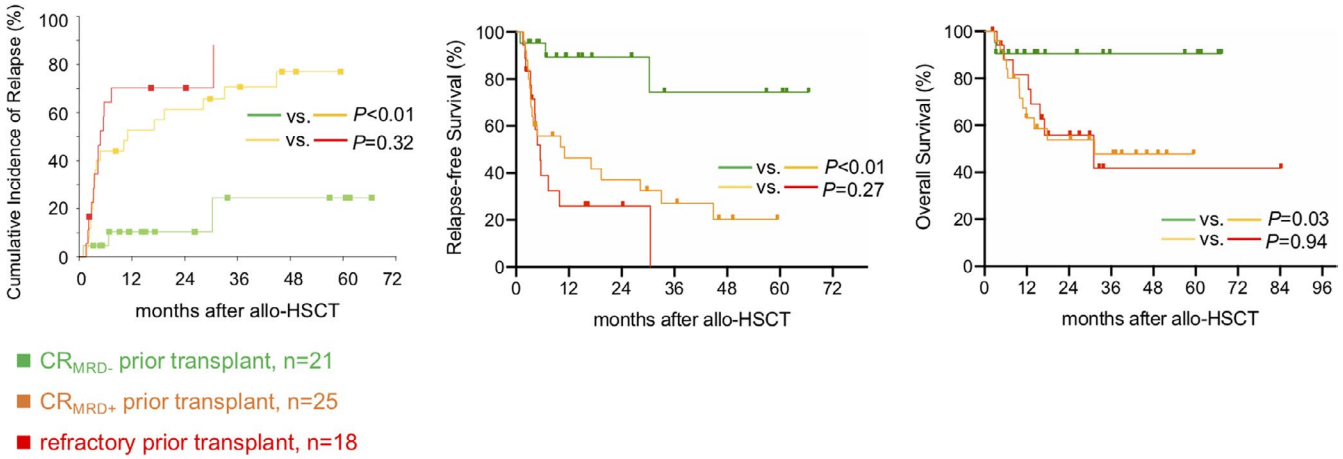

FIGURE 2 Post-transplant outcome of patients depending on MRD status assessed by PB *WT1*-mRNA expression. Figure 2 represents outcome in terms of (A) cumulative incidence of relapse, (B) relapse-free, and (C) overall survival for patients with pretransplant CR_{MRD-} (n = 21, green line), CR_{MRD+} (n = 25, orange line), and for those patients being refractory to salvage therapy prior transplant (n = 18, red line)

TABLE 3 Impact of clinical parameters on outcome after allogeneic transplantation for MDS/MPN and AML—multivariate analysis

Variable	Cumulative incidence of relapse (CIR) *P*-value HR (95% CI)	Relapse free survival (RFS)	Overall survival (OS)
Diagnosis			
MDS/MPN/sAML	-	-	ns
vs de novo AML			
Karyotype			
Complex	ns	ns	ns
vs not complex			
MRD status prior Tx[#]			
CR_{MRD+}	<0.01	<0.01	0.05
vs CR_{MRD-}	5.3 (1.6-18.4)	5.8 (1.7-19.7)	4.5 (1.0-20.2)
Donor	-	-	
Related			0.03
vs unrelated			3.4 (1.1-10.2)

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; HR, hazard ratio; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasia; MRD, minimal residual disease; ns, not significant; OS, overall survival; RFS, relapse-free survival; sAML, secondary AML; Tx, transplant; y, years; "-" means not applicable.

[#]Regarding WT1-mRNA expression (MRD+ = ≥50 WT1 copies/10^4ABL copies; MRD- = <50 WT1 copies/10^4ABL copies).

who do not carry another marker suitable for MRD monitoring in clinical routine, and thereby facilitates prediction of post-transplant relapse and outcome.

The finding that the presence of MRD prior allogeneic transplantation in patients with AML and MDS is associated with higher relapse risk and poorer survival after transplant is not new. This has already been demonstrated for the use of MFC,[8-11] PCR-based analyses in molecular defined subgroups as exemplified in NPM1-mutant AML[13,14] and NGS.[18,19] Thus, our results obtained by measurement of PB *WT1*-mRNA expression are in agreement with these above mentioned methods as well as with studies regarding *WT1*-mRNA, which employed non-standardized in-house assays or used BM as sample source.[30,31] However, determination of *WT1*-mRNA expression may have some advantages over the above mentioned methods: Molecular markers approachable by sensitive PCR assays such as NPM1 (~25%) or core-binding factor aberrations (~10%) are only present in subgroups of patients.[32] Furthermore, markers such as FLT3 mutations are instable during the course and therefore suboptimal and not recommended for MRD monitoring.[29,33] In contrast, *WT1*-mRNA overexpression is found in 80 to 90% of patients with AML and advanced MDS as well as stable during the course[20-24] and consequently recommended if no other suitable marker is

available.[29] Besides this broad applicability, the availability of a standardized assay with a reproducible cut-off value and an assay-given sensitivity of $1:10^4$ are another advantage of *WT1*-mRNA monitoring, especially in comparison with MFC. Error-corrected NGS approaches overcome some of these limitations, as they offer high sensitivity and broad applicability if several markers are used in each patient.[18,19] Nevertheless, biological aspects have to be considered and may hamper interpretation of results from NGS-based approaches. In detail, persistence of mutations in genes such as DNMT3A, TET2, and ASXL1 (so called DTA mutations) may be associated with preexisting clonal hematopoiesis and did not seem to have prognostic implications post-transplant outcome.[18,19] In other cases, persistence of these mutations may also be a consequence of persistence in differentiated cells or germ line origin. Furthermore, high technical and resource-consuming demands as reflected by a realistic turnaround time of 2-3 weeks have limited the wide use of NGS in clinical routine so far.[18] In further support of its practicability, the turnaround time for *WT1*-mRNA measurement in our routine is 4 days (range: 2-7 days, data not shown). Finally, in contrast to the other MRD methods, *WT1*-mRNA expression can be sensitively measured in PB thereby enabling frequent monitoring with sustained patient comfort.

The results from our analysis do not only indicate that measurement of PB *WT1*-mRNA expression is a practicable and valuable approach to measure MRD and to estimate post-transplant relapse risk and survival. They also point toward two other important aspects: (1) the outcome in terms of OS, RFS, and relapse incidence as well as the time to relapse in patients with hematologic remission, but detectable MRD (CR_{MRD+}) is as worse as the outcome of those refractory patients with hematologic evidence of disease. This suggests that the presence of MRD is not only a quantitative marker reflecting low detectable disease burden, but in particular also a biological property of the leukemic cells and a qualitative indicator of an intrinsic disease resistance. (2) This triggers the question how we should therapeutically address the issue of MRD positivity prior transplant in our patients in the future. One option may be the incorporation of novel compounds such as CPX-351[34] or venetoclax-based combinations[35] either as first-line therapy to induce higher rates of MRD negativity or as salvage therapy in MRD-positive patients prior transplant. Secondly, intensification of the conditioning regimen if feasible may also help to overcome the negative prognostic impact of MRD, as recently demonstrated.[19] Finally, prophylactic or preemptive treatment strategies after transplantation, for example discontinuation of immunosuppression and/or DLI,[36] hypomethylating agents,[37,38] or FLT3 inhibitors[39,40] may also reduce relapse risk and improve outcome of MRD-positive patients. The latter may explain why in our analysis the presence MRD prior transplant was associated with higher relapse incidence and lower RFS, but only with borderline significance with lower OS in multivariate analysis, since 7 CR_{MRD+} patients (=39%) relapsing after allo-SCT could be successfully treated with HMA and DLI.

In summary, measurement of PB *WT1*-mRNA expression with a standardized assay is a practicable and efficient approach to sensitively assess MRD prior transplant, which is applicable in the majority of patients with AML and MDS independent from the molecular profile, enables refined estimation of relapse risk and survival, and may help to optimize transplantation and post-transplant management.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

CR and TS conceptualized the study and writing—original draft. CR, ML, UG, and TS involved in formal analysis and investigation. CR, PJ, CF, SP, and TS involved in methodology. CR and ML visualized the study. CR, JK, RH, GK, UG, and TS involved in writing—review and editing. All authors involved in final approval of the manuscript.

DATA AVAILABILITY STATEMENT

Datasets generated and analyzed for study can be made available upon reasonable request. Decisions regarding data sharing will be made on a case-by-case basis by the corresponding author considering data protection and other applicable regulations. Proposals may be submitted by the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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