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Title: Role of pre-transplant MRD level detected by flow cytometry in recipients of allogeneic stem cell transplantation with AML.

Running title: Pre-transplant MRD level in AML patients.

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Novelty statement:

1. "Low" MRD level (0.1-0.5%) detected by flow cytometry did not have significant impact on survival outcomes in multivariate analysis related to "high" (>0.5%) MRD level.
2. The quantitative MRD detection might be less important that the qualitative one.
3. Patients with "low" pre-transplant MRD level detected with MFC represent a population with increased relapse risk compared with MRD negative patients.

Abstract

Objectives and Methods: We analyzed the impact of pre-transplant MRD level in bone marrow measured by flow cytometry using "different from normal" method on outcomes for 189 AML patients (108 males; median age, 58 (21-80) years). All patients were subdivided into negative (n=96), "low" (0.1-0.5%, n=32) and "high" MRD (>0.5%, n=61) groups.

Results: In multivariate analysis, the hazard ratios for "high" and "low" MRD levels related to MRD negativity were 7.9 (95% CI 3.5-18.1, p<0.001) and 5.4 (95% CI 2.1-14, p=0.0058) for relapse; 2.3 (95% CI 1.3-4.1, p=0.006) and 1.6 (95% CI 0.82-3.3, p=0.16) for OS; 2.8 (95% CI 1.7-4.7, p<0.001) and 2.2 (95% CI 1.1-4.2, p=0.02) for LFS, respectively. We found no significant impact of "low" MRD level on relapses (0.68, 95% CI 0.33-1.4, p=0.30), OS (0.72, 95% CI: 0.36-1.5, p=0.36) and LFS (0.79, 95% CI: 0.42-1.5, p=0.46) related to "high" MRD group.

Conclusions: Presence of detectable MRD was indicative for a high relapse risk, low LFS and OS. "Low" MRD level showed no significant impact on relapse, LFS and OS related to "high" MRD group.

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Keywords: allogeneic stem cell transplantation, acute myeloid leukemia (AML), relapse, minimal residual disease (MRD), multicolor flow cytometry (MFC)

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is an effective potentially curative post-remission treatment for patients with acute myeloid leukemia (AML). Despite substantial improvements in non-relapse mortality (NRM) during the last decades (1), up to 50% of AML patients finally develop relapses. The outcome for relapsing patients with AML is very dismal with 2-year survival rates of only 20% independent of the choice of salvage therapy (2,3).

It has been shown that residual chemotherapy-resistant leukemia cells during remission can drive leukemia relapse (4). Therefore, early and accurate detection of minimal measurable disease (MRD) has emerged as a topic of interest in the past decade. Different methods like quantitative real-time PCR (qPCR), next generation sequencing, and multiparametric flow cytometry (MFC) are most commonly used for MRD detection in AML patients (5).

Of those, MFC is a method to directly identify residual leukemic cells that can be applied in virtually all AML patients. Two separate approaches with a sensitivity of 10^{-3} to 10^{-4} were suggested: (i) the leukemia associated immunophenotype (LAIP) method which defines a disease-specific expression pattern at diagnosis and facilitates subsequent tracking of this phenotype during follow-up period (6); and (ii) the "different from normal" method (7) can be used in patients in whom the immunophenotype at diagnosis is not available. Walter *et al.* have already reported on the feasibility of the "different from normal" method in allogeneic setting (8). An international expert panel recently published a consensus for MRD detection in AML patients and recommended considering LAIP-positive cells, leukemic cells as well as "different from normal" progenitors (9).

The prognostic meaning of pre-transplant MRD level in transplant setting remains under investigation. Recently, Dillon *et al.* reported on prognostic meaning of MRD level detected with PCR in NPM1^{+} patients. After a median follow-up of 4.9 years, patients with negative, low (<200 copies per 10^5 ABL in the peripheral blood and <1000 copies in the bone marrow aspirate), and high levels of MRD had an estimated 2-year OS of 83%, 63%, and 13%, respectively (p=0.0001) (10). Regarding the MFC MRD detection, Anthias *et al.* reported on the worst outcomes due to increased relapse risk of 70% in AML patients with "high MRD" (≥1%) pre-transplant. The authors used LAIP-based method with measurements of 10^5 mononuclear cells/sample. Any level of MRD was considered as positive. Probably due to the limited number of patients there were no data on the impact of MRD level in multivariate analysis (11).

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In the present study, we assessed survival outcomes focusing on impact of pre-transplant MRD level measured using the "different from normal" flow method in 189 AML patients.

Patients and methods

Study cohort

Adult (≥18 years old) patients were included in this study if they had AML in complete remission, and underwent allo-SCT with available MRD data. We used the genetic European Leukemia Net (ELN) criteria (2017) to assign a disease-dependent risk (12). Criteria for response to therapy were used as proposed by an International Working Group (13). The conditioning was defined according to criteria published previously (14). The acute graft-versus-host disease (GVHD) were graded according to Przepiorka *et al.* (15). The chronic GVHD were classified according to Jagasia *et al.* (16). All patients consented in accordance with the Declaration of Helsinki. Follow-up was current as of May 15, 2020.

MFC detection of MRD by the "different from normal" method

Immunophenotypic analysis was performed within a median of 7 days (range 2-14) prior to allo-SCT on whole bone marrow specimens after stain-lyse-wash standard techniques. Eight-colour immunostaining with fluorochrome directly conjugated with monoclonal antibodies was performed. The extended panel consisted of (1) CD34-BV421, CD33-PE-Cy7, CD56-BV510, CD2-FITC, CD65-PE, CD14-PerCP, NG2-APC, CD45-APC-H7; (2) CD34-BV421, CD61-FITC, CD135-PE, CD14-PerCP, CD33-PE-Cy7; CD235a-APC, CD45-APC-H7; (3) CD34-PerCP, CD33-PE-Cy7, CD13-FITC, HLADR-PE, CD7-BV421, CD117-APC, CD45-APC-H7; (4) CD34-PerCP, CD33-PE-Cy7, CD19-APC,CD38-PE, CD15-FITC,CD123-BV421, CD45-APC-H7; (5) CD34-BV421, CD11b-FITC, CD65-PE, CD33-PE.Cy7, CD14-PerCP, CD117-APC, CD45-APC-H7 (="old" panel) was used in 113 (60%) patients. Up to 250,000 events per tube were acquired. In 76 (40%) patients the panel suggested by ELN (="new" panel) was used (9) with up to 2,000,000 events per tube and lower amount of used markers: (1) CD34-PerCP, CD13-PE-Cy7, CD117-APC, CD45-APC-H7, CD33-FITC, CD7-BV421, HLADR-V500, CD56-PE; (2) CD34-PerCP, CD13-PE-Cy7, CD117-APC, CD45-APC-H7, CD33-FITC, CD19-BV421, CD15-V500, CD11b-PE; (3) CD34-PerCP, CD13-PE-Cy7, CD117-APC, CD45-APC-H7, CD33-FITC, CD4-BV421, CD14-V500, CD64-PE. The aberrant blasts were defined according to asynchronous expression

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(e.g. CD34/CD15, CD34/CD64, CD34/CD11b), cross-lineage expression (e.g. CD34/CD56, CD34/CD4, CD34/CD19, CD34/CD7), loss (e.g. CD34, HLADR) and/or increased expression (e.g. CD45) of selected markers.

The comparison of both panels regarding the relapse incidences is provided in supplemental Appendix. All antibodies were obtained from Beckman-Coulter (CA, USA) or Becton Dickinson (BD Biosciences, New Jersey, USA). A CD45/SSC gating strategy was used for analysis of abnormal blasts (6). Analysis of list mode files was performed using Infinicyte™ Flow Cytometry Software (Cytognos, Salamanca, Spain). The assessments were performed using the "different from normal" strategy. The LAIP method was not used due to absence of initial phenotype data. Samples from a total of 20 healthy donors who underwent bone marrow harvest in our department were analysed according to the same strategy and were used as reference for defining the "normal" hematopoiesis. When identified, the abnormal population of granulopoietic progenitors was quantified as a percentage of the total $CD45^+$ white blood cell events. A level of at least 0.1% of cells with aberrant phenotype was considered MRD positive (MRD^+). According to the MRD status all patients were divided into three groups: MRD negative (MRD^-), "low" MRD level (0.1-0.5%) and "high" MRD level (>0.5%). There were three reasons to take the cut-off of 0.5%. First, based on relevance for survival outcomes; second, based on the fact that "old" panel was less sensitive and the cut-off of 1% mostly used in other studies may be associated with increased amount of false-negative results and third, to provide more balanced groups for uni- and multivariate analysis.

Statistical analysis

Unadjusted probabilities of overall survival (OS) and leukemia-free survival (LFS) were estimated by using the Kaplan-Meier method, and probabilities of NRM and relapse were summarized by using cumulative incidence estimates. NRM was defined as death without relapse and was considered a competing risk for relapse, whereas relapse was a competing risk for NRM. The probability of developing acute GVHD or chronic GVHD was depicted by calculating the cumulative incidence with relapse and death without relapse or acute GVHD or chronic GVHD as competing risks. Variables with a p-value of ≤0.05 were considered to be significant and were included into multivariate analysis. Multivariate analysis for OS and LFS was performed with Cox backwards regression method. The multivariate analysis for comparison of NRM and relapses was based on a subdistributive hazard model for cumulative incidence function. Categorical characteristics were compared by Pearson's or Fisher's exact test. Statistical analysis was performed with IBM SPSS

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Version 25 (SPSS, Inc.; Chicago, IL, USA) and R software (Version 3.5.1 R Foundation, Vienna, Austria) with competing risks calculated using the package 'cmprsk' (http://CRAN.R-project.org/package=cmprsk).

Results

Patients' characteristics

A total of 189 patients were included in to the analysis. The allografts were performed at University of Hamburg in the period 01/2015 to 12/2018. All patients fulfilled the criterion for CR or CRi at the time of allo-SCT. Ninety-six patients (51%) were MRD^-, whereas 32 had "low" (17%) and 61 "high" (32%) MRD level. The characteristics of the study population are summarized in Table 1. The patients with "high" MRD level had more likely adverse risk AML (41% vs 22% and 26% for "low" and MRD^- patients, respectively, p=0.06). The interval between achieving of CR and allo-SCT did not differ significantly between three groups. A total of 13 patients (7%) received donor lymphocyte infusions within 288 days (range 180-1100) post-transplant due to persisting mixed chimerism (n=6), morphologic relapse (n=5) or molecular relapse (n=2). The median dosage was $1x10^6$ kg b.w. (range $5x10^5$ – $5x10^7$) with median number of 2 infusions (range 1-3). A total of 7 (54%) patients (mixed chimerism, n=4; molecular relapse, n=1 and morphologic relapse, n=1) responded.

Impact of pre-transplant MRD status on OS, LFS, Relapse, NRM: univariate analysis

In the study cohort of 189 patients, there were a total of 58 deaths, 45 relapses, and 27 NRM events. The median follow-up among survivors was 25 months (0.3-60). The 5-year probabilities for OS and LFS were 63% (95% confidence interval [CI] 55-70%) and 55% (95% CI 52-62%), respectively. The cumulative incidences of relapse and NRM at 5 years were 31% (95% CI 22-41%) and 16% (95% CI 11-23%), respectively.

The results of univariate analysis are represented in the Table 2. The highest 5-year OS was observed for MRD negative patients compared with those with "low" and "high" MRD. The cumulative incidence of relapse at 5 years was highest in patients with "high" MRD level compared with "low" MRD level and with MRD negativity: 63% vs 39% vs 9%, p<0.001, Fig. 1a-c). There was no difference in the NRM incidence between three groups.

Among the other factors, we found male sex associated with lower 5-year due to increased relapse incidence . Older patients experienced higher NRM resulting in lower 5-year OS. Patients with extramedullary manifestations showed lower 5-year OS due to

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higher NRM. Patients with adverse ELN risk had higher incidence of relapses comparing with intermediate and favorable resulting in corresponding survival outcomes.

Cumulative incidences of acute and chronic GvHD

A total of 43 of 189 patients developed severe (grade II-IV) acute GvHD with a median of 56 days (range 8-189). The cumulative incidence of aGvHD at 1 year was 22% (95% CI 17-28%).

A total of 80 of 189 patients developed chronic GvHD (mild, n=37; moderate, n=35, severe, n=8). The cumulative incidence of chronic GvHD at 5 years was 42% (95% CI 35-50%).

Subgroup analysis regarding the conditioning

In the whole cohort, we observed higher 5-year OS for MAC comparing with RIC. Despite the higher number of patients with adverse ELN risk in the MAC group (47/133, 35% vs 10/56, 18%, p=0.008) we did not observe increased relapse incidence in MAC patients (27% vs 25%, p=0.95). On the other hand, we observed increased NRM rate in patients from RIC cohort. This was a result of higher number of older (>58 years) patients in this group (47/56, 84% vs 42/133, 32%, p<0.001).

Further, we analyzed outcomes regarding the MRD status. Due to limited patients' number, the comparison was performed between MRD^- and MRD^+ patients. First, we observed significantly higher 5-year OS (60% 95% CI 46-73% vs 36% 95% CI 17-60%, p=0.047) after MAC for MRD^+ patients. This was rather result of lower NRM (9% 95% CI 4-20% vs 28% 95% CI 12-52%, p=0.014) than lower relapse incidence in the MAC cohort (47% 95% CI 34-60% vs 34% 95% CI 16-59%, p=0.31). The 5-year LFS did not differ significantly after MAC vs RIC for these patients (35% 95% CI 20-53% vs 39% 95% CI 20-62%, p=0.45).

Second, the 5-year OS and 5-year LFS for MRD^- patients did not differ significantly after MAC (OS: 79% 95% CI 68-87%; LFS: 76% 95% CI 63-86%) and RIC (OS: 61% 95% CI 39-79%, p=0.13; LFS: 61% 95% CI 39-79%, p=0.24) as well as the relapse rate (MAC: 12% 95% CI 6-22% vs RIC: 0%, p=0.09). However, we observed higher NRM after RIC (39% 95% CI 21-61% vs MAC: 12% 95% CI 6-22%, p=0.01) allografts.

Subgroup analysis regarding the remission status

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The results of univariate analysis for all patients regarding the remission status are represented in the Table 2. In details, the data of subgroup analysis are presented in supplemental Appendix (Table 1S).

Subgroup analysis regarding the interval between achieving the remission and allo-SCT

The results of univariate analysis for all patients regarding the interval between achieving the remission and allo-SCT are represented in the Table 2. In details, the data of subgroup analysis are presented in supplemental Appendix (Table 1S).

Subgroup analysis according to ELN risk

We observed significantly higher relapse incidence in MRD^+ patients in all ELN risk groups with the highest rate in patients with adverse status. However, we did not observe any significant difference concerning the 5-year OS. This could be due to trends to increased NRM in MRD^- patients in all three groups (Table 1S). Only the 5-year LFS for patients with intermediate ELN risk was significantly higher in MRD^- patients.

Impact of pre-transplant MRD status on OS, LFS, Relapse, and NRM: multivariate analysis

The multivariate models for OS and LFS included six (patients' age, patients' sex, extramedullary involvement, ELN, conditioning and MRD status). The multivariate model for relapses included four variables (patients' sex, ELN, MRD status and conditioning); the multivariate model for NRM included three variables (patients' age, extramedullary involvement and conditioning). The results of multivariate analysis with the variables remained in final models are represented in the Table 3. The hazard ratios for "high" MRD vs. MRD negativity and "low" MRD vs. MRD negativity were significantly increased for OS, LFS and for relapses. However, we found no significant impact when comparing directly "low" MRD vs "high" MRD on relapses and OS. Also, we observed a significant negative impact of male sex on relapse incidence. Younger age (≤58 years), as well as the absence of extramedullary manifestations showed a favorable independent impact on NRM. Additionally, younger age had a favorable impact on OS and the absence of extramedullary manifestations had a favorable impact on OS. Concerning the intensity of conditioning, we observed a favorable impact of MAC on NRM but not on relapses.

Additionally, we performed a separate multivariate analysis. Concerning the MRD^- patients we observed a favorable impact of younger (≤58) age (HR 0.35 95% CI 0.14-0.88,

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p=0.026) and absence of extramedullary involvement (HR 0.19 95% CI 0.07-0.51, p=0.001) on OS and LFS (for patients ≤58 years: HR 0.38 95% CI 0.16-0.92, p=0.031 and for absence of extramedullary manifestations: HR 0.20 95% CI 0.08-0.53, p=0.001) in final models. Due to low event number (n=7) the multivariate analysis for relapses in this group was not performed. Regarding the NRM, absence of extramedullary manifestations (HR 0.86 95% CI 0.05-1.5, p=0.14) and younger age (HR 1.2 95% CI 0.26-5.5, p=0.81) remained in the final model.

Concerning the MRD$^+$ patients, the variables remained in the final model for relapses were patients' sex (male vs female: HR 1.9 95% CI 0.97-3.6, p=0.06) and ELN risk (favorable vs adverse: HR 0.96 95% CI 0.4-2.3, p=0.93; intermediate vs adverse: HR 0.53 95% CI 0.26-1.1, p=0.078; wald-test, p=0.22). Due to low NRM (n=7) the multivariate analysis for relapses was not performed. Following variables remained in the final model for OS: patients' age (≤58 vs >58, HR 0.43 95% CI 0.2-0.89, p=0.022), MAC vs RIC (HR 0.44 95% CI 0.18-1.1, p=0.068) and ELN risk (favorable vs adverse HR 0.8 95% CI 0.29-2.3, p=0.68; intermediate vs adverse HR 0.33 95% CI 0.14-0.75, p=0.008; wald-test, p=0.023). Only ELN risk (favorable vs adverse HR 1.0 95% CI 0.44-2.5, p=0.94; intermediate vs adverse HR 0.50 95% CI 0.27-0.93, p=0.029, wald-test, p=0.059) remained in the final model for LFS.

Discussion

The quantitative and qualitative determination of MRD levels using MFC during aplasia, either early after induction and/or after consolidation chemotherapy in AML patients, was proven useful to predict relapse and poor outcome outside the allogeneic setting (17-19). Further, Walter *et al.* reported on the outcomes of 99 AML patients who received allografts in CR depending on the pre-transplant MRD status using the "different from normal" method. They postulated MRD positivity prior to allo-SCT to be an independent factor for OS, DFS, and relapse (8). Other studies in AML patients (most of which were based on MRD detection by the LAIP method) confirmed these data (11,20,21). Nevertheless, the prognostic value of the pre-transplant MRD level remains to be further clarified.

In the majority of patients from our series the initial phenotype of leukemic cells was not available. Thus, the MRD assessment had been performed using the "different from normal" method with extended panel created at our institution with 250,000 events per tube ("old" panel) or according to ELN recommendations with up to 2,000,000 events per tube ("new" panel) (9). This is in sharp contrast to the majority of published studies in

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this field, which were performed using only one MFC panel. Nevertheless, comparing two different MFC panels within lineal regression method, we found significantly strong correlations for neutrophils, monocytes, lymphocytes, blasts and aberrant blasts. Also, we did not observe any difference in relapses (Appendix). The feasibility to use lower events by the MFC MRD analysis might be important in patients with hypocellular bone marrow who still not regenerated after salvage chemotherapy and undergo allo-SCT.

A proportion of 49% of the 189 patients in our study showed MRD positivity at the time of allo-SCT. This number of MRD^+ patients is slightly higher than reported in previous studies. The possible explanations for this can include different induction strategies as well as changed antigen expression in regenerative bone marrow especially in patients with CRi. Unfortunately, in our study we did not have MFC data from the regenerating bone marrow.

Regarding the clinical impact of the pre-transplant MRD status, in general, we confirmed previous observations (8,11,20,21) describing an independent prognostic significance of MRD positivity on relapse incidence, OS, LFS but not on NRM in patients with AML in CR. To further investigate the role of pre-transplant MRD level, we subdivided all patients in three groups: MRD negative, and those with “low” (0.1-0.5%) and “high” (>0.5%) MRD level. In univariate analysis, we observed higher LFS for patients with “low” MRD level due to lower relapse incidence comparing with patients with “high” MRD level. Further, we observed a trend to improved OS for the former group. In multivariate analysis, we observed an independent unfavourable impact on relapses and LFS of both “low” and “high” MRD levels vs MRD negativity. Also, “high” MRD level had a unfavourable impact on OS related to MRD negativity. However, we did not observe significant impact of “low” vs “high” MRD level on relapses, LFS or/and OS. Thus, the are suggesting that the quantitative measurements of MRD level in case of MRD positivity may be prognostically not as relevant. Several explanations could be provided in this setting. First, detection of low level of leukemic cells that may represent more aggressive clone pre-transplant can lead to fast proliferation and development of relapse post-transplant in case of failure of graft-vs-leukemia effect. Second, abnormal blast population in case of unknown LAIP can also correspond to clonal hematopoiesis, which can be associated with false-positive results (21). Third, this could be explained also due to different sensitivity of MFC approach depending on quality (e.g. blood dilution) and preparations of bone marrow samples.

In the same context, Anthias *et al.* reported on the outcomes for 88 AML patients in CR according to their pre-transplant MRD status measured by MFC according to LAIP

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method. A number of 35 (40%) of patients were MRD^+. The authors confirmed the negative independent impact of pre-transplant MRD positivity. They reported a significantly higher relapse incidence at 2 years in the high-level (≥1%) MRD^+ group compared to the low-level positive (<1%) group and the MRD^- patients at 70%, 37% and 7.6%, respectively (p<0.001) (11). These results are in line with our findings. Nevertheless, no multivariate analysis data were presented.

We found a significant favourable impact of intermediate ELN risk (2017) on relapses, LFS and OS, suggesting that cytogenetics and molecular genetics are equally important for the risk of relapse after allo-SCT. Recently, Grimm *et al.* reported on the prognostic meaning of pre-transplant ELN risk (2017) stratification on relapse risk (2.95 95% CI: 2.09-4.18, p<0.001) and OS in 234 patients with AML (23).

Further, in a randomized study by Hourigan *et al.* the authors reported improved survival with lower relapse risk for NGS positive patients independently of age (24). In our study, we observed favourable impact of MAC on LFS in multivariate analysis. However, this effect was rather due to increased NRM in RIC cohort including higher number of older patients than due to lower relapses after MAC.

Among the other factors, we found female sex to be associated with lower relapse rate in accordance with previously published studies (25). Younger (≤58 years) patients' age and absence of extramedullary manifestations were associated with improved survival due to a significantly lower NRM. The latter observation can be explained by the fact, that those patients received irradiation before the allo-SCT, which may increase the post-transplant NRM.

In conclusion, though having confirmed previous data on the significance of the pre-transplant MRD as determined by the identification of aberrant hematopoiesis by MFC on the incidence of relapses, probability of LFS and OS, our series arises some new important points. First, the MRD MFC measurements according to "different from normal" method seems to be feasible also with lower (<2,000,000 per tube) events. Second, the qualitative MRD results (positive vs negative) seems to be more prognostically important for relapse incidence, LFS and OS as its quantitative evaluation. Third, the ELN risk stratification seems to play prognostic role also in the transplant settings. Further stratification of patients according to their ELN risk pre-transplant and MRD status may be helpful to identify patients with the highest risk who might benefit from early post-transplant pre-emptive interventions such as early tapering of immunosuppression, or use pre-emptive use of donor lymphocyte infusions.

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Author contributions: EK performed the research. EK, MC, NK designed the research study. UB, AB, UFF contributed essential reagents or tools. EK, NK analysed the data. EK wrote the paper. All authors participated in the interpretation of the data.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables and Figures

Table 1. Characteristics of the patients with AML (n=189). (s, secondary; t, therapy-related; CRi, complete remission with incomplete hematologic recovery)

* only cycles of conventional chemotherapy included

Parameter	MRD$^-$ (n=96)	MRD "low" (n=32)	MRD „high" (n=61)	p-value
Patient's sex:				0.61
male	56 (58%)	20 (63%)	32 (53%)	
female	40 (42%)	12 (37%)	29 (48%)	
Patient's age				0.51
≤58	48 (50%)	16 (50%)	36 (59%)	
>58	48 (50%)	16 (50%)	25 (41%)	
Origin of disease				0.53
de novo	78 (81%)	23 (72%)	48 (79%)	
s/t-AML	18 (19%)	9 (28%)	13 (21%)	
Remission status				0.84
1 CR	63 (65%)	20 (62%)	36 (59%)	
2+ CR	11 (12%)	6 (19%)	9 (15%)	
CRi	22 (23%)	6 (19%)	16 (26%)	
Interval between remission and allograft (median, range)	61 (7-979)	49 (11-436)	65 (11-1111)	0.50
				0.77
≤ 60 days	47 (49%)	18 (56%)	30 (49%)	
> 60 days	49 (51%)	14 (44%)	31 (51%)	
Extramedullary involvement	10 (10%)	3 (9%)	3 (5%)	0.47
ELN risk score				0.06
favorable	16 (17%)	2 (6%)	10 (16%)	
Intermediate	55 (57%)	23 (72%)	26 (43%)	
adverse	25 (26%)	7 (22%)	25 (41%)	
Number of previous cycles of chemotherapy*				0.75
≤2	72 (75%)	21 (72%)	43 (71%)	
>2	20 (25%)	8 (28%)	15 (25%)	
Primary induction failure	27 (28%)	4 (13%)	18 (30%)	0.16

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Previous therapy				0.58
Chemotherapy	66 (69%)	24 (75%)	45 (74%)	
Chemotherapy + TKI	16 (17%)	4 (13%)	7 (12%)	
5-azacitidine or decitabine	5 (5%)	4 (13%)	6 (10%)	
Venetoclax in combinations	5 (5%)	-	3 (5%)	
other	3 (3%)	-	-	
Donor type:				0.28
MRD	14 (15%)	6 (19%)	16 (26%)	
MUD	61 (64%)	15 (47%)	35 (57%)	
MMUD	14 (25%)	6 (19%)	6 (10%)	
Haploidentical/Cord blood	7 (7%)	5 (16%)	4 (7%)	
CMV status (P/D)				0.07
pos/pos	57 (59%)	12 (38%)	34 (56%)	
pos/neg	13 (14%)	5 (16%)	6 (10%)	
neg/pos	4 (4%)	6 (19%)	3 (5%)	
neg/neg	22 (23%)	9 (28%)	18 (30%)	
Conditioning				0.56
myeloablative	69 (72%)	20 (63%)	44 (72%)	
reduced intensity	27 (28%)	12 (38%)	17 (28%)	
Immunosuppression				0.65
ATG	82 (86%)	25 (81%)	49 (82%)	
post-transplant cyclophosphamide	13 (14%)	6 (19%)	11 (18%)	
no	1	1	1	

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Table 2. Results of univariate analysis in the total cohort of 189 patients with AML.(s, secondary; t, therapy-related; CRi, complete remission with incomplete hematologic recovery)

Characteristic	LFS	p	OS	p	RI	p	NRM	p
Patients' sex		0.087		0.039		0.07		0.86
male (n=108)	47% (36-59%)		56% (46-65%)		35% (24-47%)		17% (11-26%)	
female (n=81)	61% (47-73%)		74% (61-84%)		24% (14-37%)		15% (9-24%)	
Patients' age		0.04		<0.001		0.22		<0.001
≤58 (n=100)	62% (50-73%)		75% (64-83%)		34% (24-46%)		4% (2-10%)	
>58 (n=89)	49% (38-61%)		50% (39-61%)		36% (13-68%)		31% (21-44%)	
Origin of disease		0.92		0.61		0.98		0.99
de novo (n=149)	51% (40-62%)		63% (54-72%)		33% (23-46%)		17% (12-24%)	
s/tAML (n=40)	56% (40-71%)		63% (47-77%)		29% (16-46%)		16% (8-31%)	
Remission status		0.51		0.25		0.28		0.65
1 CR (n=119)	54% (42-65%)		67% (57-75%)		24% (17-33%)		16% (11-23%)	
2+ CR (n=26)	55% (35-73%)		55% (35-73%)		38% (21-58%)		7% (2-23%)	
CRi (n=44)	56% (47-70%)		57% (35-75%)		26% (15-42%)		18% (9-32%)	
Interval between CR and allo-SCT		0.74		0.89		0.48		0.66
≤ 60 days (n=95)	59% (49-68%)		67% (57-76%)		29% (20-40%)		13% (7-25%)	
> 60 days (n=94)	50% (35-65%)		60% (48-71%)		33% (19-51%)		19% (11-32%)	
Extramedullary involvement:		0.041		0.019		0.89		0.03
no (n=173)	53% (41-64%)		65% (56-73%)		32% (22-46%)		15% (10-22%)	
yes (n=16)	44% (24-66%)		44% (24-66%)		25% (10-50%)		31% (13-56%)	
ELN risk score		0.012		0.014		0.08		0.36
favorable (n=28)	53% (31-74%)		57% (34-77%)		23% (9-46%)		24% (9-49%)	
intermediate (n=104)	66% (56-75%)		73% (62-82%)		22% (15-31%)		12% (7-19%)	

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adverse (n=57)	36% (22-52%)		48% (33-63%)		42% (26-60%)		21% (12-35%)	
Primary induction failure		0.94		0.68		0.62		0.53
no (n=140)	51% (40-62%)		62% (52-71%)		33% (23-46%)		16% (11-23%)	
yes (n=49)	57% (41-71%)		68% (52-81%)		25% (14-41%)		18% (9-32%)	
MRD (MFC) before allo-SCT		0.001		0.088		<0.001		0.30
negative (n=96)	72% (61-81%)		73% (62-82%)		9% (5-17%)		20% (13-29%)	
“low” (0.1-0.5%) (n=32)	51% (34-68%)		61% (44-76%)		39% (24-57%)		9% (3-24%)	
“high” (>0.5%) (n=61)	33% (20-50%)		48% (33-63%)		63% (38-83%)		15% (7-30%)	
Donor type:		0.075		0.32		0.07		0.34
MRD (n=36)	49% (27-72%)		61% (38-80%)		41% (21-64%)		10% (2-35%)	
MUD (n=11)	57% (42-71%)		67% (57-76%)		25% (14-41%)		18% (12-27%)	
MMUD (n=26)	39% (22-59%)		51% (33-69%)		45% (26-66%)		16% (6-37%)	
Haploidentical/Cord blood (n=16)	33% (11-65%)		56% (28-81%)		47% (17-79%)		21% (7-47%)	
Conditioning		0.05		0.017		0.95		0.01
myeloablative (n=133)	59% (49-68%)		69% (59-78%)		27% (18-38%)		12% (7-19%)	
reduced intensity (n=56)	48% (35-61%)		51% (38-64%)		25% (15-38%)		27% (17-49%)	

Table 3. Results of multivariate analysis (n=189).

Parameter	**OS**	**LFS**	**RI**	**NRM**
Model 1				
Patients‘ sex male vs. female	-	-	2.1 (1.1-4.0), p=0.022	-
Patients‘ age ≤58 vs. >58 years	0.42 (0.23-0.77), p=0.005	0.53 (0.33-0.85), p=0.009	-	0.12 (0.04-0.37), p=0.0002
Extramedullary manifestation:				

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no vs. yes	0.24 (0.11-0.53), p=0.001	0.35 (0.17-0.73), p=0.005	-	0.24 (0.1-0.64), p=0.0044
MRD before allo-SCT:	p=0.011	p=0.001	p<0.001	
„low“ (0.1-0.5%) vs. negative	2.1 (0.99-4.4), p=0.052	2.2 (1.1-4.2), p=0.02	6.0 (2.2-15.3), p=0.0034	
“high” (>0.5%) vs. negative	2.5 (1.4-4.6), p=0.003	2.8 (1.7-4.7), p<0.001	7.7 (3.3-17.6), p<0.0001	-
”low” (0.1-0.5%) vs. “high” (>0.5%)	0.84 (0.41-1.7), p=0.63	0.79 (0.42-1.5), p=0.46	0.78 (0.36-1.7), p=0.54	
ELN risk	p=0.008	p=0.03	p=0.25	-
intermediate vs. favorable	0.66 (0.30-1.5), p=0.31	0.65 (0.32-1.3), p=0.22	0.58 (0.25-1.3), p=0.20	
intermediate vs. adverse	0.38 (0.21-0.7), p=0.002	0.50 (0.30-0.84), p=0.008	0.55 (0.28-1.1), p=0.079	
favorable vs. adverse	0.58 (0.26-1.3), p=0.18	0.77 (0.38-1.6), p=0.47	0.89 (0.39-2.0), p=0.79	
Conditioning:				
MAC vs. RIC	0.51 (0.26-0.99), p=0.048	-	-	-
Model 2				
Patients‘ sex				
male vs. female	-	-	2.0 (1.1-3.8), p=0.025	-
Patients‘ age			-	
≤58 vs. >58 years	0.42 (0.23-0.78), p=0.006	0.54 (0.34-0.87), p=0.012		0.17 (0.05-0.58), p=0.0047
Extramedullary manifestation:			-	
no vs. yes	0.24 (0.11-0.54), p=0.001	0.36 (0.17-0.74), p=0.006		0.18 (0.1-0.48), p=0.0005
MRD before allo-SCT:				
pos vs. neg	2.3 (1.3-4.1), p=0.004	2.5 (1.5-4.1), p<0.001	7.0 (3.2-15.8), p<0.001	-
ELN risk	p=0.004	p=0.013	p=0.16	-
intermediate vs. favorable	0.64 (0.29-1.4), p=0.27	0.62 (0.31-1.2), p=0.17	0.55 (0.24-1.2), p=0.14	
intermediate vs. adverse	0.37 (0.20-0.67), p=0.001	0.47 (0.28-0.78), p=0.003	0.52 (0.27-0.99), p=0.045	
favorable vs. adverse	0.57 (0.26-1.3), p=0.18	0.76 (0.38-1.6), p=0.45	0.90 (0.39-2.1), p=0.80	

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Conditioning: MAC vs. RIC	0.51 (0.26-0.99), p=0.046	-	-	

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Figure legends:

Figure 1. Kaplan-Meier curves for all patients (n=189) according to MRD level: a) LFS, b) OS, c) RI and NRM

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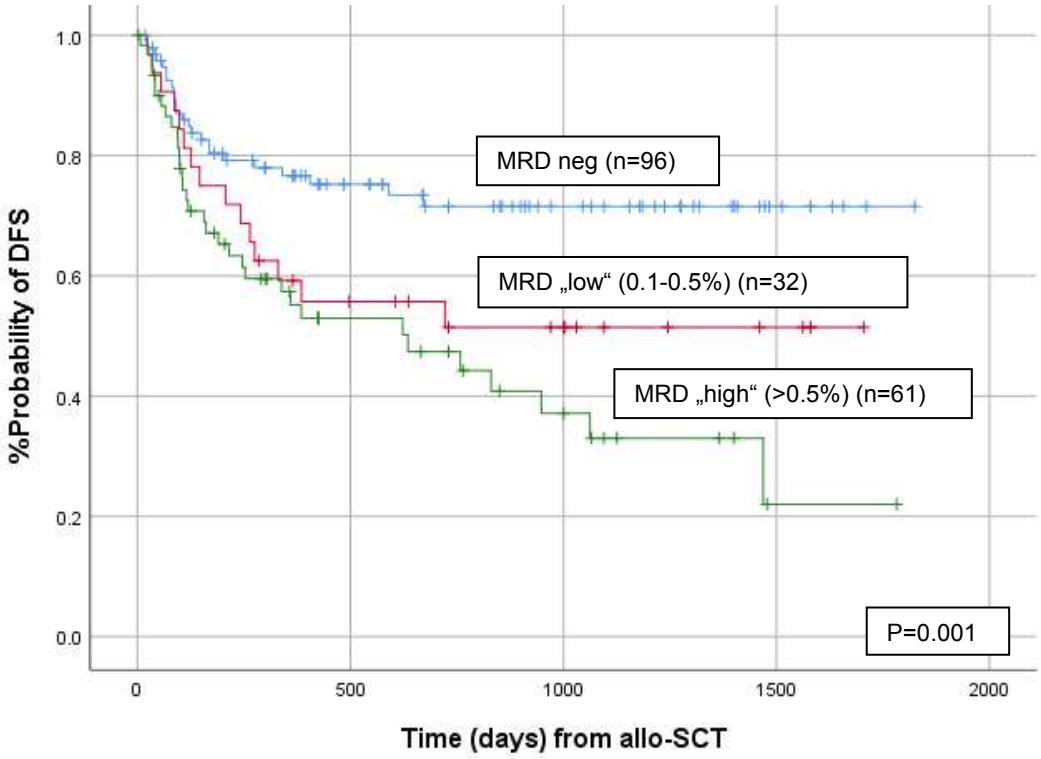
Figures:

Figure 1.

a)



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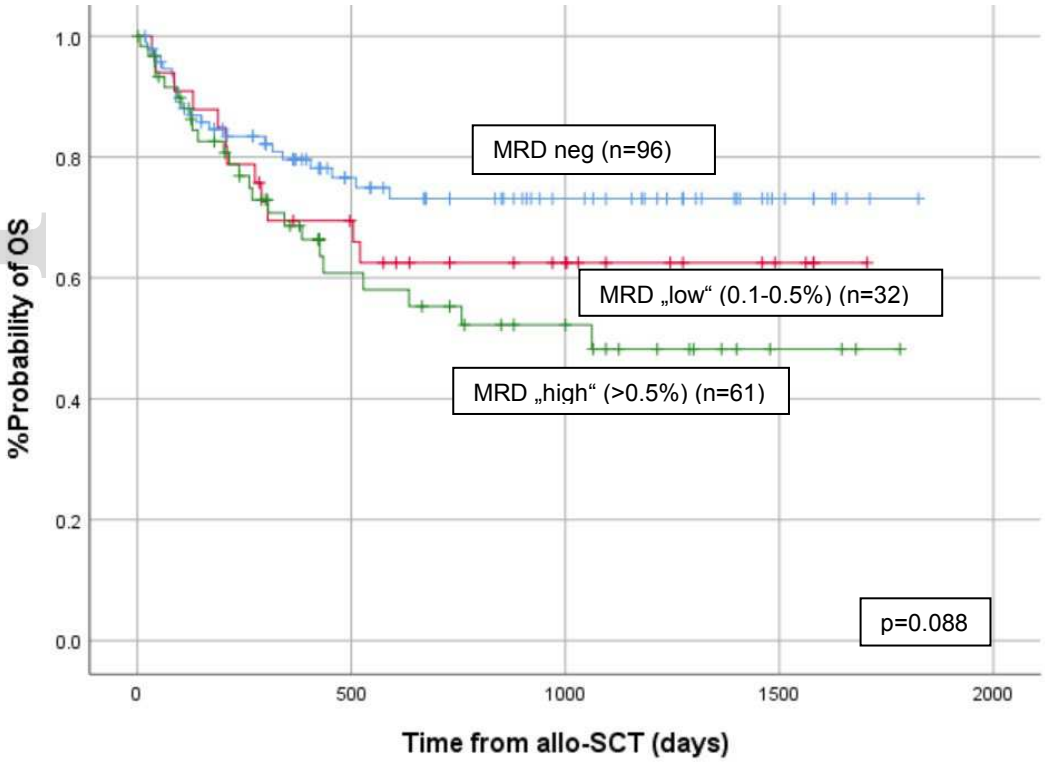

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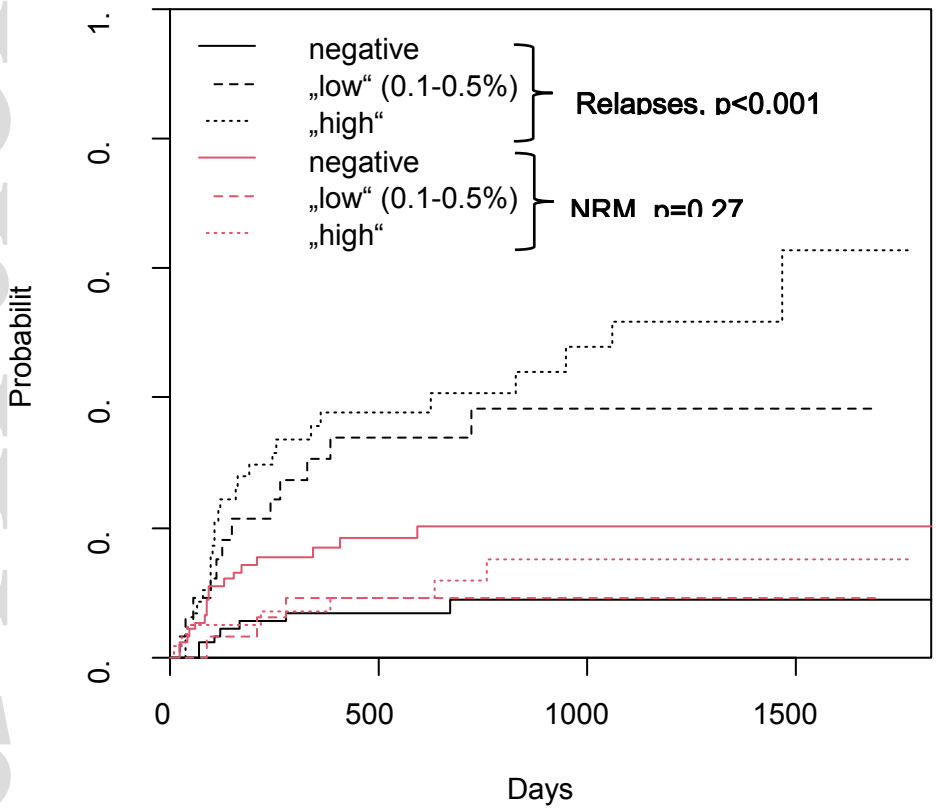
c)



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