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Peripheral Blood Tumor Associated Cell-Free DNA Testing as a Predictor for Relapse Postallogeneic Stem Cell Transplant for Acute Myelogenous Leukemia

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A B S T R A C T

Allogeneic stem cell transplantation (allo-SCT) is a curative option for acute myelogenous leukemia (AML), but relapse is a challenge. Monitoring minimal residual disease post-transplant through detection of tumor-associated circulating cell-free DNA (TA-cfDNA) in peripheral blood (PB) and bone marrow is an emerging strategy to predict relapse. Persistent mutations in TA-cfDNA may be prognostic indicators of relapse and mortality. This single-center retrospective study included 90 AML patients who received allo-SCT from 2018 to 2022, with PB TA-cfDNA tested between Day 100 and 200 after transplantation. TA-cfDNA positivity was determined using commercial genomic sequencing assays reporting tumor-associated genomic alterations with variant allele frequency above 0.01%, and TA-cfDNA negativity was considered the absence of these genomic alterations. The primary endpoints were the association of any TA-cfDNA presence with overall survival (OS) and relapse-free survival (RFS). A secondary endpoint was the association of specific mutation risk (adverse versus intermediate) with OS and RFS. Kaplan–Meier analysis revealed that patients positive for PB TA-cfDNA at Day 150 ± 50 had significantly worse OS (hazard ratio [HR] 5.4 with 95% confidence interval [CI] 2.5 to 11.8, $P < .0001$) and RFS (HR 5.2, 95% CI 2.4 to 11.3, $P < .0001$) compared to TA-cfDNA negative. Regarding mutation risk, adverse mutations at Day 150 ± 50 were linked to worse OS (HR 11.2, 95% CI 3.5 to 37.2, $P < .0001$) and RFS (HR 11.6, 95% CI 3.8 to 36.2, $P < .0001$), compared to TA-cfDNA negative. This study demonstrates that TA-cfDNA detection in PB post-allo-hematopoietic stem cell transplantation is strongly associated with increased relapse and mortality in AML patients. Persistent high-risk mutations correlate with increased risk of relapse and poor survival outcomes. These findings highlight the potential of PB TA-cfDNA as a predictive marker, potentially enabling earlier intervention to alter post-transplant treatment strategies.

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INTRODUCTION

Allogeneic stem cell transplantation is a curative option for acute myelogenous leukemia (AML), but relapse remains a challenge. The risk of relapse is influenced by a range of patient-related, disease-specific, and treatment-associated factors. Among the disease-related factors, the detection of adverse-risk mutations at the time of transplantation is associated with worse prognosis [1]. Genomic testing performed at the initial diagnosis of AML plays a critical role in stratifying patients for targeted therapies, predicting treatment response, and identifying those at elevated risk of relapse. Genomic classifications of AML have evolved over time, with the World Health Organization classification and European LeukemiaNet (ELN) regularly updating their classifications to guide diagnosis, risk stratification, and treatment selection [2–5]

The AML ELN gene classification provides a standardized framework for stratifying AML patients based on genetic abnormalities [4]. By stratifying patients into specific risk categories of favorable, intermediate, and adverse risk based on patients identified genetic abnormalities, the classification allows clinicians to personalize therapeutic strategies. The current 2022 ELN criteria includes a wide range of myelodysplasia-related gene mutations, including TP53, ASXL1, and RUNX1, and represents the advancement of genetic research and sequencing methods in finding key molecular mutations that are known drivers in AML [4].

Continuing genomic assessment after transplantation is emerging as a valuable tool for monitoring measurable residual disease (MRD). Identifying MRD using highly sensitive testing techniques may help select patients who can benefit from post-transplantation treatment modifications [1,6,7]. Notably, the detection of tumor-associated circulating cell-free DNA (TA-cfDNA) from peripheral blood (PB) is gaining interest as a non-invasive alternative. We previously demonstrated in a prospective study of 20 subjects that clinically relevant TA-cfDNA can be detected as early as Day 28 after transplantation [8]. This method allows for more frequent monitoring and avoids the limitations of bone marrow biopsies, which are invasive, may not be tolerated by older, more frail patients, and are subject to sampling error due to patchy disease involvement [9].

A growing amount of research is now examining different techniques of assessing MRD status and their ability to predict clinical outcomes [10,11]. Our study hypothesizes that the detection of MRD using PB TA-cfDNA analysis is associated with worse overall outcomes in AML patients and may serve as an early predictor for relapse after hematopoietic stem cell transplantation (HSCT). We propose that TA-cfDNA-based MRD monitoring could function as a valuable prognostic tool, ultimately guiding refinements in relapse prediction and models and informing more personalized post-HSCT management strategies.

METHODS

This retrospective study was conducted at a single institution, Hackensack University Medical Center, and focused on adult patients who underwent their first allogeneic HSCT for primary AML, transplanted between January 2018 and December 2022, allowing for up to 2-yr follow-up at the time of analysis. Eligible patients were aged 18 yr or older at the time of transplant and had a confirmed diagnosis of primary AML for which first allo-HCT was done. Patients were excluded if they were younger than the age of 18 yr at the age of transplant, had secondary AML, had previously undergone an allogeneic transplant, did not achieve remission before conditioning therapy, if they did not survive to Day 100, were in relapse at the time of testing, or if testing of TA-cfDNA at the time Day 100 to 200 was not performed. Institutional Review Board (IRB) approval was obtained from Hackensack Meridian Health. The requirement for patient-informed consent was waived by IRB as this project represented a non-interventional study utilizing routinely collected data for secondary research purposes.

We collected data on patient age at the time of transplant, ABO compatibility, Rh matching, donor and recipient CMV status, donor type, source of HSC, conditioning regimen, graft versus host disease (GvHD) prophylaxis regimen, prescence of GvHD, and the use of rabbit antithymocyte globulin (rATG) and abatacept. Conditioning regimens were classified as myeloablative, non-myeloablative, or reduced intensity. Further details regarding conditioning and post-transplant GvHD treatment are detailed in Supplementary Table 1.

The day of allogeneic stem cell transplantation infusion was considered Day 0. Cytogenetic and NGS analyses were performed on PB samples at the discretion of the treating physicians. Bone marrow sampling for MRD was also performed at the discretion of the treating physician. Due to the fact that this testing was at the discretion of the treating physician, the timing of collection for PB TA-cfDNA was not driven by a time-specific protocol. Thus, we grouped the results based on

frequency of PB TA-cfDNA collection, finding that the majority of our studied patients had PB TA-cfDNA collected between Day 100 and 200 post-transplant; therefore, the Day 150 ± 50 was chosen as the main reference timepoint in order to maximize the power of our study population.

Pre- and post-transplant molecular profiling was routinely performed by Genomic Testing Cooperative (Lake Forest, California, USA), using commercially available panels (Liquid Trace and Hematology Profile Plus) [12]. These assays utilize NGS, Sanger sequencing, and fragment length analysis to identify molecular abnormalities in DNA of 302 genes (Supplementary Table 4) associated with many hematologic neoplasms. We defined a TA-cfDNA-positive result as the presence of amplifications, deletions, single nucleotide variants, or insertion/deletions, with variant allele frequency exceeding 0.01%. TA-cfDNA negativity was defined by the absence of these genomic alterations. We excluded genes noted as germline mutations, as they are not predictive of MRD status [13].

Mutations were classified into adverse risk, intermediate risk, and favorable risk, as defined by the 2022 ELN classification, at the pretransplant setting, and at the point of interest between Day 100 and 200. Although the ELN criteria identifies genetic abnormalities by cytogenetics as well, we focused on only the molecular abnormalities to retrospectively risk-stratify our patients. Favorable mutations were CEPBA, NPM1, and MYH11, and adverse risk mutations were ASXL1, BCOR, EZH2, GATA2, RUNX1, SF3B1, SRSF2, STAG2, TP53, U2AF1, ZRSR2. Any other mutation discovered on the report was considered an intermediate risk mutation (Supplementary Table 2B). Additionally, if the assay reported multiple mutations in different risk classes, we categorized the patient by the more unfavorable mutation they carried. Supplementary Table 3 demonstrates de-identified patients with respective TA-cfDNA at diagnosis and at Day 100 to 200.

Relapse was defined as the persistence or recurrence of disease meeting the standard definition of relapse, including presence of >5% blasts in bone marrow or PB sample, and requiring the initiation of therapy or donor infusion lymphocytes (DLI). Decisions regarding post-transplant consolidation therapy, such as adjustments to immunosuppressive therapy, DLI infusions, or other interventions, were made at the discretion of the treating physician.

The primary objective was to evaluate the relationship between TA-cfDNA status and clinical outcomes, specific overall survival (OS) and relapse-free survival (RFS). RFS was defined as the time from bone marrow transplantation to the date of documented relapse or non-relapse death. Kaplan–Meier type OS and RFS were plotted using GraphPad PRISM software. Differences in survival were calculated by log-rank (Mantel-Cox) testing. Hazard ratios (HRs) were calculated by Mantel-Haenszel testing. Cox regression was used for multivariate analysis of OS and RFS. Statistical significance was set at a two-sided P value of ≤.05. Analyses were conducted in R (The R Project for Statistical Computing, http://www.r-project.org) and GraphPad Prism (GraphPad Software, San Diego, CA, USA).

RESULTS

Patient Characteristics

The study population consists of 90 patients who underwent bone marrow (24.4%) or PB (75.6%) allo-HSCT, with donor types including familial HLA haploidentical (34.4%), familial HLA matched related (14.4%), and HLA matched or mismatched unrelated donors (51.2%). The median ages at diagnosis and at transplant were 55 yr (range 22 to 76), and 56 yr (22 to 77), respectively. The full patient characteristics are shown in Table 1.

42.4% (n = 38) of patients received myeloablative conditioning, 37.8% (n = 34) of patients received non-myeloablative conditioning, and 20% (n = 18) received reduced-intensity conditioning. The details of the pretransplant conditioning regimen are described in Table 1. Table 1 describes patients who received the different GvHD prophylaxis regimens, and the number of patients who received rATG and abatacept. At a median follow-up of 21.7 mo, 65.6% of patients (n = 59) were alive, and 35.6% (n = 32) had relapsed.

TA-cfDNA Results and HSCT Outcomes

Using multivariate Cox regression analysis, we evaluated the predictive value of sex, age at transplant, ABO compatibility, Rh match, CMV match, donor type, conditioning regimen, GvHD prophylaxis regimen, and the use of rATG and abatacept, on OS and RFS. Additionally, we evaluated the predictive value of mutation risk on OS and RFS. Multivariate analysis revealed that finding adverse-risk mutations at Day 150 ± 50, compared to negative mutation status, was significantly associated with 11.2-fold risk of worse OS (P < .0001) and 11.6-fold risk of worse RFS (P < .0001). Intermediate-risk mutations found at

Table 1
Demographic Characteristics of Study Population

Characteristics	Overall ($N = 90$)
Sex, *n* (%)	
Female	41 (45.5%)
Male	49 (54.4%)
Age at transplant, yr	
Mean (SD)	54 (13.7)
Median (range)	56 (22-77)
ABO donor-recipient category, *n* (%)	
Match	47 (52.2%)
Major	14 (15.6%)
Minor	23 (25.5%)
Bidirectional	6 (6.7%)
Rh donor-recipient category, *n* (%)	
Match	51 (56.7%)
Mismatch	39 (43.3%)
CMV match, *n* (%)	
D−/R−	27 (30.0%)
D−/R+	28 (31.1%)
D+/R−	3 (3.3%)
D+/R+	32 (35.6%)
Donor type, *n* (%)	
Haploidentical	31 (34.4%)
Related	13 (14.4%)
Unrelated	46 (51.2%)
HSCT tissue, *n* (%)	
Peripheral blood	68 (75.6%)
Bone marrow	22 (24.4%)
Conditioning regimen, *n* (%)	
Myeloablative	38 (42.2%)
Non-myeloablative	34 (37.8%)
Reduced	18 (20.0%)
GvHD regimen	
Tacrolimus/methotrexate	46 (51.1%)
Tacrolimus/mycophenolate mofetil/cyclophosphamide	40 (44.4%)
Cyclosporine/sirolimus/ mycophenolate mofetil	4 (4.5%)
rATG	
No	56 (62.2%)
Yes	34 (37.8%)
Abatacept	
No	74 (82.2%)
Yes	16 (17.8%)
Relapse	
No	58 (64.4%)
Yes	32 (35.6%)
Survival	
Alive	59 (65.6%)
Dead	31 (34.4%)

Shown are the number of patients and respective percentage of patients with each category.
D− indicates donor negative; D+, donor positive; R−, recipient negative; R+, recipient positive.

Day 150 ± 50, compared to negative mutation status, also correlated with worse OS with a 5.5-fold risk ($P < .002$), and 6.6-fold risk of worse RFS ($P < .001$). Notably, the HR differences between adverse risk and intermediate risk mutations demonstrates the significance of mutation class on outcomes.

Additionally, multivariate analysis revealed significant differences in RFS specifically for major ABO compatibility status, donor type, and GvHD regimen. It revealed significant differences in donor type on RFS, where haploidentical, unrelated-match, and unrelated-mismatch were associated with increased risk of relapse compared to related donor type, demonstrating consistency with literature that matched related donor type shows improved outcomes. Otherwise, multivariate analysis did not reveal significance in regards to differences in sex, age at transplant, Rh match, CMV match, conditioning regimen, or the use of rATG or abatacept. This is shown in Table 2.

In our analysis of comparing TA-cfDNA positivity versus negativity, and stratifying mutation risk in groups, we observed significant differences in OS and RFS amongst these groups, further shown in Figure 1 with Kaplan–Meier analysis. Overall, it revealed that patients positive for any PB TA-cfDNA tumor-associated mutations at Day 150 ± 50 had significantly worse OS (HR 5.4, 95% confidence interval 2.5 to 11.8, $P < .0001$) and RFS (HR 5.2, 95% confidence interval 2.4 to 11.37, $P < .0001$), compared to TA-cfDNA negative. In looking at the 3 yr time point on Figure 1A, our data demonstrates that patients who are TA-cfDNA positive at the time point of Day 150 ± 50 have about 45% probability of survival, compared to about 85% in the TA-cfDNA negative group at the same time point. The significant difference in survival benefit is seen throughout the remainder years post-transplant course as well, indicating that the earlier test predicts for stable remission without a high risk of late relapses. In terms of RFS (Figure 1B), we see a plateau at the 2 yr post-transplant mark, showing that TA-cfDNA positive status at the 2 yr mark has a 40% probability of RFS, versus about 85% probability of relapse-free survival in the TA-cfDNA negative group.

Adverse mutations found at Day 150 ± 50 were linked to especially worse OS ($P < .0001$, Figure 1C) and RFS ($P < .0001$, Figure 1D). In these figures, we more clearly see the difference in outcomes stratified by mutation risk. In Figure 1C, which compares OS, we see a plateau at the 2-yr mark, where harboring adverse risk mutations at Day 150 ± 50 has a 30% chance of survival,

Table 2
Multivariate Analysis of Overall Survival and Relapse-Free Survival Using Cox Regression

	Overall Survival			Relapse-Free Survival		
Variable	Hazard Ratio	95% CI	*P* Value	Hazard Ratio	95% CI	*P* Value
Sex: Female versus male	1.101	0.4391-2.798	.8377	1.774	0.6796-4.818	.2474
Age at transplant (yr)	1.033	0.9854-1.087	.1969	1.025	0.9775-1.080	.3255
ABO compatibility						
Minor versus match	1.369	0.5217-3.499	.5139	0.8205	0.3039-2.122	.6867
Major versus match	0.338	0.0571-1.566	.1901	0.1363	0.0173-0.758	**.0357**
Bidirectional versus match	2.968	0.2742-17.67	.2777	1.127	0.1210-5.862	.8992
Rh match versus mismatch	1.772	0.5717-5.321	.3093	1.154	0.4052-3.147	.7817
CMV match						
D−/R+ versus D−/R−	2.886	0.7286-11.92	.1330	1.817	0.4969-6.906.	.3693
D+/R+ versus D−/R−	2.168	0.5780-8.497	.2515	1.836	0.5246-6.635	.3420
D+/R− versus D−/R−	3.490	0.1421-39.02	.3450	6.312	0.2551-74.30.	**.1680**
Donor type						
Haplo versus related	8.077	0.6821-243.0	.1412	67.76	3.256-2823	**.0121**
Unrelated-match versus related	6.211	0.5292-196.8	.2104	116.8	3.666-5972	**.0105**
Unrelated-mismatch versus related	13.51	0.9436-434.7	.0799	178.9	7.019-8035	**.0031**
Conditioning regimen						
Non-myeloablative versus myeloablative	0.854	0.1330-5.008	.8631	1.080	0.2784-11.19	.5241
Reduced versus myeloablative	0.851	0.2219-3.247	.8129	1.058	0.2840-3.977	.9320
GvHD regimen						
Tacrolimus/mycophenolate mofetil/ cyclophosphamide versus tacrolimus/methotrexate	1.177	0.1311-10.70	.8811	0.071	0.0065-0.819	
Cyclosporine/sirolimus/mycophenolate mofetil versus tacrolimus/methotrexate	1.686	0.0375-51.23	.7680	0.0283	0.0005-1.14	.0576
rATG: Yes versus no	1.341	0.1102-18.56	.8188	0.0610	0.0034-1.365	.0658
Abatacept: Yes versus no	1.815	0.5661-5.381	.2921	2.692	0.8950-7.848	.0695
Mutation risk						
Adverse versus negative	**11.18**	**3.495-37.21**	**.0001**	**11.60**	**3.750-36.25**	**.0001**
Intermediate versus negative	**5.489**	**1.818-17.06**	**.0026**	**6.647**	**2.077-22.54**	**.0017**

Significant associations observed for mutation risk, with adverse and intermediate risk mutations showing worse OS and RFS.

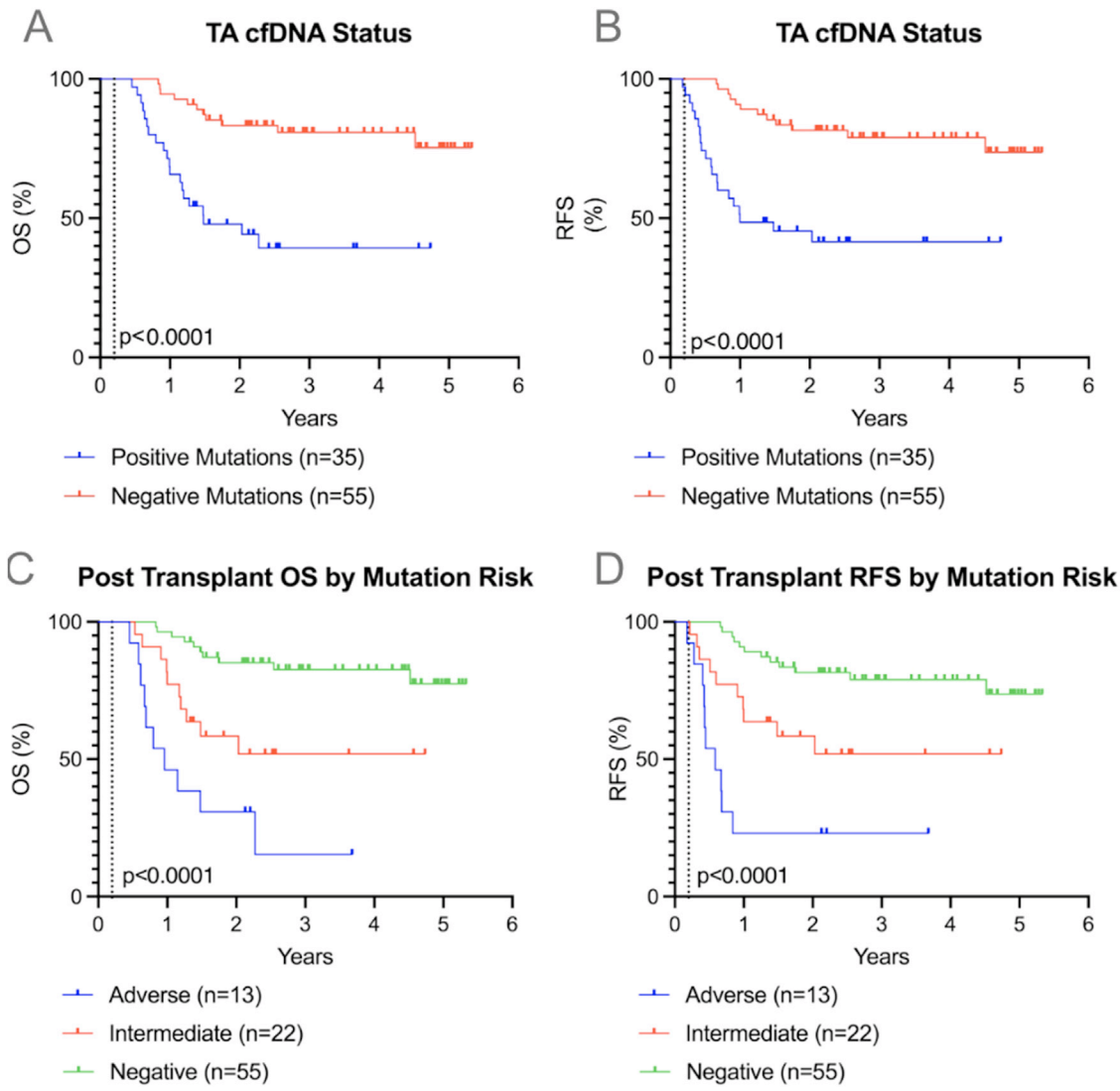

Figure 1. Kaplan–Meier curves comparing TA-cfDNA positive versus negative status at Day 150 ± 50 on OS and RFS (A and B), and Kaplan–Meier curves comparing mutation risk (adverse, intermediate, or negative) on OS and RFS (C and D). A dotted line is present at 0.2 yr, indicating the Day 100 mark of interest as to a start point of when PB TA-cfDNA was collected.

compared to intermediate risk mutations, which has a 60% chance of survival, and ultimately comparing to having no mutations has an 85% chance of survival by this time point. In Figure 1D, we see a dramatic decline in relapse-free survival for adverse risk mutation, where a plateau occurs at the 1-yr mark with about a 25% chance of relapse-free survival, versus 60% chance of RFS in intermediate risk, versus 90% chance if negative. This figure helps to delineate that if adverse risk mutations are found at Day 150 ± 50, the risk of relapse within the year is higher. These findings suggest that monitoring TA-cfDNA provides important insights into prognostication and may be an indicator to alter the post-transplant treatment plan to improve outcomes.

We also gathered data on the occurrence of acute GvHD. Our preliminary data show that 13 of the individuals with adverse risk mutation by Day 150 ± 50, 8 of them (61%), previously developed acute GvHD Grade II or higher. Of the 22 patients who had intermediate risk mutation by Day 150 ± 50, 10 (45%) had acute GvHD Grade II or higher. Of the 55 patients who cleared their mutations by Day 150 ± 50, 28 (50.9%) had acute GvHD Grade II or higher. We did not collect data on the presence or absence of chronic GvHD.

We also explored the dynamic nature of TA-cfDNA, comparing the pretransplant status to the post-transplant status at the same time-mark of Day 150 ± 50 by grouping patients via mutation risk to what they had transitioned to post-transplant (Figure 2A-F). In this analysis, we found that patients who started with adverse risk mutations pretransplant and remained with adverse risk mutations at Day 150 ± 50 had worse OS

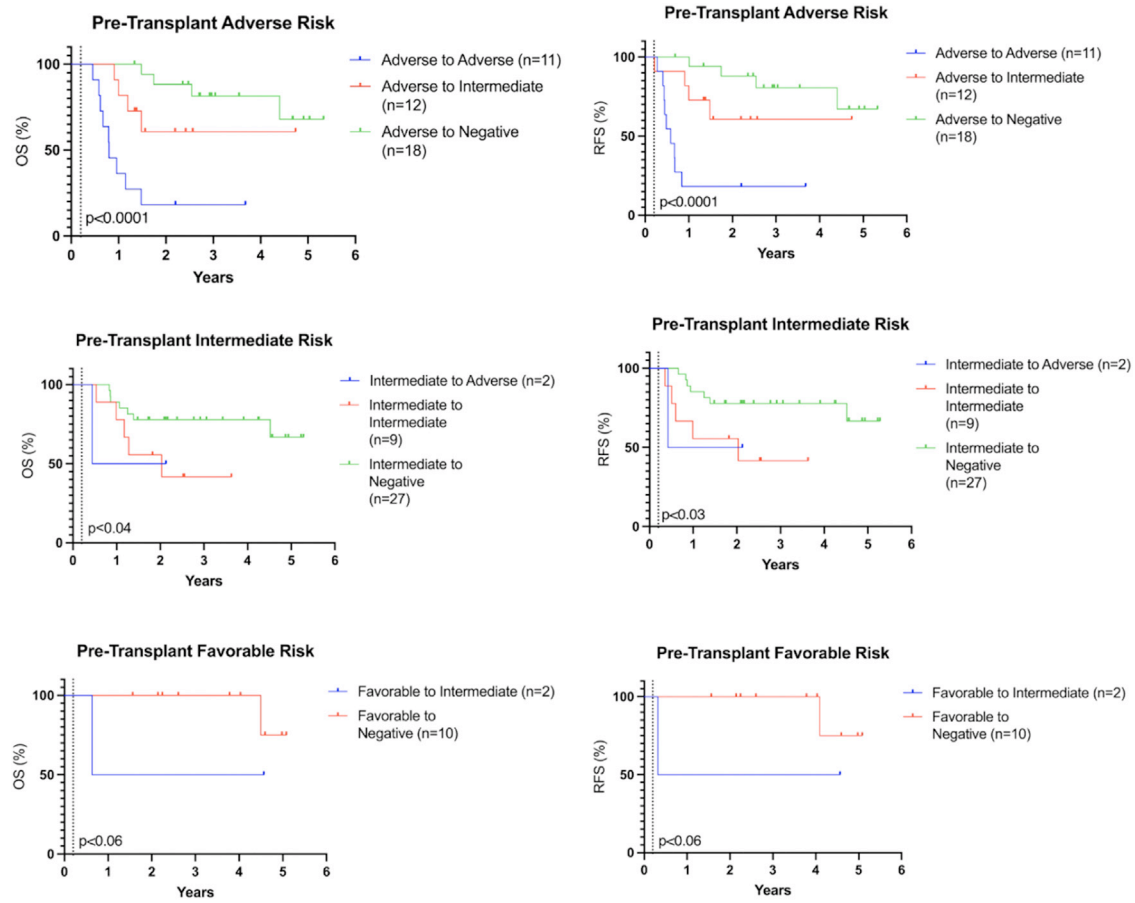

Figure 2. Kaplan–Meier curves comparing groups of patients from pretransplant mutation risk status to a post-transplant mutation risk status at Day 150 ± 50. A dotted line is present at 0.2 yr, indicating the Day 100 mark of interest as to a start point of when PB TA-cfDNA was collected. (A) Upper Left - OS of pre-transplant adverse risk mutation classification and the transition at Day 150 +/- 50 to adverse, intermediate, and negative risk mutations. (B) Upper Right - RFS of pre-transplant adverse risk classification and the transition at Day 150 +/- 50 to adverse, intermediate and negative mutations. (C) Middle Left - OS of pre-transplant intermediate risk mutation classification and the transition to Day 150 +/- 50 to respective mutation classification. (D) Middle Right - RFS of pre-transplant intermediate mutation classification and the transition to Day 150 +/- 50 to respective mutation classification. (E) Lower Left - OS of pre-transplant favorable risk mutation, and transition at Day 150 +/- 50 to respective mutation classification. (F) Lower Right - RFS of pre-transplant favorable risk mutation and the transition at Day 150 +/- 50 to respective mutation classification.

($P < .001$) and RFS ($P < .0001$), compared to individuals who "cleared" these mutations (TA-cfDNA negative). This was consistent with the other groups as well, noting worse OS and RFS in individuals who started with adverse risk mutations and then were found to have intermediate risk. Patients who started with intermediate risk mutations in the pretransplant setting, but were still found to have either adverse risk mutations, or intermediate risk mutations, had worse OS ($P < .04$) and RFS ($P < .03$), compared to negative. Lastly, patients who started with favorable mutations in the pretransplant setting but were found to have intermediate risk mutations at the time point had worse OS ($P < .06$) and RFS ($P < .06$) compared to those who transitioned from favorable to negative entirely—these data were nearly significant. In our study, we did not identify patients who obtained favorable mutations by the studied time point. Most individuals who had started with favorable risk mutations presumably cleared them after treatment and transplant, aside from two patients who started with favorable risk mutation but transitioned to having intermediate risk mutation at the studied time point.

DISCUSSION

Our study demonstrates a strong correlation between the presence of TA-cfDNA in PB post-allo-HSCT and an increased risk of relapse and mortality in patients with AML. The TA-cfDNA positivity status was determined at Day 150 ± 50, roughly corresponding to the 6-mo-mark. This point in time may serve as a critical window to

consider adjustments in post-transplant therapy, and also identifies those who may be on their way to achieving deeper remission. Our findings are consistent with previous research showing that persistent MRD after transplantation is linked to disease recurrence [8,14–19]. This study of 90 subjects builds off our smaller 20-subject prospective study of adverse-risk mutations that demonstrated that clinically relevant MRD detection using tumor-associated TA-cfDNA analysis could be performed as early as 28 days after transplantation [8]. Our data demonstrate that achieving TA-cfDNA negativity status, even for subjects with intermediate-risk genetic mutations, marks a significant survival benefit compared to any TA-cfDNA positive status. It shows that any mutation (adverse- or intermediate-risk) is evidence of MRD that predicts for relapse and is an actionable finding.

Although our data demonstrate this, there remains a small number of patients who did have a mortality event despite TA-cfDNA negativity. Out of the 12 patients who were presumed to be MRD negative but still had mortality events, 4 patients had a cause of death from primary disease or possibly from relapse, 3 patients died of GvHD-related complications, and 5 patients died from infectious etiology. Of the 4 patients who died from primary disease or the concern of relapse, 2 were found to have an intermediate risk mutation outside the time frame of our initial interest, one at Day 289, and one at Day 218. The other 2 patients did not have PB TA-cfDNA collected outside the Day 100 to 200 time frame. This demonstrates that planned serial testing after transplantation, even for patients who were initially MRD negative, is valuable to predict a risk of relapse should they become MRD positive.

We also considered the occurrence of acute GvHD, specifically with how more than 50% of individuals who “cleared” their mutations by Day 150 ± 50 had evidence of acute GvHD grade II or higher, underscoring the possibility of a graft-versus-leukemic (GvL) effect. However, individuals with both adverse risk and intermediate risk mutations at Day 150 ± 50 had similar rates of GvHD Grade II or higher, indicating a need for additional study regarding any potential GvL effect on the clearance of TA-cfDNA to assess its true statistical significance.

Additionally, our results demonstrate that the ongoing persistence of adverse risk mutations post-transplant correlates with increased risk of relapse and poor survival outcomes as well, extending to patients with the intermediate risk group as well. These outcomes may reflect the persistence, or lack of clearance, of specific leukemic subclones following transplantation. In cases where TA-cfDNA analysis identified somatic mutations that were not clearly high-risk and therefore intermediate, it is possible that the test picked up on clonal hematopoiesis of indeterminant potential (CHIP), which may have existed prior to AML onset, rather than definitive evidence of MRD. Distinguishing between CHIP and MRD remains a current diagnostic challenge. Further details about the mutation risk categorization is listed in Supplementary Table 3, where the pretransplant diagnostic NGS mutations are paired with post-transplant Day 100 to 200 NGS results for each de-identified patient. We see that the majority of patients who stayed in the same mutation class pre- and post-transplant grapple with the same mutations. Those that switch classes may have had the same mutations that were too low of variant allele frequency to be detected at initial diagnosis, but also may represent entirely different leukemic clones that arise after induction and consolidation therapy.

Distinguishing CHIP with actual MRD remains a current diagnostic challenge, especially since CHIP can potentially represent donor-transmitted mutations. Chimerism testing may mitigate this challenge, quantifying the proportion of donor and recipient cells across various hematopoietic lineages, giving that insight of donor versus recipient that TA-cfDNA testing lacks [8,20].

Overall, larger studies with a greater population of people, and possibly observing other myeloid malignancies, are needed to further define the significance of these non-adverse-risk somatic mutations after transplant, and also may lead into the discussion of donor-transmitted somatic mutations. This study is limited by a relatively small sample size, along with the heterogeneity of the donor type, conditioning regimen, GvHD prophylaxis regimen, and the variability in timing of post-transplant disease monitoring. In gathering data, we identified that the variability of timing in post-transplant varied because of the method of sampling. Generally, patients did have MRD testing at around Day 28, Day 56, and Day 84—but sample collection varied between testing BM samples and PB samples at the discretion of the treating physician. Additionally, the regularity of chimerism testing may have also influenced the timing of repeating TA-cfDNA analysis, for example, if a patient showed clearly decreasing CD3 chimerism, they receive post-

transplant interventions at the discretion of the treating physician regardless of TA-cfDNA positivity or negativity.

This study supports our hypothesis that detection of TA-cfDNA in PB samples obtained 100 to 200 days after transplantation identifies patients for whom modifications of the transplant treatment plan are required to reduce the risk of relapse. In conclusion, serial testing of TA-cfDNA is a way to monitor patients post-transplantation to search for MRD, and identifies patients at higher risk of relapse and mortality.

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Conflict of Interest Statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

ETHICAL APPROVAL AND PATIENT-INFORMED CONSENT

Institutional Review Board (IRB) approval for this study was obtained (Pro2023-0401). The study was conducted under the International Conference on Harmonization Good Clinical Practice Guidelines and according to the Declaration of Helsinki. The requirement for patient-informed consent (verbal or written) was waived by the IRB as this project represented a non-interventional study using routinely collected data for secondary research purposes.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2025.08.010.

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