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FLT3 mutational status is an independent risk factor for adverse outcomes after allogeneic transplantation in AML

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Abstract

Allogeneic HCT has been increasingly used in the setting of *FLT3* mutated AML. However, its role in conferring durable relapse-free intervals remains in question. Herein, we sought to investigate *FLT3* mutational status on transplant outcomes. We conducted a retrospective cohort study of 262 consecutive AML patients who underwent first-time allogeneic HCT (2008-2014), of whom 171 had undergone *FLT3*-ITD mutational testing. *FLT3* mutated AML was associated with

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AUTHORSHIP

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nearly twice the relapse risk (RR) compared with those without *FLT3* mutation 3 years post-HCT (63% vs. 37%, $P<0.001$), and with a shorter median time to relapse (100 vs. 121 days). *FLT3* mutational status remained significantly associated with this outcome after controlling for patient, disease, and transplant-related risk factors ($P<0.05$). Multivariate analysis showed a significant association of *FLT3* mutation with increased 3-year RR (HR 3.63, 95% CI: 2.13, 6.19, $P<0.001$), and inferior disease-free survival (HR 2.05, 95% CI: 1.29, 3.27, $P<0.01$) and overall survival (HR 1.92, 95% CI: 1.14, 3.24, $P<0.05$). These data demonstrate high risk of early relapse after allogeneic HCT for *FLT3* mutated AML that translates into adverse disease-free and overall survival outcomes. Additional targeted and coordinated interventions are needed to maintain durable remission after allogeneic HCT in this high-risk population.

Keywords

Acute myeloid leukemia; *FLT3* mutation; *FMS-like tyrosine kinase-3*; Allogeneic hematopoietic cell transplantation

INTRODUCTION

Acute myeloid leukemia (AML) is a genetically heterogeneous disease.[1] Acquired somatic mutations in the *FMS-like tyrosine kinase-3* (*FLT3*) gene occur in up to 20% to 30% of AML patients who carry the internal tandem duplication (ITD) mutation.[2-4] *FLT3*-ITD has been characterized as a gain-of-function mutation with constitutive activation of receptor tyrosine kinase FLT3.[5] This alteration has been associated with adverse prognosis in both pediatric and adult AML patients.[4, 6]

Allogeneic hematopoietic cell transplantation (HCT) is an important treatment option for patients with AML.[7] Unfortunately, disease recurrence and transplant-related toxicity remain the major causes of treatment failure.[8] Accordingly, the value of allogeneic HCT in conferring durable long-term remission free intervals continues to be an important consideration, particularly in patients with *FLT3* gene mutation.

FLT3 mutation as an independent risk factor for allogeneic transplant outcomes has previously been explored by several groups through single institution and multi-center registry studies, with inconsistent reports depending on the study population.[9-31] Unfortunately, many of these studies have been restricted to cytogenetically normal AML, small sample sizes, or specific conditioning or donor types, thereby limiting the generalizability of the findings. While allogeneic HCT with the best available donor has become widely adopted as an important therapeutic option in AML patients with *FLT3* mutation who achieve first complete remission (CR1), there may be additional patient, disease, or transplant-specific variables that increase relapse hazards.[8] Therefore, in the present study, we investigated the impact of *FLT3* mutational AML on relapse risk (RR), non-relapse mortality (NRM), disease-free survival (DFS), and overall survival outcomes following allogeneic HCT at a single institution between 2008 and 2014. The study design included a retrospective cohort analysis and detailed characterization of patient, disease, and transplant-specific factors by *FLT3* mutational status (positive *vs.* negative).

PATIENTS AND METHODS

Literature review

As the focus of this paper was on *FLT3* mutational AML in allogeneic HCT, we conducted a literature search in PubMed/MEDLINE. The search was performed in January 2015 and was restricted to studies published in English within the last 20 years (1995–2015). Three MeSH terms, 'transplantation,' '*FLT3*,' and 'acute myelogenous leukemia' were used in the search, in addition to various combinations of 'HCT,' 'FMS-like tyrosine kinase 3,' 'acute myeloid leukemia,' and 'AML.' The initial search resulted in a total of 300 articles. Two co-authors (YS and SWC) screened a random half set of the abstracts; 277 were determined as not meeting the inclusion criteria. Both authors read the full text of the remaining 23 papers. Each of these papers was reviewed in depth, with key outcomes summarized in Supplemental Table S1.

Study design

A retrospective cohort study was conducted on 262 pediatric and adult AML patients undergoing first-time allogeneic HCT between January 2008 and July 2014. The study was approved by the University of Michigan Institutional Review Board (IRBMED# HUM00095617). Informed consents were obtained from all subjects and data were collected under the IRBMED-approved protocol (IRBMED # HUM43287). Routine *FLT3* mutational testing for AML began in 2008 at the University of Michigan. Twenty-three patients who either had *FLT3*-TKD mutation without *FLT3*-ITD or who received umbilical cord blood transplantation were excluded from the study to reduce potential confounding. An additional 65 patients who did not undergo *FLT3* mutational testing were excluded from the analysis. Details of their patient, disease, and transplant-related characteristics and outcomes are provided in Supplemental Tables S2–S4. The total study population was 171 patients with known *FLT3* mutational status (positive *vs.* negative). Cytogenetic and molecular testing (*FLT3* and *NPM1*) was performed at the University of Michigan or at referring institutions.

Data abstraction of patient, disease, and transplant-related variables was performed through manual chart review of the Electronic Medical Record system (Careweb and MiChart/EPIC), supported by the University of Michigan Electronic Medical Record Search Engine (EMERSE). EMERSE is designed to comprehensively search all institutional clinical documents using specified search terms and queries.[32] Documents screened by EMERSE were examined in further detail for relevant study data. Outcomes of the study included RR, graft-versus-host disease (GVHD), NRM, DFS, and overall survival.

Patient, disease, and transplant-related characteristics

Patient characteristics included age, gender, race, ethnicity, and body mass index (BMI) at the time of allogeneic HCT. A hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score was calculated using documented clinical and laboratory data for each patient in the study, as previously described.[33] Disease features included white blood cell (WBC) count at diagnosis, Center for International Blood and Marrow Transplant Research (CIBMTR) cytogenetic risk based on karyotype at diagnosis (noting the presence or absence of complex cytogenetics [≥3 abnormalities]),[34] *NPM1* mutational status, antecedent

myelodysplastic syndrome (MDS) or myeloproliferative disorder, and therapy-related AML. Morphologic status at transplant, defined as persistent disease (≥5% blasts) *vs.* complete remission (<5% blasts), and cytogenetic remission status at transplant, defined as normal karyotype without clonal abnormalities, were also collected. Time from diagnosis to transplantation (infusion of allogeneic hematopoietic cells) was recorded (≥ *vs.* <180 days).

Transplant-related characteristics included number of pre-transplant chemotherapy cycles (induction and consolidation), conditioning intensity (myeloablative or reduced), use of thymoglobulin or alemtuzumab, total body irradiation (≥1200 cGy), stem cell source (bone marrow or peripheral blood), donor-recipient characteristics (gender, related or unrelated, human leukocyte antigen [HLA] matched [8/8] or mismatched [7/8], ABO and Rh blood type, and cytomegalovirus status), number of CD34+ cells transfused, and length of hospital stay. Additional characteristics included GVHD prophylaxis (calcineurin inhibitor [CNI]-methotrexate or CNI-mycophenolate mofetil) and time to neutrophil engraftment (defined as the first of three consecutive days with absolute neutrophil count [ANC] ≥500/mm^3).

Statistical analysis

Statistical analyses were performed using R 3.02 (GNU General Public License) with α=0.05 defining the level of statistical significance (two-sided). Summary data were calculated for patient, disease, and transplant-related variables, with medians and ranges determined for continuous variables and counts and percentages calculated for categorical variables. The cohort was then sub-divided into two groups based on their *FLT3* mutational status, and statistically significant differences between these groups were assessed using the Kruskal-Wallis test for continuous variables and the χ^2 test of association for categorical variables. The Fine-Gray method[35] was used to determine cumulative incidences with competing risks, which were then compared using the K-sample tests described by Gray.[36] The Kaplan-Meier method was used to compute overall survival.[37] Univariate regression methods (competing risks regression for RR, acute and chronic GVHD and NRM, and Cox regression for DFS and overall survival) were used to model the marginal associations of *FLT3* mutational status and other patient, disease, and transplant-related variables with clinical outcomes. Bivariate models were used to further determine the joint association of *FLT3* mutation and key variables with outcomes. Because complex cytogenetic changes are used in CIBMTR cytogenetic risk determination, this variable was excluded from bivariate and multivariate modeling. Morphologic status (persistent disease *vs.* CR) was also included in modeling because refractory disease status at the time of allogeneic HCT has previously been shown to be an independent poor prognostic factor.[27] Multivariate regression models using backward selection were used to identify best-fitting models for outcomes containing *FLT3* mutational status and other possible confounders identified in descriptive characteristics comparison and univariate and bivariate testing.

RESULTS

Characteristics by FLT3 mutational status

A total of 171 consecutive AML patients with available *FLT3* mutational testing received first-time allogeneic HCT. The median age of the study population was 55 years (range,

1−72 years). Age, gender, race, BMI, and HCT-CI distributions were similar in patient groups with *vs.* without *FLT3* mutation. The remaining patient and disease characteristics are detailed in Table 1.

The groups were also similar in morphologic status at the time of HCT (persistent disease *vs.* CR), time from diagnosis to HCT (>180 days *vs.* ≤180), and number of induction (>2 *vs.* ≤2) and combined induction and consolidation chemotherapy cycles leading to HCT (median of 3 cycles for both groups). Significantly more *FLT3* positive than *FLT3* negative patients were in cytogenetic remission at the time of HCT (94% vs. 71%, $P<0.01$). As expected, the *FLT3* mutated group had higher WBC counts (≥10,000/μL) at the time of diagnosis (70% *vs.* 36%, $P<0.001$), predominantly intermediate-risk CIBMTR cytogenetics with fewer instances of complex cytogenetics at diagnosis (0% *vs.* 21%, $P<0.001$), and higher co-occurrence with *NPM1* mutation (38% *vs.* 11%, $P<0.001$). *FLT3* mutation was less often observed in cases of preceding MDS or myeloproliferative disease (8% *vs.* 26%, $P<0.01$) and therapy-related AML (0% *vs.* 9%, $P<0.05$).

Transplant-related characteristics were also similar between the groups, including stem cell source, donor-recipient characteristics, conditioning intensity, use of thymoglobulin or alemtuzumab, total body irradiation, number of CD34+ cells transfused, length of hospital stay, GVHD prophylaxis, and time to neutrophil engraftment (Table 2).

Engraftment and neutrophil recovery

Granulocyte colony-stimulating factor was started six days after transplantation to promote neutrophil engraftment. The majority of surviving patients (>99%) engrafted within 50 days of allogeneic HCT (Table 2). There was one case of graft failure among patients without *FLT3* mutation.

Risk of relapse

Figure 1 illustrates the 3-year cumulative incidence outcomes of the patient cohort following allogeneic HCT, stratified by *FLT3* mutational status. Patients with *FLT3* mutation experienced shorter median time to relapse (100 days, range: 25−495 days) compared with those without *FLT3* mutation (121 days, range: 26−1,142 days). The risk of relapse at 3 years was significantly higher in *FLT3* mutated patients (63% *vs.* 37%, $P<0.001$). Among the other variables tested, high HCT-CI, high CIBMTR cytogenetic risk, and complex cytogenetics were also significantly associated with increased RR at 3 years (Table 3). We also performed bivariate analyses to explore the individual interactions of *FLT3* mutation with age, HCT-CI, CIBMTR cytogenetic risk, *NPM1* status, number of induction chemotherapy cycles, morphologic status, and conditioning intensity, and found that *FLT3* mutation remained a significant risk factor for relapse even after accounting for these other variables (Table 4). In the multivariate analysis, *FLT3* mutation (hazard ratio [HR] 3.63, 95% confidence interval [CI]: 2.13, 6.19, $P<0.001$), high HCT-CI (HR 1.71, 95% CI: 1.04, 2.79, $P<0.05$), high CIBMTR cytogenetic risk (HR 2.97, 95% CI: 1.52, 5.77, $P=0.001$), and persistent morphologic disease at transplant (HR 2.61, 95% CI: 1.44, 4.74, $P<0.01$) variables were significantly associated with increased RR. Myeloablative conditioning (HR 0.39, 95% CI: 0.21, 0.72, $P<0.01$) was associated with decreased RR (Table 5).

GVHD (Acute and Chronic)

The median time to onset of grade 2−4 acute GVHD in the study population was 36 days (range: 10−180 days) and the cumulative incidence at one year after HCT was 33%, which was similar in patients with *vs.* without *FLT3* mutation (30% *vs.* 34%, respectively, P=0.715). None of the other variables tested in the univariate analysis was significantly associated with 1-year acute GVHD outcomes (Table 3).

We also assessed the impact of *FLT3* mutational status on chronic GVHD. The median onset of chronic GVHD in the study population was 161 days (range: 52−580 days) with a cumulative incidence of 49% at one year following HCT, which was significantly lower among patients with *vs.* without *FLT3* mutation (36% *vs.* 54%, P<0.05). WBC count ≥10,000/μL at diagnosis was also associated with decreased risk of chronic GVHD (41% *vs.* 54%, P<0.05). In the bivariate analysis, *FLT3* mutation was associated with significantly lower incidence of chronic GVHD, even after adjusting for age, HCT-CI score, CIBMTR cytogenetic risk, number of induction cycles, morphologic status, and conditioning intensity (Table 4). Additionally, after incorporating all of these characteristics in the multivariate model, *FLT3* mutation remained the only variable significantly associated with decreased risk of chronic GVHD (HR 0.44, 95% CI: 0.25, 0.78, P<0.01, Table 5).

Non-relapse Mortality

NRM at 3 years was significantly lower in patients with *FLT3* mutation (4% *vs.* 21%, respectively, P<0.05, Figure 1). While time from AML diagnosis to HCT (>180 days *vs.* ≤180 days) did not impact the risk of NRM, patients who received >2 induction chemotherapy cycles experienced higher NRM (32% *vs.* 12%, respectively, P<0.05; Table 3). On bivariate testing, *FLT3* mutation remained significantly associated with decreased NRM after adjusting for age, HCT-CI, CIBMTR cytogenetic risk, *NPM1* status, number of induction cycles, morphologic status, and conditioning intensity (Table 4). This finding was also seen in the multivariate analysis (HR 0.21, 95% CI: 0.05, 0.92, P<0.05).

Disease-free Survival

FLT3 mutational status was associated with a trend toward inferior DFS at 3 years (*FLT3* mutation: 32% *vs.* no *FLT3* mutation: 41%, P=0.065, Figure 1). CIBMTR cytogenetic risk (high: 13% *vs.* intermediate: 45% *vs.* low: 100%, P=0.001), complex cytogenetics (≥3 abnormalities at diagnosis: 11% *vs.* <3 abnormalities: 44%, P<0.001), and number of induction cycles (>2 inductions: 15% *vs.* ≤2 inductions: 46%, P<0.01) variables at diagnosis were associated with significantly decreased DFS (Table 3). In the bivariate models, *FLT3* mutation was shown to negatively impact DFS when adjusting for CIBMTR cytogenetic risk and *NPM1* status variables only (Table 4). However, in the multivariate model that accounted for the interaction of multiple potential confounders, *FLT3* mutation (HR 2.05, 95% CI: 1.29, 3.27, P<0.01), high CIBMTR cytogenetic risk (HR 2.35, 95% CI: 1.34, 4.10, P<0.01), >2 two induction cycles (HR 1.73, 95% CI: 1.07, 2.78, P<0.05), and persistent morphologic disease at time of transplant (HR 2.52, 95% CI: 1.51, 4.21, P<0.001) were all found to be significantly associated with inferior DFS (Table 5).

Overall survival

The 3-year overall survival was decreased among patients with *vs.* without *FLT3* mutation, but the difference was not statistically significant (38% *vs.* 48%, P=0.334, Figure 1) with a median survival of 244 days (range: 57 to 2,001 days) and 368 days (range: 46 to 2,405 days), respectively. Among the other variables tested, high CIBMTR cytogenetic risk, presence of complex cytogenetics at diagnosis, and >2 induction chemotherapy cycles were each associated with significantly lower 3-year overall survival (Table 3). In the bivariate models, *FLT3* mutation negatively impacted overall survival (HR 1.69, 95% CI: 1.04, 2.75, P<0.05) after adjusting for CIBMTR cytogenetic risk at diagnosis only (Table 4). However, when accounting for all potential confounders in the multivariate model, *FLT3* mutation (HR 1.92, 95% CI 1.14, 3.24, P<0.05), high CIBMTR cytogenetic risk (HR 1.93, 95% CI 1.08, 3.48, P<0.05), >2 induction cycles (HR 1.87, 95% CI: 1.14, 3.09, P<0.05), and persistent morphologic disease at transplant (HR 2.15, 95% CI 1.23, 3.78, P<0.01) variables significantly decreased overall survival (Table 5). All causes of mortality are detailed in Supplemental Table S5.

DISCUSSION

In this study, we examined the frequency of *FLT3* mutation in children and adults with AML who underwent allogeneic HCT at our center over a 7-year study period. Patients diagnosed before 2007 were not genetically defined at diagnosis for this mutation. Given conflicting reports,[9-31] we sought to further clarify the impact of *FLT3* mutational status on HCT outcomes in a large, single institution cohort transplanted between 2008 and 2014. Consistent with the literature, *FLT3* mutated patients comprised approximately 29% of our study population.[2-4] We found that *FLT3* mutation was an independent factor for increased RR that translated into inferior DFS and overall survival after adjusting for potential confounding covariates. The low NRM was likely due to a higher RR, which is a competing risk for NRM. The present findings extend the observations from previous studies that have reported the outcomes of *FLT3* mutational AML after allogeneic HCT (Supplemental Table S1) and substantiate the need for optimizing risk stratification of AML patients at the time of transplant.

Cytogenetic characterization at diagnosis is recognized as the most powerful independent prognostic factor in AML. In recent years, risk-stratification has been refined by molecular markers, such as the *FLT3* gene. Herein, our data support the importance of carefully characterizing disease-specific variables in AML patients presenting for allogeneic transplantation. Our analyses demonstrate that, in addition to *FLT3* mutation, high-risk CIBMTR cytogenetics at diagnosis and persistent morphologic disease at transplant conferred an increased RR and lowered DFS and overall survival.

Recently, the number of induction courses required to achieve morphologic remission in AML was shown to provide independent prognostic information for outcome after transplant.[38] We therefore included this variable in our analyses and similarly demonstrated that patients who received greater than two induction cycles experienced decreased DFS and overall survival. These data provide insight into disease-specific factors that independently contribute to relapse and highlight the need to identify allogeneic HCT candidates for risk-

stratified treatment recommendations. For example, patients who are destined to relapse early after allogeneic HCT (*FLT3* mutation, high CIBMTR risk cytogenetics, and/or persistent morphologic disease) may benefit from post-transplant interventions or other non-transplant clinical trial options. Unfortunately, there remains a paucity of prospective, multi-center studies that have rigorously evaluated such strategies, such as donor leukocyte infusions,[39] targeted therapies,[40] hypomethylating agents,[41] or other immune checkpoint blockade therapy.[42] The optimal timing, dosing, duration, and type of strategy in the post-transplant setting are unknown and need to be explored.

Interestingly, *FLT3* mutated patients with intermediate CIBMTR cytogenetic risk experienced increased RR similar to those with high CIBMTR cytogenetic risk (data not shown). However, DFS and overall survival were not as comparably reduced as seen in the high CIBMTR cytogenetic risk group. These data again highlight the potential role for implementing pre-emptive FLT3-targeted therapies to reduce the relapse hazard in these molecularly defined patients.

Similar to recent studies examining the influence of age on allogeneic HCT outcomes,[50] our study did not indicate that patients >60 years of age experienced increased relapse or decreased survival. It is possible that our data represent selection of older individuals with greater fitness who met institutional criteria for allogeneic HCT or treatment according to a clinical trial. Nonetheless, we further explored other patient-specific comorbid conditions as measured by the HCT-CI[33] to help estimate outcomes following transplant and found that high HCT-CI was associated with increased RR. Although not statistically significant, this resulted in a trend toward inferior DFS and overall survival. Allogeneic HCT is increasingly offered to older patients, and identifying suitable patients could improve the effectiveness of transplantation. In our study, myeloablative conditioning was associated with lower RR and increased DFS and overall survival, suggesting a potentially protective effect in this population. It is possible that a subset of chronologically older age individuals would benefit from and tolerate increased intensity regimens. Nonetheless, we recognize that further work is needed to confirm our observations.

We recognize the limitation of our single-center, retrospective cohort study. Nonetheless, allogeneic HCT is increasingly a preferred treatment option for *FLT3* mutational AML patients, particularly with increased availability of donors and use of reduced intensity conditioning regimens, and such studies provide direction for well-designed prospective clinical trials. There is ongoing need to develop novel, preemptive post-transplant strategies in the setting of clinical research, particularly for high-risk AML, to help address malignant relapse.[52] Our study supports the recognition of *FLT3* mutation as an independent marker for high-risk disease and highlights the importance of carefully examining disease, patient, and transplant-specific variables, collectively, to identify suitable allogeneic HCT candidates and inform post-transplant expectations. Furthermore, minimal residual disease monitoring has been increasingly recognized as a source of additional valuable prognostic information that complements molecular and cytogenetic risk,[53] and combined utilization of these factors may prove crucial to optimal prognostication.

Strengths of our study include transplant practices in the current era (2008 to 2014) with detailed patient, disease, and transplant-specific variables. To our knowledge, based on the literature review (detailed in Supplemental Table S1), the present analysis represents one of the largest study populations with known *FLT3* mutational status that have undergone allogeneic HCT with the best available donor (related or unrelated) after either myeloablative or reduced intensity conditioning. At the same time, we recognize that the heterogeneity of this patient population is also a limitation, precluding us from forming generalized conclusions, such as in pediatric patients. We performed a sub-group analysis of children <18 years of age, and although the sample size was small, we observed similar trends in outcomes between the overall study population and pediatric patients (data not shown). We attempted to control for potential confounding covariates by performing both bivariate and multivariate analyses. Clearly, future studies are needed with larger populations from multi-center collaborations to confirm the observations herein.

In summary, the key finding of this study is the adverse outcomes associated with *FLT3* mutant AML. The data herein highlight the early kinetics of malignant relapse occurring within the first 100 days of transplanted patients. Therefore, it is desirable to develop novel post-transplant strategies that could effectively impact early relapse hazards.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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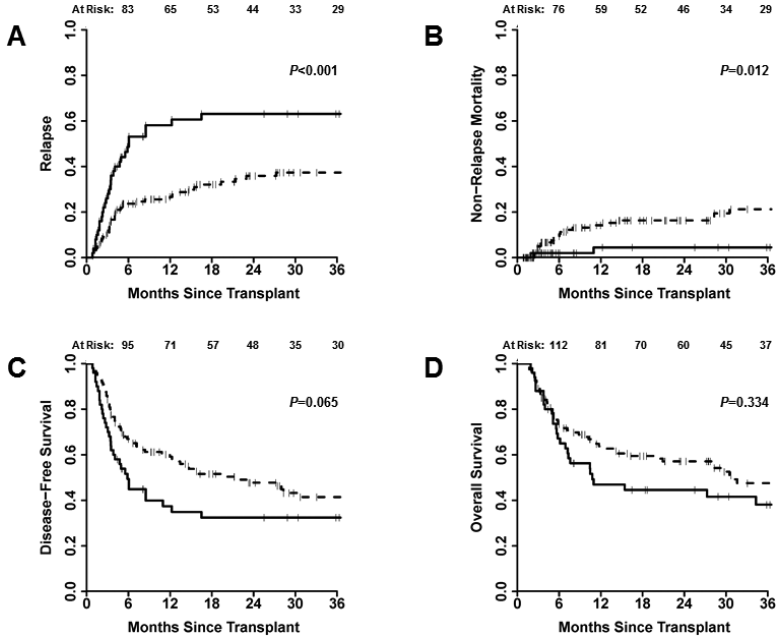

Figure 1.

Three-year Cumulative Incidence Outcomes by *FLT3* Mutational Status. A) Relapse Risk, B) Non-relapse Mortality, C) Disease-free Survival, D) Overall Survival Solid line: *FLT3* mutation positive; Dashed line: *FLT3* mutation negative

Table 1

Patient and Disease Characteristics by *FLT3* Mutational Status

	FLT3 Recorded	*FLT3* Negative	*FLT3* Positive	P
Characteristics	**N (%)**	**N (%)**	**N (%)**	
Patients (count)	171	121	50	
Age (years)				
Median [Range]	55 [1 - 72]	55 [1 - 72]	54 [3 - 71]	0.519
<60 years	117 (68)	81 (67)	36 (72)	0.518
≥60 years	54 (32)	40 (33)	14 (28)	
Gender				
Female	75 (44)	51 (42)	24 (48)	0.483
Male	96 (56)	70 (58)	26 (52)	
Race/Ethnicity				
White (Non-Hispanic)	152 (89)	109 (90)	43 (86)	0.070
White (Hispanic)	6 (4)	4 (3)	2 (4)	
Black	6 (4)	6 (5)	0 (0)	
Asian	3 (2)	1 (1)	2 (4)	
Other	4 (2)	1 (1)	3 (6)	
BMI				
<18.5 kg/m^2	5 (3)	4 (3)	1 (2)	0.900
18.5-24.9 kg/m^2	39 (23)	26 (21)	13 (26)	
25.0-29.9 kg/m^2	59 (35)	42 (35)	17 (34)	
≥30.0 kg/m^2	68 (40)	49 (40)	19 (38)	
HCT-CI				
Low Risk	34 (20)	22 (18)	12 (24)	0.630
Intermediate Risk	58 (34)	43 (36)	15 (30)	
High Risk	79 (46)	56 (46)	23 (46)	
WBC Count at Diagnosis				
<10 $\times 10^3/\mu L$	91 (53)	77 (64)	14 (28)	<0.001
≥10 $\times 10^3/\mu L$	78 (46)	43 (36)	35 (70)	
Karyotype at Diagnosis, CIBMTR Risk				
Low Risk	4 (2)	4 (3)	0 (0)	0.004
Intermediate Risk	123 (72)	82 (68)	41 (82)	
High Risk	23 (13)	23 (19)	0 (0)	
Unknown Risk	17 (10)	10 (8)	7 (14)	
Complex Cytogenetics (≥3 abnormalities) at Diagnosis				
No	142 (83)	94 (78)	48 (96)	<0.001
Yes	25 (15)	25 (21)	0 (0)	
Other Molecular Markers				
NPM1				

	FLT3 Recorded	*FLT3* Negative	*FLT3* Positive	P
Characteristics	**N (%)**	**N (%)**	**N (%)**	
Negative	104 (61)	80 (66)	24 (48)	<0.001
Positive	32 (19)	13 (11)	19 (38)	
Antecedent MDS/MPD				
No	136 (80)	90 (74)	46 (92)	0.009
Yes	35 (20)	31 (26)	4 (8)	
Therapy-related AML				
No	160 (94)	110 (91)	50 (100)	0.028
Yes	11 (6)	11 (9)	0 (0)	
Morphologic Status at Transplant				
Complete Remission	136 (80)	96 (79)	40 (80)	0.922
Persistent Disease	35 (20)	25 (21)	10 (20)	
Time to Transplant (days)				
≤ 180	117 (68)	82 (68)	35 (70)	0.775
> 180	54 (32)	39 (32)	15 (30)	
Number of Induction Cycles Before Transplant				
≤ 2	134 (78)	93 (77)	41 (82)	0.458
> 2	37 (22)	28 (23)	9 (18)	
Total Number of Chemotherapy Cycles Before Transplant				
Median [Range]	3 [0 - 10]	3 [0 - 9]	3 [1 - 10]	0.670

FLT3 mutational status (positive *vs.* negative)

BMI: body mass index; HCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index; WBC: white blood cell; CIBMTR: Center for International Blood and Marrow Transplant Research; MDS: myelodysplastic syndrome; MPD: myeloproliferative disorder; AML: acute myelogenous leukemia

Table 2

Transplantation Characteristics by *FLT3* Mutational Status

	FLT3 Recorded	*FLT3* Negative	*FLT3* Positive	P
Characteristics	**N (%)**	**N (%)**	**N (%)**	
Stem Cell Source				
Bone Marrow	18 (11)	13 (11)	5 (10)	0.885
Peripheral Blood	153 (89)	108 (89)	45 (90)	
Donor-Recipient HLA and Relation				
Matched (8/8) Related	72 (42)	51 (42)	21 (42)	0.305
Matched Unrelated	77 (45)	57 (47)	20 (40)	
Mismatched (<8/8) Related	2 (1)	2 (2)	0 (0)	
Mismatched Unrelated	20 (12)	11 (9)	9 (18)	
Donor-Recipient Gender				
Male Donor, Male Recipient	61 (36)	45 (37)	16 (32)	0.638
Male Donor, Female Recipient	50 (29)	36 (30)	14 (28)	
Female Donor, Male Recipient	34 (20)	24 (20)	10 (20)	
Female Donor, Female Recipient	25 (15)	15 (12)	10 (20)	
Donor-Recipient ABO Blood Type				
Matched	108 (63)	77 (64)	31 (62)	0.055
Anti-recipient Antibodies	31 (18)	25 (21)	6 (12)	
Anti-donor Antibodies	25 (15)	17 (14)	8 (16)	
Anti-recipient and Anti-donor Antibodies	7 (4)	2 (2)	5 (10)	
Donor-Recipient Rh Blood Type				
Matched	138 (81)	100 (83)	38 (76)	0.369
Anti-recipient Antibodies	13 (8)	7 (6)	6 (12)	
Anti-donor Antibodies	20 (12)	14 (12)	6 (12)	
Donor-Recipient CMV				
Recipient and Donor Negative	52 (30)	38 (31)	14 (28)	0.521
Recipient Negative and Donor Positive/Unknown	25 (15)	15 (12)	10 (20)	
Recipient Positive/Unknown and Donor Negative	48 (28)	33 (27)	15 (30)	
Recipient and Donor Positive/Unknown	46 (27)	35 (29)	11 (22)	
Conditioning Intensity				
Reduced Intensity	44 (26)	31 (26)	13 (26)	0.959
Myeloablative	127 (74)	90 (74)	37 (74)	
Use of Thymoglobulin or Alemtuzumab				
No	167 (98)	118 (98)	49 (98)	0.850
Yes	4 (2)	3 (2)	1 (2)	
Use of Total Body Irradiation (≥1.2Gy)				
No	165 (96)	117 (97)	48 (96)	0.822
Yes	6 (4)	4 (3)	2 (4)	
CD34 Transfused (×10^6 cells/kg)				
Median [Range]	5.5 [1.0 - 10.6]	5.6 [1.0 - 10.6]	5.3 [1.2 - 9.8]	0.232

	FLT3 Recorded	*FLT3* Negative	*FLT3* Positive	P
Characteristics	**N (%)**	**N (%)**	**N (%)**	
GVHD Prophylaxis				
CSA/Tac + MMF	69 (40)	54 (45)	15 (30)	0.072
CSA/Tac + MTX	101 (59)	67 (55)	34 (68)	
Tac + Sirolimus	1 (1)	0 (0)	1 (2)	
Length of Stay (days)				
Median [Range]	22 [17 - 97]	22 [17 - 97]	22 [18 - 34]	0.729
Time to Engraftment (days)				
Median [Range]	12 [4 - 23]	12 [4 - 23]	12 [7 - 15]	0.891

FLT3 mutational status (positive *vs.* negative)

HLA: human leukocyte antigen; CMV: cytomegalovirus; Gy: gray; GVHD: graft-versus-host disease; CSA: cyclosporine; Tac: tacrolimus; MMF: mycophenolate mofetil; MTX: methotrexate

Table 3

Univariate Analysis Results

Characteristics	N	3-year RR	P	1-year aGVHD (Grades 2–4)	P	1-year cGVHD	P	3-year NRM	P	3-year DFS	P	3-year Overall Survival	P
All *FLT3* Tested	171	45%		33%		49%		16%		39%		45%	
Age													
<60 years	117	45%	0.664	31%	0.780	46%	0.542	14%	0.514	41%	0.957	46%	0.544
≥60 years	54	44%		35%		53%		19%		37%		42%	
HCT-CI													
Low Risk	34	31%	0.033	36%	0.553	54%	0.398	14%	0.114	55%	0.139	58%	0.303
Intermediate Risk	58	37%		37%		45%		27%		37%		42%	
High Risk	79	56%		28%		49%		10%		34%		42%	
WBC Count at Diagnosis													
$<10 \times 10^3/\mu L$	91	43%	0.315	34%	0.996	54%	0.045	17%	0.707	40%	0.425	47%	0.801
$\geq 10 \times 10^3/\mu L$	78	46%		33%		41%		16%		38%		41%	
Karyotype at Diagnosis, CIBMTR Risk													
Low	4	0%	0.007	0%	0.222	67%	0.536	0%	0.284	100%	0.001	100%	<0.001
Intermediate	123	40%		32%		51%		15%		45%		49%	
High	23	73%		37%		35%		14%		13%		25%	
Unknown	17	46%		47%		44%		34%		19%		29%	
Complex Cytogenetics (≥3 abnormalities) at Diagnosis													
No	142	39%	<0.001	34%	0.924	51%	0.527	17%	0.669	44%	<0.001	48%	<0.001
Yes	25	76%		34%		37%		13%		11%		28%	
Molecular Markers Prior to Transplant													
FLT3													
Negative	121	37%	<0.001	34%	0.715	54%	0.025	21%	0.012	41%	0.065	48%	0.334
Positive	50	63%		30%		36%		4%		32%		38%	
NPM1													

Characteristics	N	3-year RR	P	1-year aGVHD (Grades 2–4)	P	1-year cGVHD	P	3-year NRM	P	3-year DFS	P	3-year Overall Survival	P
Negative	104	40%	0.525	32%	0.965	52%	0.095	18%	0.584	42%	0.801	47%	0.776
Positive	32	44%		32%		39%		16%		40%		42%	
Conditioning Intensity													
Reduced Intensity	44	53%	0.130	43%	0.156	48%	0.937	12%	0.293	35%	0.476	41%	0.431
Myeloablative	127	41%		29%		49%		17%		41%		46%	
Use of Thymoglobulin or Alemtuzumab													
No	167	45%	0.186	33%	0.205	48%	0.492	16%	0.227	39%	0.553	45%	0.748
Yes	4	0%		0%		67%		33%		67%		67%	
Time to Transplant (days)													
≤ 180	117	47%	0.347	35%	0.459	49%	0.492	13%	0.152	40%	0.978	49%	0.343
> 180	54	40%		28%		47%		22%		37%		36%	
Number of Induction Cycles Before Transplant													
≤ 2	134	43%	0.181	33%	0.704	51%	0.363	12%	0.020	46%	0.002	52%	<0.001
> 2	37	53%		32%		41%		32%		15%		19%	

FLT3 mutational status (positive *vs.* negative)

HCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index; WBC: white blood cell; CIBMTR: Center for International Blood and Marrow Transplant Research; RR: relapse risk; aGVHD: acute graft-versus-host disease; cGVHD: chronic graft-versus-host disease; NRM: non-relapse mortality; DFS: disease-free survival

Table 4

Bivariate Analysis Results

Characteristics	3-year RR		1-year cGVHD		3-year NRM		3-year DFS		3-year Overall Survival	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
***FLT3* (Positive *vs.* Negative)**	2.25 (1.40,3.61)	<0.001	0.55 (0.32,0.95)	0.031	0.20 (0.05,0.86)	0.031	1.49 (0.97,2.29)	0.066	1.26 (0.80,1.98)	0.316
Age (≥60 *vs.* <60 years)	0.93 (0.57,1.52)	0.770	1.19 (0.76,1.85)	0.450	1.24 (0.55,2.81)	0.610	1.01 (0.65,1.55)	0.981	1.16 (0.74,1.82)	0.507
***FLT3* (Positive *vs.* Negative)**	3.65 (2.11,6.33)	<0.001	0.44 (0.25,0.78)	0.005	0.19 (0.04,0.81)	0.025	2.06 (1.30,3.27)	0.002	1.69 (1.04,2.75)	0.036
Karyotype at Diagnosis, CIBMTR Risk (High *vs.* Other)	4.22 (2.32,7.67)	<0.001	0.52 (0.23,1.19)	0.120	0.64 (0.19,2.20)	0.480	2.97 (1.72,5.12)	<0.001	2.72 (1.55,4.77)	<0.001
***FLT3* (Positive *vs.* Negative)**	2.90 (1.68,5.02)	<0.001	0.69 (0.37,1.29)	0.250	0.20 (0.04,0.89)	0.034	1.75 (1.06,2.89)	0.028	1.37 (0.81,2.34)	0.245
***NPM1* (Positive *vs.* Negative)**	0.86 (0.44,1.69)	0.660	0.68 (0.35,1.32)	0.250	1.19 (0.40,3.55)	0.760	0.89 (0.50,1.57)	0.682	0.97 (0.53,1.76)	0.921
***FLT3* (Positive *vs.* Negative)**	2.25 (1.39,3.63)	<0.001	0.53 (0.31,0.92)	0.023	0.21 (0.05,0.89)	0.035	1.46 (0.95,2.24)	0.081	1.27 (0.81,2.00)	0.293
Number of Induction Cycles (>2 *vs.* ≤2)	1.43 (0.82,2.50)	0.210	0.65 (0.34,1.23)	0.190	2.30 (1.02,5.21)	0.045	1.99 (1.27,3.12)	0.003	2.29 (1.44,3.65)	<0.001
***FLT3* (Positive *vs.* Negative)**	2.23 (1.39,3.56)	<0.001	0.55 (0.32,0.94)	0.028	0.19 (0.04,0.84)	0.029	1.49 (0.97,2.28)	0.067	1.28 (0.81,2.02)	0.284
HCT-CI (High *vs.* Other)	1.83 (1.13,2.95)	0.013	1.02 (0.65,1.59)	0.930	0.51 (0.22,1.18)	0.120	1.37 (0.91,2.04)	0.130	1.31 (0.86,2.00)	0.214
***FLT3* (Positive *vs.* Negative)**	2.32 (1.44,3.75)	<0.001	0.55 (0.32,0.94)	0.028	0.20 (0.05,0.84)	0.028	1.52 (0.99,2.33)	0.057	1.27 (0.81,1.99)	0.307
Conditioning Intensity (Myeloablative *vs.* Reduced)	0.64 (0.39,1.04)	0.074	0.91 (0.57,1.46)	0.700	1.62 (0.63,4.16)	0.320	0.83 (0.53,1.28)	0.392	0.82 (0.52,1.30)	0.393
***FLT3* (Positive *vs.* Negative)**	2.31 (1.48,3.62)	<0.001	0.55 (0.32,0.94)	0.029	0.19 (0.04,0.84)	0.029	1.49 (0.97,2.28)	0.069	1.25 (0.80,1.97)	0.332
Morphologic Status (PD *vs.* CR)	2.09 (1.34,3.26)	0.001	0.65 (0.33,1.29)	0.220	2.22 (0.93,5.28)	0.071	2.65 (1.71,4.12)	<0.001	2.45 (1.55,3.88)	<0.001

FLT3 mutational status (positive *vs.* negative)

CIBMTR: Center for International Blood and Marrow Transplant Research; HCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index; PD: persistent disease; CR: complete remission (morphologic); RR: relapse risk; cGVHD: chronic graft-versus-host disease; NRM: non-relapse mortality; DFS: disease-free survival; CI: confidence interval

Hazard ratios >1 indicate greater hazard for poor outcome (i.e., increased RR, cGVHD, and NRM and inferior DFS and overall survival)

Table 5

Multivariate Analysis Results

N=167[†]	3-year RR		1-year cGVHD		3-year NRM		3-year DFS		3-year Overall Survival	
Characteristics	**Hazard Ratio (95% CI)**	**P**	**Hazard Ratio (95% CI)**	**P**	**Hazard Ratio (95% CI)**	**P**	**Hazard Ratio (95% CI)**	**P**	**Hazard Ratio (95% CI)**	**P**
***FLT3* (Positive *vs.* Negative)**	3.63 (2.13,6.19)	<0.001	0.44 (0.25,0.78)	0.005	0.21 (0.05,0.92)	0.038	2.05 (1.29,3.27)	0.003	1.92 (1.14,3.24)	0.014
Age (≥60 *vs.* <60)	0.64 (0.39,1.06)	0.081	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Karyotype at Diagnosis, CIBMTR Risk (High *vs.* Other)	2.97 (1.52,5.77)	0.001	0.52 (0.23,1.19)	0.120	N/A	N/A	2.35 (1.34,4.10)	0.003	1.93 (1.08,3.48)	0.028
Number of Induction Cycles (>2 *vs.* ≤2)	N/A	N/A	N/A	N/A	1.98 (0.90,4.34)	0.089	1.73 (1.07,2.78)	0.025	1.87 (1.14,3.09)	0.014
HCT-CI (High *vs.* Other)	1.71 (1.04,2.79)	0.034	N/A	N/A	0.49 (0.21,1.14)	0.098	N/A	N/A	N/A	N/A
Conditioning Intensity (Myeloablative *vs.* Reduced)	0.39 (0.21,0.72)	0.003	N/A	N/A	N/A	N/A	0.57 (0.35,0.95)	0.032	0.55 (0.33,0.94)	0.029
Morphologic Status (PD *vs.* CR)	2.61 (1.44,4.74)	0.002	N/A	N/A	1.98 (0.88,4.47)	0.100	2.52 (1.51,4.21)	<0.001	2.15 (1.23,3.78)	0.008

FLT3 mutational status (positive *vs.* negative)

CIBMTR: Center for International Blood and Marrow Transplant Research; HCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index; PD: persistent disease; CR: complete remission (morphologic); RR: relapse risk; cGVHD: chronic graft-versus-host disease; NRM: non-relapse mortality; DFS: disease-free survival; CI: confidence interval; N/A: not applicable (addition of characteristic did not improve fit of the multivariate model)

Hazard ratios >1 indicate greater hazard for poor outcome (i.e., increased RR, cGVHD, and NRM and inferior DFS and overall survival)

[†]Data were available for all characteristics included in the multivariate model for 167 of 171 patients with *FLT3* mutational status recorded.