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Pretransplantation MRD in Older Patients With AML After Treatment With Decitabine or Conventional Chemotherapy

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ABSTRACT

The predictive value of measurable residual disease (MRD) for survival in acute myeloid leukemia (AML) has been firmly established in younger patients treated with intensive chemotherapy. The value of MRD after treatment with decitabine in older patients is unknown. This retrospective analysis included patients ≥60 years of age with AML who received an allogeneic hematopoietic cell transplantation (alloHCT) after treatment with decitabine or intensive chemotherapy. Of the 133 consecutively transplanted patients, 109 had available pretransplantation MRD analyses (by flowcytometry [threshold 0.1%]). Forty patients received decitabine treatment (10-day schedule), and 69 patients received intensive chemotherapy (7 + 3 regimen). Patients who received decitabine were older (median 67 versus 64 years) and more often had MRD (70% versus 38%). OS after alloHCT was comparable in both groups. In the chemotherapy group, MRD-positive patients had a significantly higher relapse probability (subdistribution hazard ratio [sHR] 4.81; $P = .0031$) and risk of death (HR 2.8; $P = .02$) compared to MRD-negative patients. In the decitabine group there was no significant association between the presence of MRD and relapse (sHR 0.85; $P = .83$) or death (HR 0.72; $P = .60$). Pretransplantation MRD in patients receiving decitabine treatment does not have similar predictive value for relapse or survival in older AML patients receiving an alloHCT, compared to patients receiving intensive chemotherapy.

Acute myeloid leukemia (AML) is a phenotypically and prognostically heterogeneous disease, with relapse being the main reason for treatment failure. The quality of response to treatment, assessed by detection of measurable residual disease (MRD), has been consistently associated with prognosis and clinical outcome in AML patients treated with intensive chemotherapy [1]. MRD detectable by multiparameter flowcytometry after intensive induction chemotherapy, as well as MRD detectable before allogeneic hematopoietic cell transplantation (alloHCT), has been shown to be a powerful predictor of relapse and survival [2-7].

The majority of available data on MRD assessment in AML involves younger patients (<60-65 years) treated with intensive chemotherapy (the 7 + 3 regimen) [3-5], with only few reports describing the predictive value of MRD in older AML patients [8]. It is unknown whether MRD after treatment with hypomethylating agents (HMA) and before alloHCT has predictive power for relapse and survival. Limited data, outside the context of alloHCT, suggest that MRD status after treatment with an HMA has an impact on relapse, but not on overall survival (OS) [9]. MRD measurements are becoming part of routine clinical practice, although evidence on its value in the context of decitabine treatment is lacking.

The aim of this retrospective study is to investigate the predictive value of pretransplantation MRD status measured by flow cytometry after treatment with decitabine (5-aza-2′-deoxycytidine) or intensive chemotherapy with regard to relapse rate and survival in older patients diagnosed with AML.

PATIENTS AND METHODS

Study Design and Patient Cohort

This retrospective cohort included all consecutive AML patients older than 60 years of age who received an alloHCT after treatment with either decitabine or intensive chemotherapy between January 2013 and October 2019 in 2 academic referral centers in the Netherlands, namely the University

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Medical Center Groningen and the Radboud University Medical Center Nijmegen. Patients who previously received decitabine for myelodysplastic syndrome were excluded. Information on patient, disease, and treatment characteristics were collected by studying individual patient records. AML diagnosis was based on the World Health Organization criteria [10]. Genetic risk was defined according to the European Leukemia Net (ELN) 2017 AML risk stratification, and patients diagnosed before publication of the guideline were reclassified accordingly [11]. The treatment strategy (intensive chemotherapy or decitabine) was decided by the physician and patient, considering patient-related factors (age, performance, comorbidity), disease-related factors (genetic risk profile), and preference of the patient. Decitabine was administered according to the 10-day schedule reported by Blum et al. [12], and intensive chemotherapy according to the 7 + 3 regimen. Morphologic response was defined according to the ELN 2017 response criteria [11]. Acute and chronic graft-versus-host disease (GVHD) were diagnosed and graded according to the criteria of Harris et al. [13] and the National Institutes of Health scoring system [14]. All patients were treated in accordance with Institutional Review Board–approved protocols or standard treatment protocols and in accordance with the Declaration of Helsinki.

MRD Analysis

Eight-color flow cytometry was performed in all patients as a routine clinical test on bone marrow aspirates obtained <2 weeks before start of conditioning for alloHCT. MRD was identified using the leukemia-associated immunophenotypes approach, defined at diagnosis of AML in each specific patient. When identified, the abnormal population was quantified as a percentage of the total $CD45^+$single-cell events. An MRD level <0.1% was considered negative as previously reported

Table 1
Baseline Characteristics of Patient Cohort

	Decitabine (n = 40)	7 + 3 Chemotherapy (n = 69)	*P*
Male sex (%)	65.0	66.7	1
Age at alloHCT (median) (IQR)	67.4 (8.0)	64.4 (4.7)	.005
ELN 2017 risk - No. (%)			.74
Favorable	7 (17.5)	10 (14.5)	
Intermediate	21 (52.5)	33 (47.8)	
Adverse	12 (30.0)	26 (37.7)	
Median number of cycles (range)	4 (2-23)	2 (1-3)	<.001
Disease status prior to alloHCT – No. (%)			.06
CR	21 (52.5)	45 (65.2)	
CRi	6 (15.0)	14 (20.3)	
MLFS	5 (12.5)	7 (10.1)	
PR	2 (5.0)	2 (2.9)	
SD	6 (15.0)	1 (1.4)	
MRD status before alloHCT – No. (%)			<.001
Neg	12 (30.0)	43 (62.3)	
Pos, while in CR/CRi/MLFS	20 (50.0)	24 (34.8)	
Pos, while in PR/SD	8 (20.0)	2 (2.9)	
Days between diagnosis and alloHCT (median) (IQR)	138 (51)	120 (53)	.12
Conditioning prior to alloHCT – No. (%)			.014
Dec/Flu/TBI	17 (42.5)	23 (33.3)	
Flu/TBI	10 (25.0)	29 (42.0)	
Chemo/PT cyclo	11 (27.5)	6 (8.6)	
Chemo/ATG	2 (5.0)	11 (15.9)	
Donor source – No. (%)			.11
MUD	34 (85.0)	48 (69.6)	
SIB	6 (15.0)	19 (27.5)	
Haplo	0	1 (1.4)	
Cord blood	0	1 (1.4)	
HLA match – No. (%)			.13
5/6	0	1 (1.4)	
7/10	0	1 (1.4)	
9/10	4 (10.0)	9 (13.0)	
10/10	36 (90.0)	58 (84.1)	
Sex recipient/donor – No. (%)			.027
M/M	23 (57.5)	29 (42.0)	
M/F	3 (7.5)	17 (24.6)	
F/M	10 (25.0)	9 (13.0)	
F/F	4 (10.0)	14 (20.4)	
CMV-status recipient/donor – No. (%)			.82
Pos/pos	17 (42.5)	23 (33.3)	
Pos/neg	9 (22.5)	18 (26.1)	
Neg/pos	4 (10.0)	7 (10.2)	
Neg/neg	10 (25.0)	21 (30.4)	

ATG indicates antithymocyte globulin; CR, complete remission; CRi complete remission with incomplete blood count recovery; Chemo, chemotherapy; Cyclo, cyclophosphamide; Dec, decitabine; Flu, fludarabine; IQR, interquartile range; MLFS, morphologic leukemia free state; MUD, matched unrelated donor; PR, partial remission; PT, post-transplantation; SD, stable disease; SIB, sibling donor; TBI, total body irradiation.

[1]. Analyses were performed using the EuroFlow AML panel, FACSCanto II flow cytometer, and FacsDiva software.

Statistical Analysis

Descriptive statistics were used to characterize the cohort. Relapse probability estimates were calculated using the Fine and Gray method for competing risks, with death as a competing risk. Relapse was defined as documented relapse in patients with previously less than 5% blasts in bone marrow aspirate or as a 50% increase in blasts in the bone marrow aspirate or peripheral blood in patients with active disease according to the ELN criteria [11]. OS and GVHD-relapse-free survival (GRFS) were estimated using the Kaplan-Meier method. OS was measured from date of alloHCT to date of death or censored at last follow-up as of January 10, 2020, or after 36 months' follow-up. GRFS was measured from date of alloHCT to date of occurrence of grade 3 to 4 acute GVHD, severe chronic GVHD, relapse, death, or censored at last follow-up. Cox proportional hazards regression analyses were performed to evaluate the effect of age, ELN risk, and MRD status on survival in both treatment groups. A subdistribution hazards regression analysis was performed to estimate associations of ELN risk and MRD status with relapse accounting for death as competing risk. A P value $< .05$ was considered statistically significant. Statistical analyses were performed using R studio version 1.3.959 (R 3.6.3).

RESULTS

Characterization of Study Cohort

One hundred thirty-three older (≥60 years) AML patients received an alloHCT between January 2013 and October 2019 after treatment with intensive chemotherapy or decitabine. Two patients were excluded from analysis because they received decitabine before AML diagnosis, and 22 patients were excluded because they did not have available MRD analyses (Supplemental Table S1; Supplemental Figures S1 and S2). Of the 109 remaining patients, 40 patients received induction therapy with decitabine, and 69 patients received induction therapy with intensive chemotherapy. The ELN risk groups were balanced between both treatment groups. Patients receiving an alloHCT after decitabine therapy were older compared with those treated with intensive chemotherapy (median age 67 versus 64 years). Also, they were less likely to be in morphologic remission (ie, <5% blasts in bone marrow aspirate) at time of transplantation (80% versus 96%). Moreover, more patients were MRD-positive before alloHCT after decitabine therapy compared to intensive chemotherapy (70% versus 38%), also among patients in morphologic remission (50% versus 35%). Clinical and biologic characteristics of the patients are summarized in Table 1. Despite differences in age, remission status, and MRD status, the OS of AML patients after treatment with intensive chemotherapy or decitabine and consolidation with an alloHCT was comparable (Figure 1).

Relationship Between Pre-alloHCT MRD Status and Relapse

Twenty-four of the studied 109 patients experienced relapse after alloHCT; 6 of 40 (15%) in the decitabine group and 18 of 69 (26%) in the chemotherapy group. The median time to relapse was 3.5 months in the decitabine group and 4.2 months in the chemotherapy group. In the chemotherapy group the cumulative incidence of relapse (CIR) was significantly higher in MRD-positive patients compared with MRD-negative patients (Figure 2A). Specifically, the 1-year CIR was 50% (95% confidence interval [CI], 28%-68%) in MRD-positive and 9% (95% CI, 3%-21%) in MRD-negative patients, respectively (Table 2). Interestingly, in the decitabine group, the 1-year CIR was 11% (95% CI, 3%-26%) in MRD-positive and 17% (95% CI, 2%-43%) in MRD-negative patients, respectively (Table 2), resulting in comparable cumulative incidences of relapse among MRD-positive and MRD-negative patients after induction with decitabine (Figure 2B). A subhazards regression analysis confirmed that being MRD positive was associated with a significantly increased risk of relapse (sHR 4.81 [95% CI, 1.70-13.64]; P= .0031) in the chemotherapy group, but not in the decitabine group (sHR 0.85 [95% CI, 0.19-3.83]; P= .83) (Supplementary Table S2).

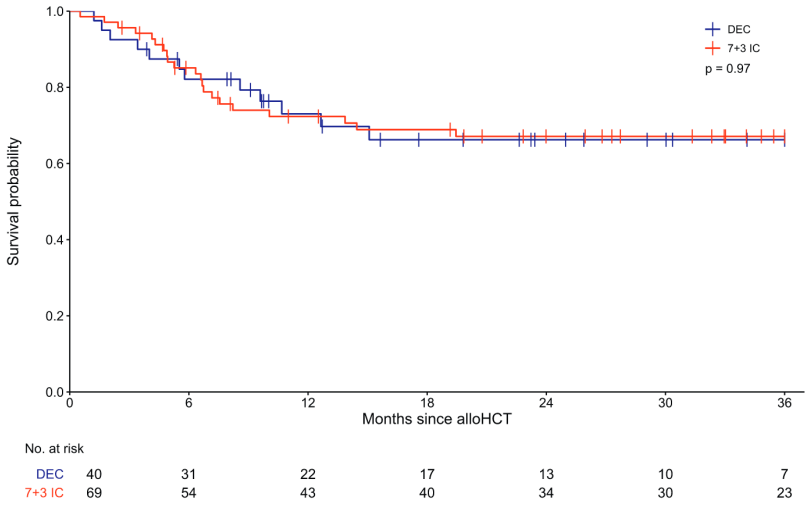

Figure 1. OS for patients treated with decitabine (DEC) versus 7 + 3 intensive chemotherapy (IC).

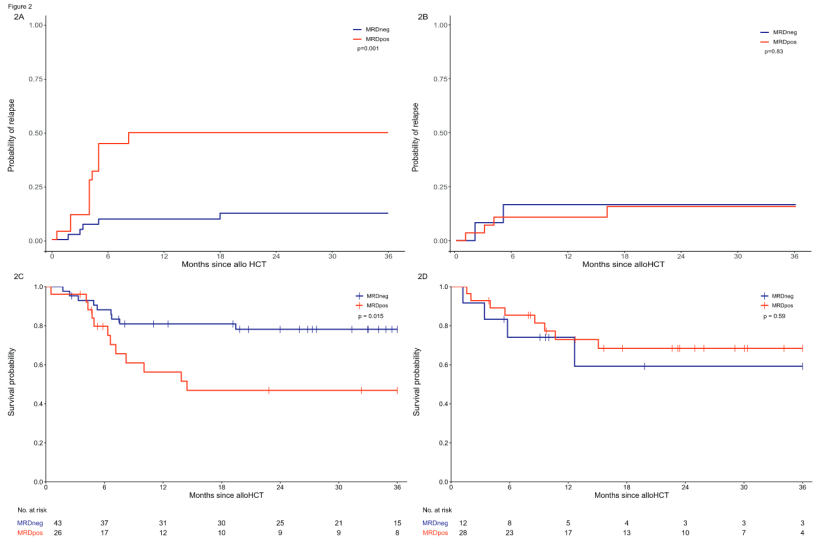

Figure 2. CIR and OS stratified for MRD status in patients treated with intensive chemotherapy or decitabine. (A) CIR in patients treated with intensive chemotherapy. (B) CIR in patients treated with decitabine. (C) OS in patients treated with intensive chemotherapy. (D) OS in patients treated with decitabine.

Table 2
Outcome Probabilities

	Decitabine		7 + 3 chemotherapy	
	MRD-neg (n = 12)	MRD-pos (n = 28)	MRD-neg (n = 43)	MRD-pos (n = 26)
OS at 2 years, % (95% CI)	59 (34-100)	68 (52-90)	78 (67-92)	47 (30-73)
CIR at 1 year, % (95% CI)	17 (2-43)	11 (3-26)	9 (3-21)	50 (28-68)
GRFS at 2 years, % (95% CI)	39 (15-99)	66 (50-87)	61 (47-78)	37 (22-61)

Relationship Between Pre-alloHCT MRD Status and OS

The median follow-up times of patients alive at last follow-up (n = 44 for the chemotherapy group and n = 26 for the decitabine group) were 34 months (range 3-85) for the chemotherapy and 23 months (range 4-56) for the decitabine group. As mentioned earlier, the OS was similar between both treatment groups, with an estimated 2-year OS of 67% (95% CI, 56%-80%) in the chemotherapy group and 66% (95% CI, 52%-84%) in the decitabine group (Figure 1). MRD-positive patients in the chemotherapy group had a significantly worse survival compared to MRD-negative patients (Figure 2C). The 2-year OS was 47% (95% CI, 30%-73%) in MRD-positive patients and 78% (95%, CI 67%-92%) in MRD-negative patients (Table 2). After decitabine therapy, no statistically significant differences in OS between MRD-positive or MRD-negative patients could be observed (Figure 2D). The 2-year OS estimates in decitabine treated patients were 68% (95% CI, 52-90%) in MRD positive patients and 59% (95% CI, 34%-100%) in MRD-negative patients (Table 2). These differences remained in an analysis including only patients in morphologic remission before transplantation (Figure 3A,B).

Univariate and multivariate models were developed to assess the effect of MRD status on OS after adjusting for age and ELN risk. In the unadjusted models, MRD positivity was associated with a significantly increased risk of death in the chemotherapy group (HR 2.8 [95% CI, 1.2-6.7]; *P*= .02), but not in the decitabine group (0.72 [95% CI, 0.22-2.4]; *P*= .60) (Table 3). After adjusting for age and ELN risk, MRD positivity remained associated with a significantly increased risk of death in the chemotherapy group (HR 2.7 [95% CI, 1.1-6.5], *P*= .02), but not in the decitabine group (HR 0.57 [95% CI, 0.16-2.0], *P*= .38) (Table 3).

Relationship Between Pre-alloHCT MRD Status and GRFS

Incidence of GVHD was comparable in both treatment groups (Supplementary Table S3). The GRFS was similar among MRD-positive and MRD-negative patients for both treatment groups (Figure 4A,B). The 2-year estimates for GRFS in the chemotherapy group were 37% (range 22%-61%) in MRD-positive and 61% (range 47%-78%) in MRD-negative patients (Table 2). The 2-year estimates for GRFS in the decitabine group were 66% (range 58%-87%) and 39% (range 15%-

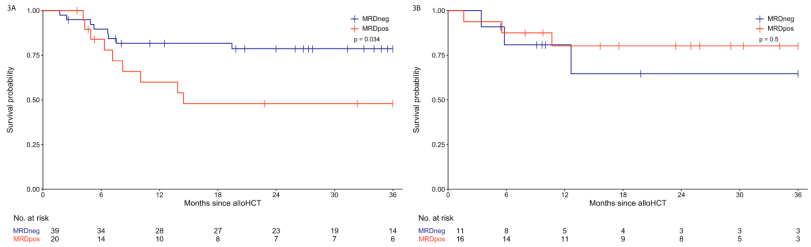

Figure 3. OS of patients in morphologic remission at time of allogeneic hematopoietic cell transplantation stratified for MRD status. (A) Patients treated with intensive chemotherapy. (B) Patients treated with decitabine.

Table 3
Regression Models for Survival Per Induction Therapy

	Decitabine		3 + 7 chemotherapy	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Univariate				
Age at alloHCT (continuous)	0.95 (0.83-1.09)	.45	1.00 (0.89-1.12)	.97
Adverse cytogenetic risk	3.52 (1.12-11.03)	.031	1.42 (0.60-3.38)	.43
MRD at alloHCT	0.72 (0.22-2.40)	.60	2.80 (1.18-6.66)	.020
Multivariate				
Age at alloHCT (continuous)	0.96 (0.83-1.10)	.54	1.01 (0.90-1.13)	.93
Adverse cytogenetic risk	3.84 (1.18-12.51)	.025	1.38 (0.58-3.30)	.46
MRD at alloHCT	0.60 (0.17-2.10)	.43	2.80 (1.16-6.62)	.023

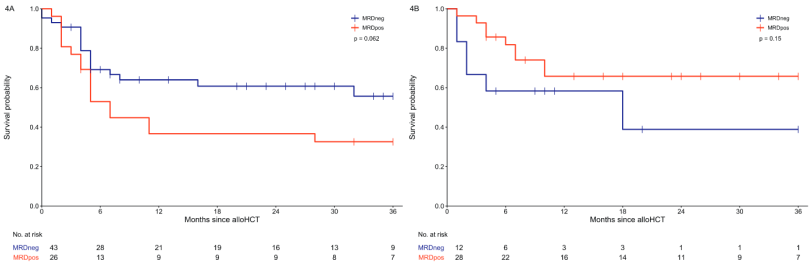

Figure 4. GFRS stratified for MRD status in patients treated with intensive chemotherapy or decitabine. (A) Patients treated with intensive chemotherapy. (B) Patients treated with decitabine.

99%) in MRD-positive and negative patients, respectively (Table 2).

DISCUSSION

This retrospective study analyzed the predictive value of MRD status measured by flow cytometry on relapse and survival in AML patients older than 60 years receiving an alloHCT after induction therapy with decitabine or intensive chemotherapy. Although the predictive value of MRD status has been firmly established in the last decade, especially in younger patients treated with intensive chemotherapy, the value of pretransplantation MRD after treatment with decitabine in older AML patients is not clear. Our data from a cohort of 109 evaluable patients confirm the predictive value of pretransplantation MRD positivity in older AML patients treated with intensive chemotherapy but, interestingly, not in the decitabine-treated patient cohort. Our data reveal no difference in relapse and OS in patients who were MRD-positive or MRD-negative receiving an alloHCT after decitabine induction therapy. Apparently, the predictive value of pretransplantation MRD measured by flow cytometry is dependent on the type of induction therapy in older AML patients.

The findings in the chemotherapy group are in accordance with reports in literature that have shown that presence of pretransplantation MRD by flow cytometry is a marker for increased risk of relapse and death [3,5,15]. The hazard ratio (HR) for overall survival (2.33) we observed in our cohort is comparable with the pooled HR found in a meta-analysis by

Buckley et al. [6] (HR 2.36), lower than HRs found in younger patients receiving an alloHCT (HR 4.06 found by Araki et al. [5] and Walter et al. [3]), but higher than the HR found in a study of older AML patients not undergoing alloHCT (HR 1.48) [8]. This indicates that MRD status is predictive for survival in older AML patients receiving an alloHCT after induction with intensive chemotherapy, although differences in outcomes are smaller than in younger patients. Similarly, a study by Buccisano et al. [7] comparing MRD status in older and younger patients (>60 versus ≤60) concluded that MRD negativity resulted in longer OS and decreased relapse in both older and younger patients, but with higher relapse rates in older patients compared with younger patients. Still, MRD analysis by flow cytometry can provide a predictive tool for risk assessment in AML and potentially guide therapeutic decision making, like immune modulation after transplantation (i.e. discontinuation of immune suppression or donor lymphocyte infusion).

In contrast, our observations suggest that the predictive value of pretransplantation MRD measured by flow cytometry is questionable when decitabine is used as induction treatment. Although small numbers in the decitabine group lead to wide confidence intervals, multivariate analysis suggests that the effect of MRD positivity is not the same as in patients treated with intensive chemotherapy. Because our data confirm that pretransplantation MRD status is predictive in older AML patients after induction with intensive chemotherapy and because the patient and disease associated risk factors (age and ELN risk profiles) were comparable among patients in our cohort, it is unlikely that differences in patients or disease characteristics can explain the lack of predictive value of MRD in the context of decitabine. A possible explanation could be that blasts detected by multicolor flow cytometry may already be primed toward differentiation caused by epigenetic changes induced by decitabine and therefore not represent "true" leukemic blasts. Another explanation could be that the immune-modulating activity of decitabine overrules the impact of presence of MRD, traditionally representing disease activity. It has extensively been reported that hypomethylating agents such as decitabine lead to expression and upregulation of epigenetically silenced tumor-associated antigens (TAAs) on leukemic cells, and subsequently promote specific T-cell responses [16-18]. This could make the residual leukemic blasts more immunogenic and therefore susceptible to clearance by the immune system. Furthermore, clonal evolution under HMAs might be another contributing factor. It is nowadays accepted that AML is a heterogeneous disease including heterogeneity of the leukemia within each patient. It has been suggested that HMAs are particularly effective against subclones (in contrast to founder clones) [19]. Interestingly, recently a cohort of 142 patients treated with decitabine did not reveal an impact on outcome by the size of the dominant clone or the number of (sub)clones (reflected by the number of mutations) [20].

This is the largest reported cohort of AML patients receiving treatment with decitabine who are subsequently consolidated with an alloHCT. Traditionally, standard intensive chemotherapy has been used to achieve complete remission before transplantation. The Freiburg group has reported their single center experience with 15 consecutive older patients (9 with AML and 6 with myelodysplastic syndrome), with a median age of 69 years, treated with decitabine at 15 mg/m^2 every 8 hours for 3 days as part of a 6-week cycle [21]. These data suggested feasibility and efficacy of decitabine as a "bridge" to alloHCT, which is confirmed in our cohort of 40 patients who were consolidated with an alloHCT after treatment with decitabine. Our evaluation of 109 subsequent patients who have been treated in two referral centers in the Netherlands allows comparing MRD in the context of induction with intensive chemotherapy and decitabine. Although not all patients were in (in) complete remission at time of transplantation, we included these patients because the antileukemic effect of decitabine and survival benefit can be reached without achievement of a formal complete remission [22]. In addition, the study by Araki et al. [5] showed that OS is comparable in MRD-positive patients and patients with active disease. Indeed, our analysis including only patients in CR/CRi showed comparable results to the analysis including the complete cohort in both treatment groups. Because of the retrospective character of this study, it was unfortunately not possible to retrieve how many patients started treatment (intensive chemotherapy or decitabine) and eventually were able to be consolidated with an alloHCT. The results of the large (n = 600) prospective randomized EORTC/GIMEMA AML-21 study, which are eagerly awaited, could potentially answer the question what the optimal strategy is to treat older, transplant-eligible AML patients (ie, induction with decitabine or intensive chemotherapy). In addition, we acknowledge that measuring MRD by flow cytometry is one method for the assessment of MRD, and other detection methods such as molecular MRD assessment should be investigated in the context of decitabine treatment.

In conclusion, in this cohort MRD status assessed by flow cytometry does not have significant predictive value for relapse or survival in older patients with AML receiving an alloHCT after induction with decitabine, in contrast to intensive chemotherapy. Therefore it suggests that the value of pretransplant MRD-positivity found in the setting of intensive chemotherapy cannot be extrapolated to the decitabine setting. Although further research is necessary to investigate the value of pretransplantation MRD in the decitabine setting, our data strongly suggest that MRD status before alloHCT should be interpreted carefully in the context of type of induction treatment.

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Authorship statement: JRH collected and analyzed the data and wrote the first version of the manuscript; LMM contributed to data interpretation and manuscript writing; WJV, ABM, CLH, MG, GC, JJS, KM, NMB contributed to data collection and critically revised the manuscript; EA and GH were involved in all aspects of the study, including design, data collection, interpretation of data, manuscript writing.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2020.12.014.

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