







## ORIGINAL PAPER

# Impact of measurable residual disease on outcomes of unrelated donor haematopoietic cell transplantation with post-transplant cyclophosphamide in AML in first complete remission

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## Summary

Pre-transplant measurable residual disease (MRD) predicts relapse and outcome of allogeneic haematopoietic cell transplantation (allo-HCT). The impact of MRD on the outcomes of post-transplant cyclophosphamide (PTCy)-based allo-HCT from a

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Presented as an oral abstract at Annual European Haematology Association (EHA) meeting, 2021.

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matched unrelated donor (UD) is unknown. This study assessed the impact of MRD in acute myeloid leukaemia (AML) in the first complete remission (CR1). A total of 272 patients (MRD negative [MRD<sup>-</sup>],  $n = 165$ ; MRD positive [MRD<sup>+</sup>],  $n = 107$ ) with a median follow-up of 19 (range: 16–24) months were studied. The incidence of grades II–IV and grades III–IV acute GVHD at day 180 was 25.2% and 25% ( $p = 0.99$ ), and 10.6% and 6.8% ( $p = 0.29$ ), respectively, and 2-year chronic GVHD was 35% and 30.4% ( $p = 0.96$ ) in MRD<sup>+</sup> and MRD<sup>-</sup> cohorts, respectively. In multivariate analysis, MRD<sup>+</sup> status was associated with a higher incidence of relapse (RI) (hazard ratio [HR] = 2.56, 95% CI: 1.39–4.72), lower leukaemia-free survival (LFS) (HR = 2.04, 95% CI: 1.23–3.39), overall survival (OS) (HR = 1.83, 95% CI: 1.04–3.25) and GVHD-free, relapse-free survival (GRFS) (HR = 1.69, 95% CI: 1.10–2.58). MRD status did not have a significant impact on non-relapse mortality (NRM), or acute or chronic GVHD risk. Among patients with AML undergoing UD allo-HCT with PTCy, pre-transplant MRD<sup>+</sup> status predicted a higher relapse rate, lower LFS, OS and GRFS.

#### KEY WORDS

acute myeloid leukaemia, allogeneic haematopoietic cell transplantation, measurable residual disease, post-transplant cyclophosphamide, unrelated donor

## INTRODUCTION

Allogeneic haematopoietic cell transplantation (allo-HCT) is an important therapeutic intervention providing durable remission from acute myeloid leukaemia (AML). Disease relapse after allo-HCT remains the main cause of death in patients with AML since survival remains dismal despite salvage therapies.<sup>1,2</sup> It is imperative to understand factors associated with increased relapse risk to improve the outcomes of allo-HCT. Traditional risk factors such as baseline cytogenetic risk and disease status at the time of transplant are routinely used for risk stratification in clinical trials and patient counselling before allo-HCT.<sup>3,4</sup> Persistent measurable residual disease (MRD<sup>+</sup>) has emerged as an important prognostic factor even after adjusting for cytogenetic risk.<sup>5–8</sup> Consolidative allo-HCT may overcome the increased relapse risk among patients with MRD<sup>+</sup> AML after induction therapy.<sup>9–11</sup> Hourigan et al. analysed the outcomes after allo-HCT for AML between patients receiving myeloablative conditioning (MAC) versus reduced intensity conditioning (RIC) regimens in the BMT CTN 0901 phase III trial, stratified by pre-transplant MRD status. It showed that the use of MAC was associated with a reduced relapse risk among patients with MRD<sup>+</sup> compared to RIC.<sup>12</sup> The relapse rate was higher in RIC than in MAC (1-year cumulative incidence, 47% vs. 15%;  $p < 0.001$ ) among MRD<sup>+</sup> patients. Previous retrospective studies have shown similar results with higher relapse risk in patients with pre-HCT MRD<sup>+</sup> compared to MRD<sup>-</sup> patients with AML.<sup>13–15</sup>

Most published studies on the impact of MRD on transplant outcomes are conducted in the setting of traditional calcineurin inhibitor (CNI)-based graft-versus-host disease (GVHD) prophylaxis. Post-transplant cyclophosphamide (PTCy) has emerged as an effective platform to prevent GVHD in the setting of partially or fully human leukocyte antigen (HLA)-matched donor allo-HCT.<sup>16–20</sup> In PROGRESS III (BMT CTM 1703), PTCy resulted in superior

GVHD-relapse-free survival (GRFS) compared to CNI-based regimen in patients receiving RIC-matched donor peripheral blood stem cell transplantation.<sup>21</sup> Similarly, BMT CTN 1301 showed comparable outcomes between PTCy versus tacrolimus/methotrexate in the setting of MAC bone marrow transplant from matched donors.<sup>22</sup> These results are supported by increasing use of PTCy in matched donor setting at many transplant centres. PTCy facilitates the selective proliferation of donor regulatory T cells (Treg) while ablating proliferating Natural Killer (NK) cells.<sup>23,24</sup> Impaired early NK cell immune reconstitution after PTCy is associated with increased relapse and lower survival.<sup>25</sup> The impact of PTCy on the graft-versus-leukaemia (GVL) effect is unknown with one registry-based study showing no protective effect of GVHD on relapse risk<sup>26</sup> contrary to what has been described with CNI-based allo-HCT.<sup>27</sup> Previous retrospective studies have shown conflicting results when it comes to the impact of pre-transplant MRD on outcomes of patients with AML who underwent haplo-identical (haplo)-HCT with PTCy.<sup>28,29</sup> A stronger GVL effect due to HLA disparity is proposed to be protective against the negative impact of MRD in patients who received haplo-HCT with PTCy. There is a lack of published literature exploring the impact of MRD in recipients of the unrelated donor (UD) allo-HCT with PTCy. Here, we used a registry-based dataset to investigate the impact of pre-transplant MRD in patients with AML after UD allo-HCT with PTCy.

## PATIENTS AND METHODS

### Study design and data collection

This was a retrospective, multicentre analysis using the dataset of the Acute Leukaemia Working Party (ALWP) of the European Society for Blood and Marrow

Transplantation (EBMT). The EBMT is a voluntary working group of more than 600 transplant centres that are required to report all consecutive stem cell transplantations and follow-ups once a year. EBMT minimum essential data forms are submitted to the registry by transplant centre personnel. Accuracy of data is assured by the individual transplant centres and by quality control measures such as regular internal and external audits. The results of disease assessments at allo-HCT were also submitted and form the basis of this report. Eligibility criteria for this analysis included adult patients ( $\geq 18$  years) with de novo AML in first complete remission (CR1), who received a first non-T cell depleted allo-HCT with PTCy from a 9/10 or 10/10 HLA-matched UD between January 2010 and June 2021 in the EBMT/ALWP registry. The exclusion criteria were secondary AML, AML not in CR1, or cases without information on cytogenetics or pre-transplant MRD status, allo-HCT from other donor types (sibling, haplo or umbilical cord blood), previous history of transplantation, use of ex vivo T-cell depleted haematopoietic cell graft. Data analysed included recipient and donor characteristics such as age, gender, cytomegalovirus (CMV) serostatus and disease-related characteristics such as cytogenetic risk per revised 2010 United Kingdom (UK)-Medical Research Council (MRC),<sup>30</sup> MRD status at transplantation, type of conditioning regimen and stem cell source. The conditioning regimen was defined as MAC or RIC based on the reports from individual transplant centres as per previously established criteria.<sup>31</sup> Techniques used for MRD assessment consisted of polymerase chain reaction (PCR) techniques alone, multiparameter flow cytometry, both techniques. The conditioning regimen was defined as MAC when containing total body irradiation (TBI) with a dose  $>6$  Gray or a total dose of busulfan  $>8$  or  $>6.4$  mg/kg when administered orally or intravenously, respectively. All other regimens were defined as RIC.<sup>31</sup> Regimens for GVHD prophylaxis were PTCy with additional immunosuppression per institutional protocol. Grading of acute GVHD was performed using established criteria.<sup>32</sup> Chronic GVHD was classified as limited or extensive according to published criteria.<sup>33</sup> The list of institutions contributing data to this study is provided in Appendix S1.

## Statistical analysis

The study endpoints were overall survival (OS), leukaemia-free survival (LFS), relapse incidence (RI), non-relapse mortality (NRM), engraftment, acute GVHD, chronic GVHD and GRFS. All endpoints were measured from the time of transplantation. Median follow-up was calculated using the reverse Kaplan–Meier (KM) method. OS was defined as time to death from any cause. LFS was defined as survival with no evidence of relapse or progression. NRM was defined as death from any cause without previous relapse or progression.<sup>34</sup> We used modified GRFS criteria. GRFS events were defined as the first event among grades III–IV acute GVHD,

extensive chronic GVHD, relapse or death from any other cause.<sup>35</sup> The median, range and interquartile range (IQR) were used for continuous variables, and frequency and percentage for categorical variables. Patient-, disease- and transplant-related characteristics were compared between the two groups (MRD– and MRD+) using the Mann–Whitney *U* test for numerical variables, and the chi-squared or Fisher's exact test for categorical variables. The probabilities of OS, LFS and GRFS were calculated using the KM estimates. The RI and NRM were calculated using cumulative incidence (CI) curves in a competing risk setting, death in remission being treated as a competing event for relapse. To estimate the CI of acute or chronic GVHD, relapse and death were considered as competing events. Univariate analyses were performed using the log-rank test for LFS, OS and GRFS while Gray's test was used for CI. Multivariate analyses were performed using the Cox proportional-hazards regression model which included variables differing significantly between the groups, factors known to be associated with outcomes, plus a centre 'frailty' effect to take account of the heterogeneity across centres. Results were expressed as the hazard ratio (HR) with a 95% confidence interval (95% CI). All tests were two-sided with the type 1 error rate fixed at 0.05. Statistical analyses were performed with SPSS 27.0 (SPSS Inc.) and R 4.1.1 (R Development Core Team, <https://www.R-project.org/>).

## RESULTS

### Patient, disease and transplantation characteristics

Table 1 shows the baseline characteristics. A total of 272 patients met the inclusion criteria: 107 (39.3%) with MRD+ and 165 (60.7%) with MRD– status before transplant. The median duration of follow-up from allo-HCT was 24 (IQR: 15–27) and 18 (IQR: 15–22) months, respectively ( $p = 0.74$ ). The median year of allo-HCT was 2019 in both the MRD+ and MRD– cohorts. The median patient age was higher in the MRD+ compared to the MRD– cohort (54.3 vs. 48.5 years,  $p = 0.002$ ). There were more patients with adverse risk cytogenetics in MRD– cohort compared to MRD+ cohort (23% vs. 13.1%,  $p = 0.013$ ). The difference in FMS-like tyrosine kinase 3-internal tandem duplication (*FTL3-ITD*) status, donor HLA matching (HLA 9/10 vs. 10/10), baseline recipient and donor CMV serotype, and distribution of graft source was not statistically significant between the cohorts. In both cohorts, most transplants were from 10/10 UD (65.8%) using peripheral blood (PB) stem cells (94.1%). Karnofsky performance status (KPS) score was  $<90$  in 21.8% versus 33% of patients in MRD– and MRD+, respectively ( $p = 0.045$ ). RIC was used more frequently in MRD+ compared to MRD– (45.8% vs. 30.3%,  $p = 0.009$ ). The most common conditioning regimens were busulfan, fludarabine (48.9%), followed by TBI-based (18%) and thiotepa-busulfan-fludarabine (TBF) (15.4%). All patients

**TABLE 1** Baseline demography according to measurable residual disease status.

	MRD negative (n = 165)	MRD positive (n = 107)	p value
Median FU (reverse KM) mo, Median (IQR)	18 [15–22]	24 [15–27]	0.74
Patient age (years), Median (min-max) [IQR]	48.5 (18.9–74.2) [36.1–57.6]	54.3 (18.2–75.8) [45.4–61.8]	0.002
Year of transplant, Median (min-max)	2019 (2012–2021)	2019 (2010–2021)	0.093
Cytogenetics risk <sup>a</sup>			
Favourable	12 (7.3%)	18 (16.8%)	0.013
Intermediate	115 (69.7%)	75 (70.1%)	
Adverse	38 (23%)	14 (13.1%)	
<i>FLT3-ITD</i> positive	47 (59.5%)	32 (50%)	0.26
Missing	86	43	
HLA match for UD			
10/10	109 (66.1%)	70 (65.4%)	0.91
9/10	56 (33.9%)	37 (34.6%)	
Patient gender			
Male	96 (58.2%)	59 (55.1%)	0.62
Female	69 (41.8%)	48 (44.9%)	
Donor gender			
Male	123 (75%)	78 (73.6%)	0.79
Female	41 (25%)	28 (26.4%)	
Missing	1	1	
Female-to-male combination	22 (13.3%)	12 (11.2%)	0.61
Time diagnosis to HCT (months), Median (min-max) [IQR]	5.5 (1.6–21.8) [4.4–7.3]	5.4 (2.9–21.9) [4.4–7.5]	0.82
Conditioning intensity			
MAC	115 (69.7%)	58 (54.2%)	0.009
RIC	50 (30.3%)	49 (45.8%)	
Conditioning regimen			
BuCy	7 (4.2%)	4 (3.7%)	0.045
BuFlu	90 (54.6%)	43 (40.2%)	
TBF	22 (13.3%)	20 (18.7%)	
FluMel	11 (6.7%)	7 (6.5%)	
FluTreo	7 (4.2%)	8 (7.5%)	
TBI based	25 (15.2%)	24 (22.4%)	
Other CT	3 (1.8%)	1 (0.9%)	
KPS ≥90	122 (78.2%)	69 (67%)	
Missing	9	4	
Cell source			
BM	7 (4.2%)	9 (8.4%)	0.15
PB	158 (95.8%)	98 (91.6%)	
Patient CMV serotype positive	126 (76.8%)	72 (67.3%)	0.084
Missing	1	0	
Donor CMV serotype positive	76 (46.1%)	47 (43.9%)	0.73
In vivo T-cell depletion			
In vivo TCD	28 (17%)	17 (15.9%)	0.81

Abbreviations: BM, bone marrow; Bu, busulfan; CMV, cytomegalovirus; Cy, cyclophosphamide; *FLT3, ITD*, FMS, like tyrosine kinase 3, internal tandem duplication; Flu, fludarabine; FU, follow up; HCT, haematopoietic cell transplant; HLA, human leukocyte antigen; IQR, interquartile range; KPS, Karnofsky performance status; MAC, myeloablative conditioning; Mel, melphalan; MRD, measurable residual disease; PBSC, peripheral blood stem cells; RIC, reduced, intensity conditioning; TBF, thiotepa busulfan fludarabine; TBI, total body irradiation; TCD, T cell depletion; Treo, treosulfan; UD, unrelated donor.

<sup>a</sup>Per UK MRC.

received PTCy. Most patients received either cyclosporin with mycophenolate mofetil (MMF) (31.6%) or tacrolimus with MMF (25%) as additional immunosuppression for GVHD prevention as the most patient received a PB graft. A full list of additional immunosuppressive drugs used in each study cohort is provided in [Table S1](#). In vivo T cell depletion was used in 17 (15.9%) MRD+ patients and 28 (17%) MRD- patients ( $p = 0.81$ ). MRD testing methodology varied between the centres with most centres using a combination of polymerase chain reaction (PCR) and flow cytometry (45.4%) per a recent ALWP survey,<sup>36</sup> however, this information was missing for most study participants (56.3%).

## Transplantation outcomes

Graft failure was reported in 1.9% (MRD+) versus 2.5% (MRD-) of patients. The incidence of grades II-IV (25.2%; 95% CI: 17.2-34 and 25%; 95% CI: 18.5-31.9) and grades III-IV (10.6%; 95% CI: 5.6-17.5 and 6.8%; 95% CI: 3.6-11.5) acute GVHD at day 180 was not statistically different between MRD+ and MRD- cohorts, respectively. The incidence of overall and extensive chronic GVHD at 2 years was 35% (95% CI: 24.2-46.1) and 14.9% (95% CI: 7.5-24.7) in MRD+ patients versus 30.4% (95% CI: 22.1-39.1) and 7.9% (95% CI: 3.8-13.8) in MRD- patients ( $p = 0.96$  and  $0.40$ , respectively) (results not shown in tables). Two-year relapse was higher among MRD+ patients (32.4% [95% CI: 22.4-42.8] vs. 19.7% [95% CI: 13.1-27.2]; HR = 2.56 [95% CI: 1.39-4.72];  $p = 0.056$ ). The median time to relapse after allo-HCT was comparable between the study cohorts (MRD+, 5.8 months [range 2.5-41.5]; MRD-, 5.6 months [range 1.5-55]). MRD+ patients experienced a lower 2-year LFS (56.5% [95% CI: 44.8-66.7] vs. 70.2% [95% CI: 61.2-77.5]; HR = 2.04 [95% CI: 1.23-3.39];  $p = 0.006$ ), OS (64.9% [95% CI: 53.1-74.4] vs. 76.5 [95% CI: 67.8-83.1]; HR = 1.83 [95% CI: 1.04-3.25];  $p = 0.037$ ) and GRFS (42% [95% CI: 30.9-52.7] vs. 59.8% [95% CI: 50.7-67.8]; HR = 1.69 [95% CI: 1.1-2.58];  $p = 0.016$ ) compared to MRD- patients. There was no significant difference in NRM (11% [95% CI: 5.4-18.9] vs. 10.2% [95% CI: 5.7-16.1]; HR = 1.29 [95% CI: 0.49-3.37];  $p = 0.61$ ) between study cohorts ([Figure 1](#)). The following factors were identified as having an independent prognostic impact on outcomes in the multivariate analysis. Adverse-risk cytogenetics was associated with the highest RI, which resulted in lower LFS, OS and GRFS compared to favourable/intermediate-risk cytogenetics. The type of conditioning regimen (RIC vs. MAC) did not impact RI, LFS or OS. A transplant from HLA 9/10 UD was associated with a higher risk of chronic GVHD compared to HLA 10/10 UD. A longer time from diagnosis to HCT was associated with a lower relapse and improved LFS. Older patients had a higher NRM, lower grades II-IV acute GVHD and a lower OS ([Table 2](#)). There was no statistically significant interaction between the development of grades II-IV acute GVHD

or chronic GVHD and LFS in either study cohorts (data not shown).

## Cause of death

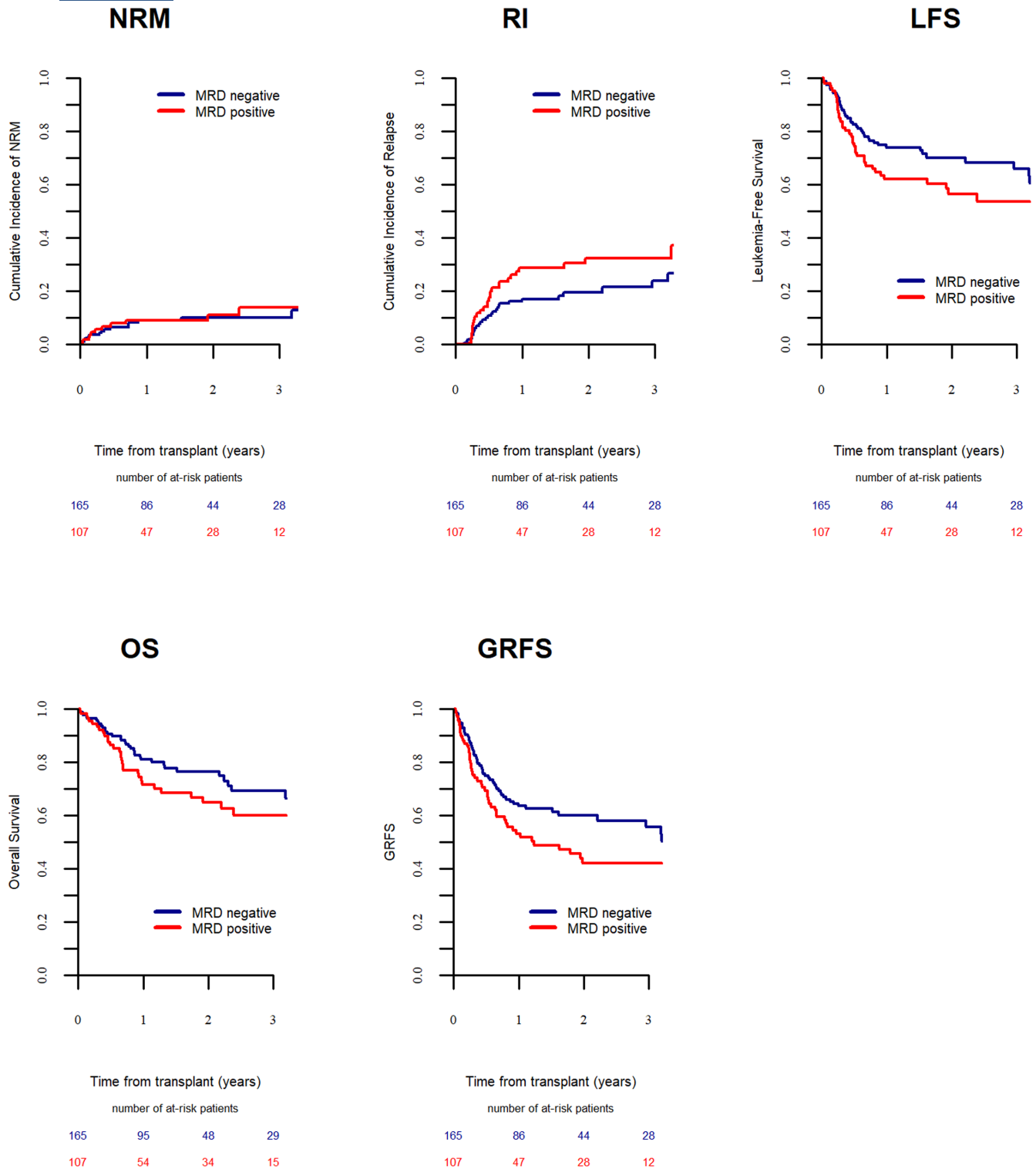
[Table 3](#) shows the cause of death. A total of 68 (25%) patients died during the study period, comprising 32 and 36 of the MRD+ and MRD- cohorts, respectively. Disease relapse was the main cause of death in both groups (58.2%), followed by infection (25.4%) and GVHD (7.5%).

## DISCUSSION

In this study, we showed a clinically meaningful impact of pre-transplant MRD in recipients of UD allo-HCT with PTCy for AML. Persistent MRD was associated with higher relapse and lower LFS and GRFS after adjusting for other transplant-related variables. In this analysis, neither graft source nor the intensity of the conditioning regimen affected RI, LFS or OS. Adverse risk cytogenetics maintained its negative impact on RI and was associated with lower LFS and OS even after adjusting for MRD status.

MRD represents a persistent or re-emergence of low-level of cancer cells or malignant clone in patients with morphological remission. In AML, persistent MRD at the end of induction chemotherapy or pre-transplant is associated with higher relapse risk.<sup>5,6,9,11</sup> The use of MAC may be able to partially overcome the negative impact of pre-transplant MRD as shown by Hourigan et al. who used an NGS-based MRD assay in prospectively collected samples from the BMT CTN0901 trial.<sup>12</sup> Similarly, our previous EBMT analysis of AML CR1 patients showed that the protective effect on relapse with MAC was limited to MRD+ patients <50 years of age compared to RIC/non-MAC. In that study, most patients received a matched sibling or UD transplant using conventional GVHD prophylaxis.<sup>15</sup>

Factors affecting GVL such as peri-transplant immunosuppression may further increase the relapse risk among MRD+ patients. We previously showed that the use of in vivo T cell depletion with antithymocyte globulin (ATG) did not impact the relapse risk in patients who were MRD+ and was associated with a lower risk of chronic GVHD.<sup>37</sup> Interaction between PTCy and MRD status is mainly investigated in the setting of haplo-HCT. In a previous EBMT study, pre-transplant MRD+ was associated with higher relapse risk and lower LFS after T cell-replete haplo-HCT with PTCy.<sup>29</sup> Similar results were reported by the Brazilian Bone Marrow Transplantation Society in acute leukaemia paediatric patients receiving PB grafts from haplo donors.<sup>38</sup> In contrast, a single-centre, retrospective study from M.D. Anderson showed no significant interaction between MRD status and relapse risk after haplo-HCT with fludarabine-melphalan



**FIGURE 1** Transplantation outcome—non-relapse mortality (NRM), relapse incidence (RI), leukaemia-free survival (LFS), overall survival (OS) and GVHD-free, relapse-free survival (GRFS) in AML patients with (+) or without (–) measurable residual disease (MRD) receiving an HCT from an unrelated donor (UD) with post-transplant cyclophosphamide (PTCy) as graft-versus-host disease (GVHD) prophylaxis. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

conditioning with PTCy.<sup>28</sup> These results highlight the unique influence of PTCy on donor immune reconstitution and the GVL effect. In the current study, most patients

received a PB graft from a UD after MAC and MRD maintained its prognostic significance. Interestingly, 2-year RI in the MRD+ cohort was favourable (32%) to what

**TABLE 2** Multivariate analysis of transplant outcomes.

Variables <sup>a</sup>	RI		NRM		LFS		OS		GRFS		Acute GVHD, grades II–IV		Chronic GVHD	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
MRD neg. (reference)	1		1		1		1		1		1		1	
MRD pos	2.56 (1.39–4.72)	0.003	1.29 (0.49–3.37)	0.61	2.04 (1.23–3.39)	0.006	1.83 (1.04–3.25)	0.037	1.69 (1.1–2.58)	0.016	1.08 (0.64–1.81)	0.78	1.32 (0.76–2.32)	0.33
Cytogenetics risk group		0.005		0.18		0.02		0.001		<0.001		0.50		0.54
Favourable cytogenetics (reference)	1		1		1		1		1		1		1	
Intermediate	1.72 (0.54–5.47)	0.36	0.58 (0.15–2.25)	0.43	1.07 (0.45–2.52)	0.88	2.09 (0.6–7.27)	0.25	1.01 (0.5–2.01)	0.99	1.51 (0.59–3.85)	0.39	1.07 (0.47–2.42)	0.88
Adverse	4.67 (1.32–16.5)	0.017	1.51 (0.32–7.17)	0.6	2.92 (1.13–7.57)	0.027	5.78 (1.52–22.01)	0.01	2.72 (1.24–5.97)	0.012	1.1 (0.36–3.33)	0.87	1.64 (0.57–4.75)	0.36
Age per 10 years	0.97 (0.77–1.21)	0.78	1.95 (1.27–3.01)	0.002	1.17 (0.97–1.43)	0.11	1.45 (1.16–1.82)	0.001	1.12 (0.95–1.33)	0.18	1.09 (0.89–1.34)	0.39	1.17 (0.94–1.46)	0.15
UD HLA 10/10 (reference)	1		1		1		1		1		1		1	
UD HLA 9/10	0.67 (0.35–1.28)	0.23	1.55 (0.6–4)	0.37	0.87 (0.51–1.48)	0.61	0.98 (0.54–1.8)	0.96	1.28 (0.83–1.99)	0.26	1.11 (0.65–1.87)	0.71	1.8 (1.03–3.13)	0.038
MAC (reference)	1		1		1		1		1		1		1	
RIC vs. MAC	1.11 (0.54–2.28)	0.78	0.52 (0.18–1.54)	0.24	0.85 (0.47–1.55)	0.6	0.62 (0.32–1.22)	0.17	0.87 (0.53–1.43)	0.59	1.02 (0.58–1.8)	0.94	0.71 (0.37–1.35)	0.3
Female D to male R	1.41 (0.62–3.23)	0.41	1.04 (0.28–3.86)	0.95	1.14 (0.57–2.27)	0.7	1.24 (0.59–2.6)	0.57	1.31 (0.73–2.36)	0.37	1.44 (0.74–2.77)	0.28	1.46 (0.71–3)	0.31
KPS ≥ 90	0.9 (0.43–1.89)	0.79	0.44 (0.16–1.2)	0.11	0.69 (0.39–1.23)	0.21	0.79 (0.41–1.54)	0.49	0.77 (0.47–1.27)	0.3	1.54 (0.82–2.9)	0.18	1.18 (0.6–2.32)	0.64
In vivo TCD	0.77 (0.35–1.69)	0.51	0.2 (0.02–1.55)	0.12	0.56 (0.28–1.14)	0.11	0.58 (0.25–1.37)	0.22	0.9 (0.52–1.54)	0.69	0.69 (0.32–1.45)	0.32	1.07 (0.55–2.05)	0.85
Time from diagnosis to HSCT	0.88 (0.79–0.99)	0.028	0.96 (0.84–1.09)	0.5	0.9 (0.82–0.98)	0.013	0.92 (0.84–1)	0.064	0.98 (0.92–1.04)	0.49	0.91 (0.83–1)	0.049	0.99 (0.92–1.07)	0.79

Abbreviations: CI, confidence interval; GRFS, graft-versus-host disease-free, relapse-free survival; GVHD, graft-versus-host disease; HLA, human leukocyte antigen, OS, overall survival; HR, hazard ratio; KPS, Karnofsky performance status, UD, unrelated donor; LFS, leukaemia-free survival; MAC, myeloablative conditioning; MRD, measurable residual disease; RI, relapse incidence; RIC, reduced-intensity conditioning.

<sup>a</sup>Variables differing significantly between the groups and factors known to be associated with outcomes.

TABLE 3 Cause of death.

Cause of death	MRD negative (n = 36)	MRD positive (n = 32)	Overall (n = 68)
Original disease	20 (57.1%)	19 (59.4%)	39 (58.2%)
Infection	11 (31.4%)	6 (18.8%)	17 (25.4%)
GVHD	1 (2.9%)	4 (12.5%)	5 (7.5%)
Multiorgan failure	1 (2.9%)	0 (0%)	1 (1.5%)
CNS toxicity	0 (0%)	1 (3.1%)	1 (1.5%)
Other HCT related	2 (5.7%)	1 (3.1%)	3 (4.5%)
Non-HCT related	0 (0%)	1 (3.1%)	1 (1.5%)
Unknown	1	0	1

Abbreviations: CNS, central nervous system; GVHD, graft-versus-host disease; HCT, haematopoietic cell transplantation; MRD, measurable residual disease; neg, negative; pos, positive.

was reported after haplo-HCT (37%) in a previous EBMT analysis.<sup>29</sup> Similarly, in our previous analysis of patients who received CNi-based GVHD prophylaxis after MUD PBSCT, 2-year relapse rate was 38% in MRD+ cohort.<sup>37</sup> Three-year relapse incidence was 42% in BMT CTN 0901 among MRD+ patients.<sup>12</sup> Similar trend was seen in BMT CTN 1301 where PTCy was associated with less relapse compared to FK/MTX (2-year relapse: 13.9% vs. 25.6%,  $p = 0.076$ ) after matched donor BM graft.<sup>22</sup> There is a possibility that PTCy allows better immune-reconstitution and early discontinuation of immunosuppression after matched donor PBSCT, hence improving GVL and reducing the relapse risk for MRD+ patients. This needs to be explored in future prospective studies. Strategies to improve the outcomes of MRD+ disease may include additional therapies before transplant, preferential use of MAC and post-transplant therapies. Impaired NK cell reconstitution after HCT with PTCy is associated with increased relapse risk.<sup>25</sup> This has led to various strategies to enhance NK cell function such as infusing ex vivo expanded donor-derived NK cells.<sup>39–41</sup>

Being registry-based, this analysis has several limitations including the lack of complete genomic information at diagnosis and details on upfront pre- and post-transplant therapies. Information regarding MRD testing methodology, detection sensitivity cut-off and MRD status after allo-HCT was missing for most study patients. There was heterogeneity in specific conditioning regimens and concurrent immunosuppressive drugs (in addition to PTCy) based on centre preferences. However, our study cohorts more accurately represent the contemporary standard of care across EBMT centres and real-world outcomes, and we were able to demonstrate that pre-transplant MRD remains an important prognostic factor in patients with AML in CR undergoing UD allo-HCT with PTCy. Novel therapies are urgently needed to optimize transplant platforms and post-transplant therapies to improve the outcomes for these patients.

## ACKNOWLEDGEMENTS

We thank all the European Society for Blood and Marrow Transplantation (EBMT) centres and national registries for contributing patients to this study (Table S1). We also thank the data managers for their excellent work and the patients who contributed their data.

## CONFLICT OF INTEREST STATEMENT


BD—Institutional research funding: Janssen, Angiocrine, Pfizer, Poseida, MEL, Orcabio, Wugen, Allovir. Consultancy/Advisor: Jazz, Gamida Cell, MJH BioScience, Arivan Research, BEAM therapeutics, Janssen, Atheneum. The other authors declare no relevant COI. Authors report no relevant conflict of interest in relation to this work.

## DATA AVAILABILITY STATEMENT


Please contact the EBMT for the raw data used for this study ([www.ebmt.org](http://www.ebmt.org)).


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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Nagler A, Labopin M, Dholaria B, Blaise D, Bondarenko S, Vydra J, et al. Impact of measurable residual disease on outcomes of unrelated donor haematopoietic cell transplantation with post-transplant cyclophosphamide in AML in first complete remission. *Br J Haematol*. 2023;201(6): 1169–1178. <https://doi.org/10.1111/bjh.18765>