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Prognostic value of FLT3 mutations among different cytogenetic subgroups in acute myeloid leukemia

Fabio P S Santos, MD¹, Dan Jones, MD², Wei Qiao³, Jorge E. Cortes, MD¹, Farhad Ravandi, MD¹, Elihu E Estey, MD⁴, Dushyant Verma, MD¹, Hagop Kantarjian, MD¹, and Gautam Borthakur, MD¹

¹Department of Leukemia, University of Texas – M. D. Anderson Cancer Center, Houston, Texas, USA

²Department of Hematopathology, University of Texas – M. D. Anderson Cancer Center, Houston, Texas, USA

³Department of Biostatistics, University of Texas – M. D. Anderson Cancer Center, Houston, Texas, USA

⁴University of Washington Department of Medicine and Member of Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Abstract

Background—Impact of FLT3 mutations and mutation burden in cytogenetic subgroups of acute myeloid leukemia (AML) other than normal karyotype (NK-AML) is unclear.

Methods—Patients with newly diagnosed AML were divided among three cytogenetic subgroups: Core-Binding Factor (CBF) AML, NK-AML, Poor Risk AML.

Results—A total of 481 patients were included (CBF-AML – 13%, NK-AML – 57%, poor risk – 30%). *FLT3* mutations (any) frequency was 20%, 32% and 7.6% in respective cytogenetic subgroups. *FLT3* mutation did not impact event free survival (EFS) in patients with CBF (P=0.84) and poor-risk AML (P=0.37). In NK-AML, EFS was worse in the *FLT3*-internal tandem duplication (ITD) group (20 vs. 41 weeks, P<0.00001) but not in *FLT3* tyrosine kinase domain (TKD) point mutation group (61 vs. 41 weeks, P=0.15). Patients with NK-AML and higher *FLT3*-ITD burden had worse EFS and overall survival (OS), but not so with *FLT3*-TKD mutation. In multivariate analysis, *FLT3*-ITD mutation was prognostic for EFS in NK-AML patients (hazard ratio 3.1, P=0.03).

Conclusion—*FLT3* mutations did not have a prognostic impact in AML patients with good and poor-risk karyotype. In patients with NK-AML, *FLT3*-ITD mutations led to worse survival, more so in patients with high mutation burden.

Corresponding Author: Gautam Borthakur, M.D., Department of Leukemia, University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 0428, Houston, Texas 77030, Phone (713) 563-1586, gborthak@mdanderson.org.

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Keywords

Acute myeloid leukemia; FLT3; cytogenetics; prognostic factors

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease¹, and its outcome is directed by the presence of several prognostic factors.^{2, 3} The most important ones include age, cytogenetics, performance status (PS) and response to treatment.^{2, 3} Most recently, several groups have identified molecular abnormalities that have an important prognostic impact in AML, such as mutations of *NPM1*, *FLT3*, *WT1* and *CEBPA* genes.⁴ The perception that genetic and molecular abnormalities define unique subtypes of leukemias with important clinical and prognostic features has led to an overall change of direction in the classification of AML, going from a pure morphological classification to a more genetic and molecular-based one, as seen in the most recent WHO classification.⁵

FLT3 (FMS-like tyrosine kinase 3) is a receptor tyrosine kinase (RTK) that belongs to the class III of RTK (which also includes *KIT*).⁶ *FLT3* is expressed in early hematopoietic stem cells and a subset of dendritic cell progenitors.⁷ *FLT3* signaling activates intracellular pathways (e.g. Ras-Raf-Mek, PI3K-AKT) that promote proliferation and inhibition of apoptosis.⁶

The most common *FLT3* mutation described in AML is the internal tandem duplication (ITD) mutation of the juxtamembrane (JM) segment.^{4, 6} This mutation leads to loss of the autoinhibition exerted by the JM domain over the tyrosine kinase domain (TKD)⁶, generating a constitutively active *FLT3* molecule. *FLT3-ITD* mutations are found in 20–30% of patients with AML, being more common in normal karyotype (NK)-AML, acute promyelocytic leukemia and AML with t(6;9)(p23;q34).^{8–13} Patients with *FLT3-ITD* positive NK-AML have higher leukocyte count, a similar CR rate to *FLT3-ITD* negative patients, but lower disease free survival (DFS) and overall survival (OS), mainly due to frequent relapses.^{9–11} The allele burden of *FLT3-ITD* is important, with patients with higher burden having a worse prognosis.¹⁴ Another class of *FLT3* mutations is point mutations in the TKD.^{11, 15–20} The most common point mutation is on aspartic acid residue at position 835 (D835).^{16, 17, 19} Point mutations of TKD shift the activation loop to a permanently open configuration and lead to constitutive signaling.²¹ *FLT3-TKD* mutations are present in 5–10% of patients with NK-AML.^{16, 17, 19} Their prognostic significance is still controversial, and it seems to depend on the presence of other mutations.^{16, 17, 19}

While common in NK-AML, *FLT3* mutations are less common in other well-defined cytogenetic subgroups of AML, such as core binding factor (CBF) AML (e.g. t(8;21)(q22;q22) and inv16/t(16;16)) and AML with poor-risk cytogenetics (such as –5/del(5q), –7/del(7q) and 11q23 translocations). *FLT3-ITD* mutations have been described in 5–10% of patients with CBF-AML, 3–7% of patients with AML with chromosomes 5 and/or 7 abnormalities and 3% of AML patients with 11q23 translocation.^{8–11} *FLT3-TKD* mutations seem to be more common in patients with inv(16) (24%), but are uncommon in other cytogenetic subtypes of AML.¹⁷ The independent prognostic role of *FLT3* in these

cytogenetic subgroups is unclear. While RTK mutations (*KIT* mutations) are known to result in worse DFS and OS in patients with CBF AML^{22–24}, the prognostic influence of *FLT3* mutations in non-NK AML and non-APL AML is unclear.

In this study, we retrospectively evaluated the prognostic impact on survival of *FLT3* mutations in well defined cytogenetic subgroups of patients with AML.

Patients and Methods

Patients

We retrospectively reviewed the records of patients with newly diagnosed AML (except APL) from 2003 until 2007 treated at University of Texas – M.D. Anderson Cancer Center (UT-MDACC) and had one of the following karyotypes: t(8;21), inv(16)/t(16;16), Diploid/-Y, -5/del(5q), -7/del(7q) and 11q abnormalities. A diagnosis of AML was based on the World Health Organization definition.⁵ Patients were treated on front-line studies conducted at UT-MDACC. Studies were approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to study entry. Patients received different treatment regimens according to the period of diagnosis and prevailing studies. The treatment regimens were broadly divided into: Group 1 – Regimens with cytarabine (ara-C); group 2 – Regimens without ara-C. Most common regimens utilized in group 1 included fludarabine + ara-C (FA), fludarabine, ara-C and G-CSF (FLAG), fludarabine, ara-C, idarubicin (FAI), fludarabine, ara-C and topotecan (FAT)^{25–27}; idarubicin and ara-C (IA) and combinations of IA with other agents (IL-11, tipifarnib, sorafenib, pravastatin, bevacizumab)^{28–31}; single agent ara-C or in combination with other agents (laromustine, clofarabine, topotecan, triapine)^{32, 33}. In most studies Ara-C was used in high doses (1.5–2.0 g/m²/dose). Most common treatments in group 2 included single agent clofarabine, laromustine, hypomethylating agents (5-azacitidine, decitabine), tyrosine kinase inhibitors (PKC412, AG013736) and histone deacetylase inhibitors (vorinostat)^{33–38}.

Cytogenetic Data

Cytogenetic analysis was performed with standard methods. Pts were divided among three cytogenetic subgroups: CBF-AML (t(8;21), inv(16)/t(16;16)); NK-AML (Diploid,-Y) and Poor Risk Karyotype AML (-5/del(5q), -7/del(7q), 11q abnormalities).

Detection and quantification of FLT3 Mutations

FLT3 ITD and codon 835/838 TKD mutations were determined from genomic DNA extracted from bone marrow aspirate using a short-cycle PCR-based method, with subsequent restriction-digest with EcoRV for the TKD reaction and size fractionation by capillary electrophoresis, as described³⁹. Quantification of mutation burden was performed by summing the area under the curve for all TKD or ITD peaks divided by the total peak areas for mutant and unmutated peaks. For some analyses, blast-normalized FLT3 mutation burdens were also calculated by dividing the FLT3 mutation ratio by the percentage of blasts counted on a parallel bone marrow aspirate sample. The assay sensitivity was determined to

be 2% mutation-bearing cells based on dilution mixing studies with a run-to-run precision of the quantitative measurement of $\pm 3\text{--}9\%$.

Criteria for Response and Survival Definitions

Complete remission (CR) was defined by the presence of less than 5% blasts in the bone marrow (BM) with more than $1 \times 10^9/\text{L}$ neutrophils and more than $100 \times 10^9/\text{L}$ platelets in the peripheral blood (PB).^{40, 41} A relapse was defined by more than 5% blasts in a BM aspirate unrelated to recovery or by the presence of extramedullary disease. Event free survival (EFS) was calculated from the beginning of treatment until an event. An event was defined as relapse, resistance to induction therapy or death. Induction death was defined as death occurring before achievement of CR or confirming resistant disease. DFS was calculated from the moment of CR until relapse or death in CR. OS was calculated from the time of diagnosis until death. Patients alive at last follow-up were censored.

Statistical analysis

Categorical and continuous variables were compared by the Fischer's exact test and Wilcoxon test, respectively.⁴² Kaplan-Meier product-limit survival probability estimates of OS, DFS and EFS were calculated⁴³ and log-rank tests⁴⁴ were performed to compare the time to events between *FLT3* mutation positive and *FLT3* mutation negative group for each karyotype risk group (CBF-AML, NK-AML, or Poor Risk AML). Since the frequencies of *FLT3* mutations were lower in CBF- and poor-risk subgroups, ITD/TKD mutations were considered together in the analysis, while in the intermediate risk group they were analyzed separately. Among the cohort of NK-AML, patients with double mutation (both *FLT3-ITD* and *FLT3-TKD*) were grouped together with those with *FLT3-ITD* as their survival clustered with *FLT3-ITD* patients. To avoid collinearity, comparisons of *FLT3* mutation burden (continuous value) across categorical variables (like, gender, race, and treatment group, etc.) were done using Wilcoxon tests and Spearman correlation was calculated between *FLT3* and the other continuous variables. Separate models were fit for each of time-to-event outcomes (OS and EFS) in each cytogenetic subgroup. For each fitted Cox regression model, non-significant variables in univariate analyses were eliminated using in a step-down fashion of a p-value cut-off of $p = .10$.⁴⁵ In addition, the clinical factors that indicated significant association with *FLT3* mutation were excluded from the final multivariate to avoid collinearity. To evaluate for possible difference of impact of *FLT3* across different treatments (ara-C vs. non-ara-C), an interaction term was added in the final variate model to adjust for the different effect of treatments on *FLT3* value. All computations were done in SAS version 9.1 (Cary, NC), S-Plus version 7.0 (Palo Alto, CA) and SPSS version 17.0 (Chicago, IL).

Results

Patients Characteristics and Survival Outcomes

Data from 481 patients was analyzed. There were 65 patients (13%) with CBF-AML, 272 patients with NK-AML (57%) and 144 patients (30%) with Poor-Risk AML. Prevalence of *FLT3* mutations by cytogenetic subgroup is shown in Table 1. There were 111 patients with *FLT3* mutation (23%). Patients with *FLT3* mutation were unequally divided among the three

cytogenetic subgroups, being more common in NK-AML (32%) compared to CBF AML (20%) and Poor Risk AML (7.6%) ($p < 0.001$). Median follow-up of all patients was 95 weeks (range 0–249).

Core Binding Factor AML—There were 65 patients with CBF AML. Their baseline characteristics are summarized in Table 2(a). There were 13 patients with *FLT3* mutation (20%), including two with *FLT3-ITD* (3%) and 8 with *FLT3-TKD* (12%). Three patients (5%) had both ITD and TKD mutation. The median *FLT3* mutation burden was 12% (range 1%–49%). The median blast-normalized *FLT3* mutation burden was 20% (range 5–60%), with 6 patients (46%) having high *FLT3* mutation burden ($>20\%$). *FLT3* mutations were more common in patients with *inv*(16) abnormality than in patients with *t*(8;21)(q22;q22) [11/36 (31%) vs 2/29 (7%), $p=0.02$]. *FLT3-TKD* mutations were found in 10 (28%) patients with *inv*(16) and in 1 (3.5%) patient with *t*(8;21)(q22;q22). *FLT3-ITD* mutations were found in 4 (11%) patients with *inv*(16) and in 1 (3.5%) patient with *t*(8;21)(q22;q22). All three patients with double mutations were found in the *inv*(16) group. Patients with *FLT3*-mutated CBF-AML had a higher WBC count than wild-type (wt) *FLT3* patients and a higher BM blast percentage. All patients with CBF AML were treated with chemotherapeutic regimens that contained high-dose Ara-C and majority (88%) were treated with fludarabine and Ara-C based combination. *KIT* mutation analysis was done in 9 patients only, and no patients were positive.

There was no difference in CR rate among patients with and without *FLT3* mutations (92% vs. 90%; $p = 1.0$). Survival results are summarized in table 3(a), figures 1(a) and 2(a). In our cohort, *FLT3*-mutations did not impact survival in patients with CBF-AML.

Normal Karyotype AML—There were 272 patients with NK-AML. There were 59 patients (22%) with isolated *FLT3-ITD*, 18 patients (7%) with isolated *FLT3-TKD* and 10 patients (4%) had both ITD and TKD mutations. Patients with *FLT3-ITD* and *FLT3-TKD* mutations were analyzed separately, and their survival outcomes compared to patients with NK-AML who had wt-*FLT3* (negative for both mutations). The baseline characteristics of patients with NK-AML are summarized in Table 2(b).

Patients with *FLT3-ITD* mutations had higher WBC and higher BM blast percentage than patients with wt-*FLT3*. Data on *FLT3-ITD* mutation ratio was available for 66 patients. The median *FLT3-ITD* mutation ratio was 36% (range 0.8%–81%). Median blast-normalized *FLT3-ITD* mutation ratio was 51% (range 1–235%), and 54 patients (82%) had a high *FLT3-ITD* ratio (> 0.2). There was no difference in CR rate among patients with *FLT3-ITD* mutation and wt-*FLT3* (58% vs. 68%; $p = 0.17$). Survival results are shown in table 3(b), figure 1(b) and 2(b). *FLT3-ITD* mutations were associated with inferior EFS, DFS and OS. There was a trend for a higher EFS in patients with low *FLT3-ITD* burden compared to high *FLT3-ITD* burden (median 36 weeks vs. 16 weeks, $p=0.08$). Similarly, patients with low *FLT3-ITD* burden had higher OS (median 63 weeks vs. 30 weeks, $p=0.05$). There was no difference in EFS ($p=0.55$) and OS ($p=0.54$) among patients with low *FLT3-ITD* allele burden and wt-*FLT3*.

Similar to patients with *FLT3-ITD* mutations, patients with NK-AML who carried *FLT3-TKD* mutations had a higher presenting WBC and higher BM blast percentage than patients with wt-*FLT3*. Twelve patients (66%) had a high mutation burden (> 0.2). There was no statistically significant difference in the proportion of patients with *FLT3-TKD* mutations receiving treatment with Ara-C containing regimens (94%) compared to wt-*FLT3* (86%) ($p = 0.47$). There was no difference in the CR rate (78% vs. 68%, $p = 0.59$). Survival outcomes are summarized in table 3b and figure 1b. *FLT3-TKD* mutations did not impact survival in patients with NK-AML. There was no difference in EFS and OS by *FLT3-TKD* burden (median not reached [NR] [low] vs. 59 weeks [high], $p=0.59$, and median NR vs. 74 weeks, $p=0.72$, respectively). However, the number of patients analyzed in each group was small.

Poor Prognosis AML—There were 144 patients with poor-risk karyotype AML including 11 (7.5%) patients with *FLT3* mutation, 3 with *FLT3-ITD* (2%) and 8 with *FLT3-TKD* (5.5%). Their baseline characteristics are summarized in Table 2(c). No patients had double mutations. The median *FLT3* mutation burden was 21% (range 2%–56%). The median blast-normalized *FLT3* mutation burden was 37% (range 8–158%), including 9 patients (82%) with high burden. In contrast to our findings in patients with CBF-AML and NK-AML, in patients with poor prognosis cytogenetics, *FLT3* mutations were not associated with higher WBC counts or higher BM blast percentages. Patients with *FLT3*-mutated AML in this group had a lower CR rate than patients with wt-*FLT3* but without statistical significance (18% vs. 43%; $p=0.20$). Only two patients achieved CR in the *FLT3*-mutated group and so we didn't conduct an analysis of DFS. EFS and OS are summarized in table 3(c), figure 1(c) and 2(c). There was no statistically significant difference in EFS and OS by *FLT3*-mutation status.

Multivariate Analysis for EFS

Core Binding Factor AML—In the univariate analysis for EFS in patients with CBF-AML, the following covariates were significantly associated with lower EFS: lower platelets, higher bilirubin levels and higher performance status (PS). In the multivariate analysis for EFS (table 4), only PS was prognostic for EFS, with no impact of *FLT3* mutations. Similarly, *FLT3* mutations were not associated with worse OS in the multivariate analysis (data not shown).

Normal Karyotype AML—Two Cox models were fit in patients with NK-AML. In the first model the population of patients included patients that were *FLT3-ITD* mutated or had wt-*FLT3*. In the univariate analysis for EFS, the following covariates were found to be significantly associated with shorter EFS: higher age, non-white race, higher WBC, lower platelets, higher serum creatinine, *FLT3-ITD* mutation, presence of antecedent hematological disorder (AHD), history of prior chemotherapy or radiation, higher performance status and treatment without Ara-C. The results of the multivariate analysis showed that *FLT3-ITD* (as a continuous variable) was associated with worse EFS (table 4) and worse OS (data not shown). There was no interaction between Ara-C regimens and *FLT3-ITD* mutations in the multivariate analysis.

In the second model we included patients who were positive for *FLT3-TKD* mutation or had *wt-FLT3*. In the univariate analysis, the following factors were associated with shorter EFS: higher age, non-white race, higher creatinine, presence of AHD, history of chemotherapy or radiation therapy and treatment without Ara-C. By multivariate analysis, *FLT3-TKD* mutations in NK-AML did not influence EFS (table 4) or OS (data not shown). Also, there was no interaction between Ara-C regimens and *FLT3-TKD* mutations in the multivariate analysis.

Poor Risk AML—In the univariate analysis for EFS in patients with poor risk karyotype AML, the following covariates were significantly associated with lower EFS: higher age, male sex, higher serum creatinine levels, and treatment without Ara-C regimens. In the multivariate analysis (Table 4), only age was prognostic for EFS, with no impact of *FLT3* mutations or treatment regimen.

Discussion

As clinical outcome of patients with AML largely depends on cytogenetic subgroups, we analyzed the impact of *FLT3* mutations in the outcome of patients within specific cytogenetic subgroups of AML and that of allele burden of mutant *FLT3* particularly in patients with NK-AML. Ours is one of the larger cohorts of poor-risk and CBF-AML patients with *FLT3* mutations and in concordance with published reports, we found a low prevalence of *FLT3* mutation (both ITD and TKD) in these patients.^{9, 11, 17} However, limited number of patients did not allow us to analyze the impact of allele burden in poor-risk cytogenetics and CBF-AML. *FLT3* mutations (mostly TKD mutations) were more common in patients with Inv(16) CBF-AML than in t(8;21), as previously reported.¹⁷ *FLT3* mutations (ITD and TKD mutations analyzed together) did not impact survival outcomes in patients with CBF-AML and Poor-Risk karyotype AML. *FLT3-ITD* mutations were more prevalent in patients with NK-AML, and we found that *FLT3-ITD* but not *FLT3-TKD* mutation impacted survival in this subgroup of patients largely treated with high-dose ara-C based induction regimens.

FLT3 mutations may play an important role in the leukemogenesis of CBF-AML as it has been shown that *FLT3* cooperates with both *AML1-ETO* and *CBFB-MYH11* to induce the leukemic phenotype in mouse models.^{46, 47} Boissel et al reported that *FLT3* mutations lead to lower EFS and OS in patients with CBF-positive AML, particularly due to an excess occurrence of early events.⁴⁸ In that report the authors estimated that only approximately 47% of patients received high-dose ara-C during consolidation courses. This may have impacted the results as in our population 100% of patients were treated with regimens that contained high-dose Ara-C, the majority (87%) having received regimens that contained fludarabine + ara-C, a combination that was recently reported to improve the outcomes of patients with CBF-AML.²⁵ Thus it is conceivable that effective therapy may overcome the negative prognostic impact of *FLT3* mutations. In a gene expression profiling study of patients with CBF-AML no correlation was found between *FLT3-ITD* and a gene signature that predicted worse survival in *KIT*-mutation negative patients with CBF-AML.⁴⁹

In AML with poor-risk karyotype, the incidence of *FLT3* mutations is even lower than in CBF-AML, and its prognostic impact is less studied. Indeed, *FLT3* mutations appear to be less common in patients who present with secondary AML, therapy-related AML and AML with complex karyotype.^{50, 51} Patients with *FLT3* mutations in this subgroup did not have higher WBC or higher BM blast counts.

In NK-AML, the role of *FLT3* mutations is better defined. We did confirm in our population that *FLT3-ITD* was associated with a worse survival compared to patients with wt-*FLT3*. Contrary to previous reports,¹⁹ but in accordance with others, we didn't find that *FLT3-TKD* mutations impacted survival in patients with NK-AML.^{16, 17}

In our cohort, patients with CBF-AML with *FLT3* mutation had a lower blast-normalized mutation ratio (46%) compared to patients with NK-AML (80%) and poor-risk AML (82%) ($p=0.027$). In patients with NK-AML, we confirmed previous findings that a high *FLT3-ITD* mutation burden is associated with a worse survival. The same report also suggested that even low burden of *FLT3* mutations (1–24% of total *FLT3* allele) resulted in worse outcome compared to wt-*FLT3*.¹⁴ Our Cox analysis demonstrated that the risk associated with *FLT3-ITD* increased progressively with an increasing burden of the mutation and that patients who had a low mutation burden (< 20%) of *FLT3-ITD* had similar EFS and OS compared to patients with wt-*FLT3*. In view of recent reports recommending allogeneic SCT for patients harboring *FLT3-ITD* mutations,⁵² we do believe that information about allele level should be taken into account when making therapeutic decisions. We did not find *FLT3-TKD* allele burden to influence outcome in patients with NK-AML. However, the number of patients with TKD mutation was small, and we must be careful about drawing conclusions from such subgroup analysis.

Our study has several limitations. First and foremost, it is a retrospective analysis and subgroup analysis may lack the power to detect differences in survival. Due to the low prevalence of *FLT3* mutations in patients with CBF-AML and poor-risk AML, we combined *FLT3-ITD* and *FLT3-TKD* mutations in this analysis. This may have influenced our results, since these are biologically distinct mutations, a fact that was indicated by our own analysis in NK-AML patients. As expected in a retrospective analysis, there is a degree of heterogeneity of treatment regimens administered, particularly in the subgroups of NK-AML and poor-risk AML. Patients who received non-Ara-C containing regimens were older than patients who received ara-C regimens (median age 69 vs. 57 years, $p<0.00001$). Finally, data is emerging about the impact and interaction of different mutations (e.g. *NPM1*, *KIT*, *CEBPA*) in AML but unfortunately comprehensive analysis of these mutations was not available for all our patients.¹⁶

In conclusion, in our cohort of patients, *FLT3* mutations did not impact survival in CBF and poor risk cytogenetics AML. In patients with NK-AML, *FLT3-TKD* mutations had no impact on survival, while a high *FLT3-ITD* allele burden was associated with worse survival. Analysis of merged data from large databases may help to better discern the prognostic value of different *FLT3* mutations among different cytogenetic subtypes of AML.

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(b).

Figure 1.
Event Free Survival (EFS) curves.
(a). Core Binding Factor associated acute myeloid leukemia (AML).
(b). Normal Karyotype-AML (wt-*FLT3*, *FLT3-ITD* and *FLT3-TKD*).
(c). Poor Risk AML.

(a).

(b).

Figure 2.

Overall Survival (OS) curves.

(a). CBF-AML

(b). NK-AML (wt-*FLT3*, *FLT3-ITD* and *FLT3-TKD*).

(c). Poor Risk AML.

Table 1Prevalence of *FLT3* mutations across cytogenetic subgroups

Cytogenetics	<i>FLT3</i> -All, n (%)	<i>FLT3</i> -ITD, n (%)	<i>FLT3</i> -TKD, n (%)	Double Mutants, n (%)
CBF-AML (N = 65)	13 (20)	2 (3)	8 (12)	3 (5)
NK-AML (N = 272)	87 (32)	59 (22)	18 (7)	0 (4)
Poor Risk AML (N = 144)	11 (7.5)	3 (2)	8 (5.5)	0 (0)

Abbreviations: ITD – internal tandem duplication; TKD – tyrosine kinase domain.

Table 2

Characteristics of Patients with AML by FLT3 mutation

a. Patients with CBF-AML					
Parameter	Median [Range] or No. (%)			p	
	FLT3-Mutated (N = 13)	wt-FLT3 (N = 52)			
Age, years	50 [19–80]	49.5 [18–88]			0.95
Male Sex	9 (69)	27 (52)			0.35
White Race	9 (69)	33 (63)			0.76
ECOG PS 3–4	0 (0)	1 (2)			1.0
Hb, g/dL	8.5 [6.7–10.6]	8.8 [5.1–13.1]			0.54
WBC, × 10 ⁹ /L	37 [3.9–89.5]	14 [0.6–14.6]			0.019
Platelets, × 10 ⁹ /L	38 [17–135]	42.5 [4–330]			0.66
BM Blasts, %	76 [21–91]	50.5 [10–92]			0.006
Creatinine, mg/dL	1.0 [0.5–1.7]	1.0 [0.4–5.2]			0.82
Bilirubin, mg/dL	0.6 (0.3–1.0)	0.5 (0.1–4.7)			0.41
Cytogenetics					0.02
• t(8;21)	2 (15)	27 (52)			
• inv(16)/t(16;16)	11 (85)	25 (48)			
AHD	4 (31)	9 (17)			0.27
Previous Chemo/XRT	0 (0)	5 (10)			0.57
Treatment Regimens					1.0
• With Ara-C	13 (100)	52 (100)			
CR Rate	92%	90%			1.0

b. Patients with NK-AML				
Parameter	Median [Range] or No. (%)			p (TKD vs WT)
	FLT3-ITD (N = 69) ^f	FLT3-TKD (N = 18)	wt-FLT3 (N = 185)	
Age, years	58 [17–84]	62 [28–78]	61 [18–83]	0.12
Male Sex	33 (48)	10 (55)	99 (54)	0.48
White Race	52 (75)	17 (94)	148 (80)	0.49
				0.20

b. Patients with NK-AML						
Parameter	Median [Range] or No. (%)				p (ITD vs WT)	p (TKD vs WT)
	<i>FLT3-ITD</i> (N = 69) ^f	<i>FLT3-TKD</i> (N = 18)	wt- <i>FLT3</i> (N = 185)			
ECOG PS 3–4	0 (0)	2 (11)	3 (2)		0.56	0.05
Hb, g/dL	8.2 [5–11.7]	7.7 [5–10.6]	8.4 [4.0–14.2]		0.43	0.03
WBC, × 10 ⁹ /L	18.1 [1–166]	22.5 [1.2–291]	3.3 [0.4–204]		< 0.001	< 0.001
Platelets, × 10 ⁹ /L	50 [9–302]	57.5 [18–119]	64 [3–469]		0.13	0.27
BM Blasts, %	70 [8–96]	65 [24–91]	44 [3–93]		< 0.001	0.01
Creatinine, mg/dL	0.9 [0.5–2.0]	1.0 [0.6–2.1]	0.9 [0.4–6.8]		0.20	0.38
Bilirubin, mg/dL	0.5 [0.1–2.3]	0.45 [0.2–1.4]	0.5 [0.1–10.3]		0.44	0.51
AHD	27 (39)	4 (22)	88 (47.6)		0.25	0.04
Previous Chemo/XRT	2 (3)	3 (17)	22 (11.9)		0.02	0.47
Treatment Regimens					0.03	0.47
• With Ara-C	51 (74)	17 (94)	159 (6)			
• Without Ara-C	18 (27)	1 (6)	26 (14)			
CR Rate	58%	78%	68%		0.17	0.59

(c). Patients with Poor Risk-AML			
Parameter	Median [Range] or No. (%)		p
	<i>FLT3-mutated</i> (N = 11)	wt- <i>FLT3</i> (N = 133)	
Age, years	59 [34–83]	60 [19–85]	0.9
Male Sex	9 (82)	61 (46)	0.03
White Race	8 (73)	101 (76)	0.73
ECOG PS 3–4	0 (0)	4 (3)	1.0
Hb, g/dL	8.5 [5.9–10.4]	8 [2–14.2]	0.95
WBC, × 10 ⁹ /L	8.4 [1.6–119]	4 [0.8–433]	0.16
Platelets, × 10 ⁹ /L	45 [16–117]	39 [7–762]	0.86
BM Blasts, %	55 [10–96]	40 [5–97]	0.33
Creatinine, mg/dL	1.1 [0.7–1.5]	0.9 [0.5–4.5]	0.02
Bilirubin, mg/dL	0.6 [0.3–2.0]	0.6 [0.1–6.2]	0.53
Cytogenetics			0.18

(c). Patients with Poor Risk-AML			
Parameter	Median [Range] or No. (%)		p
	FLT3-mutated (N=11)	wt-FLT3 (N = 133)	
• 11q23	3 (28)	17 (13)	
• -5/del(5q) and/or -7/del(7q)	8 (72)	116 (87)	
AHD	6 (55)	69 (52)	1.0
Previous Chemo/XRT	3 (27)	36 (27)	1.0
Treatment Regimens			0.03
• With Ara-C	6 (55)	111 (4)	
• Without Ara-C	5 (45)	22 (16)	
CR Rate	18%	43%	0.20

Abbreviations: AHD, antecedent hematological disorder; BM, bone marrow; Chemo, chemotherapy; Hb, hemoglobin; PS, performance status; WBC, white blood cell count; XRT, radiotherapy.

Abbreviations: AHD, antecedent hematological disorder; BM, bone marrow; Chemo, chemotherapy; Hb, hemoglobin; PS, performance status; WBC, white blood cell count; XRT, radiotherapy.

¹Includes 10 patients with double mutations.

Abbreviations: AHD, antecedent hematological disorder; BM, bone marrow; Chemo, chemotherapy; Hb, hemoglobin; PS, performance status; WBC, white blood cell count; XRT, radiotherapy.

Table 3

Survival Results by FLT3 Status

(a). Patients with CBF-AML				
Parameter (95% CI)	FLT3-Mutated (N =13)	wt-FLT3 (N = 52)	p	
DFS, 2 years	50% (14–95%)	64% (47–83%)	0.66	
Median, weeks	NR (82-NR)	NR (84-NR)		
EFS, 2 years	50% (12–88%)	58% (41–75%)	0.84	
Median, weeks	100 (86-NR)	NR (73-NR)		
OS, 2 years	92% (76–100%)	74% (59–88%)	0.57	
Median, weeks	NR (112-NR)	NR (112-NR)		

(b). Patients with NK-AML				
Parameter (95% CI)	FLT3-ITD (N = 69) ^I	FLT3-TKD (N = 18)	wt-FLT3 (N = 185)	p (ITD vs WT)
DFS, 2 years	10% (0–21%)	49% (14–84%)	40% (30–50%)	0.001
Median, weeks	28 (21–35)	102 (45-NR)	63 (41–85)	
EFS, 2 years	6% (0–11%)	48% (23–74%)	26% (19–34%)	< 0.00001
Median, weeks	20 (12–24)	61 (0–132)	41 (29–52)	
OS, 2 years	13% (2.5–23%)	43% (13–73%)	47% (39–56%)	< 0.00001
Median, weeks	33 (29–37)	84 (58–110)	90 (53–126)	

(c). Patients with Poor Risk-AML			
Parameter (95% CI)	FLT3-Mutated (N =11)	wt-FLT3 (N = 133)	p
EFS, 2 years	0% (0%)	6% (1–10%)	0.37
Median, weeks	8 (5–11)	11 (7–15)	
OS, 2 years	0% (0%)	11% (5–17%)	0.44
Median, weeks	55 (35–74)	24 (18–30)	

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; OS, overall survival; NR, not reached.

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; OS, overall survival; NR, not reached.

^IIncludes 10 patients with double mutations

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; OS, overall survival; NR, not reached.

Table 4

Multivariate Analysis for EFS

Parameter	Multivariate		
	HR	95% CI	p
A. CBF AML			
<i>FLT3</i> (continuous variable)	1.28	0.04–37.78	0.88
PS	1.77	1.07, 2.91	0.03
B. NK-AML (<i>FLT3-ITD</i> and <i>wt-FLT3</i>)			
Age	1.02	1.01–1.03	0.005
Race (White vs Non-White)	0.56	0.39–0.81	0.002
Creatinine	1.39	1.09–1.77	0.007
<i>FLT3-ITD</i> (continuous variable)	3.10	1.09–8.78	0.03
Treatment (Ara-C vs. No-Ara-C)	1.65	1.05–2.59	0.03
Interaction Term			
• <i>FLT3-ITD</i> *Treatment	0.66	0.30–1.47	0.31
C. NK-AML (<i>FLT3-TKD</i> and <i>wt-FLT3</i>)			
Age	1.03	1.01–1.04	0.0001
Race (White vs. Non-White)	0.47	0.30–0.73	0.001
Creatinine	1.47	1.03–2.09	0.003
Prior Chemotherapy/XRT	1.98	1.22–3.22	0.005
<i>FLT3-TKD</i> (continuous variable)	0.57	0.11–2.76	0.48
Treatment (Ara-C vs. No Ara-C)	0.74	0.45–1.23	0.25
Interaction Term			
• <i>FLT3-TKD</i> *Treatment	0.88	0.32–2.46	0.81
D. Poor Risk AML			
Age	1.02	1.01–1.04	0.01
<i>FLT3</i> (continuous variable)	4.24	0.15–117.87	0.39
Treatment (Ara-C vs. No Ara-C)	1.26	0.77–2.04	0.35
Interaction Term			
• <i>FLT3</i> Mut* <i>Treatment</i>	0.52	0.07–3.79	0.52