REGULAR ARTICLE

blood advances

Co-occurrence of *FLT3*-TKD and *NPM1* mutations defines a highly favorable prognostic AML group

Prajwal Boddu, Hagop Kantarjian, Gautam Borthakur, Tapan Kadia, Naval Daver, Sherry Pierce, Michael Andreeff, Farhad Ravandi, Jorge Cortes, and Steven M. Kornblau

Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX

Key Points

- *FLT3*-TKD/*NPM1* double mutation status is associated with superior relapse-free survival compared with *NPM1*-only–mutated AML.
- Allogeneic stem cell transplant in complete responders does not improve outcomes in *FLT3*-TKD/*NPM1* AML patients.

Although *FLT3* internal tandem duplication (ITD) mutations in acute myeloid leukemia (AML) confer an adverse prognosis, co-occurrence with a nucleophosphomin (*NPM1*) mutation partially improves response and survival outcomes. In contrast, simultaneous *NPM1* and *FLT3* tyrosine kinase domain (TKD) mutations were reported to improve response over that of an isolated *NPM1* mutation in one as yet unverified report. To validate this, we explored the impact of the co-occurrence of *FLT3*-TKD and *NPM1* mutations on clinical outcomes. Study populations included 21 patients (8%) with *FLT3*-TKD^{+}*NPM1*$^{+}$ mutated, 18 patients (7%) with *FLT3*-TKD-only–mutated, 117 patients (44%) with *NPM1*-only–mutated, and 107 patients (41%) with *FLT3*-ITD^{+}*NPM1*–mutated AML. Compared with *NPM1*$^{+}$-only–mutated AML, *FLT3*-TKD/*NPM1* double mutation status was associated with a significantly superior relapse-free survival (median, not reached vs 18.3 months; $P = .03$) and a trend toward improved overall survival (OS). The presence of *FLT3*-TKD/*NPM1* double mutation status was an independent positive predictor in multivariable analysis. Allogeneic stem cell transplant did not improve outcomes in the *FLT3*-TKD/*NPM1* cohort. Consistent with historical data, the co-mutation status defined a highly favorable prognostic group characterized by high response rates and prolonged disease-free and OS. These study findings substantiate previous data describing this intriguing paradoxical cooperative effect. Our results emphasize the need for elucidating the mechanistic links between *FLT3*-TKD and *NPM1* in future molecular and murine model studies.

Introduction

Nucleophosphomin (*NPM1*) is a ubiquitously expressed nucleocytoplasmic shuttling protein that plays an active role in ribosomal protein assembly, chromatin remodeling, and DNA repair, replication, and transcription.[1-3] Mutations in the *NPM1* gene are noted in ~35% of acute myeloid leukemia (AML) cases.[4] The favorable prognosis conferred by *NPM1* mutations was most recently confirmed in a meta-analysis, which demonstrated *NPM1* mutations to be associated with higher complete remission (CR) rates and prolonged disease-free and overall survival (OS).[5] In regards to FMS-like tyrosine kinase 3 (*FLT3*), the more common internal tandem duplication (ITD) is associated with short remission durations and high relapse rates after conventional induction therapy,[6] whereas the less frequent tyrosine kinase domain (TKD) mutations are of unclear prognostic relevance. The incidence of ITD and TKD mutations in *FLT3*-mutated AML vary slightly according to age, clinical risk groups, and cytogenetic profile.[6] Although both classes result in

Submitted 2 June 2017; accepted 12 July 2017. DOI 10.1182/bloodadvances.2017009019.

The full-text version of this article contains a data supplement.

constitutive activation of the FLT3 receptor, differences in receptor activation likely account for the different clinical and biological features observed for these mutations.[7] In vitro studies have demonstrated that *FLT3*-ITD and *FLT3*-TKD mutants harbor differing gene expression profiles, especially in regards to *STAT5* target gene expression.[8]

Murine models have shown mutant *NPM1* and *FLT3*-ITD to exhibit a marked and potent molecular synergy toward driving AML pathogenesis.[9] Patients with *FLT3*-ITD and *NPM1* mutations have improved response rates and disease-free and OS compared with those who only have the *FLT3*-ITD mutation, although outcomes are inferior to *NPM1*-only–mutated AML.[5] In a previous study, Bacher et al[10] showed that, in contrast to the prognostic impact of *FLT3*-ITD on *NPM1*, *FLT3*-TKD has an additional favorable impact on outcomes in *NPM1*-mutated AML, suggesting a cooperative positive effect. This favorable effect of *FLT3*-TKD-NPM1 on OS compared with the overall AML population was recently confirmed by Pappaemanuil et al.[11] However, case numbers in this particular group in the Bacher et al[10] study were few. We therefore sought to validate this observation in our sample cohort.

Methods

Adult (≥18 years of age) patients newly diagnosed with AML (by standard criteria) who were treated at MD Anderson Cancer Center from January 2009 to January 2017 were evaluated. The study was approved by the institutional internal review board. Informed consent was obtained following institutional guidelines, and patients were treated in accordance with the rules of the Declaration of Helsinki. Molecular mutation analyses on *FLT3* and *NPM1* mutations, from January 2010 to September 2012, were performed by using a single gene testing approach of polymerase chain reaction amplification followed by Sanger sequencing, using previously described methodology.[12] From September 2012 on, *NPM1* and *FLT3* testing was performed within our panel-based, next-generation sequencing hematologic malignancy platform, as previously described.[13] *FLT3* AML cases for which *NPM1* mutation analysis was not performed, and vice versa, were excluded. Minimal residual disease elimination status, assessed by clearance of *NPM1*,[14] was also collected. The study population was grouped into 4 study cohorts: (1) *FLT3*-ITD$^+$*NPM1*$^+$, (2) *FLT3*-TKD$^+$*NPM1*$^+$, (3) *NPM1* only, and (4) *FLT3*-TKD only. Patients were treated with a variety of frontline protocols active during this period, and these were broadly classified into: (1) high- or intermediate-intensity idarubicin with cytarabine–based regimens, and (2) low-intensity hypomethylating agent– and low-dose cytarabine–based regimens. Response on hypomethylating agents was classified based on achieving CR or partial response; stable disease was classified as nonresponse. Categorical variables were compared by using the χ^2/Fisher's exact test, and continuous variables were compared by using the Mann-Whitney *U* test. Response criteria were graded according to international working group criteria.[15] OS was measured from time of treatment to date of death or censored at last follow-up date. Relapse-free survival (RFS) was calculated from time of remission to date of death or relapse, or censored at last follow-up date. Response-survival relationships were studied by multivariable Cox regression analysis, and covariates were chosen by stepwise variable selection procedures. Statistical significance was defined as a 2-sided *P* value of $<.05$.

Results

A total of 1319 patients were evaluated during this period, and 1282 had an *FLT3* mutational analysis available; of these, 239 patients (19%) had ITD and 60 patients (5%) had TKD mutations. *NPM1* mutational analysis was available for 1215 patients, and 239 patients (20%) were positive for the mutation. Study populations included 21 patients (8%) with *FLT3*-TKD$^+$*NPM1*$^+$ mutated, 18 patients (7%) with *FLT3*-TKD only mutated, 117 (44%) with *NPM1* only mutated, and 107 (41%) with *FLT3*-ITD$^+$*NPM1*–mutated AML. Ten cases in the *FLT3*-ITD$^+$*NPM1* cohort had an additional *FLT3*-TKD mutation, whereas the *FLT3*-TKD$^+$*NPM1*$^+$ cohort excluded cases with concurrent *FLT3*-ITD mutation. Of note, in regards to TKD mutations, all were *FLT3*-D835 point mutations except one case with a TKD I836 deletion. The median age of the entire cohort was 62 years (range: 17-88 years); 55% of patients were aged ≥60 years. Patient and disease features and differences in baseline characteristics between cohorts are outlined in Table 1.

We next evaluated the effect of TKD mutation on outcome. The CR rate did not differ between patients with a TKD domain mutation, whether they had a *NPM1* mutation (81%; reference) or not (77%, $P > .99$), as compared to those with an *NPM1* mutation only (90%; $P = .27$), those with both an ITD and *NPM1* mutation (84%; $P = .75$). The CR rate was similar in patients with *FLT3*-TKD$^+$*NPM1*$^+$ and *NPM1*$^+$ only mutated (81% vs 90%; $P = .27$). However, there were marked differences in the relapse rates and the median remission durations. Only 24% (4 of 17 patients) of *FLT3*-TKD$^+$*NPM1*$^+$ patients relapsed compared with 70% (10 of 14 patients) of *TKD*$^+$-only cases ($P = .01$), 58% (61 of 105 patients) of *NPM1*$^+$-only cases ($P = .01$), and 50% (45 of 90 patients) ITD$^+$*NPM1* mutant cases ($P = .06$), suggesting that *FLT3*-TKD$^+$*NPM1*$^+$ patients are significantly less likely to relapse. Likewise, RFS was not reached (NR) in the *FLT3*-TKD$^+$*NPM1*$^+$ mutant patients compared with 18.3 months for *NPM1*$^+$only, 23.4 months for *FLT3*-ITD$^+$*NPM1*$^+$, and 7.2 months for TKD$^+$*NPM1* wild-type cases ($P = .03$).

OS was superior in the *FLT3*-TKD$^+$*NPM1*$^+$ cohort when compared with the *FLT3*-TKD$^+$-only cohort (median, NR vs 13.8 months; $P = .03$) (Figure 1A); there were trends toward improved OS in ITD$^+$*NPM1* mutant cases (NR vs 25.8 months; $P = .07$). Survival was not statistically significant when compared with *NPM1*$^+$-only mutated AML patients (median, NR vs 33.7 months; $P = .29$). A similar and more obvious pattern was observed in a subset analysis of patients treated with idarubicin with cytarabine–based regimens (Figure 1B; supplemental Table 1). RFS was significantly superior in *FLT3*-D835$^+$*NPM1*$^+$ AML patients compared with all other study cohorts, *NPM1*$^+$-only mutated patients included (Figure 1C-D). Because transplantation strategies may have influenced outcomes, RFS was evaluated after censoring at the time of transplant and showed a trend toward superior survival in the *FLT3*-TKD$^+$*NPM1*$^+$ cohort when compared with *NPM1*$^+$-only patients (median, NR vs 24.6 months; $P = .08$; data not shown).

Although numbers were small, we noticed a similar trend among double *FLT3* mutants (*FLT3*-ITD and TKD), where those without NPM1 performed poorly, with survival outcomes equivalent to those with *FLT3*-TKD only mutations (12.3 vs 13.8 months, $P = .71$). Furthermore, triple (TKD$^+$ITD$^+$*NPM1*$^+$)-mutant status was associated with equivalent survival compared with *NPM1*$^+$-only status (supplemental Figure 2). Eight patients proceeded to transplant after achieving their first CR; there was no difference in pretransplant

Table 1. Baseline patient and disease characteristics

Characteristics	*FLT3*-TKD$^+$*NPM1*$^+$ (N = 21)	*FLT3*-TKD$^+$ only (N = 18)	*P* (*FLT3*-TKD$^+$*NPM1*$^+$ vs *FLT3*-TKD$^+$)	*NPM1*$^+$ only (N = 117)	*P* (*FLT3*-TKD$^+$*NPM1*$^+$ vs *NPM1*$^+$)	*FLT3*-ITD$^+$*NPM1*$^+$ (N = 107)	*P* (*FLT3*-TKD$^+$*NPM1*$^+$ vs *FLT3*-ITD$^+$*NPM1*$^+$)
Age, median (range), y	60 (45-83)	58 (28-84)	.34	63 (17-88)	.10	60 (23-86)	.44
Sex, male, n (%)	13 (62)	8 (44)	.34	58 (50)	.63	38 (36)	.03
WBC count, median (range), $\times 10^9$/L	3 (1-69)	17 (1-103)	.16	7 (0-100)	.23	8 (0-378)	.17
Platelet count, median (range), $\times 10^9$/L	25 (2-111)	46 (12-217)	.045	45 (3-535)	.01	30 (1-326)	.45
Hemoglobin, median (range), g/dL	10 (8-13)	10 (8-12)	.84	9 (7-14)	.67	9.5 (5-12)	.35
ANC, median (range), $\times 10^9$/L	1 (0-13)	0 (0-22)	.85	1.1 (0-23.3)	.40	.4 (0-36)	.29
BM blast, median (range), %	82 (55-95)	82 (22—94)	.52	69 (26-92)	.03	75 (21-97)	.45
Absolute peripheral blast count, median (range), $\times 10^9$/L	37 (0-94)	4.6 (0-95)	.59	19 (0-95)	.94	3 (0-367)	.06
LDH, median (range), IU/L	796 (483-3193)	842 (390-9508)	.98	817 (315-11912)	.36	990 (424-6639)	.61
Therapy-related AML status	6 (29)	6 (33)	>.99	21 (20)	.25	13 (12)	.09
Cytogenetics							
Adverse, n (%)	1 (5)	4 (22)	.18	6 (5)	.99	0 (0)	.16
Intermediate, n (%)	18 (95)	14 (78)		106 (95)		97 (100)	
Favorable, n (%)	0 (0)	0 (0)		0 (0)		0 (0)	
Unevaluable metaphases, n	2	0		5		10	
Mutations, n/N (%)							
RAS	5/21 (24)	7/18 (39)	.49	33/116 (28)	.79	10/104 (10)	.13
CEBPA	2/19 (11)	0/11 (0)	.51	10/87 (11)	.99	10/83 (12)	.99
DNMT3A	4/14 (29)	2/7 (29)	1.00	20/64 (31)	1.00	21/69 (30)	.99
IDH1/2	5/21 (24)	2/11 (22)	>.99	30/91 (33)	.60	31/92 (34)	.45
TP53	0/15 (0)	0/7 (0)	>.99	3/56 (5)	>.99	2/59 (3)	>.99
Induction regimens, n (%)							
High/intermediate-intensity cytarabine-based regimens	17 (81)	12 (67)	.46	72 (62)	.14	75 (70)	.43
Low-intensity–based regimens	4 (19)	6 (33)		45 (38)		32 (30)	
Consolidation treatments among responders, n (%)							
Chemotherapy	8/17 (47)	6/14 (43)	>.99	89/105 (85)	.001	49/90 (54)	.61
Transplant	9/17 (53)	8/14 (57)		16/105 (15)		41/90 (46)	

ANC, absolute neutrophil count; BM, bone marrow; LDH, lactate dehydrogenase. WBC, white blood cell.

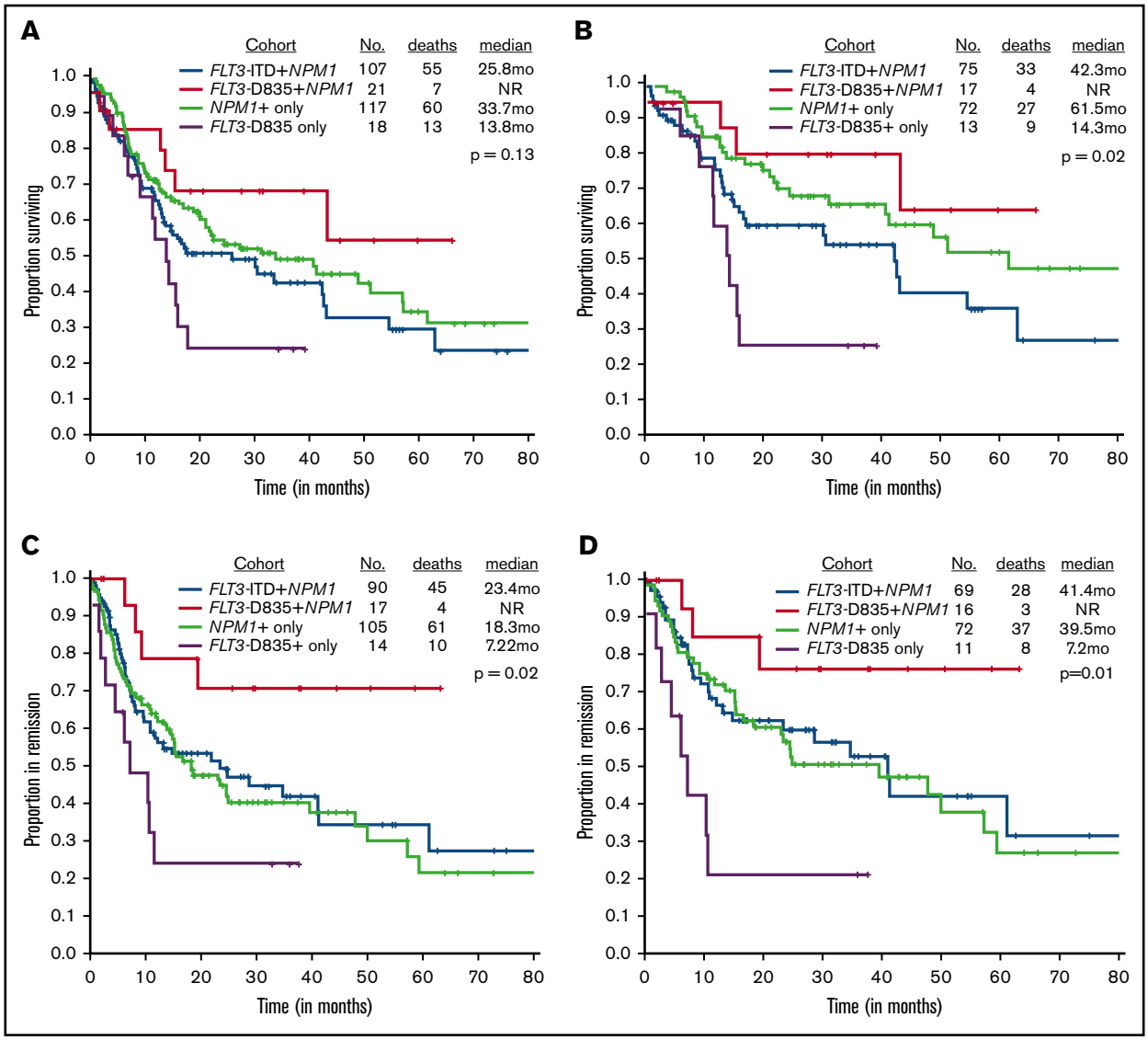

Figure 1. Kaplan-Meier survival curves comparing study cohorts. OS by FLT3 and NPM1 status in (A) all cases and (B) patients treated on intensive cytarabine-based treatment regimens. RFS by FLT3 and NPM1 status in (A) all cases and (B) patients treated on intensive idarubicin and cytarabine–based treatment regimens.

minimal residual disease status or age in the cohort of patients undergoing transplant versus those treated only with chemotherapy. Importantly, undergoing transplant after CR did not improve RFS (supplemental Figure 1). In multivariable cox regression analysis in *NPM1*$^+$-mutated AML, *FLT3*-TKD$^+$ status was an independent favorable prognostic factor for RFS, even after accounting for transplant (supplemental Table 2). However, *FLT3*-TKD$^+$*NPM1*$^+$ mutation status did not emerge as a significant predictor of OS on multivariable analysis (supplemental Table 2).

Discussion

Results from our study are consistent with data from previous studies, which have similarly shown *FLT3*-TKD$^+$*NPM1*$^+$ mutation status to be associated with superior RFS compared with *NPM1*$^+$ alone.[10] The superior RFS in our study cohort did not translate to an improved OS, which was likely due to the small sample size estimates. More intriguing, however, is the paradoxical cooperative favorable effect on *NPM1* conferred by *FLT3*-TKD, a phenomenon not observed between *FLT3*-ITD and *NPM1* (Figure 1).[5] One may surmise, based on the similar response rates observed between *FLT3*-TKD$^+$*NPM1*$^+$ and *NPM1*$^+$-only mutation cohorts, that favorable effects are likely due to durable CRs effected by chemotherapy on an inherently biologically indolent disease. As alluded above, *FLT3*-ITD and *FLT3*-TKD mutants show distinct gain-of-function phenotypes with distinct differences in signaling properties and gene expression patterns.[8,16] Our results emphasize the need for elucidating the mechanistic links between *FLT3*-TKD and *NPM1* in future molecular and murine model studies.

Acknowledgments

This work was supported in part by MD Anderson Cancer Center Support Grant CA016672 and award P01 CA049639 from the National Institutes of Health, National Cancer Institute.

Authorship

Contribution: P.B. and S.M.K. designed the study, collected data, and wrote and reviewed the manuscript; S.P. provided data for the study and reviewed the manuscript; and H.K., G.B., T.K., N.D., M.A., F.R., and J.C. provided patients for the study and were involved in writing and reviewing the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Steven M. Kornblau, Department of Leukemia, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Box 448, Houston, TX 77030; e-mail: skornblau@mdanderson.org.

References

1. Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. Nucleophosmin regulates the stability and transcriptional activity of p53. *Nat Cell Biol*. 2002;4(7):529-533.
2. Borer RA, Lehner CF, Eppenberger HM, Nigg EA. Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell*. 1989;56(3):379-390.
3. Lindstrom MS. NPM1/B23: A multifunctional chaperone in ribosome biogenesis and chromatin remodeling. *Biochem Res Int*. 2011;2011:195209.
4. Verhaak RG, Goudswaard CS, van Putten W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. *Blood*. 2005;106(12):3747-3754.
5. Liu Y, He P, Liu F, et al. Prognostic significance of NPM1 mutations in acute myeloid leukemia: A meta-analysis. *Mol Clin Oncol*. 2014;2(2):275-281.
6. Levis M. FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013? *Hematology Am Soc Hematol Educ Program*. 2013;2013:220-226.
7. Laine E, Chauvot de Beauchêne I, Perahia D, Auclair C, Tchertanov L. Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region: implications for dimerization and activation mechanisms. *PLOS Comput Biol*. 2011;7(6):e1002068.
8. Janke H, Pastore F, Schumacher D, et al. Activating FLT3 mutants show distinct gain-of-function phenotypes in vitro and a characteristic signaling pathway profile associated with prognosis in acute myeloid leukemia. *PLoS One*. 2014;9(3):e89560.
9. Mupo A, Celani L, Dovey O, et al. A powerful molecular synergy between mutant Nucleophosmin and Flt3-ITD drives acute myeloid leukemia in mice. *Leukemia*. 2013;27(9):1917-1920.
10. Bacher U, Haferlach C, Kern W, Haferlach T, Schnittger S. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters–an analysis of 3082 patients. *Blood*. 2008;111(5):2527-2537.
11. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209-2221.
12. Patel KP, Ravandi F, Ma D, et al. Acute myeloid leukemia with IDH1 or IDH2 mutation: frequency and clinicopathologic features. *Am J Clin Pathol*. 2011;135(1):35-45.
13. DiNardo CD, Ravandi F, Agresta S, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. *Am J Hematol*. 2015;90(8):732-736.
14. Ivey A, Hills RK, Simpson MA, et al; UK National Cancer Research Institute AML Working Group. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016;374(5):422-433.
15. Gotlib J, Pardanani A, Akin C, et al. International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) & European Competence Network on Mastocytosis (ECNM) consensus response criteria in advanced systemic mastocytosis. *Blood*. 2013;121(13):2393-2401.
16. Choudhary C, Schwäble J, Brandts C, et al. AML-associated Flt3 kinase domain mutations show signal transduction differences compared with Flt3 ITD mutations. *Blood*. 2005;106(1):265-273.