FLT3 Internal Tandem Duplication in Acute Myeloid Leukemia with Normal Karyotype

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Background: The presence of FLT3 internal tandem dupulication (FLT3/ITD) in patients with acute myeloid leukemia (AML) with normal karyotype was investigated in order to evaluate its clinical and prognostic significance.

Methods: The FLT3/ITD was studied by PCR assay in bone marrow samples obtained from 123 patients at diagnosis. Ninety patients who received intensive induction chemotherapy were evaluated.

Results: Of total 123 patients, forty-seven (38.2%) demonstrated the aberrant FLT3/ITD. Patients with FLT3/ITD had significantly higher leukocyte counts at presentation than did patients without FLT3/ITD (P=0.04). By multivariate analysis, the FLT3/ITD was an independent prognostic factor of leukemic-free survival (LFS) (P=0.01) in AML patients with normal karyotype.

Conclusion: This study demonstrated that the presence of the FLT3/ITD was a significant factor for poor prognosis in AML patients with normal karyotype. ***(Korean J Hematol 2007;42:250-257.)***

Key Words: FLT3/ITD, Acute myeloid leukemia, Normal karyotype

INTRODUCTION

The *fms*-like tyrosine kinase 3 (FLT3) belongs to the class III receptor tyrosine kinase (RTK) family, which also includes the KIT, FMS, and Platelet-derived growth factor (PDGF) receptors. FLT3 is expressed in early hematopoietic progenitors and its dimerization by FLT3 ligand (FL) induces growth control signals in normal hematopoiesis. The gene encoding FLT3 maps to chromosome band 13q12,[1,2] and an internal tandem duplication (ITD) of the gene (FLT3/ITD) can be detected in 20%～30% of young adults with acute myeloid leukemia (AML).[3,4] The duplication involves a segment of the juxtamembrane (JM)-domain-coding sequence, which frequently in-

접수 : 2007년 7월 27일, 수정 : 2007년 8월 31일
승인 : 2007년 9월 4일
교신저자 : 김형준, 전라남도 화순군 화순읍 일심리 160
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This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health&Welfare, Republic of Korea (01-PJ10- PG6-01GN16-0005).

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volves exon 14 and, less commonly, intron 14 or exon 15, and is always in-frame.[5] In support of a potential role of this mutation in leukemogenesis, FLT3/ITD from AML patients has been shown to induce autonomous proliferation in cytokine-dependent cell lines.[6,7]

Nonetheless, controversy exists as to the prognostic significance of FLT3/ITD. Previous studies have suggested that the mutation is an independent prognostic factor in AML patients,[4,8] but an association with poor outcome has not been established in highly heterogeneous patient populations, such as with respect to age, karyotype, and treatment regimens. Several studies have also shown that FLT3/ITD predicted a poor clinical outcome in AML patients with normal karyotype;[9,10] but it is still a matter of debate as to whether the mutation plays an independent role in the prognosis of those patients.[11]

The primary objective of this study was to determine the prognostic influence of FLT3/ITD in AML patients with normal karyotype.

MATERIALS AND METHODS

1. Patients

Bone marrow (BM) samples from 123 adult AML patients at five different hospitals in Korea were used in this study and were retrospectively analyzed for the presence of FLT3/ITD by PCR on genomic DNA. The 123 patients had all been diagnosed with *de novo* AML with normal karyotype. Of the patients participating in the study, ninety patients received intensive induction chemotherapy and were included in the analysis of survival data. The details of the patients' age, gender, peripheral blood (PB) leukocyte counts,

Table 1. Clinical and biological features in 123 acute myeloid leukemia (AML) patients with normal karyotype according to FLT3/ITD expression

	FLT3/ITD$^+$	FLT3/ITD$^-$	*P*-value
No. of patients (%)	47 (38.2%)	76 (67.5%)	
Age, median (range)	47 (16～79)	53 (18～84)	0.19
Sex			0.50
Male, No. (%)	19 (40.4%)	28 (36.8%)	0.45
Female, No. (%)	36 (76.6%)	40 (52.6%)	
FAB subtypes			0.001
M0	1 (2.1%)	2 (2.6%)	
M1	8 (17.0%)	5 (6.6%)	
M2	13 (27.7%)	41 (53.9%)	
M4	5 (10.6%)	14 (18.4%)	
M5	7 (14.9%)	7 (9.2%)	
M6	8 (17.0%)	0 (0%)	
Unknown	5 (10.6%)	7 (9.2%)	
WBC (/L), median (range)	28.6 (0.9～292,9)	11.0 (0.8～127.2)	0.04
Hb. (g/dL), median (range)	8.3 (14.5～13.1)	7.8 (3.1～14.8)	0.93
Platelets (/L), median (range)	83.0 (28.0～231,0)	75.0 (2.8～261.0)	0.67
BM blasts (%), median (range)	85 (20～95)	75 (7.5～95)	0.74
Remission induction regimens (n=90)			0.853
IDA/BH-AC	20 (33.3%)	20 (80%)	
IDA/AraC	14 (23.3%)	19 (38%)	
IDA/VP-16/AraC	2 (3.3%)	3 (6%)	
IDA/BH-AC/6TG	2 (3.3%)	5 (6%)	
IDA/AraC/G-CSF priming	2 (3.3%)	3 (6%)	

Abbreviations: Hb, hemoglobin; BM, bone marrow; IDA, idarubicin; BH-AC, N^4-behenoyl-1-D-arabinofuranosylcytosine; VP-16, etoposide; 6TG, 6-thioguanine.

and percentages of BM blasts are given in Table 1.

2. Treatment

Of the 90 patients, 40 (44.4%) patients received remission induction therapy consisting of 3 days of idarubicin (IDA) at 12mg/m^2/day and 7 days of N^4-behenoyl-1-D-arabinofuranosylcytosine (BH-AC) (300 mg/m^2/day for patients younger than 40 years old, 200mg/m^2/day for patients older than 40 years old). Thirty-three patients (36.7%) received 3 days of IDA at 12mg/m^2/day and 7 days of cytarabine (Ara- C) at 100mg/m^2/day. Etoposide, 6-thioguanine, or G-CSF was added to the treatment protocols for the remaining patients. If patients failed to achieve complete remission (CR) after the first round of induction chemotherapy, they received reinduction chemotherapy with same regimen. Patients achieving CR received three to four courses of combination chemotherapy or two to three courses of high-dose Ara-C chemotherapy for consolidation. Patients achieving CR were allowed to proceed to allogeneic or autologous stem cell transplantation, but in this study the survival data were evaluated until the day of transplantation.

3. Polymerase chain reaction (PCR) analysis of the FLT3/ITD

Genomic DNA was isolated from pellets of BM samples, such as cryopreserved mononuclear cells, paraffin- or plastic-embedded BM sections, or BM smear slides, at the time of diagnosis using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea) according to the manufacturer's protocol. PCR amplification of genomic DNA was carried out with the appropriate primers using the thermal cycler (PTC 200, MJ Research, Waltham, Mass, USA). A 328-bp fragment encompassing exon 14 and 15 of the wild-type FLT3 gene was amplified by genomic PCR using the primers 5'- CAATTTAGGTATGAAAGCC-3' (sense) and 5'-CTTTCAGCATTTTGACGGC AACC-3' (anti-sense). The reaction contained 2.5mm dNTP, 2.5mm MgCl2, 1×PCR buffer, 0.5 μm of each primer, and 1U Taq. polymerase in a total volume of 20 μL. Samples were amplified using standard PCR conditions: 5-min initial denaturation at 95°C followed by 10 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min; followed by 25 cycles of 95°C for 1 min, 47°C for 30 sec, and 72°C for 1 min; and extension at 72°C for 10 min. PCR products (10 μL) were resolved on 5% polyacrylamide gels using the MADGE system (MadgBio, Grantham and Southampton, UK), stained with ethidium bromide and photographed under ultraviolet light. FLT3/ITD was detected as an abnormal, longer product (Fig. 1).

4. Statistical analysis

The response to initial therapy was evaluated after induction chemotherapy. According to the NCI criteria,[12] CR was defined as a normocellular BM containing less than 5% blasts and showing evidence of normal maturation of other BM elements; an absolute neutrophil count of 1.5×10^9/L or more; a platelet count of 100×10^9/L or more; no blasts in the peripheral blood; and no extramedullary leukemia. Overall survival (OS) was defined as the time from diagnosis to death or transplantation. Leukemic-free survival (LFS) was calculated as survival without relapse, death, or transplantation from the date of first CR. Cytogenetic features were classified according to the classification of the MRC AML 10 trial and the Southwest Oncology Group (SWOG) study.[13,14] The favorable-risk group included patients with inv (16) or t (8;21). Cytogenetics were defined as adverse if they included at least five unrelated abnormalities

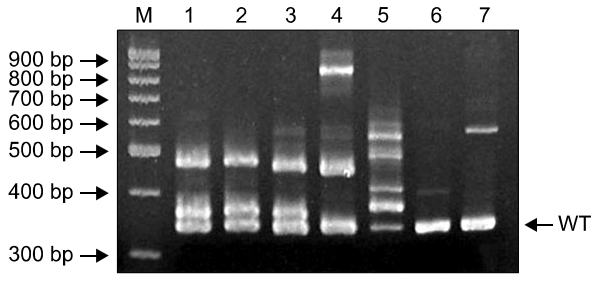

Fig. 1. PCR products of the FLT3/ITD, analyzed by gel electrophoresis. Reverse view of ethidium-bromide-stained 6% polyacrylamide gel. FLT3/ITD was detected as extra-bands in addition to normal band of 328 bp. Lanes 1 to 7 show PCR results in seven patients with FLT3/ITD. M: molecular weight markers, WT: wild type.

(complex karyotype) or one of the following abnormalities: −5, −7, del (5q), abnormal 3q, or t (9;22). An intermediate prognosis was assumed in the presence of either a normal karyotype or any other chromosomal change not encompassed by the favorable or adverse group.

Categorical data were compared for patients with and without FLT3/ITD by means of Fisher's exact test, and continuous variables were compared using the Mann-Whitney *U* test. OS and LFS were analyzed by means of Kaplan-Meier survival curve estimates and log-rank tests for comparison of differences in the distribution of survival for patients with and without FLT3/ITD. OS is defined for all patients in a trial, and measured from the date of diagnosis onto a study until death from any cause. For a patient who is not known to have died by the end of study follow-up, observation of OS is censored on the date he or she was last known to be alive. For a patient underwent allogeneic or autoloug stem cell transplantation, OS is censored on the date of transplantation.

LFS is defined only for patients who achieve CR, and is measured from the date of attaining the leukemia-free state until the date of AML relapse or death from any cause, whichever occurs first. For a patient who is not known to have relapsed or died by the end of study follow-up, observation of LFS is censored on the date of his or her last follow-up examination. For a patient underwent allogeneic or autoloug stem cell transplantation, LFS is censored on the date of transplantation.

Univariate and multivariate Cox's proportional- hazards models were used to analyze the influence of age, gender, leukocyte counts, and peripheral or BM blasts percentage, treatment protocol, and cytogenetics. A *P* value less than 0.05 was considered to be statistically significant; 95% confidence intervals are given. All statistical computations were performed using SPSS software version 12.0. (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Baseline characteristics of the FLT3/ITD$^+$ patients

The mean age of the 123 patients was 49.4 years (range, 16～84 years). In the total group of 123 patients, 47 (38.2%) had an FLT3/ITD. Details of the presenting features of the FLT3/ITD-positive group are given in Table 1. The presence of the mutation was not related to age (P=0.19) or gender (P=0.50). The median peripheral blood (PB) leukocyte counts at diagnosis were increased in patients with FLT3/ITD (28.6×10^9/L) compared with those in patients without FLT3/ ITD (11.0×10^9/L). Pair-wise comparisons showed that this difference between patients with and without FLT3/ ITD was statistically significant (P= 0.04). In contrast, there was no difference between the two groups regarding the percentage of BM blasts (75% vs. 80%, P=0.74). There was also no significant difference regarding other clinical characteristics, such as hemoglobin level (P=0.93) or platelet count (P=0.67) at diagnosis.

2. Outcome of patients with FLT3/ITD

To analyze the response to or outcome of therapy, 90 patients who received intensive induction chemotherapy were evaluated. Median follow-up duration was 7.5 months (range, 0.2～93.3 months). There was no significant difference between patients with and without FLT3/ITD according to the treatment protocols (P=0.85) (Table 1).

Of the 90 patients, 73 (81.1%) achieved CR. Univariate analysis showed that there was no difference in the response rate to induction chemotherapy between patients with and without FLT3/ITD (FLT3/ITD$^+$ 85.4% vs. FLT3/ITD$^-$ 77.6%, P=0.35). The proportion of patients who needed two rounds of induction chemotherapy to achieve complete remission was higher in patients with the FLT3/ITD than in patients without the mutation (23.9% vs. 13.3%, P=0.15).

With respect to the relapse rate according to the presence of FLT3/ITD, there was a significantly high-

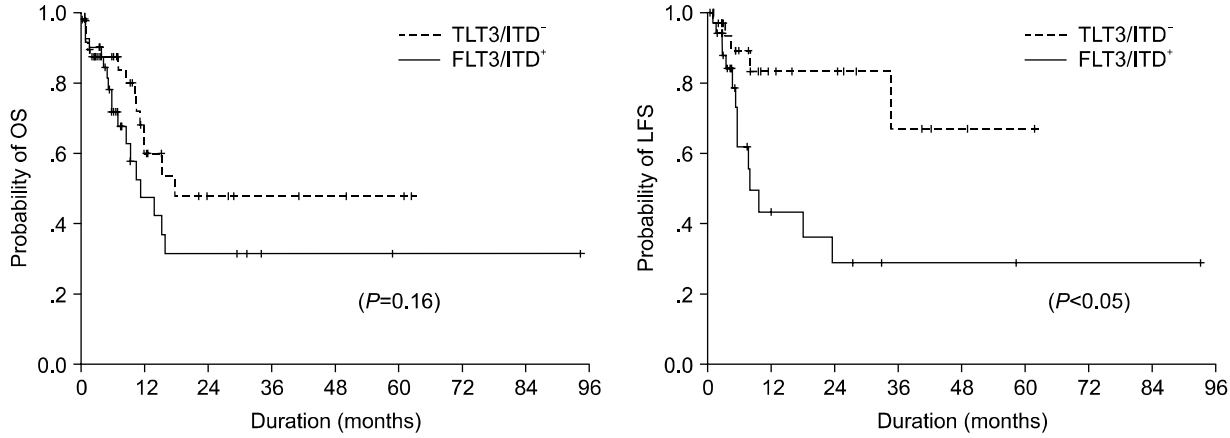

Fig. 2. Overall survival (OS) (upper panel) and leukemic-free survival (LFS) (lower panel) of AML patients with normal karyotype before transplantation. A trend for longer survival was observed in patients without FLT3/ITD compared with those with FLT3/ITD, but there was no statistical significance. A significant difference in LFS was found between FLT3/ITD-positive and FLT3/ITD-negative patients (OS: $P=0.16$; LFS: $P=0.01$ by the log rank test with pooled over strata).

Table 2. Multivariate analysis of prognostic factors for LFS

	P-value	OR	95% CI
Age	0.71	1.00	0.967~1.02
Sex (male)	0.40	0.70	0.295~1.618
FLT3/ITD mutation			
Negative	Reference		
Positive	0.04	1.079~6.120	1.125~7.463
PB WBC	0.34	1.0	1.00~1.00
BM blast	0.77	1.0	0.977~1.018
Remission induction regimens			
Ida/BH-AC (n=40)	Reference		
Ida/AraC (n=33)	0.18	2.0	0.727~5.480
Miscellaneous* (n=17)	0.535	1.50	0.414~5.463

Abbreviations: LFS, Lekemic-free survival; OR, odds ratio; CI, confidence interval; PB, peripheral blood; BM, bone marrow. *Miscellaneous regimens included etoposide, 6-thioguanine, or G-CSF in addition to the IDA/BH-AC or IDA/AraC regimens.

er relapse rate in FLT3/ITD$^+$ patients than in FLT3/ITD$^-$ patients (55.6% vs. 16.7%, P=0.01).

In addition, as shown in Fig. 2, the presence of FLT3/ITD was also associated with a lower leukemic-free survival (LFS) rate in patients with normal karyotype (median duration of LFS, 7.9±2.1 months vs. not available, P=0.01). While the overall survival (OS) in these patients seemed to show a similar trend, there was no statistical significance (median duration of OS, 11.3±3.1 months vs. 17.7±0 months, P=0.16).

In multivariate analyses with Cox proportional hazard model, FLT3/ITD was found to be an independent prognostic factor of LFS in AML patients with normal karyotype (Table 2).

DISCUSSION

The RTKs are classified based on the presence of five immunoglobulin-like domains, a JM domain and two domains that are separated by a kinase insert domain. The FLT3 RTK domains are activated by an allosteric dimerization process through binding with its natural ligand (FL).[15-17] FLT3 is normally ex-

pressed on the surface of early bone marrow hematopoietic progenitor cells and has been demonstrated to have an important role in the survival and/or differentiation of multipotent stem cells.[15-17] Furthermore AML cells have been shown to express FLT3, and exogenous FL can enhance their survival and proliferative responsiveness.[15-17]

The mechanisms by which this FLT3/ITD contributes to AML remain unclear. In experimental systems, the FLT3/ITD leads to ligand-independent FLT3 dimerization and constitutive activation of the TK domains via autophosphorylation.[5,18,19] This in turn appears to constitutively activate signal transducers and activators of transcription 5 and mitogen-acitvated protein kinase and introduces autonomous cell growth in cytokine-dependent cell lines.[6,7,20]

Since Kiyoi et al.[21] first reported the clinical outcome of AML patients with FLT3/ITD, many studies have found that the presence of the mutation correlated with a poor prognosis for these patients.[4,8,9,22] Those results are still a source of debate. Frohling et al.[23] grouped patients according to karyotype and showed that the presence of FLT3/ITD was related to shorter overall survival (OS) and leukemic free survival (LFS) in normal karyotype. This was a noteworthy study because patients with normal cytogenetics comprise a heterogeneous molecular group and the molecular differences were likely to correlate with prognosis.

A previous study also suggested that FLT3/ITD was associated with a significant increase in the relapse rate rather than a decrease in the CR. Kottaridis et al.[4] reported that the presence of FLT3/ITD was the worst prognostic factor for CR duration and relapse-free survival, based on multivariate analysis. In the present study, the CR rate was not affected by the presence of FLT3/ITD (P=0.35), although there was a significant difference in the relapse rate and LFS between the two groups (P=0.01 and P=0.01, retrospectively).

In the previous study, there was a significant association between FLT3/ITD and leukocytosis, indicating that the presence of FLT3/ITD enhances biological proliferation.[24] In this study, patients with FLT3/ITD had higher leukocyte counts at diagnosis (P=0.04), but other known prognostic factors, such as BM blast percentage at diagnosis and age, were not associated with the presence of FLT3/ITD. Furthermore, according to multivariate analyses including these variables, FLT3/ITD was an independent prognostic factor in these patients (P=0.01).

In the present study, there are some limitations. First, recent studies have shown that overrepresentation of the ITD relative to the wild-type FLT allele was especially predictive of an adverse outcome.[4,25] The present study did not demonstrate the FLT3 allele ratio in individual patients. To make the FLT3/ITD assessment truly semiquantitative, a previous study suggested that GeneScan analysis should be used at diagnosis for better prognostification.[25] Second, in the present study, 22 and 6 patients underwent allogeneic and autologous stem cell transplantations, retrospectively. Because the number of patients who underwent transplantation was too small to be analyzed, their survival data after transplantation were not assessed in the present study because of possible selection bias.

In conclusion, this study demonstrated that for AML patients with normal karyotype, the presence of the FLT3/ITD has important implications with respect to disease management. The assessment of this mutation allows the identification of those patients who have a probability of shorter LFS and higher rate of relapse, which may contribute to the stratification of treatment AML patients with normal karyotype.

요 약

배경: FLT3/ITD 유전자 변이는 급성골수성백혈병 환자에서 가장 많이 발현되는 유전자 변이 중의 하나이다. 저자들은 정상 핵형을 갖는 것으로 진단된 급성골수성백혈병 환자에서 FLT3/ITD 변이가 예후에 미치는 영향에 관하여 조사하였다.

방법: 정상 핵형의 급성골수성백혈병으로 진단된 총 123명 환자의 진단시 골수 표본을 이용하여 중합효소연쇄반응을 통해 FLT3/ITD 변이를 확인하였다. 이들 중 관해유도 항암화학요법을 받은 90명의 환자들에서 완전관해율 및 생존 기간을 분석하였다.

결과: 47명(38.2%)의 환자에서 FLT3/ITD 변이가 관찰되었으며, FLT3/ITD 변이를 가지고 있는 환자는 없는 환자에 비해 진단시 백혈구치가 유의하게 높았다 (P=0.04). FLT3/ITD 변이 유무는 완전관해율 및 평균 생존기간에는 유의한 차이가 없었으나 이 변이가 나타난 군에서 유의하게 높은 재발률을 보이며(P=0.01), 무병생존기간 역시 유의하게 짧은 것을 확인하였다 (P=0.01).

결론: 본 연구에서는 FLT3/ITD 변이가 정상 핵형을 가진 급성골수성백혈병 환자에서 불량한 예후를 나타내는 중요한 예후인자임을 확인할 수 있다.

REFERENCES

1) Rosnet O, Schiff C, Pebusque MJ, et al. Human FLT3/FLK2 gene: cDNA cloning and expression in hematopoietic cells. Blood 1993;82:1110-9.
2) Small D, Levenstein M, Kim E, et al. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. Proc Natl Acad Sci USA 1994;91:459-63.
3) Abu-Duhier FM, Goodeve AC, Wilson GA, et al. FLT3 internal tandem duplication mutations in adult acute myeloid leukemia define a high-risk group. Br J Haematol 2000;111:190-5.
4) Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752-9.
5) Kiyoi H, Towatari M, Yokota S, et al. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia 1998; 12:1333-7.
6) Hayakawa F, Towatari M, Kiyoi H, et al. Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene 2000;19: 624-31.
7) Tse KF, Mukherjee G, Small D. Constitutive activation of FLT3 stimulates multiple intracellular signal transducers and results in transformation. Leukemia 2000;14:1766-76.
8) Ciolli S, Vannucchi AM, Leoni F, et al. Internal tandem duplications of Flt3 gene (FLT3/ITDs) predicts a poor post-remission outcome in adult patients with acute non-promyelocytic leukemia. Leuk Lymphoma 2004;45:73-8.
9) Kainz B, Heintel D, Marculescu R, et al. Variable prognostic value of FLT3 internal tandem duplications in patients with de novo AML and a normal karyotype, t(15;17), t(8;21) or inv(16). Hematol J 2002;3:283-9.
10) Beran M, Luthra R, Kantarjian H, Estey E. FLT3 mutation and response to intensive chemotherapy in young adult and elderly patients with normal karyotype. Leuk Res 2004;28:547-50.
11) Chillon MC, Fernandez C, Garcia-Sanz R, et al. FLT3-activating mutations are associated with poor prognostic features in AML at diagnosis but they are not an independent prognostic factor. Hematol J 2004;5:239-46.
12) Cheson BD, Cassileth PA, Head DR, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. J Clin Oncol 1990;8:813-9.
13) Grimwade D, Walker H, Harrison G, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 2001;98:1312-20.
14) Slovak ML, Kopecky KJ, Cassileth 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.
15) Birg F, Rosnet O, Carbuccia N, Birnbaum D. The expression of FMS, KIT and FLT3 in hematopoietic malignancies. Leuk Lymphoma 1994;13:223-7.
16) Shurin MR, Esche C, Lotze MT. FLT3: receptor and ligand. Biology and potential clinical application. Cytokine Growth Factor Rev 1998;9:37-48.
17) McKenna HJ, Stocking KL, Miller RE, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood 2000;95:3489-97.
18) Lisovsky M, Estrov Z, Zhang X, et al. Flt3 ligand stimulates proliferation and inhibits apoptosis of acute myeloid leukemia cells: regulation of Bcl-2 and Bax. Blood 1996;88:3987-97.
19) Fenski R, Flesch K, Serve S, et al. Constitutive

activation of FLT3 in acute myeloid leukaemia and its consequences for growth of 32D cells. Br J Haematol 2000;108:322-30.
20) Mizuki M, Fenski R, Halfter H, et al. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. Blood 2000;96:3907-14.
21) Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 1999;93:3074-80.
22) Ozeki K, Kiyoi H, Hirose Y, et al. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. Blood 2004;103:1901-8.
23) Frohling S, Schlenk RF, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood 2002;100:4372-80.
24) Au WY, Fung A, Chim CS, et al. FLT-3 aberrations in acute promyelocytic leukaemia: clinicopathological associations and prognostic impact. Br J Haematol 2004;125:463-9.
25) Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res 2001;61:7233-9.