fms Like Tyrosine kinase3- Internal Tandem Duplication (FLT3-ITD) in Acute Myeloid Leukemia, Mutation Frequency and its Relation with Complete Remission, 2007- 2008

Emami AH,[1] Shekarriz R,[1] Meysamie A,[2] Sharifian RA,[1] Safaei R,[1] Toogeh GhR,[1] Keihani M,[1] Shekarriz R[3]

[1]Hematology-Oncology Department, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2]Community Medicine Department, Faculty of Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
[3]Community Medicine Department, Faculty of Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Shekarriz Ramin, MD; Fellowship of Hematology-Oncology.
Hematology ward, Imam Khomeini Hospital, Keshavarz Blv., Tehran, Iran
E-mail: raminshf@gmail.com
Tel: 09123061858

Abstract:

Introduction: About half of acute myeloid leukemia (AML) adult patients have no cytogenetic abnormalities as a main determinant of complete remission after treatment, so other markers are needed such as FLT3-ITD (Fms-like Tyrosine kinase3-internal tandem duplication) mutations in patients with normal karyotype. The objective of this study was assessing the frequency of FLT3-ITD mutations and its relation with complete remission in different FAB (French- American- British) and cytogenetic subgroups of AML patients who had been hospitalized at Tehran Imam Khomeini Hospital, hematology ward.

Methods: The current study, was a cross sectional descriptive study which was performed during the years 2007-2008. Population frame were consecutive patients whose diseases were confirmed and who had been hospitalized in Tehran Imam Khomeini Hospital, hematology ward. Contemporary, flowcytometry, cytogenetic and chromosomal studies were performed for the cytogenetic subgroup assessment and to investigate the presence of FLT3-ITD mutation. Finally, complete remission achievement after induction chemotherapy were assessed. The obtained data was entered onto the information forms and analyzed by statistical tests.

Results: Out of 40 patients who participated in this study, 18 (45%) were female and 22 (55%) were male. The median age of the patients with mutation was 33 years of age, and the ones without mutation were 39.5. M1, M2 and M_4 FAB subgroups, with respectively 60, 37.5 and 35.7%, had the most occurrence of mutation. There was no significant relationship between mutation and the FAB subgroups (P=0.45). Favorable, intermediate and adverse cytogenetic risk groups had respectively 10, 37 and 66.7% mutations and 69.2% of the patients were in the normal karyotype group. Seventeen (42.5%) of the 40 patients achieved complete remission. 17.6% of them had mutations. There was no relationship between mutation and complete remission (P=0.085).

Conclusion: There was no relationship between the presence of FLT3-ITD mutation and complete remission achievement following chemotherapy.

Keywords: Acute Myeloid Leukemia, FLT3-ITD Mutation, FAB Subgroups, Cytogenetic Risk Groups

Received: 25, Aug., 2009
Accepted: 26, Sep., 2009

Introduction:

The development of cancer in human beings is the consequence of genetic changes accumulating in the malignant cells, frequently detectable as gain or loss of genetic material, chromosomal translocations, chromosomal deletions, or even characterized by genomic instability. However, several malignancies are considerably different because they lack gross genomic alterations. As an example of this, about half of all adult patients with acute myelogenous leukemia (AML) lack any detectable cytogenetic abnormalities and thus display a normal karyotype.(1)

Patients with acute myeloid leukemia (AML) are divided into three cytogenetic groups: Favorable, intermediate and adverse. AML patients with normal karyotype usually are in an intermediate risk group and clinical outcome of them is quite

variable. Moreover, the choice of appropriate consolidation therapy after an achievement of complete remission in these patients is not clear. So, additional markers with prognostic importance are required in order to detect clinically relevant subgroups in AML patients with normal karyotype.(2-4)

FLT3-ITD mutation (fms-like tyrosine kinase-internal tandem duplication) are located on the juxtamembrane regions of the class III receptor tyrosine kinase family and FLT3 plays an important role with FLT3 ligand in the early stages of hematopoiesis. Their interaction regulates the growth of pluripotent hematopoietic stem cells, early progenitor cells and immature lymphocytes as well as leukemic cells. It has been demonstrated that FLT3 receptor activation causes a proliferation of AML cells in vitro, as it appears to both stimulate activation and inhibit apoptosis of the cells.(5) FLT3-ITD as a somatic mutation in FLT3 genes has been reported in about 20-23% of adult patients with AML.(6)

AML patients with FLT3-ITD mutation commonly have similar characteristics like normal cytogenetic, leukocytosis, high marrow blast percentages and monocytic differentiations.(7,8) In the majority of reports, presence of FLT3-ITD mutation in AML patients has been associated with an increased relapse rate and decreased overall survival rate.(9)

Generally, it is reported that there is no difference between AML patients with or without mutant FLT3 in the achievement of complete remission. However most studies concerning this clinical parameter have used the results of patients treated with intensive chemotherapy regimens and available data suggests that conventional 7+3 regimen result in fewer remission rate in this group of patients.(10)

In FLT3-ITD(+) patients small molecule FLT3 inhibitors develop. When blast cells with this mutation are more sensitive to these inhibitors in vitro, they especially benefit from addition of FLT3 inhibitors to conventional chemotherapy.(11-12)

Moreover, it has been suggested that FLT3-ITD is a poor prognostic marker and that must be used as an indication for transplantation and other investigational therapies.(13-14)

According to the NCCN (National Comprehensive Cancer Network) guidelines, AML patients with normal karyotype and isolated FLT3-ITD mutations are ranked in the poor risk group.(15-16)

The aim of this cross-sectional study was to identify FLT3-ITD mutation frequency and its association with complete remission attainment in different FAB and cytogenetic subgroups of AML patients who had been admitted to the hematology ward of Tehran Imam Khomeini Hospital.

By identifying this mutation in acute myeloid leukemia patients, and, by placing them in a more adverse risk subgroup, we can recommend them for SCT (Stem Cell Transplantation). Through follow up with these patients, we can estimate relapse rate, disease free survival rate and overall survival rate in further studies.

Methods and Materials:

The current study was a cross-sectional descriptive study which was performed during the years 2007-2008. The study population was consecutive patients with confirmed acute myeloid leukemia who had undergone peripheral blood smear, bone marrow aspiration and biopsy. The samples were studied by hematologist-medical oncologist and pathologist. Then the patients were admitted to the hematology ward of Tehran Imam Khomeini hospital. Contemporary, flowcytometry (to identify immunophenotype and subgroups according to FAB classification), cytogenetic assessment and chromosomal studies to identify cytogenetic risk groups, as well as the investigation of the presence of FLT3-ITD mutation were performed on bone marrow aspiration samples. Each of them was performed in a separate laboratory unit. Criteria for inclusion in this study were patients with newly diagnosed AML in the age group of 15 to 60. Performance status was based on ECOG$\leq$ 2, who for those who were admitted to the hematology ward of Tehran Imam Khomeini hospital. Patients out of this age range, with PS> 2, EF< 50%, serum creatinine levels of more than 2 miligram per deciliter, bilirubin concentration of equal or more than 5 miligram per deciliter, patients with relapsed AML and patients with chronic myeloid leukemia blast crisis and MDS patients were excluded from the study. AML patients, those in the other than M_3 group, were treated with remission induction regimen including seven days continuous intravenous infusion of cytosine arabinoside (100mg/m2/day) and three days intravenous infusion of daunorobicin (45mg/m2/day). Bone marrow aspiration was performed on days 14 and 28 to identify the existence of residual disease and the achievement of remission, respectively.

AML-M_3 patients were treated with ATRA and daunorobicin protocol. In the recovery phase of the blood cell count (between days 28- 35), a bone marrow aspiration was performed to observe maturation toward mature myeloid cells and to

achieve of remission. Dependent variable in this study was complete remission following induction chemotherapy and independent variables were FLT3-ITD mutation, cytogenetic abnormalities, age and gender. The definition of acute myeloid leukemia based on the WHO definition was assumed the presence of equal or more than 20% myeloblasts in bone marrow aspiration. The definition of acute promyelocytic leukemia was based on morphology and the dominancy of promyelocytes (more than 30%) in bone marrow aspiration. The presence of t(15,17) (being positive for PML-RARα) in cytogenetic assessment and the absence of HLA-DR in flowcytometry were also considered for a confirmation of acute promyelocytic leukemia. Complete remission was defined based on bone marrow blast percentage < 5% with equal or more than 20% cellularity on day 28 after the beginning of treatment, in addition to the absence of evidence of extramedullary disease, a platelet count of more than 100,000 per microliter and a neutrophil count more than 1,000 per microliter. Patients with inv(16), t(8,21), t(15,17) were considered as favorable cytogenetic group and patients with complex karyotypes (equal to or more than, 3 chromosomal abnormalities), monosomy 5 and 7, del(5q), abn(3q), del(7q), t(6, 9), t(9, 22) and 11q23 abnormalities except for t(9, 11) were considered to be in the poor cytogenetic risk group (based on NCCN guidelines). Patients with normal karyotype, trisomy 8, t(9, 11) and other abnormalities were not placed in the favorable or poor cytogenetic risk groups. They were considered to be in the intermediate risk group. DNA assessment for the presence or absence of FLT3-ITD mutation in bone marrow aspirate samples was performed through the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism), the widely accepted and standard method for this purpose.(17) All data and demographic characterizations were recorded on a specifically designed information form. Using SPSS software (version 11.5) the data was analyzed with Chi-square and One-way ANOVA statistical tests.

Ethical Considerations: All the patients were ensured that the obtained information would be used just for investigational purposes. No diagnostic procedure was done without obtaining their personal consent.

Results

Out of forty patients who participated in this study, 18 (45%) were female and 22 (55%) were male. The mean age of the participants was 37.8 years old ± 11.9 SD. The mean of the participants' leukocyte count was 30992/5 ± 34134/9 SD. The mean of serum LDH level was 851.25 ± 369.9 SD and the mean of the bone marrow blast percentage was 66.95% ± 17.3 SD. No one had lymphadenopathy. Five (12.5%) of the patients had hepatomegaly and 20 (50%) patients had splenomegaly.

Considering the AML subgroups based on the FAB classification, no patient was in M_0, M_6, M_7 subgroups. Five patients (12.5%) were in M_1, 8 (20%) in M_2, 8 (20%) in M_3, 14 (35%) in M_4 and 5 patients (12.5%) were in the M5 subgroup.

Seventeen (42.5%) of patients had cytogenetic abnormalities. regarding cytogenetic risk group, 10 patients (25%) were in the favorable group, 27 (67.5%) were in an intermediate group and 3 (7.5%) were in an adverse group.

Thirteen patients (32.5%) had FLT3-ITD mutations. Seventeen patients (42.5%) achieved complete remission.

Table- 1, compares the clinical and laboratory manifestations of AML patients with and without FLT3-ITD mutation. According to the table, there was no significant relationship between variables including gender, age, presence of hepatomegaly, presence of splenomegaly, leukocyte count, bone marrow blast percentage and serum LDH level as well as FLT3-ITD mutations. In this table, mean and median age in the FLT3-ITD (+) group were less than in the FLT3-ITD (-) group, but this difference is not significant statistically. Moreover, the mean of the leukocyte count, bone marrow blast percentage and serum LDH level was higher in the FLT3-ITD(+) group than in the FLT3-ITD(-) group but these differences are not significant, either (Table-1 P- values).

Table- 2 shows a frequency distribution of FLT3-ITD mutation based on the FAB classification subgroup, cytogenetic risk subgroup and complete remission. The most relative frequency of FLT3-ITD mutation in the FAB classification subgroups is in M_1 (60%). The relative frequency of FLT3-ITD mutation in M_2, M_4, M_5 and M_3 are 37.5, 35.7, 20 and 12.5%, respectively while there is no significant statistical relationship between FAB subgroup and the FLT3-ITD mutation.

According to Figure- 1, which shows a frequency distribution for each of the FAB subgroups in FLT3-ITD(+) and in the FLT3-ITD(-) groups, 38.5% of patients with FLT3-ITD (+) were in the M_4 subgroup and patients within the M_1, M_2 FAB subgroup each of them was 23%. In the subgroup M_3, M_5 each one was 7.7%.

Table- 1: Comparing the clinical and laboratory features of AML patients with and without FLT3-ITD mutation

		FLT3-ITD$^{(+)}$ (n=13)	FLT3-ITD$^{(-)}$ (n=27)	P-value
Gender	**Male** Frequency (%)	9 (69.2)	13 (48.1)	0.209
	Female Frequency (%)	4 (30.8)	14 (51.9)	
Age (y)	Mean ± SD	34.46 ± 12.24	39.27 ± 11.74	0.215
	Median	33	39.5	
	Range	15-55	17-60	
WBC	Mean ± SD	44930 ± 31725	25100 ± 34148	0.073
Serum LDH level	Mean ± SD	1007.7 ± 361.6	786.5 ± 358.7	0.063
Bone marrow blast percentage	Mean ± SD	72.46 ± 18.31	64.04 ± 16.7	0.165
Hepatomegaly	Frequency (%)	3 (23.1)	2 (7.4)	0.307
Splenomegaly	Frequency (%)	9 (69.2)	11 (40.7)	0.091

Table- 2: The frequency distribution of FLT3-ITD mutation based on FAB classification subgroup and cytogenetic risk subgroup

			FLT3-ITD$^{(+)}$ (n=13)	FLT3-ITD$^{(-)}$ (n=27)	Pvalue
FAB subgroup	M_1	Frequency(%)	3 (60)	2 (40)	0.45
	M_2	Frequency(%)	3 (37.5)	5 (62.5)	
	M_3	Frequency(%)	1 (12.5)	7 (87.5)	
	M_4	Frequency(%)	5 (35.7)	9 (64.3)	
	M_5	Frequency(%)	1 (20)	4 (80)	
Cytogenetic risk subgroup	favorable	Frequency(%)	1 (10)	9 (90)	0.125
	intermediate	Frequency(%)	10 (37)	17 (63)	
	adverse	Frequency(%)	2 (66.7)	1 (33.3)	

Table No-3: The frequency distribution of complete remission based on cytogenetic risk subgroup and presence of FLT3-ITD mutation

			Complete Remission(n=17)	No Complete Remission(n=23)	P- value
cytogenetic risk subgroup	favorable	Frequency(%)	8 (80)	2 (20)	0.021
	intermediate	Frequency(%)	8 (29.6)	19 (70.4)	
	adverse	Frequency(%)	1 (33.3)	2 (66.7)	
FLT3-ITD mutation	Yes	Frequency(%)	3 (23.1)	10 (76.9)	0.085
	No	Frequency(%)	14 (51.9)	13 (48.1)	

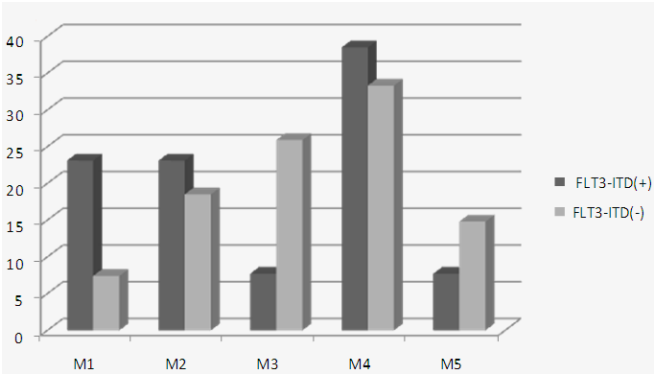

Figure- 1: Comparing the frequency distribution of FAB Subgroups in FLT3-ITD+ group & FLT3-ITD-

According to Table- 2, 66.7% of the patients in the adverse cytogenetic risk subgroup had FLT3-ITD mutation. This rate in the intermediate and favorable subgroups was 37 and 10%, respectively, but there was no significant statistical relationship between the cytogenetic risk subgroup and the FLT3-ITD mutation.

Table- 3 shows the frequency distribution of complete remission based on the cytogenetic risk subgroup and the presence of FLT3-ITD mutation. With respect to the cytogenetic risk subgroup, 80% of patients in the favorable group had achieved complete remission. This percentage in the intermediate and adverse group was 29.6 and 33.3, respectively.

Also, 23.1% of the patients with FLT3-ITD mutation had achieved complete remission. This percentage in the patients without FLT3-ITD mutation was 51.9%. It should be noted that there is a statistically significant relationship between the cytogenetic risk subgroup and complete remission (α=0.05), but, there is no statistically significant relationship between the FLT3-ITD mutation and complete remission.

In individuals with normal karyotype (23 cases), 6 people (26.1%) achieved complete remission and 17 people (73.9%) had not. With respect to these people, there was no significant relationship between the mutation and complete remission achievement (P=0.565). Among those who had the mutation (9 people), two of them (22.2%) had

achieved complete remission and among those who did not have the mutation (14 people), four (28.6%) had achieved complete remission.

Discussion

According to the results of the current study, 69.2% of the AML patients with FLT3-ITD mutation were male and 30.8% of them were female. The male/female ratio was 2.25/1 and there was no significant relationship between gender and FLT3-ITD mutation (Pvalue=0.209). According to the results of AMLCG study published in the Blood journal (2002), mutation in females was more prevalent than in males (1.36/1, P=0.023) and according to UKMRC $AML_{10,12}$ study published in the Blood journal (2001), 52% of AML patients with FLT3-ITD (+) were female and 48% of them were male, which was not significant (P= 0.5). It seems that there was no significant statistical relationship between gender and FLT3-ITD mutation.(18)

In this current study, the median age of the AML patients with FLT3-ITD was 33 years of age and in AML patients without FLT3-ITD, the median age was 39.5 of age, in a published study in journal of Clinical Cancer Research (2005), the median age of AML patients with FLT3-ITD was 52 of age and in AML patients without FLT3-ITD, it was 48 years old (with non-significant Pvalue). In AMLCG study, the median age of AML patients with FLT3-ITD was 54.9 of age and in AML patients without FLT3-ITD, the median age was 57.6 years (with non-significant P-value). Overall, it seems that there is no significant statistical relationship between FLT3-ITD mutation and the median age.

In our study, the WBC count mean at the time of diagnosis in patients with FLT3-ITD and patients without FLT3-ITD were 44,930 and 25,100 respectively, which was consistent with previous studies. Unlike previous studies, in the study, there was no significant statistical relationship between FLT3-ITD mutation and the leukocyte count.

In this current study the mean percentage of bone marrow blasts at the time of diagnosis of the disease, in patients with FLT3-ITD and patients without FLT3-ITD was 72.46% and 64.04% respectively. The results were similar to the results of the AMLCG study, the UKMRC $AML_{10,12}$ study and others. Unlike previous studies, in this study there was no significant statistical relationship between the FLT3-ITD mutation and the bone marrow blast percentage.

In the current study, 38.5% of the patients with FLT3-ITD(+) were in the M_4 subgroup. Patients within the M_1, M_2 FAB subgroup were 23% and M_3, M_5 was 7.7%. The M_1, M_2 and M_4 subgroups with 60%, 37.5% and 35.7%, respectively, had the most incidence of FLT3-ITD mutation. In AMLCG study, M_{3V} (M_3 variant), M_1, M_{5b} and M_4 subgroups had the most incidence of FLT3-ITD mutation respectively, while in the UKMRC $AML_{10,12}$ study M_3, M_4 and M_1 subgroups with 36%, 29% and 27% respectively, had the most incidence of FLT3-ITD mutation.

It seems that in the current study, similar to previous studies, there is no significant statistical relationship between the FLT3-ITD mutation and the AML FAB subgroup.

Regarding the cytogenetic risk subgroups, as it has been seen in previous studies, the FLT3-ITD mutation are more prevalent in normal cytogenetic (karyotype) patients. In this study, 39.1% (9 out of 23) patients with normal cytogenetic (karyotype) had FLT3-ITD mutation and favorable, intermediate and poor cytogenetic risk subgroups had 10%, 37% and 66.7% FLT3-ITD mutation respectively. It is significant that in this study 69.2% of the patients with FLT3-ITD mutation were in the normal karyotype group. The fewer number of patients in the adverse cytogenetic subgroup could be the possible reason for the difference in the presence of mutation between the cytogenetic groups.

In the current study, 17 out of 40 treated patients (42.5%) achieved complete remission. three out of thirteen (23%) patient with FLT3-ITD mutation achieved complete remission while complete remission achievement in patients without FLT3-ITD mutation was 51.9%. 17.6% of the patients who achieved complete remission had FLT3-ITD mutation and 82.4% did not have this mutation. There is no significant statistical relationship between the FLT3-ITD mutation and complete remission achievement, while there is a significant statistical relationship between the cytogenetic risk subgroups and complete remission (α=0.05). There is also no significant statistical relationship between the FLT3-ITD mutation and the complete remission achievement in patients with normal karyotype (23 people). Although in previous studies and literary reviews regarding univariate analysis, there was a borderline significant relationship between the FLT3-ITD mutation and complete remission achievement. With multivariate analysis done in different studies, as mentioned above, the presence of FLT3-ITD mutation does not predict complete remission achievement and the results of the study

is supported by similar studies conducted in this respect.

Considering the importance of parameters like mutation of CEBPA (CCAAT/enhancer binding protein-alfa),(19) different levels of BAALC (Brain and Acute Leukemia cytoplasmic) expression,(20) mutation of NPM1 (nucleophosmin 1) and MLL-PTD (Mixed lineage leukemia-Partial tandem duplication) mutation in clinical outcome of AML patients especially with normal karyotype,(21) it would be necessary to evaluate the above parameters in addition to the FLT3-ITD mutation in order to estimate relapse rate, disease free survival rate and overall survival rate in further studies.

Conclusion:

This study showed that there were no relationships between age, gender and FLT3-ITD mutation. Although the mean of the WBC count and bone marrow blast percentage at the time of disease diagnosis in patients with FLT3-ITD mutation were greater than in patients without mutation, there were no statistically significant relationships between them. This study did not show any relationship between the FLT3-ITD mutation and morphologic FAB classification subgroups. In this study also, a significant relationship between the favorable, intermediate and poor cytogenetic risk groups and FLT3-ITD mutation was not seen, although 62% of patients with FLT3-ITD mutation in this study were in the normal karyotype group.

Similar to previous studies, in this study, there was no statistically significant relationship between the FLT3-ITD mutation and complete remission achievement. The presence of FLT3-ITD mutation did not predict complete remission achievement. Considering that in previous studies with a longer follow up period, FLT3-ITD mutation coincided with higher relapse rate and lower disease free survival and overall survival rates, it seems that longer follow up of patients after a complete remission achievement is essential in determining of relapse rate, disease free survival and overall survival rates in order to identify the negative effect of this mutation on clinical outcomes. By increasing study sample size, especially, the relationship between patients with normal karyotype (in intermediate subgroup) and FLT3-ITD mutation will become clearer. By considering the negative effect of this mutation on long-term clinical outcomes, we will be able to recommend patients with FLT3-ITD mutation after complete remission achievement for Hematopoietic Stem Cell Transplantation (HSCT) or investigational therapies such as FLT_3 inhibitors.

References

1. Steudel C, Wernke M. Comparative Analysis of MLL Partial Tandem Duplication and FLT-3 Internal Tandem Duplications in 956 Adult Patients with AML. GENES, CHROMOSOMES & CANCER, 2003; 37: 237- 251.
2. Grimwade D, Walker H, Oliver F, et al. The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1612 Patient Entered into the MRC AML 10 Trial. Blood, 1998; 92: 2322-33.
3. Suciu S, Mandelli F, De witte T, et al. Allogenic Compared with Autologous Stem Cell Transplantation in the Treatment of Patient Younger than 46 Years with Acute Myeloid Leukemia (AML) in First Complete Remission (CR_1): An Intention- to- Treat Analysis of the EORTC/GIMEMA AML-10 Trial. Blood, 2003; 102: 1232- 40.
4. Slovak ML, Kopecky Ky, Casssileth PA, et al. Karyotypic Analysis Predicts Outcome of Preremission and Postremission Therapy in Adult Acute Myeloid Leukemia: A Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood, 2000; 96: 4075- 83.
5. Dina Yassin, Iman Sidhom. Internal Tandem Duplication of FLT3 Gene in Egyptian Pediatric Acute Myeloid leukemia and Acute Lymphoblastic Leukemia. Journal of the Egyptian Nat. Cancer Ins. 2000; 15 (1): 7-23.
6. Levis M, Smell D: FLT3: It does Matter in Leukemia. Leukemia, 2003; 17 (9): 1738- 52.
7. Gilliland DG, Griffin JD: The Roles of FLT3 in Hematopoiesis and Leukemia. Blood 2002; 100(5): 1532-42.
8. Kottaridis PD, Gale RE, et al. The Presence of a FLT3 Internal Tandem Duplication with AML Adds Important Prognostic to the First Cycle of Chemotherapy: Analysis of 854 Patients from the UKMRC AML 10 and 12 Trials. Blood, 2001; 98(6): 1752-9.
9. Yanada M, Matsuo K, Suzuki T. Prognostic Significance of FLT3 Internal Tandem Duplication and Tyrosine Kinase Domain Mutations for Acute Myeloid Leukemia: A Meta-analysis. Leukemia, 2005; 19(8): 1345-9.
10. Wang L, Lin D, Zhang X, et al: Analysis of FLT3 Internal Tandem Duplication and D835 Mutations in Chinese Acute Leukemia Patients. Leuk Res, 2005; 29(12): 1393-8.
11. Brown P, Meshinchi S, Levis M, et al. Pediatric AML Primary Samples with FLT3-ITD Mutations are Preferentially Killed by FLT3 Inhibition. Blood, 2004; 104: 1841- 1849.
12. Knapper S, Mills KJ, Gilkes AF, et al. The Effects of Lestauritinib (CEP701) and PKC412 ON Primary AML Blasts: The Induction of Cytotoxicity Varies with Dependence on FLT3 Signaling in both FLT3- mutated and Wild- type Cases. Blood, 2006; 108: 3494-3503.
13. Meshinchi S, Arceci RJ, Sanders JE, et al. Role of Allogenic Stem Cell Transplantation in FLT3/ITD– Positive AML. Blood, 2006; 108: 400.
14. Bornhauser M, Illmer T, Schaich M, et al. Improved Outcome after Stem Cell Transplantation in FLT3- ITD-positive AML. Blood, 2007; 2264- 2265.
15. Frohling S, Schlenk RF, Breitruck J, et al. Prognosic Significance of Activating FLT3 Mutations in Younger Adults with Acute Myeloid Leukemia and Normal Cytogenetics: A Study of the AML Study Group ULM. Blood, 2002; 100: 4372- 4380.
16. Bienz M, Ludwig M, Leibundgut EO,et al. Risk assessment in patient with AML and a normal Karyotype . Clinical Cancer Res, 2005; 11: 1416- 1424.

17. Mills KI, Gilkes AF, Walsh V. Rapid and Sensitive Detection of Internal Tandem Duplication and Activating Loop Mutations of FLT3. Br J Haematol, 2005; 130(2):203-8.
18. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 Length Mutations in 1003 Patients with AML: Correlation to Cytogenetics, FAB Subtype, and Prognosis in the AMLCG Study and Usefulness as a Marker for the Detection of Minimal Residual Disease. Blood, 2002; (100): 59- 66.
19. Van Waalwijk B, van Doorn-Khosorvani S, Erpelinck C, et al. Biallelic Mutations in the CEBPA Gene and Low CEBPA Expression Levels as Prognostic Markers in Intermediate- Risk AML. Hematol J, 2003; 31-40.
20. Baldus CD, Tanner SM, Ruppert AS, et al. BAALC Expression Predicts Clinical Outcome of de novo Acute Myeloid Leukemia Patients with Normal Cytogenetics: A Cancer and Leukemia Group B Study. Blood, 2003; 102: 1613- 8.
21. Schlenk RF, Döhner K, Krauter J, et al. Mutations and Treatment Outcome in Cytogenetically Normal Acute Myeloid Leukemia. N Engl J Med, 2008; 358: 1909-18.