Journal Pre-proof

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PII: S2666-6367(21)00952-0
DOI: https://doi.org/10.1016/j.jtct.2021.06.003
Reference: JTCT 56424

To appear in: *Transplantation and Cellular Therapy*

Received date: 15 April 2021
Accepted date: 2 June 2021

Please cite this article as: Evgeny Klyuchnikov , Anita Badbaran , Radwan Massoud , Ulrike Fritsche-Friedland , Dietlinde Janson , Francis Ayuk , Christine Wolschke , Ulrike Bacher , Nicolaus Kröger , Enhanced immune reconstitution of $\gamma\delta$ T cells after allograft overcomes negative impact of pre-transplant MRD positive status in AML patients., *Transplantation and Cellular Therapy* (2021), doi: https://doi.org/10.1016/j.jtct.2021.06.003

Enhanced immune reconstitution of γδ T cells after allograft overcomes negative impact of pre-transplant MRD positive status in AML patients.

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Short running title: γδ T cells and MRD+ AML

Keywords: allogeneic stem cell transplantation, γδ T cells, minimal/measurable residual disease, AML

Specifications:

Abstract: 373 words

Main body of text: 3912 words

Tables/Figures: 5 Tables /2 Figures

References: 56

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Highlights

- γδ T cells in MRD+ AML patients leads to lower relapses and higher survival
- pt-Cy is associated with lower post-transplant levels of γδ T cells compared with ATG
- γδ T cells is not associated with development of acute GvHD

Abstract

Background: Minimal residual disease (MRD) prior to allogeneic stem cell transplantation (allo-SCT) in AML is a poor risk factor for outcome. The γδ T cells represents a unique minority lymphocyte population which is preferentially located in peripheral tissues, can recognize antigens in non-MHC restricted manner and plays a "bridging" role between innate and adaptive immune system.

Objectives: In this study, we investigated a potential graft-vs-leukaemia effect of γδ T cells reconstitution post-transplant in AML patients with pre-transplant positive minimal/measurable disease status (MRD+).

Study design: We investigated a potential graft-vs-leukaemia effect of γδ T cells reconstitution post-transplant in AML patients with pre-transplant positive MRD+. MRD assessment was performed in 202 patients (MRD+, n=100) with multicolored flow cytometry ("different from normal" strategy). Analysis for absolute concentrations of CD3+, CD4+, CD8+, NK, and γδ T cells were performed by flow cytometry according to an internal protocol at day +30 and +100 post-transplant. Differences between categorical and continuous variables were determined by Chi-square and Student's T-test, respectively. The Mann-Whitney test was used to compare medians of continuous variables. Spearman correlation was used for nonparametric assessment of correlation between different cell subsets during immune reconstitution. Kaplan Meier survival analysis and Cox regression analysis were used to investigate the associations between immune reconstitution and survival outcomes. Grays' analysis was used to compute incidences of relapses, non-relapse mortality (NRM) and graft-vs-host disease (GvHD).

Results: Follow-up for survivors was 28 months (3-59). Younger age (≤58) of recipient and donor (<30), sex mismatch, matched donors, CMV reactivation and ATG were associated with a faster γδ T cell reconstitution. In multivariable analysis for MRD+ patients, higher than median level of γδ T cells on days +30 and +100 resulted in a significant improved leukaemia-free (HR 0.42, p=0.007 and HR 0.42, p=0.011, respectively) and overall survival (HR 0.44, p=0.038 and HR 0.33, p=0.009, respectively). Further, higher γδ T cell level on day +30 led to significant reduced

risk of relapse (HR 0.36, p=0.019). No impact of γδ T cell level on day +30 and +100 could be seen in MRD- patients and no correlation with occurrence of graft-*versus*-host disease could be observed.

Conclusion: An enhanced immune reconstitution of γδ T cells post-transplant may overcome the higher relapse risk of pre-transplant MRD+ patients with AML.

Introduction

Allogeneic stem cell transplantation (allo-SCT) is a curative treatment for several hematological malignancies including acute myeloid leukemia (AML). Considerable frequency of post-transplant relapses and non-relapse mortality (NRM) caused by Graft- versus- host diseases (GVHD), organ toxicity, and infectious complications represent limiting factors for success of this approach. Studies evaluating the impact of measurable residual disease (MRD) have been expanding in the last decades and clearly demonstrated higher relapses and lower survival in pre-transplant MRD+ AML patients. (ref. 1-3) Recently, Hourigan et al. (ref. 4) showed that in AML MRD + patients myeloablative conditioning (MAC) is associated with less relapses in comparison to reduced intensity conditioning regimens. Nevertheless, the 3-year OS for pre-transplant MRD+ (assessed by next-generation sequencing) patients after reduced intensity (RIC) conditioning were still around 50%. The meaning of other factors that might be relevant in this setting still remains unclear.

Post-transplant immune reconstitution plays an important role in the development of infections, GVHD and relapses. (ref. 5) The cells of the innate immune system, which are not MHC restricted, could be crucial for development of the graft-*versus*-leukemia (GvL) effect without development of GVHD. (ref. 6) Of those, γδ T cells represent a unique population counting up to 20% of circulating CD3+ lymphocytes and constituting the major subset of resident T cells in skin and mucosa. (ref. 7,8) Together with NK cells they do not express CD4 and CD8, recognize peptide- and non-peptide antigens and regenerate quickly after allo-SCT. (ref. 6,9) Additionally, these cells play a “bridging” role between innate and adaptive immune system modulating dendritic cells (ref. 10), NK cells (ref. 11), B (ref. 12) and other T cells. (ref. 13) Moreover, γδ T cells can exert effective anti-tumor activity against various solid tumors (ref. 14) and hematologic malignancies, such as lymphoma (ref. 15), multiple myeloma (ref. 16) and AML. (ref. 17-19) Of interest, a subset of γδ T cells expands upon CMV reactivation and these CMV-induced γδ T cells are able to recognize and lyse leukemic blasts. (ref. 20) This phenomenon can at least partly

explain the association between low post-transplant relapses and CMV reactivation after allo-SCT observed in some studies. (ref. 21)

The role of γδ T cells in developing GVHD remains controversial. Pabst et al. (ref. 22) reported on an association between an increased number of γδ T cells in the graft with development of acute GVHD after allo-SCT from unrelated donors. In contrast, Lamb et al. (ref. 23), reported that γδ T cells are not substantially activated in *in vitro* allogeneic mixed lymphocyte cultures. Though other authors observed no association between γδ T cells and occurrence of GVHD (ref. 24, 25), γδ T cells can participate in GVHD being activated by αβ T cells. (ref. 26)

Some studies showed an association between improved outcomes and increased post-transplant levels of γδ T cells in adult and pediatric patients with acute leukemia. (ref. 27-30) In a recently published meta-analysis, Arruda et al (ref. 31) observed that high γδ T cell level after allo-SCT were associated with less relapses (HR 0.58, 95% CI 0.40-0.84; p=0.004), fewer viral infections (HR 0.59, 95% CI 0.43-0.82; p=0.002) and better OS (HR 0.28, 95% CI 0.18-0.44; p<0.00001) and DFS (HR 0.29, 95% CI 0.18-0.48; p<0.00001) without any association with acute GVHD incidence (HR 0.72, 95% CI 0.41-1.27, p=0.26). In addition, Galimberti et al (ref. 32) reported on a changed T cell receptor profile of γδ T cells that was associated with achieving MRD negativity in patients with multiple myeloma after allo-SCT.

To analyze the role of this unique cell population in the context of post-transplant outcomes for MRD+ AML patients, we hypothesize that increased post-transplant levels of γδ T cells may overcome the negative impact of pre-transplant MRD+ without an increased rate of acute GVHD.

Patients and Methods

Study cohort

Adult (≥18 years old) AML patients in complete remission (CR) who underwent allo-SCT with available pre-transplant MRD data were included in this retrospective study. European Leukemia Net (ELN) criteria (2017) were used to assign a disease-dependent risk. (ref. 33) Response to therapy was documented according to International Working Group criteria. (ref. 34) The conditioning intensity was defined according to criteria published previously. (ref. 35)

Flow cytometry analysis of immune reconstitution

Routine analyses for absolute concentrations of CD3+, CD4+, CD8+, NK, and γδ T cells were performed by flow cytometry according to an internal protocol: (1) CD4-APC, CD8-PE, Multitest (CD3 FITC, CD16+56 PE, CD45 PerCP, CD19 APC); (2)

CD4-APC, CD45-V450, Multitest (CD45RA FITC, CD45RO PE, CD3 PerCP, CD8 APC) ; (3) CD45-V450, CD3-PerCPl, anti-TCR-PE, anti-HLA DR-APC in peripheral blood samples. All antibodies were obtained from Becton Dickinson (BD Biosciences, New Jersey, USA). Up to 5 000 events (25 000 per sample) were acquired per tube. Sample acquisition was performed using a BDTM FACS-Canto flow cytometer with the BDTM FACSDiva software which was also used for data analyses. The data for γδ T cells reconstitution were collected on days +30 and +100 according to dynamics of their reconstitution. (ref. 9, 36)

Flow cytometry analysis for MRD assessment

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. Our MRD assessment approach is published in detail in our previous work. (ref. 3) The sensitivity of the method was 10^{-4} to 10^{-5}. The cut-off for MRD positivity was 0.1%. 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. (ref. 37) 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 following the ELN consensus as published by Schuurhuis et al. (ref. 38)

Statistical analysis

Kaplan Meier survival analysis and Cox regression analysis were used to investigate the associations between immune reconstitution and OS and LFS. In addition to cell populations, pre-transplant factors thought to have a possible impact on OS and LFS were included in the analysis. Variables with a $p \leq 0.1$ in univariate analysis were included in the multivariable models. Differences between categorical and continuous variables were determined by Chi-square and Student’s T-test, respectively. The Mann-Whitney test was used to compare medians of continuous variables. Spearman correlation was used for nonparametric assessment of correlation between different cell subsets during immune reconstitution.

The cumulative incidence of GVHD and death from relapse, was determined using Gray’s competing risks analysis. NRM was defined as death from all causes other than relapse. Competing risk for death from relapse was non-relapsed mortality (NRM). The probability of developing acute (grade II-IV) GVHD and chronic GVHD was depicted by calculating the cumulative incidence with death without GVHD as competing risks.

Statistical analysis was performed with IBM SPSS 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 202 patients with AML in CR before allogeneic SCT were included in the study (median age, 58 years, range, 21 to 80 years; 116 males). One hundred patients were flow MRD+ before SCT, whereas 102 were MRD-. All allografts (140 MAC, 62 RIC) were performed at University of Hamburg from 01/2015 to 01/2020. Of the 202 patients included into the study 163 received ATG and 36 post-transplant cyclophosphamide. In general, the immunosuppression with post-transplant cyclophosphamide is determined for haploidentical allografts (n=17) and ATG for other donors (n=162). As part of clinical studies, post-transplant cyclophosphamide was given to eleven patients transplanted with MUD, five patients transplanted with MRD and three patients transplanted with MMUD. The immunosuppression with ATG is determined for patients transplant from MRD, MUD or MMUD. As part of clinical study, ATG was given to one patient after haploidentical transplantation.

All patients consented in accordance with the Declaration of Helsinki. Follow-up was current as of February 15, 2020. The characteristics of the study population are summarized in Table 1.

<u>Pre-transplant MRD+ patients</u>

<u>Immune reconstitution in MRD+ patients</u>

The data on immune reconstitution were obtained on days +30 for 187 (MRD+, n=93) and on +100 for 167 patients (MRD+, n=86). Immune reconstitution was assessed using absolute amounts of cells on days +30 and +100 post-transplant. We observed a strong positive correlation of CD3+ and CD3+CD4+ (r^2=0.66, p<0.001), CD3+CD8+ (r^2=0.96, p<0.001), and a weak positive correlations between CD3+ cells and NK (r^2=0.26, p<0.001) and γδ T cells (r^2=0.53, p<0.001) on day +30. The same was documented for day +100 (CD3+CD4+, r^2=0.65, p<0.001; CD3+CD8+, r^2=0.98, p<0.001; NK cells, r^2=0.28, p<0.001; γδ T cells, r^2=0.41, p<0.001). The data on immune recovery on days +30 and +100 were available in 169 patients. The stratification into "low" and "high" subgroups in this setting was performed according

the median γδ T cell number on corresponding days in available patients, independently of the MRD status. Of 84 patients with "low" (≤18/μl) 62 (74%) experienced "low" (≤26/μl) level on day +100, whereas 22 (26%) converted from "low" to "high" status (p<0.001). Of 85 patients with "high" (>18/μl) level of γδ T cells on day +30, 59 (69%) experienced "high" level, whereas 26 (31%) experienced decrease of these cells on day +100.

For day +30, the MRD+ patients with γδ T cells number >13x10^6/L (which was representing the median) were proposed to belong to the "high" group and those ≤13x10^6/L to the "low" group, respectively. For day +100, MRD+ patients with γδ T cells numbers >23x10^6/L were proposed to belong to the "high" group and those ≤23x10^6/L to the "low" group. We observed no significant differences of the different lymphocyte subsets including γδ T cells on days +30 and +100 between MRD+ and MRD- patients (Table 2).

Regarding day +30 (n=93), we observed a positive correlation between patients' sex (female), patient/donor sex status, donors' age, matched donor type, CMV reactivation, type of immunosuppression (ATG) and higher number of γδ T cells in MRD+ patients (Table 3).

Regarding day +100 (n=86), we observed positive correlations between patients' sex (female), patient/donor sex status, younger patients' age, type of immunosuppression (ATG) and higher number of γδ T cells in MRD+ patients.

Female patients were more likely to receive ATG as immunosuppression compared with male patients (38/44, 86% vs 39/54, 72%, p=0.072).

The correlations for MRD- patients are represented in Table S1 of supplemental files.

Survival, relapses and NRM in MRD+ patients

The median follow up for survivors was 28 months (range 3-59). There were 39 deaths, 39 relapses and 11 NRM events.

The 5-year OS and LFS were 55% (95% CI 44-66%) and 35% (95% CI 22-50%), respectively. Relapses and NRM at 5 years after allo-SCT were 52% (95% CI 37-67%) and 13% (95% CI 7-23%).

We observed a significant higher 5-year OS (RR 0.38, 95% CI 0.18-0.79, p=0.01) and LFS (RR 0.44, 95% CI 0.23-0.82, p=0.009) for patients, who had "high" level of γδ T cells on day +30, due to a significantly lower risk of relapses at 5 years after allo-SCT (RR 0.42, 95% CI 0.22-0.83, p=0.012, Table 4, Fig. 1a-c).

Similarly, we observed a significantly higher 5-year OS (RR 0.32, 95% 0.14-0.73, p=0.007) and LFS (RR 0.41, 95% CI 0.21-0.80, p=0.009) for patients, who had a "high" level of γδ T cells on day +100 also due to lower relapse incidence (RR 0.52,

95% 0.26-1.0, p=0.063), although significance was not reached. Importantly, the "high" and "low" categories for the other lymphocyte subsets did not correlate with OS and LFS in univariate analysis.

Among other factors, younger patient age (≤ 58 years) was associated with lower NRM and higher OS. MAC was associated with a better OS as compared to RIC. This was possibly a result of significantly older patients (>58 years) included in the RIC cohort (25/32, 78% vs 20/68, 29%, p<0.001).

Frequency of acute and chronic GVHD

For the MRD+ patients, the rate of severe (II-IV) acute GVHD at 1 year was 16% (95% CI 10-25%). We observed no correlation between the level of γδ T cells on days +30 ("high" vs "low": RR 1.9, 95% CI 0.7-5.2, p=0.21) and +100 ("high" vs "low": RR 0.48, 95% CI 0.48 (0.1-2.6, p=0.39) and the rate of acute GVHD.

The rate of chronic GVHD at 5 years for MRD+ patients was 41% (95% 32-51%). There were no correlations between the rate of chronic GVHD and the level of γδ T cells on days +30 and +100.

Multivariate analysis

The following parameters were included into multivariate models: patients' sex, patient/donor sex constellation, patients' age, conditioning intensity, levels of γδ T cells on days +30 and +100 as well as the level of CD3+CD8+ cells on day +100. Though non-significant in univariate analysis, we added post-transplant immunosuppression to the multivariate models as there was a significant correlation between use of ATG and "high" post-transplant levels of γδ T cells on days +30 and +100. We analysed and provided two separate models, one for day +30 and another for day +100, respectively (Table 5).

In the first model (day +30) we observed a significant better OS for younger patients' age (≤ 58 years; HR 0.39: 95% CI: 0.19-0.82, p=0.012) and for "high" level of γδ T cells (HR 0.44: 95% CI: 0.20-0.96, p=0.038) and a lower OS for patient/donor gender matched allo-SCT (HR 2.3: 95% CI: 0.99-5.11, p=0.052) (Table 5). A "high" level of γδ T cells was the only significant factor in the MVA for LFS (HR 0.42: 95% CI: 0.22-0.79, p=0.007) and for relapse (HR 0.36: 95% CI: 0.21-0.88, p=0.019), respectively. (Table 5).

For the second multivariate model (day +100) the only significant factor for OS and LFS was a "high" level of γδ T cells on day +100: HR 0.33: 95% CI: 0.15-0.76, p=0.009) and HR 0.42: 95% CI: 0.21-0.82, p=0.011, respectively and a non-significant lower risk of relapse: HR: 0.5: 95%: 0.21-1.1, p=0.08)

No impact for any outcome in the multivariate analysis was seen for patients sex, immunosuppression and the intensity of the conditioning regimen. A multivariate analysis was not performed for NRM due to low event number (n=11).

Pre-transplant MRD- patients

Outcomes for MRD- patients

The 5-year OS and LFS were 75% (95% CI 64-83%) and 70% (95% CI 59-79%). The relapse rate and NRM at 5 years after allo-SCT were 12% (95% CI 6-22%) and 19% (95% CI 12-28%). No difference in 5-year OS and LFS for patients, who had "high" level of γδ T cells on day +30 ("high" vs "low": OS, RR 0.8, 95% CI 0.3-2.1, p=0.65; LFS: 0.97, 95% CI 0.4-2.2, p=0.95; relapses: RR 0.78, 95% CI 0.23-2.6, p=0.69; NRM, RR 1.3, 95% CI 0.43-3.9, p=0.65) was observed. Improved OS was seen (RR 0.39, 95% CI 0.15-1.0, p=0.051) and LFS (RR 0.45, 95% CI 0.20-1.1, p=0.06) in patients who had "low" (≤86x10^6/L) CD3+CD8+ cells on day +30. No difference in 5-year OS and LFS for patients, who had "high" level of γδ T cells on day +100 ("high" vs "low": OS, RR 1.1, 95% CI 0.4-3.1, p=0.89; LFS, RR 0.93, 95% CI 0.4-2.3, p=0.88; relapses, RR 2.0, 95% CI 0.53-7.9, p=0.30; NRM, RR 0.62, 95% CI 0.18-2.1, p=0.45) was observed (Table S2 of Supplement files, Fig. 2a-c).

Discussion

In this study we investigated on the role of post-transplant reconstitution of γδ T cells and its impact on post-transplant outcomes for AML patients with a MRD+ status before allo-SCT.

Because of the observed GvL effect of the innate immune system by γδ T levels we hypothesized that a faster γδ T cell reconstitution after allo-SCT could reduce the high relapse risk in MRD+ AML patients. According to our retrospective results, we were able to confirm this hypothesis showing an independent significant favorable impact of “high” γδ T levels on day +30 on relapses, OS and LFS in MRD+ patients and also an independent significant favorable impact of “high” γδ T levels on day +100 on OS and LFS. Similarly, we observed a higher level of γδ T cells on day +100 in those patients who did not relapse after allo-SCT. In contrast, we found no significant impact of γδ T cell levels on post-transplant outcomes for MRD- patients suggesting that an improved reconstitution of γδ T levels after allo SCT may overcome the negative impact in MRD+ AML patients. Moreover, the "high" and "low" categories for the other lymphocyte subsets did not correlate with OS and LFS.

γδ T cells represent a unique population of T cells belonging to innate immunity. Furthermore, these cells are also involved in the activation of the adaptive immune system (ref. 6) representing a “bridging” event between the innate and adoptive immune system. Being in general non-MHC restricted, these cells can induce a GvL effect without inducing GVHD. (ref. 39) Though the first reports on γδ T cells reconstitution did not deal with their impact on survival, (ref. 40-42) later studies showed a favorable impact of increased levels of γδ T cells on post-transplant outcomes in adult (ref. 28, 30) and pediatric patients. (ref. 29) However, most of these reports included patients with different hematologic malignancies and remission status. For instance, Godder et al. (ref. 28) reported on a higher 5-year LFS (54.4 vs 19.1%; p=0.0003) in young acute leukemia patients (ALL, n=77, AML, n=76; median age 22, 1-59) with “increased” γδ T cells level (vs “normal/decreased”) after receiving *in vivo* T cell depleted bone marrow allografts from mismatched donors. The threshold for defining the “increased” group was ≤1.75×10^5 γδ T cells/ml at two consecutive measurements within the first year post-transplant. More recently, Minulescu et al. (ref. 30) reported on worse survival (HR 5.16, 95% CI 1.94–13.7, p=0.001) due to higher relapses (HR 2.7, 95% CI 1.32–5.53, p=0.007) in patients with different hematologic malignancies and low level of γδ T cells (<21×10^6/L) on day +56 post-transplant. Interestingly, the authors found a significant correlation between high levels of γδ T cells and lower incidence of acute GVHD. A recently published meta-analysis by Arruda et al. (ref. 31) showed that high γδ T-cell levels

after allo-SCT were associated with less relapses (0.58, 95% 0.40-0.84; p=0.004), fewer viral infections (0.59, 95% CI 0.43-0.82; p=0.002), higher OS (0.28, 95% CI 0.18-0.44, p=0.00001) and DFS (0.29, 95% CI 0.18-0.48, p=0.00001).

The literature regarding the role of γδ T cells reconstitution according to pre-transplant MRD status is scarce. Galimberti et al (ref. 32) reported on a different spectrum of γδ T cell receptors in pre-transplant MRD+ and MRD- patients with multiple myeloma after non-myeloablative conditioning. The reported data show an association of high level of γδ T cells and less relapse which can be explained with an enhanced GvL effect in case of persisting leukemic cells in pre-transplant MRD+ AML patients.

If a higher γδ T cell level prevent relapse in AML factors that drive a fast reconstitution are crucial in the setting of post-transplant improvement of γδ T cell reconstitution.

In line with Minulescu et al. (ref. 30), we found a significant correlation between higher γδ T cell levels and younger (<30 years) donors as well as HLA matched grafts. Moreover, female sex, mismatched patient/donor sex constellation, younger patients' age (≤ 58 years), CMV reactivation and the use of ATG instead of post-transplant cyclophosphamide were associated with higher post-transplant γδ T cell levels as well.

Younger donors' and patients' age are associated with preserved thymic function resulting in better post-transplant immune reconstitution.

In our study female sex was significantly associated with pre-transplant ATG rather than with post-transplant cyclophosphamide. This may have led to better survival for females taking into account that both estrogens and androgens have a negative impact on thymic function and post-transplant immune reconstitution. (ref. 43)

Also, we observed an unfavorable impact of matched sex allografts on OS due to a trend to more relapses. Taking together the association of sex mismatched transplantations with higher level of γδ T cells, we suggest that such allografts can be associated with development of GvL effect without GVHD through increased number of γδ T cells in the early post-transplant period. In this line , Nakasone et al. (ref. 44) reported on reduced relapses (HR 0.64, p<0.01) in male recipients transplanted with female donors after total lymphoid irradiation with antithymocyte globuline due to development of H-Y antibodies.

Post-transplant CMV reactivation is also known to be associated with activation and reconstitution of γδ T cells. (ref. 9, 30)

Interestingly, the use of post-transplant cyclophosphamide was associated with low level of γδ T cells levels on days +30 and +100 in the present study. In our

previous work (ref. 45) in 599 patients with different hematological malignancies who underwent allo-SCT from MRD (n=105), MUD (n=360), MMRD (n=17) and MMUD (n=117) after MAC, we observed faster reconstitution of γδ T cells and lower frequency of infections after ATG compared to post-transplant cyclophosphamide.

One of the limitations of our study was the absence of data on graft composition and levels of γδ T cells in the grafts. Based on their analysis of the γ-chain repertoire, Arruda et al. (ref. 46) described distinct clonotypes in grafts associated with sustained clinical remission after allo-SCT. Ravens et al. (ref. 9) showed that regenerated γδ T cell repertoires after transplantation were qualitatively comparable to the hosts' repertoires before transplantation. Nevertheless, displayed clonotypes were very different from the pre-transplant hosts' repertoires, suggesting that they were generated *de novo* in the host thymus from donor stem cells. Unfortunately, the present study cannot answer the question whether host or donor γδ T cells are responsible for the GvL effect in AML patients.

The role of γδ T cells in the development of GVHD is controversial. Though some studies showed an association with development of acute GVHD (ref. 47-49), others including the recently published meta-analysis found no evidence for such association. (ref. 32, 40, 50) Tsuji et al. (ref. 26) showed that γδ T cells can be recruited into the donor αβ T cell-initiated lesions, playing a secondary role in the development of GVHD. In the present study, we found no difference in the incidence of acute GVHD in MRD+ patients regarding the level of γδ T cells at days +30 and +100 post-transplant. This was in contrast to Minulescu et al. (ref. 30) who reported on an even decreased incidence of acute GVHD in patients with higher numbers of γδ T cells. Our data are in accordance with Arruda et al. (ref. 31) who found no association between the levels of γδ T cells and the incidence of acute GVHD in a meta-analysis.

To our knowledge, this is the first study focusing on the role of post-transplant reconstitution of γδ T cells in AML patients regarding their pre-transplant MRD status as determined by the "different from normal" approach following ELN guidelines. (ref. 38) In recent years, new approaches of cellular therapies have been developing very fast. In this context, the results of our study seem to be interesting. The data from the pediatric and the adult haplo-setting postulated an effective and safe use of negatively selected (ref. 51) γδ T cell allografts. (ref. 52, 53) GVHD rates and relapse-free survival outcomes were shown to be better after T cell depleted haplo-identical transplantation as compared with that from unrelated donors at least in pediatric patients. (ref. 25) Moreover, adoptive transfer of haploidentical γδ T cells can lead to remission in patients with advanced/refractory hematologic malignancies.

(ref. 54) All these factors pave the path to new strategies for clinical use of these cells such as *in vivo* expansion with zolendronic acid in acute leukemia patients, (ref. 55) adoptive transfer of *ex vivo* expanded γδ T cells, (ref. 54) and chimeric antigen strategies. (ref. 56)

In conclusion, we could show that pre-transplant MRD+ patients with AML may benefit from higher levels of post-transplant γδ T cells which lower the risk of relapses and lead to improved leukaemia-free and overall survival. Finally, patients with low post-transplant levels of γδ T cells might be candidates for *in vivo* expansion or adoptive transfer of (un-)modified γδ T cells.

Acknowledgements: EK, RM and NK designed study, performed the analysis and wrote the manuscript; UFF performed the laboratory measurements; AB performed the laboratory measurements and participated in data interpretation; UB provided critical suggestions for improving the manuscript; DJ, FA and CW provided patents' data and supported by manuscripts' preparation. All authors read the final manuscript and participated in the discussion of results.

Financial disclosure statement: The authors have no financial interests in relation to the work

Data statement: The data are available by request

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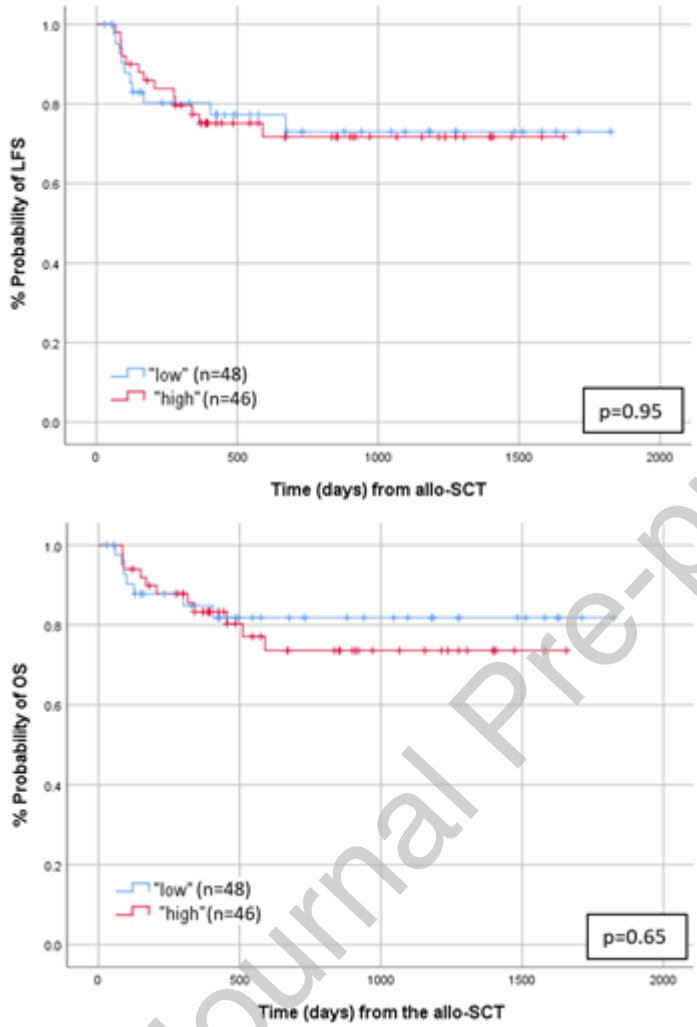
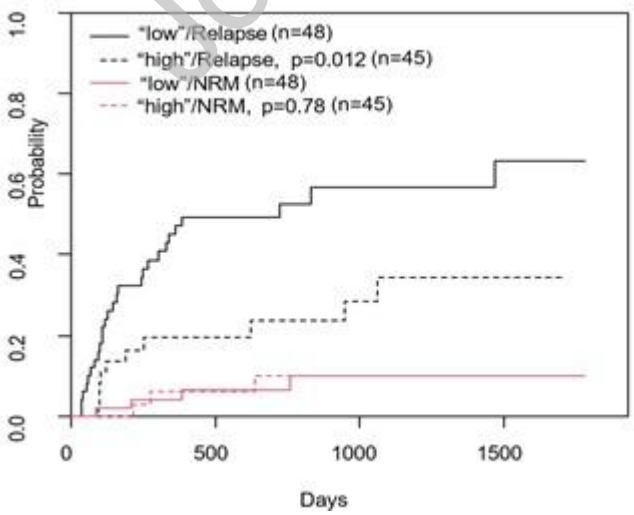
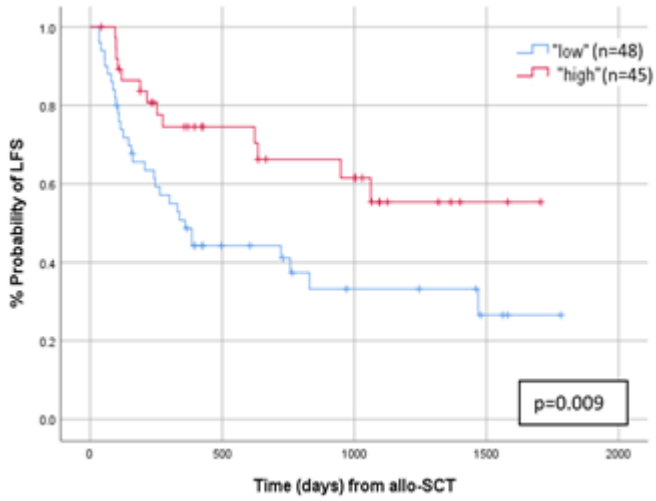
Figure Legends:

Figure 1. Overall survival a), leukemia-free survival b), relapse and NRM c) incidences for pre-transplant MRD+ patients according to γδ T cell level on day +30.

Figure 2. Overall survival a), leukemia-free survival b), relapse and NRM c) incidences for pre-transplant MRD- patients according to γδ T cells on day +30.







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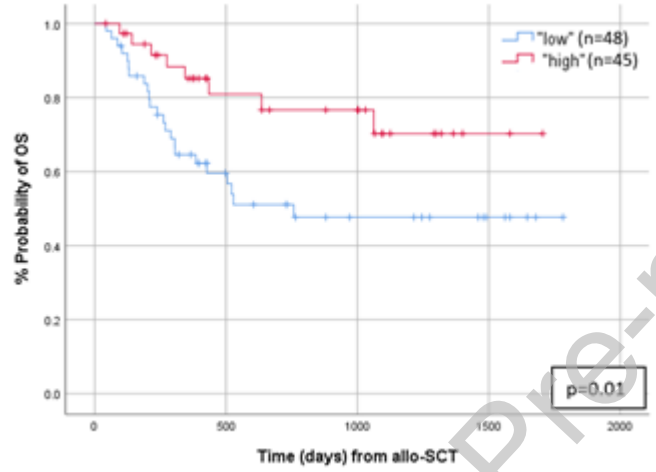

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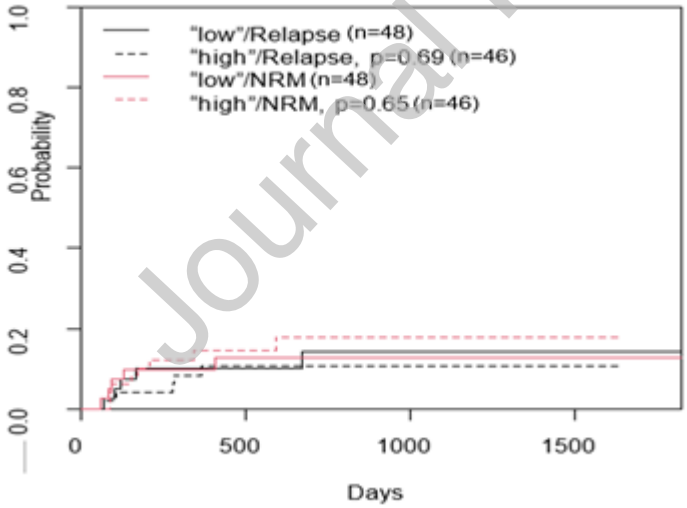

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Tables and Figures

Table 1. Patients' characteristics according to pre-transplant MRD status (n=202) (M, male; F, female; s/tAML, secondary/therapy-associated AML; CR(i), complete remission with incomplete hematologic recovery; TKI, tyrosine-kinase inhibitors; P/D, Patient/Donor; post-Cy, post-transplant cyclophosphamide)

Characteristics	MRD pos, n, %	MRD neg, n, %	p
Patients' sex:			0.40
M	56 (56%)	60 (59%)	
F	44 (44%)	42 (41%)	
Sex match (P/D)			0.54
match	64 (64%)	65 (64%)	
mismatch	36 (36%)	37 (36%)	
			0.93
M>M	47 (47%)	51 (50%)	
M>F	9 (9%)	9 (9%)	
F>M	27 (27%)	28 (28%)	
F>F	17 (17%)	14 (14%)	
Patients' age			0.67
median (range)	58 (21-80)	60 (24-77)	
Donor age			0.47
median (range)	31 (18-79)	30 (19-69)	
Origin of disease			0.18
de novo	74 (74%)	82 (80%)	
s/tAML	26 (26%)	20 (20%)	
Remission status			0.68
1st CR	61 (61%)	65 (64%)	
≥2nd CR	16 (16%)	12 (12%)	
CRi	23 (23%)	25 (25%)	
ELN risk score			0.32
favorable	12 (12%)	16 (16%)	
intermediate	53 (53%)	60 (59%)	
adverse	35 (35%)	26 (26%)	
Previous therapy			0.18
chemotherapy	76 (76%)	69 (68%)	
chemotherapy + TKI	11 (11%)	17 (17%)	
azacytidine/decitabine monotherapy	10 (10%)	5 (5%)	
venetoclax in combinations	3 (3%)	7 (7%)	
other	-	4 (4%)	
Donor code:			0.53
matched	77 (77%)	78 (77%)	
mismatched	23 (23%)	24 (23%)	
			0.40
MRD	22 (22%)	14 (14%)	
MUD	55 (55%)	64 (63%)	
MMUD	12 (12%)	15 (15%)	
Haploidentical/Cord blood	11 (11%)	9 (9%)	
CMV constellation (P/D)			0.41
pos/pos	52 (52%)	59 (58%)	
pos/neg	11 (11%)	14 (14%)	
neg/pos	9 (9%)	4 (4%)	
neg/neg	28 (28%)	25 (25%)	
CMV reactivation			0.44
yes	45 (45%)	48 (47%)	
no	55 (55%)	54 (53%)	
Conditioning			0.40
MAC	68 (68%)	72 (71%)	
RIC	32 (32%)	30 (29%)	
Immunosuppression			0.15
ATG	77 (79%)	86 (84%)	
post-Cy	21 (21%)	15 (15%)	
no	2	-	
both	-	1 (1%)	

Table 2. Mean and median numbers of lymphocytic cell populations according to pre-transplant MRD status on days +30 and +100 post-transplant.

	Day +30				Day +100			
	Mean*, /µL	**Median**, /µL**	**Range, /µL**	**p**	**Mean*, /µL**	**Median**, /µL**	**Range, /µL**	**p**
CD3+								
MRD+	332	143	3-2624	p=0.56*	663	453	6-4126	p=0.66*
MRD-	381	147	1-3438	p=0.73**	620	421	8-2808	p=0.61**
CD3+CD4+								
MRD+	76	30	0-815	p=0.43*	155	100	0-1266	p=0.13*
MRD-	63	32	0-489	p=0.70**	120	93	0-432	p=0.29**
CD3+CD8+								
MRD+	229	70	0-2368	p=0.79*	479	318	3-3899	p=0.75*
MRD-	248	86	0-3182	p=0.56**	452	255	6-2427	p=0.73**
NK cells								p=0.64*
MRD+	175	122	2-1623	p=0.57*	203	172	2-876	p=0.93**
MRD-	193	124	1-1280	p=0.76**	216	159	16-1097	
γδ T cells								
MRD+	34	13	0-459	p=0.39*	39	23	0-257	p=0.29*
MRD-	43	20	0-530	p=0.20**	48	26	0-350	p=0.42**

For further analysis patients were separated into two groups ("high" and "low") according to the median:
Day +30: CD3+ (MRD+, "low": ≤143/µL, "high": >143/µL; MRD-, "low": ≤147/µL, "high": >147/µL), CD3+CD4+ (MRD+, "low": ≤30/µL, "high": >30/µL; MRD-, "low": ≤32/µL, "high": >32/µL), CD3+CD8+ (MRD+, "low": ≤70/µL, "high": >70/µL; MRD-, "low": ≤86/µL, "high": >86/µL), NK cells (MRD+, "low": ≤122/µL, "high": >122/µL; MRD-, "low": ≤124/µL, "high": >124/µL and γδ T cells (MRD+, "low": ≤13/µL, "high": >13/µL; MRD-, "low": ≤20/µL, "high": >20/µL).
Day +100: CD3+ (MRD+, "low": ≤453/µL, "high": >453/µL; MRD-, "low": ≤421/µL, "high": >421/µL), CD3+CD4+ (MRD+, "low": ≤100/µL, "high": >100/µL; MRD-, "low": ≤93/µL, "high": >93/µL), CD3+CD8+ (MRD+, "low": ≤318/µL, "high": >318/µL; MRD-, "low": ≤255/µL, "high": >255/µL), NK cells (MRD+, "low": ≤172/µL, "high": >172/µL; MRD-, "low": ≤159/µL, "high": >159/µL and γδ T cells (MRD+, "low": ≤23/µL, "high": >23/µL; MRD-, "low": ≤26/µL, "high": >26/µL).

Table 3. Factors associated with increased amount of γδ T cells at day +30 and +100 in MRD+ patients. (M, male; F, female; s/tAML, secondary/therapy-associated AML; CR(i), complete remission with incomplete hematologic recovery; post-Cy, post-transplant cyclophosphamide)

Factor	Day +30			Day +100		
	"low" (n=48)	**"high" (n=45)**	**p**	**"low" (n=42)**	**"high" (n=44)**	**P**
Patient's sex:			**0.025**			**0.064**
M	**32 (67%)**	**20 (44%)**		**26 (62%)**	**19 (43%)**	
F	**16 (33%)**	**25 (56%)**		**16 (38%)**	**25 (57%)**	
Patient/donor sex match			**0.09**			**0.035**
match	**34 (71%)**	**25 (56%)**		**30 (71%)**	**22 (50%)**	
mismatch	**14 (29%)**	**20 (44%)**		**12 (19%)**	**22 (50%)**	
			0.32			0.62
F>M	3 (6%)	5 (11%)		4 (9%)	4 (9%)	
other	45 (94%)	30 (89%)		38 (91%)	40 (91%)	
Patient's age			0.22			**0.026**
≤58	24 (50%)	27 (60%)		**19 (45%)**	**30 (68%)**	
>58	24 (50%)	18 (40%)		**23 (55%)**	**14 (32%)**	
Donor age			**0.057**			0.26
≤ 30	**21 (44%)**	**28 (62%)**		20 (48%)	25 (57%)	
>30	**27 (56%)**	**17 (38%)**		22 (52%)	19 (43%)	
Origin of disease			0.093			0.26
de novo	34 (71%)	38 (84%)		31 (74%)	36 (82%)	
s/tAML	14 (29%)	7 (16%)		11 (26%)	8 (18%)	
Remission status			0.68			0.15
1 CR	27 (56%)	29 (64%)		21 (50%)	31 (71%)	
2+ CR	8 (17%)	7 (16%)		9 (21%)	5 (11%)	
CRi	13 (27%)	9 (20%)		12 (29%)	8 (18%)	
ELN risk score			0.49			0.92
favorable	6 (12%)	4 (9%)		5 (12%)	5 (11%)	
Intermediate	24 (50%)	28 (62%)		23 (55%)	26 (59%)	
adverse	18 (38%)	13 (29%)		14 (33%)	13 (31%)	
Donor code:			**0.062**			0.12
match	**33 (69%)**	**38 (84%)**		30 (71%)	37 (84%)	
mismatch	**15 (31%)**	**7 (16%)**		12 (29%)	7 (16%)	

			0.30			0.16
MRD	8 (17%)	12 (26%)		8 (19%)	11 (25%)	
MUD	25 (52%)	26 (59%)		22 (52%)	26 (59%)	
MMUD	7 (15%)	5 (11%)		4 (10%)	6 (14%)	
Haploident/Cord blood	8 (17%)	2 (4%)		8 (19%)	1 (2%)	
Donors' CMV status			0.40			0.34
pos	18 (38%)	19 (42%)		23 (55%)	27 (61%)	
neg	30 (62%)	26 (58%)		19 (45%)	17 (39%)	
Patients' CMV status			0.51			0.35
pos	18 (38%)	16 (36%)		25 (60%)	29 (66%)	
neg	30 (62%)	29 (64%)		17 (40%)	15 (34%)	
CMV reactivation			**0.055**			0.58
yes	**22 (46%)**	**29 (64%)**		19 (45%)	20 (45%)	
no	**26 (54%)**	**13 (36%)**		23 (55%)	24 (55%)	
Conditioning			0.33			0.20
MAC	35 (73%)	30 (67%)		26 (62%)	32 (73%)	
RIC	13 (33%)	15 (33%)		16 (38%)	12 (27%)	
Immunosuppression*			**0.007**			**0.003**
ATG	**32 (68%)**	**40 (91%)**		**28 (68%)**	**41 (93%)**	
post-Cy	**15 (32%)**	**4 (9%)**		**13 (32%)**	**3 (7%)**	

* two patients who did not received neither ATG nor post-Cy as well as one patients received both medications are not shown.

Table 4. Results of univariate analysis for pre-transplant MRD+ patients. (OS, overall survival; LFS, leukemia-free survival; NRM, non-relapsed mortality; M, male; F, female; s/tAML, secondary/therapy-associated AML; CR(i), complete remission with incomplete hematologic recovery; post-Cy, post-transplant cyclophosphamide)

Factors	OS (95% CI), p	LFS (95% CI), p	Relapse (95% CI), p	NRM (95% CI), p
Patient's sex:				
M vs F	**2.1 (1.1-4.3), p=0.035**	**1.5 (0.8-2.6), p=0.21**	1.6 (0.8-3.0), p=0.15	0.93 (0.3-3.0), p=0.9
Patient's age				
≤58 vs >58	**0.40 (0.2-0.8), p=0.007**	**0.76 (0.44-1.3), p=0.33**	1.5 (0.8-2.8), p=0.25	**0.07 (0.01-0.6), p=0.013**
Patient/donor sex status:				
match vs mismatch	**2.0 (0.99-4.2), p=0.053**	**1.7 (0.91-3.1), p=0.098**	**2.1 (1.1-4.1), p=0.037**	0.68 (0.2-2.2), p=0.52
F>M vs others	0.91 (0.18-2.6), p=0.86	0.98 (0.41-2.3), p=0.95		
Donor age				
≤ 30 vs >30	0.90 (0.47-1.7), p=0.74	0.92 (0.52-1.6), p=0.76		
Origin of disease				
de novo vs s/tAML	0.63 (0.3-1.3), p=0.18	0.76 (0.41-1.4), p=0.38		
Remission status				
1 CR vs CRi	0.82 (0.38-1.8), p=0.63	1.1 (0.6-2.3), p=0.72		
2+ CR vs CRi	0.99 (0.35-2.8), p=0.98	1.2 (0.5-3.0), p=0.74		
2+ CR vs 1 CR	1.2 (0.48-3.0), p=0.70	1.0 (0.43-1.8), p=0.72		
ELN risk score				
favorable vs adverse	1.0 (0.38-2.9), p=0.94	0.99 (0.4-2.3), p=0.98	0.7 (0.3-1.8), p=0.5	1.7 (0.3-9.1), p=0.56
intermediate vs adverse	**0.55 (0.27-1.1), p=0.09**	**0.51 (0.3-0.94), p=0.03**	**0.5 (0.3-0.9), p=0.04**	**0.8 (0.2-2.8), p=0.70**
intermediate vs favorable	0.53 (0.19-1.4), p=0.21	0.52 (0.22-1.2), p=0.14	0.7 (0.3-1.6), p=0.39	0.5 (0.1-2.5), p=0.37
Conditioning				
MAC vs RIC	**0.46 (0.23-0.92), p=0.027**	**0.74 (0.39-1.4), p=0.36**	**1.6 (0.7-3.9), p=0.26**	**0.2 (0.1-0.6), p=0.005**
Donor type				
matched vs mismatched	0.8 (0.4-1.7), p=0.58	0.8 (0.4-1.5), p=0.52		
Immunosuppression				
ATG vs post-Cy	1.3 (0.54-3.1), p=0.56	1.0 (0.51-2.1), p=0.93		
CMV status patient				
neg vs pos	1.6 (0.9-3.1), p=0.14	1.1 (0.6-2.0), p=0.71		
CMV status donor				
neg vs pos	0.9 (0.5-1.7), p=0.69	0.7 (0.4-1.3), p=0.27		
CMV reactivation				
yes vs no	1.2 (0.6-2.3), p=0.56	1.0 (0.6-1.7), p=0.99		
CD3+ at day +30 (*10^6/L)				
high (>143) vs low (≤143)	0.63 (0.32-1.3), p=0.20	0.83 (0.46-1.5), p=0.54		

CD3+CD4+ at day +30 (*10^6/L) high (>30)vs low (≤30)	0.9 (0.45-1.8), p=0.77	0.92 (0.51-1.7), p=0.77		
CD3+CD8+ at day +30 (*10^6/L) high (>71) vs low (≤71)	0.62 (0.31-1.3), p=0.19	0.72 (0.40-1.3), p=0.29		
NK cells at day +30 (*10^6/L) high (>122) vs low (≤122)	0.8 (0.40-1.6), p=0.52	0.72 (0.40-1.3), p=0.29		
γδ TCR at day +30 (*10^6/L) high (>13) vs low(≤13)	**0.38 (0.18-0.79), p=0.01**	**0.44 (0.23-0.82), p=0.009**	**0.42 (0.22-0.83), p=0.012**	0.75 (0.19-3.5), p=0.78
CD3+ at day +100 (*10^6/L) high (>453) vs low (≤453)	0.57 (0.27-1.2), p=0.14	0.95 (0.51-1.8), p=0.88		
CD3+CD4+ at day +100 (*10^6/L) high (>100) vs low (≤100)	0.9 (0.44-1.9), p=0.78	0.86 (0.41-1.6) p=0.63		
CD3+CD8+ at day +100 (*10^6/L) high (>318) vs low (≤318)	**0.47 (0.22-1.9), p=0.054**	**0.62 (0.32-1.2), p=0.15**	0.70 (0.34-1.4), p=0.32	0.48 (0.1-2.5), p=0.38
NK cells at day +100 (*10^6/L) high (>172) vs low (≤172)	0.83 (0.40-1.8), p=0.63	0.62 (0.32-1.2), p=0.15		
γδ TCR at day +100 (*10^6/L) high (>23) vs low (≤23)	**0.32 (0.14-0.79), p=0.007**	**0.41 (0.21-0.80), p=0.009**	**0.52 (0.26-1.0), p=0.06**	0.19 (0.03-1.6), p=0.12

Table 5. Results of multivariate analysis for pre-transplant MRD+ patients. (OS, overall survival; LFS, leukemia-free survival; M, male; F, female; post-Cy, post-transplant cyclophosphamide)

Factor	OS	LFS	Relapse
Model 1			
Patients' age: ≤ 58 vs >58 years	0.39 (0.19-0.82), p=0.012	-	-
Patient/donor sex status match vs mismatch	2.3 (0.99-5.1), p=0.052	-	-
γδ **TCR day +30** high vs low	0.44 (0.20-0.96), p=0.038	0.42 (0.22-0.79), p=0.007	0.36 (0.21-0.88), p=0.019
Model 2*			
γδ **TCR day +100** high vs low	0.33 (0.15-0.76), p=0.009	0.42 (0.21-0.82), p=0.011	0.5 (0.21-1.1), p=0.08

* following adjustment to all other variables

Supplemental files

Table S1. Results of univariate analysis for pre-transplant MRD- patients. (OS, overall survival; LFS, leukemia-free survival; M, male; F, female; s/tAML, secondary/therapy-associated AML; CR(i), complete remission with incomplete hematologic recovery; post-Cy, post-transplant cyclophosphamide)

Factors	OS (95% CI), p	LFS (95% CI), p
Patient's sex:		
M vs F	1.5 (0.64-3.8), p=0.33	1.8 (0.8-4.1), p=0.17
Patient/donor sex match		
match vs mismatch	0.77 (0.3-1.8), p=0.55	0.91 (0.4-2.0), p=0.82
Patient's age		
≤58 vs >58	**0.4 (0.2-0.98), p=0.045**	**0.5 (0.2-1.1), p=0.099**
Donor age		
≤ 30 vs >30	0.71 (0.3-1.6), p=0.42	0.9 (0.4-1.9), p=0.78
Origin of disease		
de novo vs s/tAML	1.6 (0.5-5.4), p=0.46	1.1 (0.4-3.0), p=0.81
Remission status		
1 CR vs CRi	0.53 (0.2-1.3), p=0.18	0.6 (0.3-1.4), p=0.22
2+ CR vs CRi	0.83 (0.2-3.2), p=0.79	0.7 (0.2-2.7), p=0.61
2+ CR vs 1 CR	1.6 (0.5-5.6), p=0.48	1.2 (0.4-4.1), p=0.77
ELN risk score		
favorable vs adverse	0.7 (0.2-2.1), p=0.47	0.5 (0.2-1.7), p=0.28
intermediate vs adverse	0.4 (0.2-0.99), p=0.048	0.4 (0.2-0.97), p=0.04
intermediate vs favorable	0.6 (0.2-2.0), p=0.41	0.8 (0.3-2.5), p=0.70
Conditioning		
MAC vs RIC	**0.5 (0.2-1.1), p=0.09**	**0.7 (0.3-1.5), p=0.34**
Donor code:		
match vs mismatch	**0.4 (0.2-0.9), p=0.028**	**0.29 (0.1-0.6), p=0.002**
Immunosuppression		
ATG vs post-Cy	0.6 (0.2-1.8), p=0.37	0.8 (0.3-2.3), p=0.65
CMV patient		
neg vs pos	**0.3 (0.1-1.1), 0.079**	**0.5 (0.2-1.2), p=0.13**
CMV donor		
pos vs neg	0.6 (0.2-1.4), p=0.22	**0.5 (0.2-1.2), p=0.13**
CMV reactivation		
yes vs no	**0.5 (0.2-1.1), p=0.098**	**0.6 (0.3-1.2), p=0.13**
γδ TCR at day +30 (*10^6/L)		
≤20 vs >20	0.87 (0.36-2.1), p=0.75	0.90 (0.4-2.0), p=0.79
CD3+ at day +30 (*10^6/L)		
≤147 vs >147	0.61 (0.25-1.5), p=0.28	0.53 (0.23-1.2), p=0.13
CD3+CD4+ at day +30 (*10^6/L)		
≤32 vs >32	0.94 (0.39-2.3), p=0.88	0.92 (0.42-2.1), p=0.85
CD3+CD8+ at day +30 (*10^6/L)		
≤86 vs >86	**0.39 (0.15-1.0), p=0.051**	**0.45 (0.20-1.1), p=0.06**
NK cells at day +30 (*10^6/L)		
≤124 vs >124	0.92 (0.38-2.2), p=0.86	1.3 (0.58-2.9), p=0.53
γδ TCR at day +100 (*10^6/L)		
≤26 vs >26	1.0 (0.38-2.7), p=0.99	0.76 (0.3-1.8), p=0.55
CD3+ at day +100 (*10^6/L)		
≤422 vs >422	0.55 (0.20-1.5), p=0.23	0.46 (0.19-1.2), p=0.10
CD3+CD4+ at day +100 (*10^6/L)	0.90 (0.35-2.3), p=0.83	0.62 (0.26-1.5), p=0.28
≤93 vs >93		
CD3+CD8+ at day +100 (*10^6/L)	0.63 (0.24-1.7), p=0.34	0.51 (0.21-1.2), p=0.13
≤256 vs >256		
NK cells at day +100 (*10^6/L)		
≤160 vs >160	1.1 (0.41-2.8), p=0.89	1.3 (0.54-3.0), p=0.58

Table S2. Results of univariate analysis for pre-transplant MRD- patients. (OS, overall survival; LFS, leukemia-free survival; M, male; F, female; s/tAML, secondary/therapy-associated AML; CR(i), complete remission with incomplete hematologic recovery; post-Cy, post-transplant cyclophosphamide)

Factors	OS (95% CI), p	LFS (95% CI), p
Patient's sex: M vs F	1.5 (0.64-3.8), p=0.33	1.8 (0.8-4.1), p=0.17
Patient/donor sex match match vs mismatch	0.77 (0.3-1.8), p=0.55	0.91 (0.4-2.0), p=0.82
Patient's age ≤58 vs >58	**0.4 (0.2-0.98), p=0.045**	**0.5 (0.2-1.1), p=0.099**
Donor age ≤ 30 vs >30	0.71 (0.3-1.6), p=0.42	0.9 (0.4-1.9), p=0.78
Origin of disease *de novo* vs s/tAML	1.6 (0.5-5.4), p=0.46	1.1 (0.4-3.0), p=0.81
Remission status		
1 CR vs CRi	0.53 (0.2-1.3), p=0.18	0.6 (0.3-1.4), p=0.22
2+ CR vs CRi	0.83 (0.2-3.2), p=0.79	0.7 (0.2-2.7), p=0.61
2+ CR vs 1 CR	1.6 (0.5-5.6), p=0.48	1.2 (0.4-4.1), p=0.77
ELN risk score		
favorable vs adverse	0.7 (0.2-2.1), p=0.47	0.5 (0.2-1.7), p=0.28
intermediate vs adverse	0.4 (0.2-0.99), p=0.048	0.4 (0.2-0.97), p=0.04
intermediate vs favorable	0.6 (0.2-2.0), p=0.41	0.8 (0.3-2.5), p=0.70
Conditioning MAC vs RIC	**0.5 (0.2-1.1), p=0.09**	**0.7 (0.3-1.5), p=0.34**
Donor code: match vs mismatch	**0.4 (0.2-0.9), p=0.028**	**0.29 (0.1-0.6), p=0.002**
Immunosuppression ATG vs post-Cy	0.6 (0.2-1.8), p=0.37	0.8 (0.3-2.3), p=0.65
CMV patient neg vs pos	**0.3 (0.1-1.1), 0.079**	**0.5 (0.2-1.2), p=0.13**
CMV donor pos vs neg	0.6 (0.2-1.4), p=0.22	**0.5 (0.2-1.2), p=0.13**
CMV reactivation yes vs no	**0.5 (0.2-1.1), p=0.098**	**0.6 (0.3-1.2), p=0.13**
γδ TCR at day +30 (*10^6/L) ≤20 vs >20	0.87 (0.36-2.1), p=0.75	0.90 (0.4-2.0), p=0.79
CD3+ at day +30 (*10^6/L) ≤147 vs >147	0.61 (0.25-1.5), p=0.28	0.53 (0.23-1.2), p=0.13
CD3+CD4+ at day +30 (*10^6/L) ≤32 vs >32	0.94 (0.39-2.3), p=0.88	0.92 (0.42-2.1), p=0.85
CD3+CD8+ at day +30 (*10^6/L) ≤86 vs >86	**0.39 (0.15-1.0), p=0.051**	**0.45 (0.20-1.1), p=0.06**
NK cells at day +30 (*10^6/L) ≤124 vs >124	0.92 (0.38-2.2), p=0.86	1.3 (0.58-2.9), p=0.53
γδ TCR at day +100 (*10^6/L) ≤26 vs >26	1.0 (0.38-2.7), p=0.99	0.76 (0.3-1.8), p=0.55
CD3+ at day +100 (*10^6/L) ≤422 vs >422	0.55 (0.20-1.5), p=0.23	0.46 (0.19-1.2), p=0.10
CD3+CD4+ at day +100 (*10^6/L) ≤93 vs >93	0.90 (0.35-2.3), p=0.83	0.62 (0.26-1.5), p=0.28
CD3+CD8+ at day +100 (*10^6/L) ≤256 vs >256	0.63 (0.24-1.7), p=0.34	0.51 (0.21-1.2), p=0.13
NK cells at day +100 (*10^6/L) ≤160 vs >160	1.1 (0.41-2.8), p=0.89	1.3 (0.54-3.0), p=0.58