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Measurable residual *KMT2A* partial tandem duplication before allogeneic stem cell transplantation in acute myeloid leukemia

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Data availability statement

The data generated and analyzed during this study are not publicly available due to potential risks to patient privacy and the protection of sensitive clinical information. However, they are available

upon reasonable request from the corresponding author, subject to approval from the institutional review board of Peking University People's Hospital.

Competing interests

The authors declare no competing interests.

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Author contributions

Lulu Wang contributed to conceptualization, data curation, formal analysis, and writing—original draft. Haixia Fu contributed to conceptualization, methodology, project administration, resources, and supervision. Peng Zhao contributed to data curation, formal analysis, and validation. Zhuoyu An contributed to data curation and validation. Yazhen Qin, Xiaosu Zhao, Yun He, Qiusha Huang, Yuhong Chen, and Meng Lv contributed to investigation and validation. Jun Kong, Zhidong Wang, and Wei Han contributed to investigation. Yuanyuan Zhang, Xiaodong Mo, Tingting Han, Chenhua Yan, Yingjun Chang, Fengrong Wang, Xiangyu Zhao, Yu Wang, and Lanping Xu contributed to resources. Xiaojun Huang contributed to supervision. Xiaohui Zhang contributed to conceptualization, methodology, project administration, resources, supervision, and writing—review and editing. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

One supplementary file that is associated with the manuscript

Abstract

KMT2A partial tandem duplication (*KMT2A*-PTD) is a recurrent genetic alteration in acute myeloid leukemia (AML), but its role as a measurable residual disease (MRD) marker before allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains unclear. We retrospectively analyzed 180 *KMT2A*-PTD AML patients undergoing allo-HSCT at Peking University People's Hospital between 2015 and 2025. Pre-transplant *KMT2A*-PTD MRD was quantified by real-time quantitative PCR, with positivity defined as ≥1500 copies/10^5 ABL1. Among 158 patients transplanted in complete remission (CR), 144 had pre-transplant MRD assessment, and 34 (24%) were MRD-positive. Compared with MRD-negative patients, MRD-positive patients had significantly inferior 3-year overall survival (OS, 49% vs. 80%; p<0.001) and event-free survival (EFS, 42% vs. 73%; p<0.001), as well as a higher cumulative incidence of relapse (CIR, 42% vs. 22%; p=0.01). Multivariable analysis confirmed pre-transplant MRD positivity as an independent predictor of OS, EFS, CIR and non-relapse mortality. Exploratory comparison showed no significant outcome differences between MRD-positive patients and those with refractory disease. Combined analysis of *KMT2A*-PTD MRD and multiparameter flow cytometry-MRD further refined risk stratification. These findings establish pre-transplant *KMT2A*-PTD MRD as an important prognostic biomarker and support MRD-guided risk stratification in *KMT2A*-PTD AML undergoing allo-HSCT.

Keywords: acute myeloid leukemia, *KMT2A*-PTD, measurable residual disease, allogeneic hematopoietic stem cell transplantation, *FLT3*-ITD

Introduction

Lysine methyltransferase 2A (*KMT2A*), formerly known as the mixed-lineage leukemia (*MLL*) gene, is located on chromosome 11q23 and is a critical epigenetic regulator of *HOX* gene expression during hematopoiesis.[1-5] In acute myeloid leukemia (AML), *KMT2A* alterations mainly comprise chromosomal rearrangements (*KMT2A*-r) that generate oncogenic fusion genes and partial tandem duplication (*KMT2A*-PTD). Unlike classical *KMT2A*-r leukemias, which are driven by oncogenic fusion proteins, *KMT2A*-PTD is an intragenic in-frame duplication of *KMT2A* exons in a 5′–3′ orientation that does not involve a fusion partner, resulting in an elongated protein with gain-of-function properties.[2, 6-9]

KMT2A-PTD is detected in approximately 5%–10% of adult AML cases, with a particularly high prevalence among patients with cytogenetically normal AML. Approximately half of *KMT2A*-PTD-positive patients harbor concurrent *FLT3* internal tandem duplication (*FLT3*-ITD) mutations,[10-14] and other frequent co-occurring alterations include mutations in *DNMT3A*, *RUNX1*, and other recurrent myeloid genes, delineating a distinct molecular landscape. Despite its recognition as a recurrent genetic lesion, the precise mechanism by which *KMT2A*-PTD contributes to leukemogenesis remains incompletely understood. Mouse models have shown that *KMT2A*-PTD alone fails to induce overt leukemia,[6, 7] but instead exhibit distinct hematopoietic abnormalities including reduced hematopoietic stem cells numbers, enhanced self-renewal, myeloid differentiation blockade, and lineage bias,[6] In contrast, crossing these mice with transgenic mice carrying a second mutation, such as *FLT3*-ITD, rapidly precipitated leukemia,[4, 7, 8] indicating that *KMT2A*-PTD functions as a gain-of-function lesion requiring cooperating mutations to drive leukemogenesis.

The prognostic implications of *KMT2A*-PTD in AML have been a subject of debate. While some

studies have associated *KMT2A*-PTD with dismal survival,[15] others have suggested that it lacks prognostic significance.[16] Importantly, *KMT2A*-PTD has emerged as a reliable marker for measurable residual disease (MRD) monitoring, with quantitative PCR-based assays enabling early detection of molecular relapse, often weeks to months before hematologic relapse[17-19]-an ability comparable to established markers such as CBFB-*MYH11* and *NPM1*.[17, 20] Yet its clinical application is complicated by the occasional detection of *KMT2A*-PTD transcripts at very low levels in healthy individuals,[15, 17, 21] indicating that molecular detectability alone does not necessarily reflect clinically relevant residual disease. Therefore, a clinically meaningful quantitative threshold is needed to distinguish background-level detection from MRD associated with adverse clinical outcomes. This issue may be particularly relevant in the transplant setting, where persistent *KMT2A*-PTD transcripts have been observed in patients remaining in continuous complete remission (CR) after allogeneic hematopoietic stem cell transplantation (allo-HSCT),[17] However, a clinically informative pre-transplant *KMT2A*-PTD cutoff for predicting relapse and survival after allo-HSCT has not been established.

Although allo-HSCT is an effective consolidation strategy for patients with *KMT2A*-PTD AML,[22, 23] large-cohort studies focusing on transplant outcomes in this molecular subtype remain scarce. In this study, we analyzed a cohort of 180 patients with *KMT2A*-PTD AML who underwent allo-HSCT to determine the prognostic significance of pre-transplant *KMT2A*-PTD MRD, identify a clinically relevant pre-transplant molecular cutoff for risk stratification, and evaluate the impact of co-occurring mutations on post-transplant outcomes.

Methods

Study cohort

This retrospective study included 180 patients with *KMT2A*-PTD AML who underwent allo-HSCT at Peking University People's Hospital between January 1, 2015, and October 1, 2025. Patients were aged ≥16 years at transplant. *KMT2A*-PTD was confirmed at diagnosis by real-time quantitative PCR (RQ-PCR) or next-generation sequencing (NGS). The study was approved by the Peking University People's Hospital Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained.

KMT2A-PTD and mutation analysis

Bone marrow (BM) aspirates for pre-transplant *KMT2A*-PTD MRD assessment were collected within two months before allo-HSCT and before conditioning. K*MT2A*-PTD transcripts were quantified by TaqMan-based RQ-PCR with an analytical sensitivity of 10^{-4}, normalized to ABL1, and reported as copies per 10^5 ABL1. The previously validated threshold of 80 copies/10^5 ABL1 defined *KMT2A*-PTD positivity at diagnosis.[19, 21] For prognostic analyses, MRD levels were evaluated across prespecified thresholds and as a continuous variable; low-level values, including those below 80 and between 80 and 1499 copies/10^5 ABL1, were retained. The study-derived cutoff of 1500 copies/10^5 ABL1 was selected (Figure S1). For dichotomized analyses, values <1500 copies/10^5 ABL1 were classified as MRD-negative, whereas values ≥1500 copies/10^5 ABL1 were classified as MRD-positive. *FLT3*-ITD was determined at diagnosis by PCR-based fragment analysis or NGS.

Transplantation procedures and definitions

Allo-HSCT was performed according to institutional protocols.[24-26] Detailed conditioning, antithymocyte globulin (ATG), donor, graft-source, graft-versus-host disease (GVHD) prophylaxis, FLT3 inhibitor, maintenance, and donor lymphocyte infusion details are provided in Supplementary.

Multiparameter flow cytometry (MFC)-MRD was assessed as previously described, with positivity defined as ≥0.01% aberrant cells.[27, 28] The CR category included conventional CR, CR with incomplete platelet recovery (CRp), and CR with incomplete hematologic recovery (CRi). Conventional CR required BM blasts <5%, no circulating blasts or extramedullary disease, ANC ≥1.0 × 10^9/L, and platelets ≥100 × 10^9/L; CRp or CRi allowed incomplete platelet or hematologic recovery, respectively. Overall survival (OS) was defined as time from transplantation to death from any cause; event-free survival (EFS) as time to relapse or death, with censoring at the last follow-up; and non-relapse mortality (NRM) as death without prior relapse.

Statistical analysis

Patient characteristics were summarized. Categorical variables were compared using the Chi-square test and continuous variables using the Mann-Whitney U or Kruskal-Wallis test, as appropriate. OS and EFS were estimated by Kaplan-Meier analysis and compared using the log-rank test; CIR and NRM were estimated using competing-risk methods and compared with Gray's test. Cox models were used for OS and EFS, and Fine-Gray models for CIR and NRM. Pre-transplant *KMT2A*-PTD MRD status was included a priori in all multivariable models as the primary variable of interest. Additional covariates were restricted to pre-transplant variables with $p<0.05$ in univariable analysis. Cutoff exploration was performed across candidate values for OS, EFS, and CIR. Restricted cubic spline (RCS) analysis based on Cox proportional hazards models assessed the continuous association between log10-transformed *KMT2A*-PTD transcript levels and relapse risk, while maximally selected rank statistics (maxstat) analysis identified outcome-associated cutoff values. Analyses were performed using R 4.5.2 and SPSS 27.

Results

Characteristics of study cohort

This study included 180 patients with *KMT2A*-PTD AML who underwent allo-HSCT. The cohort comprised 90 males (male-to-female ratio, 1:1). Median age at transplantation was 45 years (range, 16–74 years), with the majority of patients aged ≤50 years (64%). Median white blood cell (WBC) count at diagnosis was 4.8×10^9/L (range, 0.7–176.9×10^9/L), and median BM blast infiltration was 50% (range, 13%–98%). Among the 180 *KMT2A*-PTD AML patients, 27 (15%) had secondary AML, mostly (n=19) following a known history of myelodysplastic syndrome. Based on the 2017 European LeukemiaNet (ELN) risk classification,[29] 87 patients were classified as intermediate risk, and 93 as adverse risk. The majority of patients (n=133) had a normal karyotype. The most frequent cytogenetic abnormalities were trisomy 8 (n=15, 8%) and trisomy 11 (n=11, 6%). The *FLT3*-ITD status was measured in all *KMT2A*-PTD AML patients. A *FLT3*-ITD was present in 56 of the 180 patients (31%). Among 80 patients with available NGS data at diagnosis (Figure 1), a median of 2 mutations (range, 0–9) was detected. The most frequent concomitant mutations were *FLT3*-ITD (n=28), *DNMT3A* (n=19), *U2AF1* (n=19), and *RUNX1* (n=17). Additional mutations frequently involved *WT1*, *STAG2*, *TET2*, and *ASXL1*.

Donor types included matched sibling donors (n=37), matched unrelated donors (n=8), and haploidentical donors (n=135). Disease status at transplantation was first CR1 in 146, second CR2 in 12 and refractory disease (RD) in 22 patients. The number of prior chemotherapy cycles differed significantly across the three groups, being highest in the CR2 (median 4; range, 2–11) group, followed by RD (median 3; range, 1–14) and CR1 (median 3; range, 1–10; p=0.009) groups, other

baseline characteristics were comparable among the three groups (Table 1).

Evaluation of MRD

Of the 158 patients in CR prior to allo-HSCT, pre-transplant MRD was assessed in 144 patients. MRD was measured within one month (n=106, 74%) or two months (n=38, 26%) before transplantation. Pre-transplant *KMT2A*-PTD transcript levels among the 144 patients ranged from 10 to 173,800 copies/10^5 ABL1, with a median of 90 copies/10^5 ABL1. To identify a clinically meaningful threshold for pre-transplant *KMT2A*-PTD MRD positivity, we first performed hazard ratio–based cutoff exploration analyses for OS, EFS, and CIR. Across all three endpoints, thresholds approximately centered around 1500 copies/10^5 ABL1 consistently demonstrated prognostic discrimination (Figures S1A–C). We next analyzed *KMT2A*-PTD levels as a continuous variable. RCS analysis demonstrated a continuous positive association between increasing pre-transplant *KMT2A*-PTD levels and relapse risk (Figure S1D). In addition, maxstat analysis based on CIR identified a relatively stable high-risk range beginning around 1500 copies/10^5 ABL1 (Figure S1E). Collectively, these findings supported the selection of 1500 copies/10^5 ABL1 as a clinically meaningful threshold for defining pre-transplant *KMT2A*-PTD MRD positivity. Baseline characteristics of MRD-negative and MRD-positive patients are summarized in Table 2.

Survival analysis of study cohort

The median follow-up for surviving patients among the 180 *KMT2A*-PTD AML cases was 41.6 months (95% CI, 33.3–49.8). The estimated 3-year OS, EFS, CIR, and NRM were 70%, 63%, 27%, and 10%, respectively. Among patients transplanted in remission, outcomes were similar between those transplanted in CR1 (3-year OS 73%, EFS 64%) and CR2 (3-year OS 67%, EFS 58%), with no

significant differences observed. Patients with RD had a 3-year OS and EFS of 57% for both endpoints (Figure S2). Patients transplanted in CR tended to have higher OS and lower NRM than those transplanted with RD (OS: 72% vs. 57%; NRM: 10% vs. 24%), whereas CIR (28% vs. 19%) and EFS (64% vs. 57%) did not differ significantly (Figure S3). The estimated 3-year CIR for CR1, CR2, and RD were 28%, 33%, and 19%, respectively, and the corresponding NRM rates were 8%, 8%, and 24%, respectively (Figure S2).

Outcomes were comparable between CR patients with (n=144) and without (n=14) pre-transplant MRD assessment (3-year OS, 74% vs. 68%; p=0.42). Using the study-derived threshold of 1500 copies/10^5 ABL1 for molecular positivity, MRD-negative patients (n=110) had significantly lower 3-year CIR than MRD-positive patients (n=34; 22% vs. 42%; p=0.01). In line with the increased relapse risk, MRD-negative patients also achieved superior 3-year OS (80% vs. 49%; p<0.001) and EFS (73% vs. 42%; p<0.001), as well as lower NRM (6% vs. 16%; p=0.067; Figure 2). In an exploratory comparison, no statistically significant differences were detected between MRD-positive patients and patients transplanted with RD in 3-year OS (49% vs. 57%; p=0.75), EFS (42% vs. 57%; p=0.46), CIR (42% vs. 19%; p=0.16), or NRM (16% vs. 24%; p=0.49). However, given the small group sizes, this comparison was underpowered and should not be interpreted as evidence of equivalent outcomes.

Subgroup analysis by *FLT3*-ITD status

FLT3-ITD status at diagnosis was classified as positive (*FLT3*-ITD+) or negative (*FLT3*-ITD–). Overall, *FLT3*-ITD+ patients exhibited inferior post-transplant outcomes compared with *FLT3*-ITD– patients, including significantly worse 3-year OS (62% vs. 79%; p=0.015), EFS (55% vs. 72%; p=0.012), and

higher CIR (38% vs. 20%; p=0.003), whereas NRM was comparable between the two groups (7% vs. 9%; p=0.70; Figure S4). To further investigate the interaction between *FLT3*-ITD and residual disease burden, we stratified patients according to pre-transplant *KMT2A*-PTD MRD status. Among MRD-negative patients, *FLT3*-ITD retained prognostic relevance primarily through increased relapse risk. Although differences in 3-year OS (74% vs. 83%; p=0.16) and EFS (64% vs. 77%; p=0.062) did not reach statistical significance, *FLT3*-ITD+ patients had a significantly higher 3-year CIR compared with *FLT3*-ITD– patients (33% vs. 16%; p=0.014), while NRM remained similar between groups (3% vs. 7%; p=0.50; Figure S5). In contrast, among MRD-positive patients, the prognostic impact of *FLT3*-ITD appeared attenuated. No significant differences were observed between *FLT3*-ITD– and *FLT3*-ITD+ patients in 3-year OS (60% vs. 37%; p=0.16), EFS (49% vs. 37%; p=0.27), CIR (35% vs. 50%; p=0.22), or NRM (17% vs. 13%; p=0.83; Figure S6).

Among the 47 *FLT3*-ITD+ patients, 33 (70%) received a *FLT3* inhibitor during induction and/or consolidation chemotherapy before allo-HSCT. In exploratory analyses, no statistically significant differences were observed between patients without and with *FLT3* inhibitor exposure during pre-transplant chemotherapy in 3-year OS (57% vs. 65%; p=0.16), EFS (42% vs. 61%; p=0.084), CIR (44% vs. 36%; p=0.41), or NRM (14% vs. 3.1%; p=0.14). After transplantation, 6 patients received prophylactic *FLT3* inhibitor maintenance, whereas 4 received *FLT3* inhibitor therapy after molecular relapse. Given the small numbers and heterogeneous indications, the impact of post-transplant *FLT3* inhibitor use on outcomes was not separately evaluated.

Multivariable model for prediction of posttransplant outcome

We performed univariable and multivariable regression analyses to identify factors associated with

post-transplant outcomes. In univariable analysis, pre-transplant *KMT2A*-PTD MRD positivity was significantly associated with inferior OS (HR=3.38; 95% CI, 1.75–6.54; p<0.001), inferior EFS (HR=2.97; 95% CI, 1.63–5.40; p<0.001), and higher CIR (HR=2.50; 95% CI, 1.25–5.01; p=0.01), and showed a trend toward higher NRM (HR=2.91; 95% CI, 0.90–9.45; p=0.075; Table 3). In multivariable analysis, pre-transplant *KMT2A*-PTD MRD positivity remained independently associated with inferior OS (HR=3.73; 95% CI, 1.87–7.45; p<0.001), inferior EFS (HR=3.23; 95% CI, 1.74–6.01; p<0.001), higher CIR (HR=2.20; 95% CI, 1.07–4.54; p=0.032), and higher NRM (HR=3.69; 95% CI, 1.12–12.18; p=0.031). Age >50 years was also independently associated with inferior OS (HR=3.86; 95% CI, 1.96–7.60; p<0.001), inferior EFS (HR=2.94; 95% CI, 1.61–5.34; p<0.001), and higher NRM (HR=6.62; 95% CI, 1.78–24.56; p=0.005; Table 4).

As a sensitivity analysis, 78 patients with sufficient cytogenetic and molecular data for 2022 ELN risk classification were analyzed separately (intermediate risk, n=34; adverse risk, n=44), including 22 patients with pre-transplant *KMT2A*-PTD MRD positivity and 56 with MRD negativity. After adjustment for 2022 ELN risk classification, pre-transplant *KMT2A*-PTD MRD positivity remained significantly associated with inferior OS (HR=2.63; 95% CI, 1.03–6.71; p=0.043) and EFS (HR=2.69; 95% CI, 1.17–6.17; p=0.020). The associations with CIR (HR=2.31; 95% CI, 0.90–5.92; p=0.081) and NRM (HR=2.78; 95% CI, 0.41–18.84; p=0.29) were directionally consistent with the primary analysis but did not reach statistical significance in this smaller subset (Table S1).

Combined molecular and immunophenotypic MRD assessment

To evaluate the prognostic value of combining molecular and immunophenotypic MRD assessment, we stratified patients according to pre-transplant *KMT2A*-PTD MRD status (positive vs. negative)

and MFC-MRD status (positive vs. negative). Patients were classified into four categories: *KMT2A*-PTD-/MFC-MRD- (n=96), *KMT2A*-PTD+/MFC-MRD- (n=14), *KMT2A*-PTD-/MFC-MRD+ (n=14), and *KMT2A*-PTD+/MFC-MRD+ (n=20). Significant differences in post-transplant outcomes were observed across the four groups. Patients with double negativity achieved the best outcomes (3-year OS, 80%; EFS, 73%), followed by those with isolated MFC-MRD positivity (OS, 79%; EFS, 71%). Patients with isolated *KMT2A*-PTD positivity had the lowest observed OS and EFS (OS, 21%; EFS, 21%), while those with double positivity showed intermediate outcomes (OS, 60%; EFS, 50%; $p<0.001$ for OS, $p<0.001$ for EFS). Relapse rates followed a similar pattern, with CIR of 21%, 29%, 36%, and 50%, respectively ($p=0.024$). NRM was highest in the isolated *KMT2A*-PTD positive group (44%), intermediate in the double-negative group (6%), and absent in both MFC-MRD-positive groups (0%; $p<0.001$; Figure 3). These findings suggest that combined *KMT2A*-PTD MRD and MFC-MRD assessment may provide complementary prognostic information; however, the results of the smaller subgroups should be interpreted cautiously, especially the unexpectedly high NRM observed in the *KMT2A*-PTD+/MFC-MRD− group.

Discussion

KMT2A alterations in AML include *KMT2A*-r and *KMT2A*-PTD, which represent biologically distinct entities.[30, 31] The 2022 ELN classification explicitly excludes *KMT2A*-PTD from the adverse-risk category assigned to *KMT2A*-rearranged AML, underscoring the need to evaluate its prognostic significance separately.[32] In this study, we demonstrated that pre-transplant *KMT2A*-PTD MRD, defined using a threshold of 1500 copies/10^5 ABL1, was an important prognostic marker of post-transplant outcomes in patients with *KMT2A*-PTD AML. MRD-positive patients had significantly inferior survival and higher relapse rates than MRD-negative patients. Although no statistically

significant differences were detected between MRD-positive patients and those transplanted with refractory disease, the small sample sizes and limited statistical power preclude any conclusion regarding equivalence. Nevertheless, the poor outcomes observed among MRD-positive patients support the clinical significance of persistent molecular disease despite morphological remission and suggest that conventional morphological response criteria may not fully capture residual disease risk. Moreover, the integration of *KMT2A*-PTD MRD with MFC-MRD further refined risk stratification, highlighting the value of an integrated MRD framework for refining pre-transplant risk assessment.

Although achieving morphological CR prior to transplantation improved OS and reduced NRM, it failed to discriminate relapse risk, highlighting the limitations of morphology-based assessment in detecting subclinical disease. In contrast, molecular MRD assessment using *KMT2A*-PTD provided clear prognostic stratification. Using continuous risk and cutoff exploration analyses, we identified a clinically relevant high-risk interval centered around 1500 copies/10^5 ABL1. Interestingly, Ommen et al.[17] previously used an *KMT2A*-PTD/ABL1 threshold of 15×10^{-3}, corresponding to 1500 copies/10^5 ABL1, to define molecular relapse. Although derived for a different purpose and in a cohort including both transplanted and non-transplanted patients, this threshold is consistent with the cutoff independently identified in our study. This is particularly relevant given that *KMT2A*-PTD transcripts can be detected at low levels in healthy individuals,[15, 17] complicating interpretation of MRD positivity. Our findings identify a clinically informative, study-derived threshold for pre-transplant *KMT2A*-PTD MRD in this cohort, which requires external validation before broader clinical application.

We further characterized the cytogenetic and mutational landscape of our *KMT2A*-PTD AML cohort.

Consistent with previous reports, a normal karyotype predominated, with trisomy 8 abnormalities and trisomy 11 being the most frequent cytogenetic alterations.[33-36] *KMT2A*-PTD rarely occurs as a sole lesion, with the majority of patients harboring additional somatic mutations.[35] In our cohort, *FLT3*-ITD was the most prevalent co-occurring mutation. Among patients who achieved CR, *FLT3*-ITD at diagnosis was associated with an increased risk of relapse, consistent with prior studies.[17] The differential impact of *FLT3*-ITD according to pre-transplant MRD status offers insight into the interplay between mutational biology and residual disease burden. In MRD-negative patients, *FLT3*-ITD retained prognostic relevance primarily through relapse risk, as *FLT3*-ITD+ patients had a significantly higher 3-year CIR than *FLT3*-ITD– patients (33% vs. 16%; p=0.014). This suggests that when leukemic burden is low, *FLT3*-ITD–driven proliferative signaling—mediated in part through constitutive *FLT3* receptor activation and downstream STAT5/RAS pathways—may promote relapse from residual leukemic cells.[14] In contrast, among MRD-positive patients, the prognostic impact of *FLT3*-ITD was attenuated, with no significant difference in 3-year CIR between *FLT3*-ITD+ and *FLT3*-ITD– subgroups (50% vs. 35%; p=0.22), despite a numerical trend toward higher relapse in the *FLT3*-ITD+ group. Together, these findings highlight that the prognostic value of *FLT3*-ITD is context-dependent and may be modulated by *KMT2A*-PTD MRD status at transplantation. In the absence of detectable *KMT2A*-PTD MRD, *FLT3*-ITD remains useful for identifying patients at increased relapse risk who may benefit from intensified monitoring or post-transplant maintenance strategies. This observation is consistent with the ELN MRD Working Party recommendation that *KMT2A*-PTD be combined with a second MRD marker,[37, 38] such as *FLT3*-ITD, particularly in patients with undetectable *KMT2A*-PTD MRD. However, once *KMT2A*-PTD MRD positivity is established, therapeutic decision-making may be driven more by the presence of residual disease than by the

specific mutational profile of the persistent clone, as the dominant prognostic effect of measurable disease may overshadow the incremental value of additional markers.

Analysis of the broader mutational landscape in the 80 patients with available NGS data at diagnosis revealed additional recurrent co-occurring mutations, with *DNMT3A* (23.8%), *U2AF1* (23.8%) and *RUNX1* (21.2%) being the most prevalent. These findings are largely consistent with previous reports. Hinai et al. identified *DNMT3A* (38.8%), *FLT3*-ITD (34.9%), and *IDH2* (21.2%) as the most frequent co-mutations.[14] Sun et al. reported high frequencies of *FLT3* (46%), *DNMT3A* (25%), *IDH1/2* (31%), and *RUNX1* (23%),[39] and Kao et al. observed *DNMT3A* mutations in 32.7% of patients, which were associated with inferior prognosis.[35] Notably, the frequency of *U2AF1* mutations in our cohort was higher than those reported by Hinai et al. (9.4%)[33] and Sun et al. (12.5%),[39] which may reflect differences in cohort composition, sequencing platforms, or patient selection. According to the stepwise model proposed by Sun et al., mutations in *DNMT3A*, *IDH1/2*, *TET2*, and *U2AF1* arise first, followed by *KMT2A*-PTD, with proliferative mutations such as *FLT3*-ITD and *RAS* occurring later as subclonal events. This model is supported by the observation that *DNMT3A* and *U2AF1* mutations often persist in remission, whereas *KMT2A*-PTD and *FLT3*-ITD are typically cleared.[39] In this context, our findings further suggest that *KMT2A*-PTD MRD more specifically reflects residual leukemic burden at the level of the disease-defining clone, whereas early founder mutations may persist without indicating active disease. In summary, our findings confirm the recurrent mutational landscape of *KMT2A*-PTD AML reported across multiple studies and support the stepwise model of leukemogenesis in this high-risk subtype.

Integrating molecular and immunophenotypic MRD may provide complementary prognostic information. The distinct prognostic patterns observed across the combined *KMT2A*-PTD MRD and

Table 1. Baseline characteristics of *KMT2A*-PTD AML patients according to pre-transplant remission status

Remission status prior to allogeneic HSCT	Overall (n=180)			CR1 (n = 146)			CR2 (n = 12)			RD (n = 22)			p-value
				No.		%	No.		%	No.		%	
Median age (at time of HSCT; years)		45			44			45			50		0.085
Range		16-74			17-71			16-63			26-74		
Age category													
≤50 years	115		64	95		65	8		67	12		55	0.60
>50 years	65		36	51		35	4		33	10		45	
Gender													
Male	90		50	73		50	6		50	11		50	1.00
Female	90		50	73		50	6		50	11		50	
Type of AML													
de novo	153		85	125		86	11		92	17		77	0.55
secondary	27		15	21		14	1		8	5		23	
WBC count, $x10^9$/L Median		4.8			4.6			6.1			3.8		0.29
Range		0.7-176.9			0.7-176.9			1.9-152.0			0.7-161.6		
Platelet count, $x10^9$/L Median		59			61			67			44		0.25
Range		2-1405			2-1405			18-533			3-856		
Percentage of BM blasts, Median		50			50			60			42		0.17
Range		13-98			13-98			24-95			17-80		
2017 ELN risk classification													
Intermediate	87		48	71		49	4		33	12		55	0.51
Adverse	93		52	75		51	8		67	10		45	
FLT3-ITD mutated	56		31	48		33	3		25	5		22	0.64
Donor type													0.85

MFC-MRD subgroups further highlight the heterogeneity of residual disease. Notably, patients with isolated *KMT2A*-PTD MRD positivity (*KMT2A*-PTD+/MFC-MRD−) had the poorest survival and the highest NRM. However, this subgroup was small (n=14), and these findings should be interpreted cautiously. Although no statistically significant differences in the assessed baseline characteristics were identified among the four groups, some numerical imbalances were present (Table S2), and clinically relevant differences cannot be excluded given the limited sample size. Furthermore, five of the eight deaths in this subgroup were non-relapse deaths; four were infection-related, including three pulmonary infections and one Klebsiella pneumoniae sepsis, whereas one was due to gastrointestinal GVHD (Table S3). Therefore, the unexpectedly high NRM and poor survival observed in this subgroup should be interpreted cautiously, as the observed differences may reflect subgroup heterogeneity, treatment-related factors, competing risks, or random variation. These findings should be considered exploratory and require validation in larger independent cohorts.

From a therapeutic perspective, the molecular characteristics of *KMT2A*-PTD AML may also provide a rationale for exploring targeted therapeutic strategies. Although *KMT2A*-PTD AML is molecularly distinct from *KMT2A*-r AML, the two share certain HOX-related transcriptional programs.[23, 40, 41] This partial biological overlap, together with the preclinical activity of the menin inhibitor bleximenib (JNJ-75276617) observed in the *KMT2A*-PTD EOL-1 cell line, provides a biologically plausible rationale for investigating menin inhibition.[42] However, clinical evidence supporting menin inhibition specifically in *KMT2A*-PTD AML remains lacking. Whether molecular MRD assessment may serve as a biomarker in future targeted therapy studies warrants further investigation.

First, although this study represents one of the largest cohorts of patients with *KMT2A*-PTD AML

undergoing allo-HSCT, subgroup analyses remained limited by sample size, particularly for less frequent co-mutations. Second, the MRD threshold identified in this study was derived from a single-center retrospective cohort and has not been externally validated; therefore, its generalizability remains uncertain and it should not yet be regarded as a universal cutoff. Third, the retrospective single-center design may have introduced selection bias, and treatment heterogeneity, together with the predominance of haploidentical transplantation, may limit the generalizability of our findings to other transplant platforms.

In conclusion, in this cohort, pre-transplant *KMT2A*-PTD MRD positivity defined using a study-derived threshold of 1500 copies/10^5 ABL1 was associated with adverse post-transplant outcomes. Integration of MRD status with mutational and immunophenotypic data enables refined risk stratification and supports the development of MRD-guided therapeutic strategies. As targeted therapies continue to evolve, standardized and sensitive MRD assessment will play a critical role in optimizing patient selection, guiding therapeutic intervention, and ultimately improving outcomes in this high-risk AML subtype.

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MSD Haplo MUD	37 135 8		21 75 4	32 107 7		22 73 5	1 11 0		8 92 0	4 17 1		18 77 5	
Prior chemotherapy cycles		3 1-14			3 1-10			4 2-11			3 1-14		0.009
Trisomy 8	15		8	12		8	2		17	1		5	0.43
Trisomy 11	11		6	10		7	0		0	1		5	1.00
Donor gender Male Female	 65 115		 36 64	 52 94		 36 64	 4 8		 33 67	 9 13		 41 59	 0.87
Stem cell source PB BM+PB	 116 64		 64 36	 94 52		 64 36	 9 3		 75 25	 13 9		 59 41	 0.66
Major ABO incompatibility	50		28	43		30	3		25	4		18	0.59

Abbreviations: CR, complete remission; CR1, first complete remission; CR2, second complete remission; RD, refractory disease; WBC, white blood cell; MSD, matched sibling donor; Haplo, haploidentical donor; MUD, matched unrelated donor; PB, peripheral blood; BM, bone marrow.

Table 2. Baseline characteristics of patients stratified by pre-transplant *KMT2A*-PTD MRD status

	KMT2A-PTD MRD negative (n=110)			*KMT2A*-PTD MRD positive (n=34)			p-value
	No.		%	No.		%	
Median age (at time of HSCT; years) Range		44 16-71			40 17-67		0.38
Age category ≤50 years >50 years	71 39		65 35	25 9		74 26	0.33
Gender Male Female	57 53		52 48	15 19		44 56	0.43
Type of AML de novo secondary	96 14		87 13	29 5		85 15	0.77
Remissionstatus CR1 CR2	105 5		95 5	28 6		82 18	0.012
Chemotherapy cycles to achieve CR		1 1-9			2 1-7		<0.001
WBC count, $x10^9$/L Median Range		3.6 0.7-176.9			6.9 1.1-156.9		0.022
Platelet count, $x10^9$/L Median Range		61 9-717			72 2-1405		0.92
Percentage of BM blasts, Median Range		48 13-93			59 13-95		0.15

2017 ELN risk classification							
Intermediate	58		53	12		35	0.075
Adverse	52		47	22		65	
FLT3-ITD mutated	32		29	15		44	0.10
Donor type							
MSD	22		20	6		18	0.46
Haplo	84		76	25		73	
MUD	4		4	3		9	
Prior chemotherapy cycles		3 1-11			3 1-7		0.61
Trisomy 8	12		11	0		0	0.069
Trisomy 11	6		5	4		12	0.25
Donor gender							
Male	69		63	21		62	0.92
Female	41		37	13		38	
Stem cell source							
PB	66		60	27		79	0.039
BM+PB	44		40	7		21	
Major ABO incompatibility	38		35	14		41	0.48

Abbreviations: CR, complete remission; CR1, first complete remission; CR2, second complete remission; WBC, white blood cell; MSD, matched sibling donor; Haplo, haploidentical donor; MUD, matched unrelated donor; MRD, measurable residual disease; PB, peripheral blood; BM, bone marrow.

Table 3. Univariable analysis of factors associated with post-transplant outcomes

	OS		EFS		CIR		NRM	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
Sex, Male vs. Female	0.77 (0.40-1.49)	0.44	0.84 (0.47-1.51)	0.56	0.74 (0.38-1.46)	0.39	1.21 (0.37-3.93)	0.75
Age, years, >50 vs. ≤50	3.36 (1.73-6.53)	<0.001	2.57 (1.43-4.63)	0.002	1.73 (0.88-3.39)	0.11	5.68 (1.52-21.21)	0.01
WBC count, $\times 10^9$/L	1.00 (1.00-1.01)	0.27	1.00 (1.00-1.01)	0.55	1.01 (1.00-1.01)	0.075	0.97 (0.93-1.01)	0.11
Percentage of blast at diagnosis, %	1.02 (1.00-1.03)	0.055	1.01 (0.99-1.02)	0.32	1.00 (0.99-1.02)	0.68	1.01 (0.99-1.03)	0.17
Pre-HSCT *KMT2A*-PTD MRD, positive vs. negative	3.38 (1.75-6.54)	<0.001	2.97 (1.63-5.40)	<0.001	2.50 (1.25-5.01)	0.01	2.91 (0.90-9.45)	0.075
***FLT3*-ITD status, *FLT3*-ITD+ vs. *FLT3*-ITD–**	2.23 (1.15-4.31)	0.018	2.10 (1.16-3.80)	0.014	2.71 (1.39-5.27)	0.003	0.77 (0.21-2.85)	0.69
Donor type								
MSD	Ref.		Ref.		Ref.		Ref.	
Haplo	0.64 (0.31-1.34)	0.24	0.77 (0.39-1.54)	0.46	0.84 (0.38-1.84)	0.66	0.62 (0.16-2.32)	0.48
MUD	1.03 (0.22-4.72)	0.97	1.51 (0.42-5.45)	0.53	1.28 (0.28-5.93)	0.75	1.45 (0.15-14.02)	0.75
Stem cell source, PB vs. PB+BM	0.82 (0.41-1.64)	0.57	0.70 (0.37-1.32)	0.27	0.45 (0.20-1.01)	0.054	2.16 (0.67-6.95)	0.20
Major ABO incompatibility	1.19 (0.61-2.33)	0.61	0.91 (0.49-1.69)	0.77	0.73 (0.35-1.54)	0.41	1.50 (0.46-4.89)	0.50
2017ELN, Adverse vs. Intermediate	2.05 (1.03-4.05)	0.04	1.80 (0.99-3.28)	0.055	2.22 (1.12-4.41)	0.023	0.82 (0.25-2.63)	0.73

Abbreviations: OS, overall survival; EFS, event-free survival; CIR, cumulative incidence of relapse; NRM, non-relapse mortality; HR, hazard ratio; CI, confidence interval; WBC, white blood cell; MRD, measurable residual disease; MSD, matched sibling donor; Haplo, haploidentical donor; MUD, matched unrelated donor; ELN, European LeukemiaNet; PB, peripheral blood; BM, bone marrow.

Table 4. Multivariable analysis of factors associated with post-transplant outcomes

	OS		EFS		CIR		NRM	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
Age, years, >50 vs. ≤50	3.86 (1.96-7.60)	<0.001	2.94 (1.61-5.34)	<0.001			6.62 (1.78-24.56)	0.005
Pre-HSCT *KMT2A*-PTD MRD, positive vs. negative	3.73 (1.87-7.45)	<0.001	3.23 (1.74-6.01)	<0.001	2.20 (1.07-4.54)	0.032	3.69 (1.12-12.18)	0.031
***FLT3*-ITD status, *FLT3*-ITD+ vs. *FLT3*-ITD–**	1.46 (0.61-3.48)	0.40	1.74 (0.95-3.21)	0.075	2.15 (0.93-4.96)	0.074		
2017ELN, Adverse vs. Intermediate	1.33 (0.55-3.21)	0.53			1.26 (0.53-3.00)	0.60		

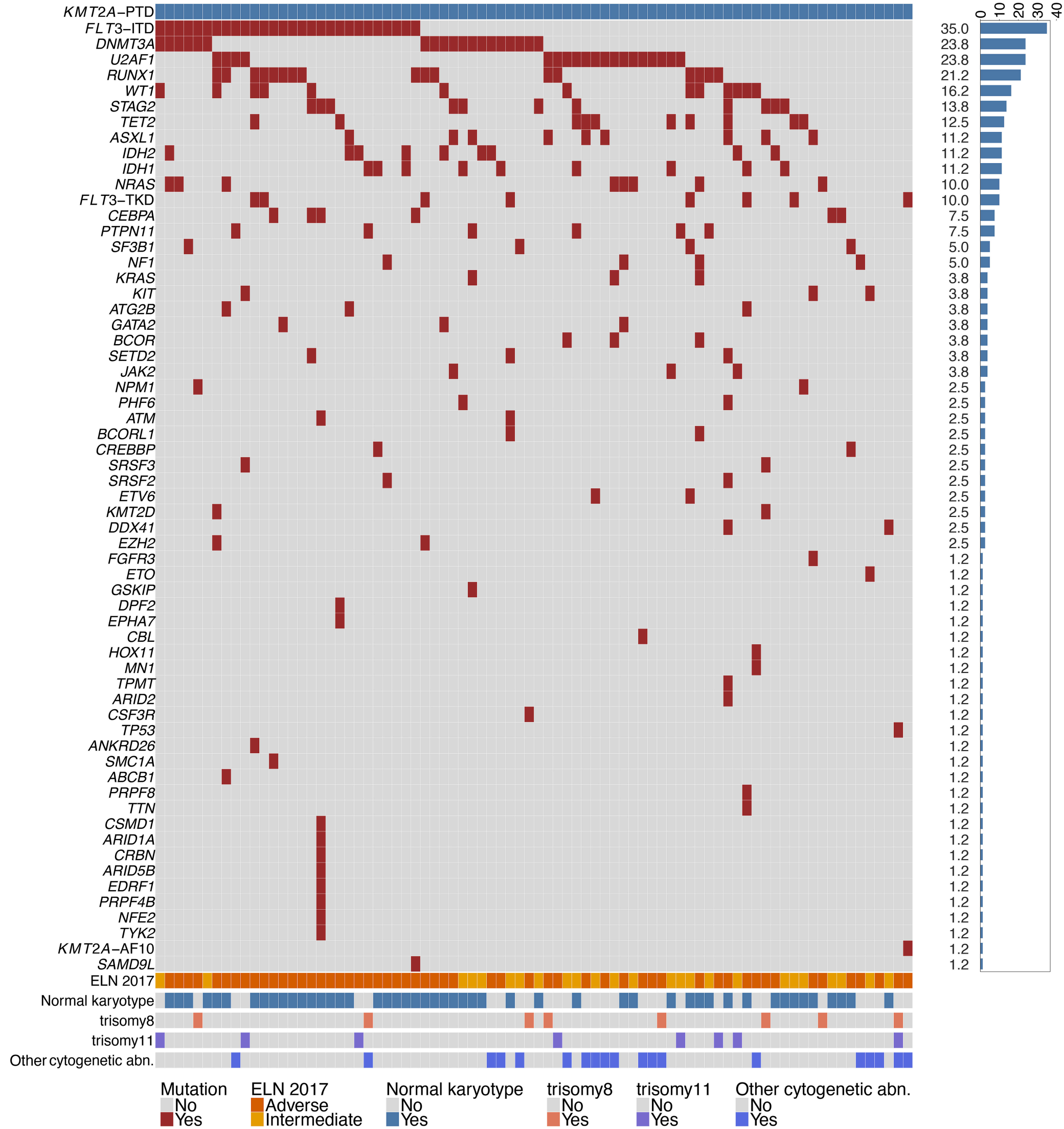
Abbreviations: OS, overall survival; EFS, event-free survival; CIR, cumulative incidence of relapse; NRM, non-relapse mortality; HR, hazard ratio; CI, confidence interval; MRD, measurable residual disease; ELN, European LeukemiaNet. Pre-transplant *KMT2A*-PTD MRD status was included a priori in all multivariable models.

Figure legend.

Figure 1. Mutational landscape and cytogenetic features of patients with *KMT2A*-PTD AML.

Figure 2. Post-transplant outcomes according to pre-transplant *KMT2A*-PTD MRD status. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

Figure 3. Post-transplant outcomes according to combined pre-transplant *KMT2A*-PTD MRD and MFC-MRD status. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).


KMT2A–PTD
FLT3–ITD
DNMT3A
U2AF1
RUNX1
WT1
STAG2
TET2
ASXL1
IDH2
IDH1
NRAS
FLT3–TKD
CEBPA
PTPN11
SF3B1
NF1
KRAS
KIT
ATG2B
GATA2
BCOR
SETD2
JAK2
NPM1
PHF6
ATM
BCORL1
CREBBP
SRSF3
SRSF2
ETV6
KMT2D
DDX41
EZH2
FGFR3
ETO
GSKIP
DPF2
EPHA7
CBL
HOX11
MN1
TPMT
ARID2
CSF3R
TP53
ANKRD26
SMC1A
ABCB1
PRPF8
TTN
CSMD1
ARID1A
CRBN
ARID5B
EDRF1
PRPF4B
NFE2
TYK2
KMT2A–AF10
SAMD9L
35.0
23.8
23.8
21.2
16.2
13.8
12.5
11.2
11.2
11.2
10.0
10.0
7.5
7.5
5.0
5.0
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10
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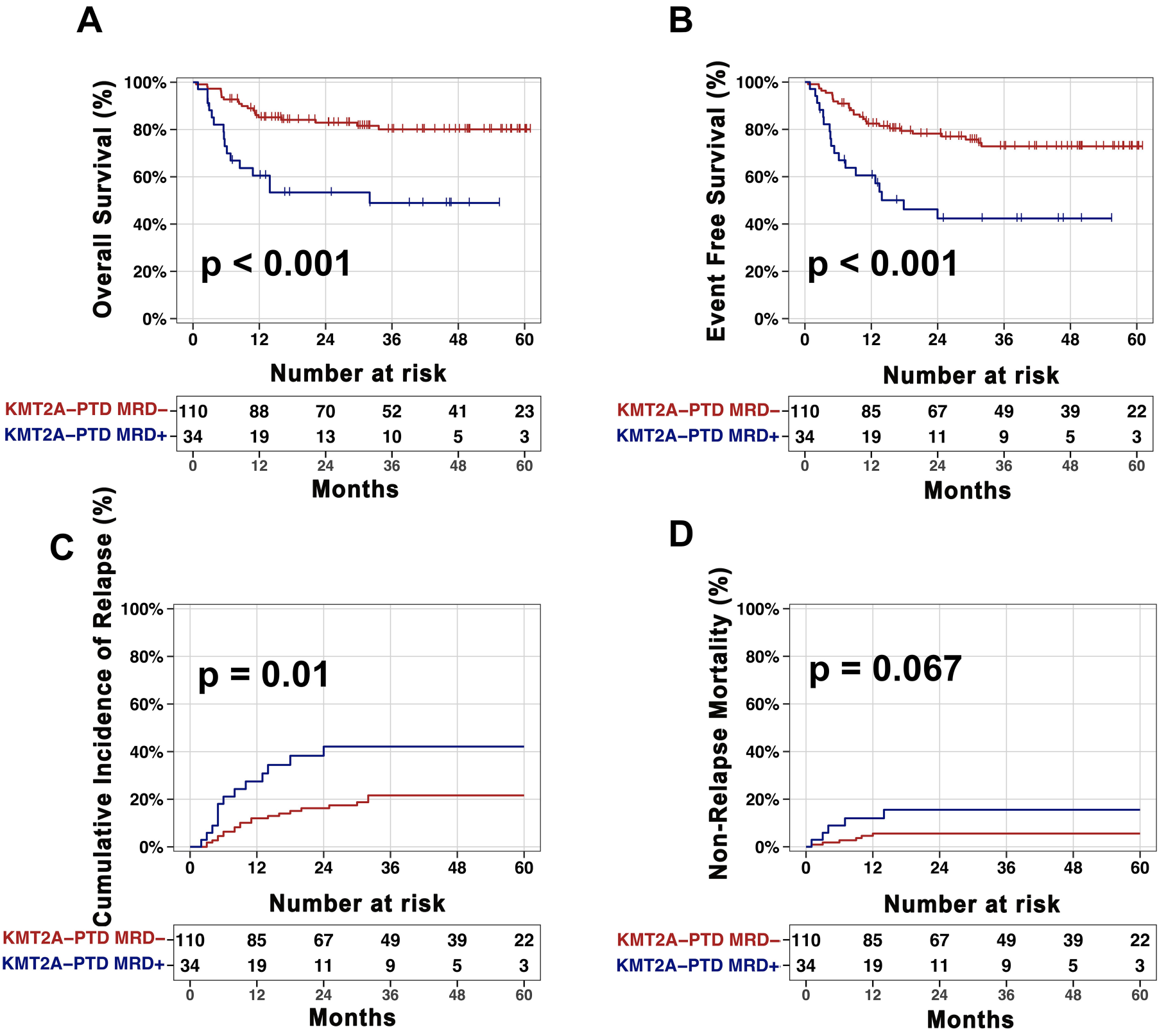
30
40
ELN 2017
Normal karyotype
trisomy8
trisomy11
Other cytogenetic abn.
Mutation
No
Yes
ELN 2017
Adverse
Intermediate
Normal karyotype
No
Yes
trisomy8
No
Yes
trisomy11
No
Yes
Other cytogenetic abn.
No
Yes


A
Overall Survival (%)
100%
80%
60%
40%
20%
0%
p < 0.001
0
12
24
36
48
60
Number at risk
KMT2A–PTD MRD–
KMT2A–PTD MRD+
110 88 70 52 41 23
34 19 13 10 5 3
0
12
24
36
48
60
Months
B
Event Free Survival (%)
100%
80%
60%
40%
20%
0%
p < 0.001
0
12
24
36
48
60
Number at risk
KMT2A–PTD MRD–
KMT2A–PTD MRD+
110 85 67 49 39 22
34 19 11 9 5 3
0
12
24
36
48
60
Months
C
Cumulative Incidence of Relapse (%)
100%
80%
60%
40%
20%
0%
p = 0.01
0
12
24
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48
60
Number at risk
KMT2A–PTD MRD–
KMT2A–PTD MRD+
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34 19 11 9 5 3
0
12
24
36
48
60
Months
D
Non-Relapse Mortality (%)
100%
80%
60%
40%
20%
0%
p = 0.067
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60
Number at risk
KMT2A–PTD MRD–
KMT2A–PTD MRD+
110 85 67 49 39 22
34 19 11 9 5 3
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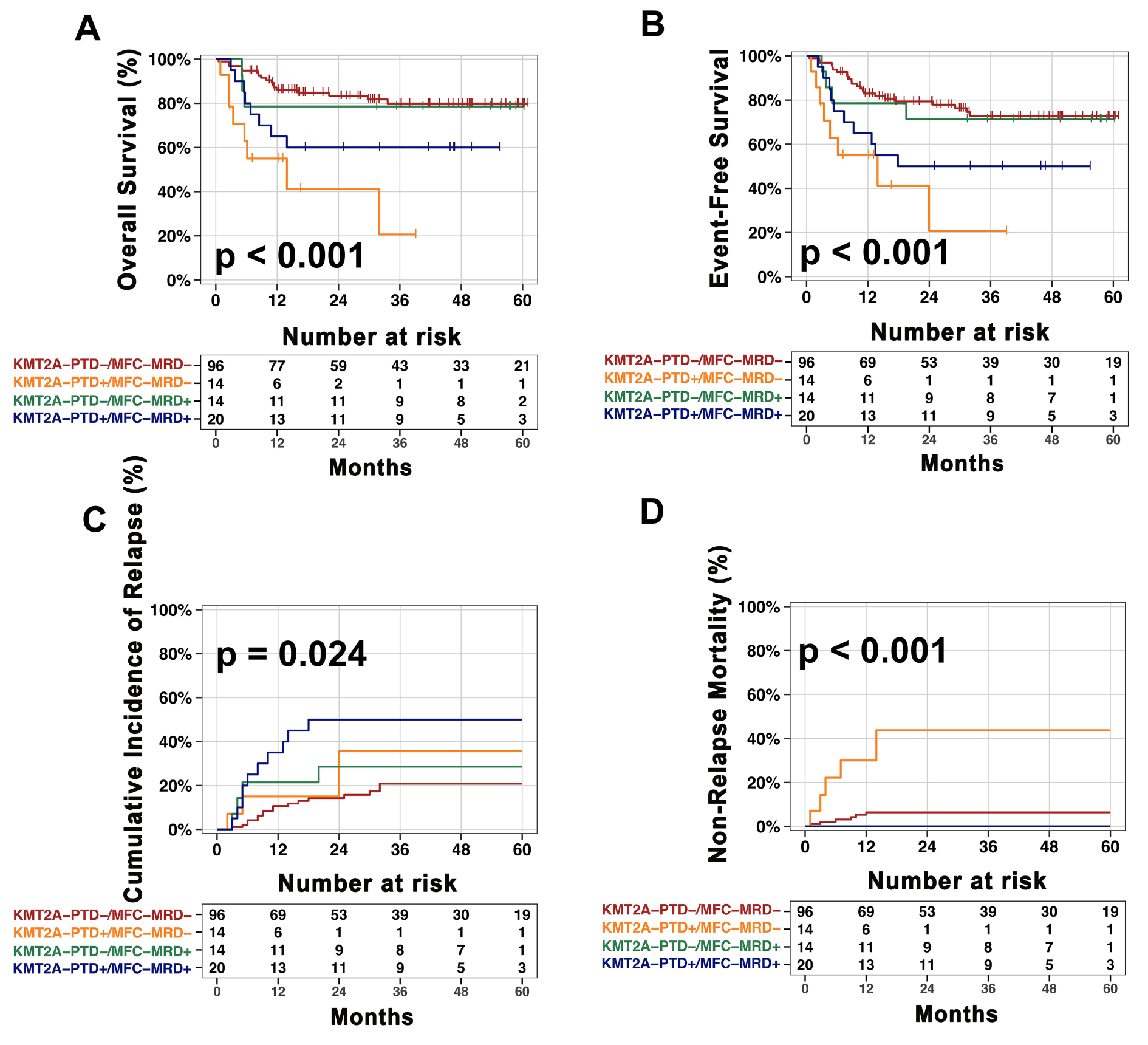
60
Months


A
Overall Survival (%)
100%
80%
60%
40%
20%
0%
p < 0.001
0
12
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60
Number at risk
KMT2A–PTD–/MFC–MRD– 96 77 59 43 33 21
KMT2A–PTD+/MFC–MRD– 14 6 2 1 1 1
KMT2A–PTD–/MFC–MRD+ 14 11 11 9 8 2
KMT2A–PTD+/MFC–MRD+ 20 13 11 9 5 3
Months
B
Event-Free Survival
p < 0.001
Number at risk
KMT2A–PTD–/MFC–MRD– 96 69 53 39 30 19
KMT2A–PTD+/MFC–MRD– 14 6 1 1 1 1
KMT2A–PTD–/MFC–MRD+ 14 11 9 8 7 1
KMT2A–PTD+/MFC–MRD+ 20 13 11 9 5 3
Months
C
Cumulative Incidence of Relapse (%)
p = 0.024
Number at risk
KMT2A–PTD–/MFC–MRD– 96 69 53 39 30 19
KMT2A–PTD+/MFC–MRD– 14 6 1 1 1 1
KMT2A–PTD–/MFC–MRD+ 14 11 9 8 7 1
KMT2A–PTD+/MFC–MRD+ 20 13 11 9 5 3
Months
D
Non-Relapse Mortality (%)
p < 0.001
Number at risk
KMT2A–PTD–/MFC–MRD– 96 69 53 39 30 19
KMT2A–PTD+/MFC–MRD– 14 6 1 1 1 1
KMT2A–PTD–/MFC–MRD+ 14 11 9 8 7 1
KMT2A–PTD+/MFC–MRD+ 20 13 11 9 5 3
Months

Supplementary Materials

Transplantation procedures

Allo-HSCT was performed according to institutional protocols. Donors included matched sibling donors (MSD), matched unrelated donors (MUD), and haploidentical donors (Haplo). Conditioning regimens were selected according to donor type, patient characteristics, disease status, and other clinical considerations. Most patients received myeloablative busulfan-based conditioning regimens. The most commonly used conditioning regimen consisted of cytarabine, busulfan, cyclophosphamide, and simustine. Cytarabine was administered according to donor type: 2 g/m²/day on day −9 for MSD transplantation, 4 g/m²/day on days −10 to −9 for Haplo transplantation, and 2 g/m²/day on days −10 to −9 for MUD transplantation. Busulfan was administered at 3.2 mg/kg/day on days −8 to −6, cyclophosphamide at 1.8 g/m²/day on days −5 to −4, and simustine at 250 mg/m² on day −3. Rabbit ATG was administered at 2.5 mg/kg/day on days −5 to −2 to recipients of Haplo or MUD grafts but was not routinely used in MSD transplantation. Grafts consisted of peripheral blood stem cells alone or a combination of bone marrow and peripheral blood stem cells. GVHD prophylaxis consisted of cyclosporine, mycophenolate mofetil, and short-course methotrexate.

***FLT3* inhibitor use**

The use of *FLT3* inhibitors during chemotherapy was permitted but not mandatory and was determined after discussion between the treating physicians and patients. In patients with *FLT3*-ITD-positive AML, sorafenib or gilteritinib could be administered during induction and/or consolidation chemotherapy before allo-HSCT. After transplantation, prophylactic *FLT3* inhibitor maintenance was considered following hematopoietic recovery, generally between days 31 and 60 after allo-HSCT. Sorafenib or gilteritinib could be used for maintenance. The choice of agent, dose, initiation time, and treatment duration was individualized according to clinical status, adverse events, and treatment tolerance. *FLT3* inhibitors could also be administered as preemptive treatment after molecular relapse. Information on *FLT3* inhibitor exposure was retrospectively collected from medical records.

Donor lymphocyte infusion

Donor lymphocyte infusion (DLI) was administered according to previously described institutional protocols. Indications for DLI included hematologic relapse treated with chemotherapy followed by DLI, persistent or recurrent molecular disease in the absence of active GVHD, and graft failure.

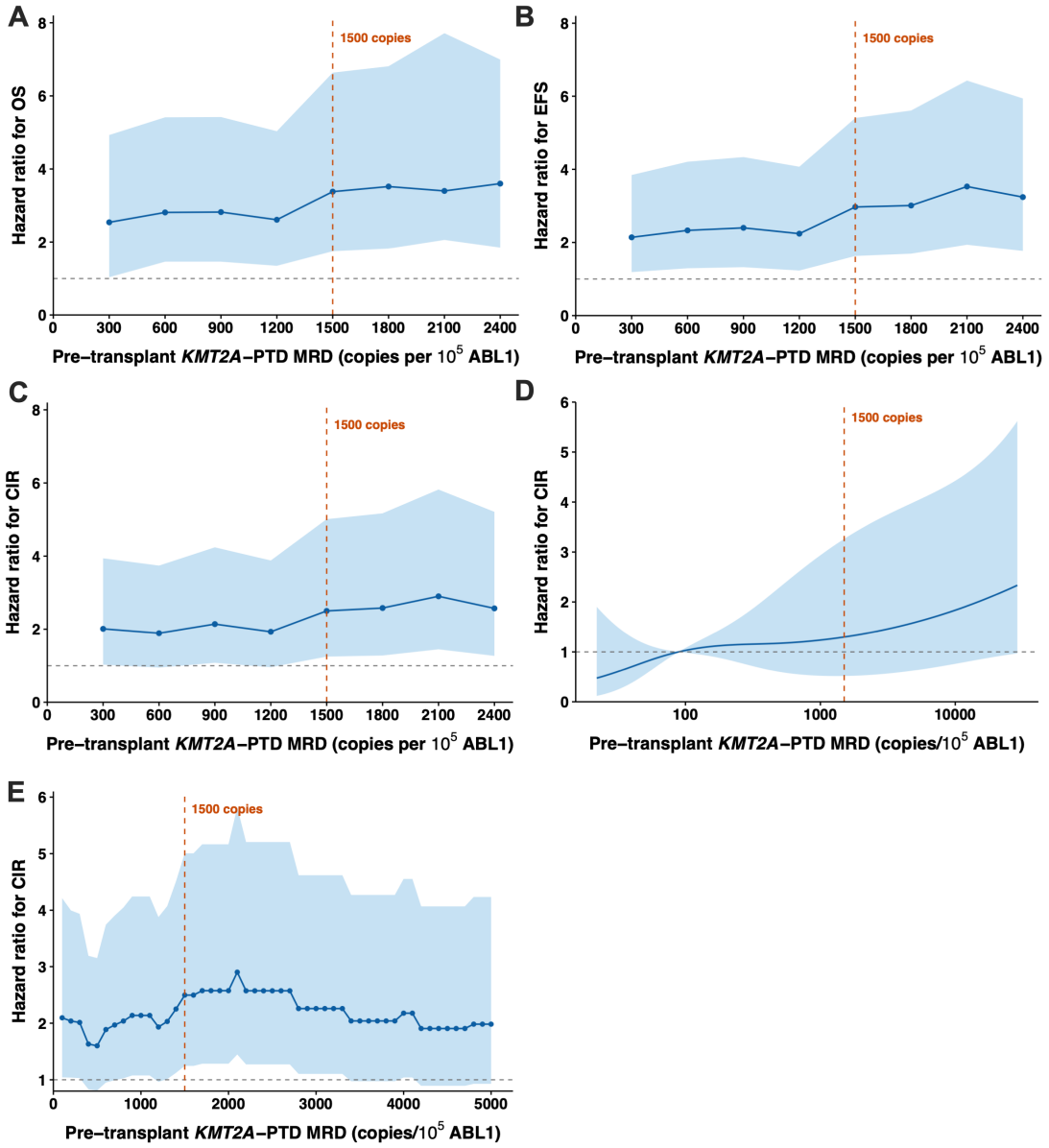

Figure S1. Statistical evaluation of the clinically meaningful threshold for pre-transplant *KMT2A*-PTD MRD. (A–C) Hazard ratio–based cutoff exploration analyses for overall survival (OS), event-free survival (EFS), and cumulative incidence of relapse (CIR) across a range of candidate MRD thresholds. (D) Restricted cubic spline analysis based on CIR. (E) Maxstat analysis based on CIR. Shaded areas represent 95% confidence intervals. The dashed vertical line indicates the selected threshold of 1500 copies/10^5 ABL1.

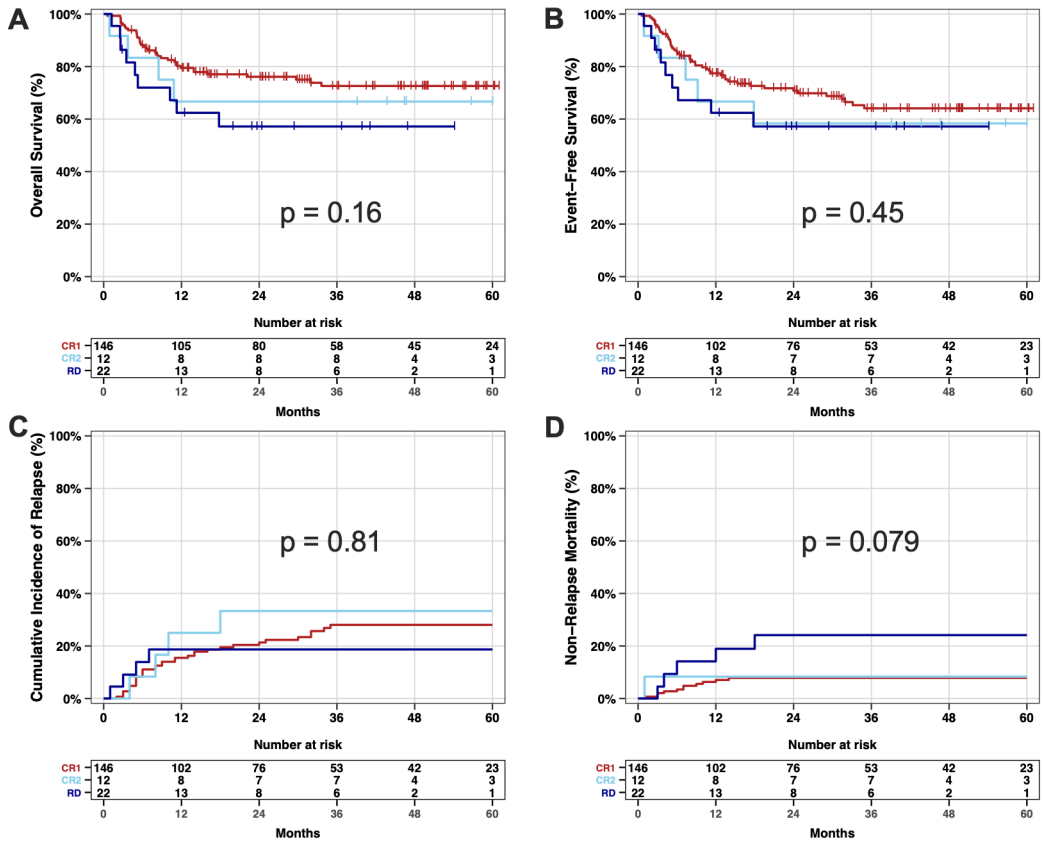

Figure S2. Post-transplant outcomes according to pre-transplant remission status (CR1, CR2, and RD). CR1, first complete remission; CR2, second complete remission; RD, refractory disease. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

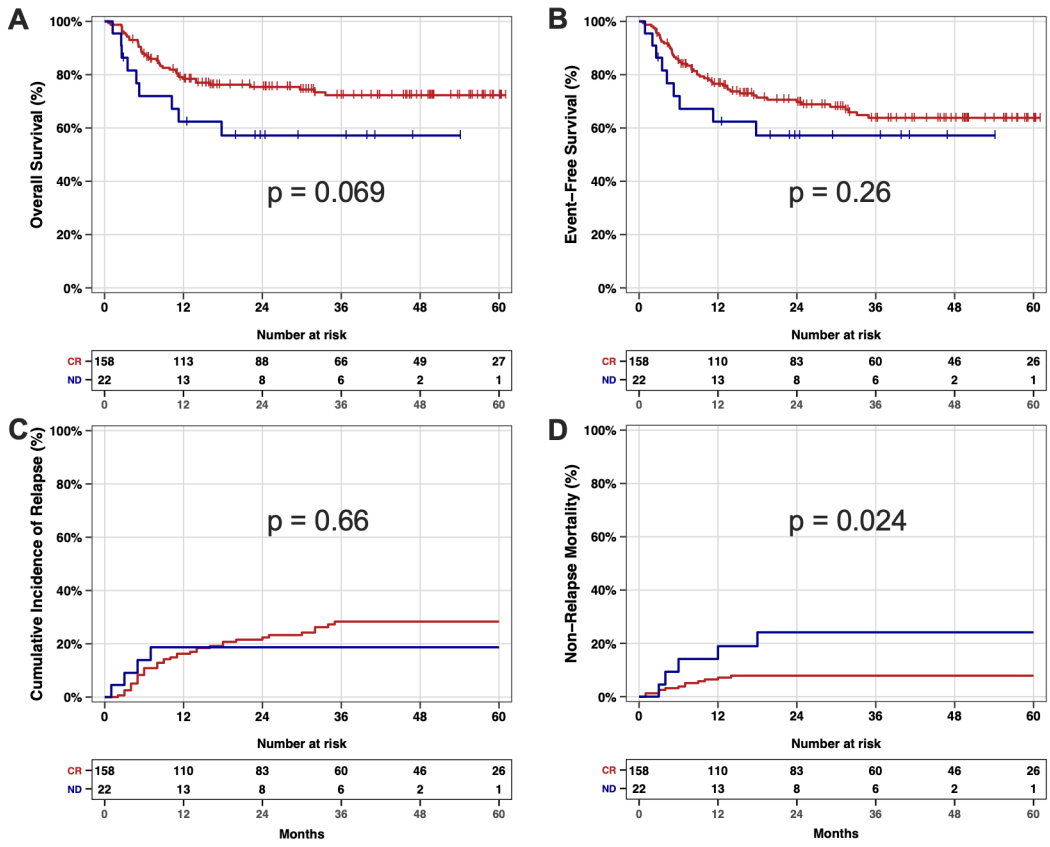

Figure S3. Post-transplant outcomes comparing patients transplanted in CR versus RD. CR, complete remission; RD, refractory disease. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

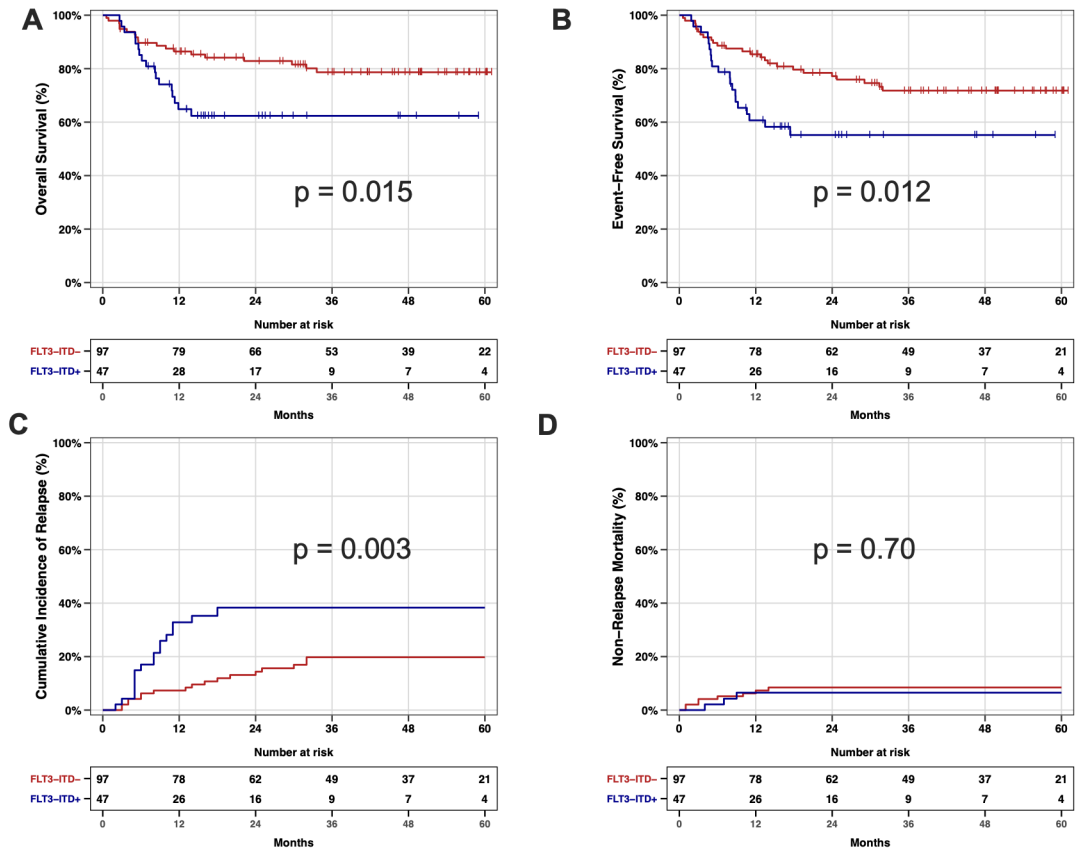

Figure S4. Post-transplant outcomes according to *FLT3*-ITD status. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

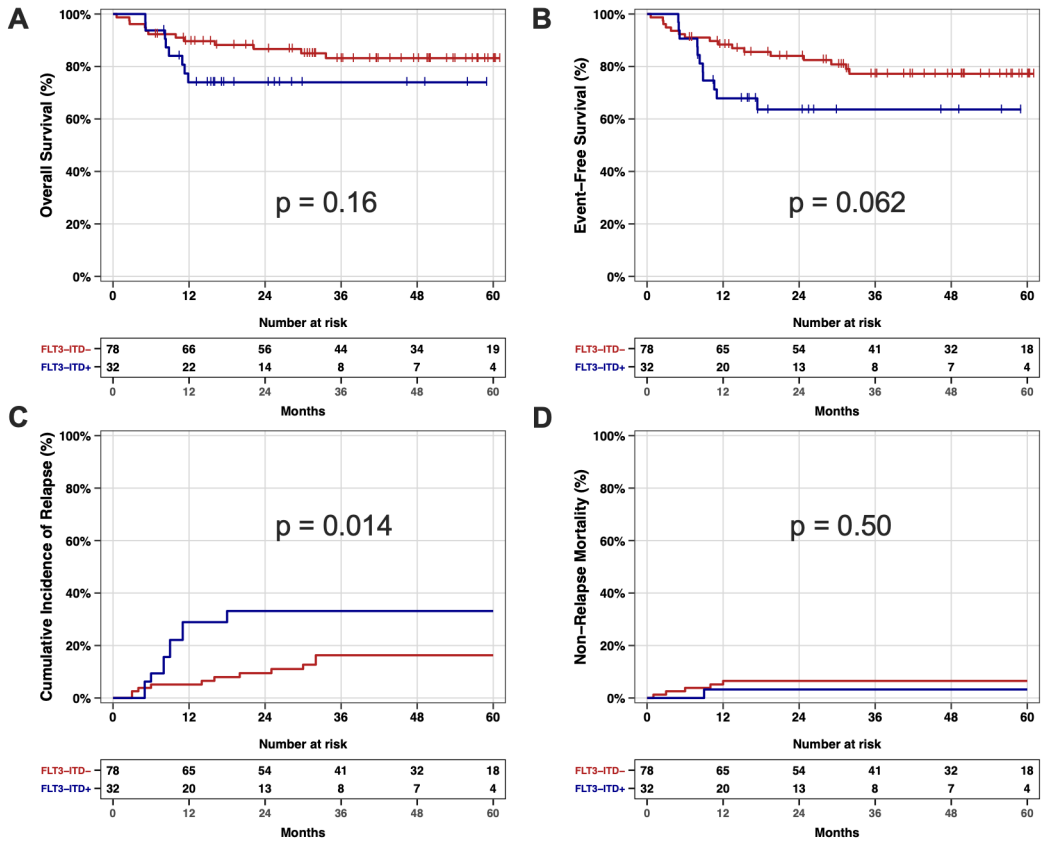

Figure S5. Post-transplant outcomes among *KMT2A*-PTD MRD-negative patients stratified by *FLT3*-ITD status. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

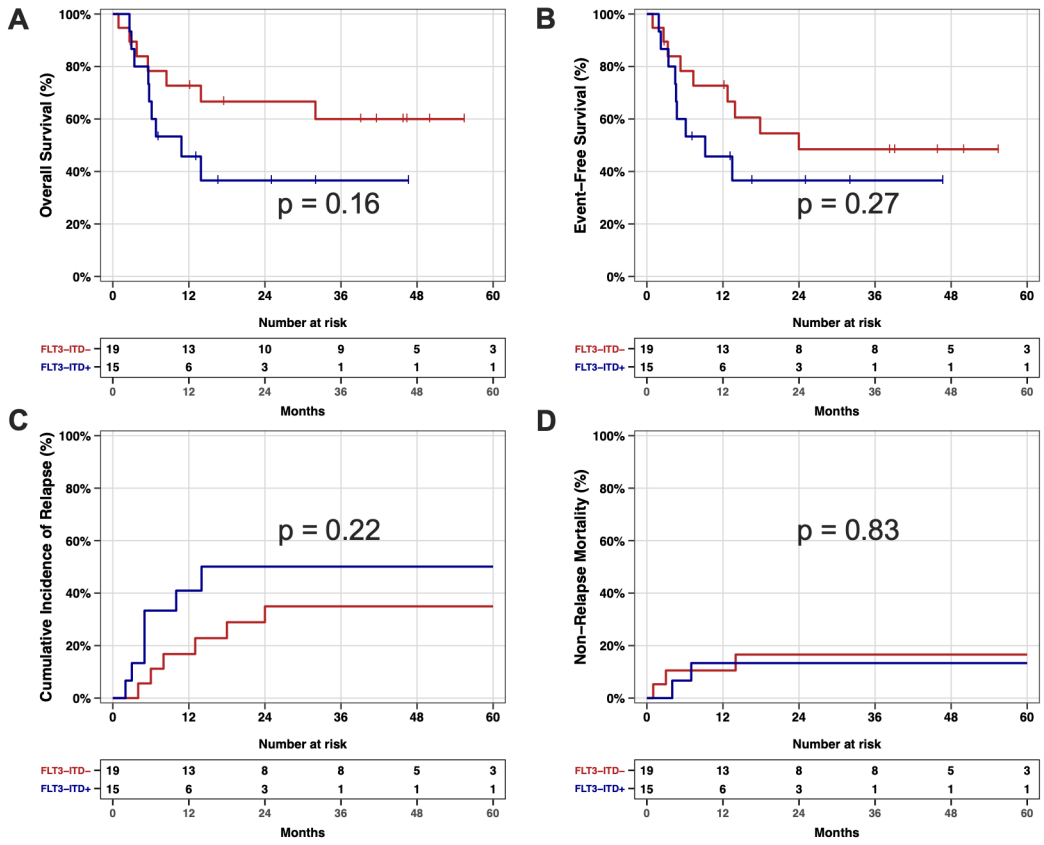

Figure S6. Post-transplant outcomes among *KMT2A*-PTD MRD-positive patients stratified by *FLT3*-ITD status. (A) Overall survival (OS); (B) Event-free survival (EFS); (C) Cumulative incidence of relapse (CIR); (D) Non-relapse mortality (NRM).

Table S1. Univariable and multivariable sensitivity analyses of pre-transplant *KMT2A*-PTD MRD using the 2022 ELN risk classification

Univariable analysis								
	OS		EFS		CIR		NRM	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
2022ELN, Adverse vs. Intermediate	0.762 (0.30-1.92)	0.57	0.69 (0.30-1.57)	0.38	0.69 (0.29-1.67)	0.42	0.73 (0.11-5.10)	0.75
Multivariable analysis								
2022ELN, Adverse vs. Intermediate	0.70 (0.28-1.77)	0.45	0.63 (0.28-1.43)	0.27	0.65 (0.26-1.61)	0.35	0.72 (0.10-5.05)	0.74
Pre-HSCT *KMT2A*-PTD MRD, positive vs. negative	2.63 (1.03-6.71)	0.043	2.69 (1.17-6.17)	0.02	2.31 (0.90-5.92)	0.081	2.78 (0.41-18.84)	0.29

Abbreviations: OS, overall survival; EFS, event-free survival; CIR, cumulative incidence of relapse; NRM, non-relapse mortality; HR, hazard ratio; CI, confidence interval; MRD, measurable residual disease; ELN, European LeukemiaNet.

Table S2. Baseline characteristics according to combined pre-transplant *KMT2A*-PTD MRD and MFC-MRD status

Combined MRD status prior to allogeneic HSCT	*KMT2A*-PTD-/MFC-MRD- (n=96)			*KMT2A*-PTD+/MFC-MRD- (n=14)			*KMT2A*-PTD-/MFC-MRD+ (n=14)			*KMT2A*-PTD+/MFC-MRD+ (n=20)			p-value
	No.		%	No.		%	No.		%	No.		%	
Median age (at time of HSCT; years) Range		44 16-71			43 21-59			43 17-64			40 17-67		0.64
Age category ≤50 years >50 years	61 35		64 36	9 5		64 36	10 4		71 29	16 4		80 20	0.54
Gender Male Female	47 49		49 51	9 5		64 36	10 4		71 29	6 14		70 30	0.075
Type of AML de novo secondary	86 10		90 10	12 2		86 14	10 4		71 29	17 3		85 15	0.31
WBC count, $x10^9$/L Median Range		4.2 0.7-176.9			9.7 1.6-130.3			2.4 0.8-29.6			6.9 1.1-156.9		0.07
Platelet count, $x10^9$/L Median Range		61 9-717			79 8-269			70 22-172			48 2-1405		0.84
Percentage of BM blasts, Median Range		50 13-93			60 24-84			31 22-75			55 13-95		0.12
2017 ELN risk classification Intermediate Adverse	48 48		50 50	4 10		29 71	10 4		71 29	8 12		40 60	0.12

FLT3-ITD mutated	31		32	7		50	1		7	8		40	0.089
Donor type													
MSD	21		22	1		7	1		7	5		25	0.27
Haplo	71		74	11		79	13		93	14		70	
MUD	4		4	2		14	0		0	1		5	
Prior chemotherapy cycles		3 1-11			3 2-5			4 2-10			3 1-7		0.52
Donor gender													
Male	62		65	11		79	7		50	10		50	0.27
Female	34		35	3		21	7		50	10		50	
Stem cell source													
PB	59		62	11		79	7		50	16		80	0.18
BM+PB	37		38	3		21	7		50	4		20	
Major ABO incompatibility	32		33	7		50	6		43	7		35	0.62

Table S3. Causes of death according to combined pre-transplant *KMT2A*-PTD MRD and MFC-MRD status

Combined MRD group	Total deaths	Death after relapse	Non-relapse deaths and causes
KMT2A-PTD−/MFC-MRD−	19	13	6: pulmonary infection (n=3), central nervous system and pulmonary infections (n=1), pulmonary tuberculosis (n=1), viral encephalitis with pulmonary infection (n=1)
KMT2A-PTD+/MFC-MRD−	8	3	5: pulmonary infection (n=3), gastrointestinal GVHD (n=1), sepsis due to Klebsiella pneumoniae (n=1)
KMT2A-PTD−/MFC-MRD+	3	3	0
KMT2A-PTD+/MFC-MRD+	9	9	0

Abbreviations: MRD, measurable residual disease; MFC, multiparameter flow cytometry; GVHD, graft-versus-host disease