Is FLT3 internal tandem duplication significant indicator for allogeneic transplantation in acute myeloid leukemia? An analysis of patients from the Czech Acute Leukemia Clinical Register (ALERT)

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Received May 2, 06

To assess the prognostic relevance of activating mutations of FLT3 gene on outcome of allogeneic transplantations in AML patients, we performed an analysis of all patients with FLT3 mutations registered in the Czech Acute Leukemia Clinical Register (ALERT) from 2003 till the end of 2005. Within the mentioned period 170 patients with AML of median age 56 years (23-77) were investigated for FLT3 mutation, within them 36 cases (21 %) with FLT3 mutations (32 FLT3 ITD and 4 FLT3 D835) were found.Out of FLT3 ITD positive patients 13 had allogeneic transplantation, 20 patients with mutations of FLT3 were treated with chemotherapy without transplantation. Results of the treatment of these patients were compared with the results of the group of patients without FLT3 mutation, which was according to other characteristics identical with the group of patients with FLT3 mutations (n=134). Median overall survival (OS) was significantly shorter for patients with FLT3 ITD (34.8 weeks) than for those without FLT3 mutations (67.7 weeks; P=0.028). Median OS of patients with FLT3 ITD who had allogeneic transplantation was 42.5 weeks; median OS of patients with FLT3 ITD treated only with chemotherapy was 29.6 weeks (P=0.362). After allogeneic transplantation, median OS of FLT3 mutations negative patients was similar to FLT3 ITD positive patients (46.7 versus 42.5 weeks; P=0.443).

Our results suggest that at present there is no strong evidence that FLT3 status alone should influence the decision to proceed to allogeneic transplantation in AML patients. Decision to proceed to alogeneic transplantation should not be based on the FLT3 status only, but it should also consider other prognostic factors.

Key words: acute myeloid leukaemia; allogeneic transplantation; FLT3; ITD; FLT3 mutations; treatment

Prognostic assessment is important for the management of acute myeloid leukemia (AML), since treatments can be optimized on the basis of accurate estimation of outcome [1, 2]. Karyotype is widely recognized as one of the most important prognostic factors in AML [2, 3]. Although the use of karyotype analysis for risk stratification of AML is widely accepted, prognosis of AML is not sufficiently predictable as yet [2, 3]. So that additional prognostic markers are required.

In AML patients, a prognostic genetic marker is the FMS-like tyrosin kinase 3 (FLT3), a member of the class 3 receptor tyrosin kinase family, which plays an important role in hematopoiesis. FLT3 is preferentially expressed on hematopoietic progenitor cells and mediates stem cell differentiation and proliferation [4, 5, 6, 7, 8].

Two main types of activating FLT3 mutations have been described in AML. Internal tandem duplication (ITD) of the FLT3 gene can be detected in 20% to 30% of younger adults with AML [9, 10, 11, 12]. Point mutation within the activation loop of the tyrosin kinase domain (TKD), which mostly affects asparate 835 (D835), have been described in 7% adult AML patients [13]. Many studies have shown that presence of FLT3 ITD correlates with poor outcome of AML patients [10]. The prognostic relevance of D835 mutation is less clear, although most likely it also has a negative prognostic effect

on the patients with AML [10]. So far, it is not perspicuous how to treat the patients with FLT3 ITD and D835 mutations compared to patients without these mutations and whether these patients benefit from allogeneic blood stem cells transplantation. Some data suggest that at present there is no strong evidence that FLT3 status should influence the decision to proceed to transplantation [14].

To assess the prognostic relevance of activating mutations of FLT3 gene on outcome of allogeneic transplantations in Czech AML patients, we performed an analysis of all patients with FLT3 mutations registered in the Czech Acute Leukemia Clinical Register (ALERT) from 2003 till the end of 2005. ALERT registers all adult patients diagnosed in 6 main hematology centers in the Czech Republic: Institute of Hematology and Blood Transfusion (Praha), Charles University Hospital Kralovské Vinohrady (Praha), Charles University Hospital (Plzeň), Charles University Hospital (Hradec Králové), Palacký University Hospital (Olomouc), and Masaryk University Hospital (Brno).

Patients and methods

Patients

Within the mentioned period there were 449 new patients registered in the ALERT with diagnosis of AML. Median age was 57 years (19-82). 170(38%) of these patients of median age 56 years (23-77) were investigated for FLT3 mutation, within them 36 cases (21%; 18 men and 18 women) with FLT3 mutations (32 FLT3 ITD and 4 FLT3 D835) were found.Out of these patients 13 had allogeneic transplantation (4 unrelated donors; 9 HLA identical siblings), 20 patients with mutations of FLT3 were treated with chemotherapy without transplantation, and 3 patients received only palliative treatment. Results of the treatment of these patients were compared with the results of the group of patients without FLT3 mutation, which was according to other characteristics identical with the group of patients with FLT3 mutations (n=134).

The incidence of an FLT3 ITD was significantly higher in patients with a normal karyotype (25 patients, 69%). Unfavorable cytogenetics was observed in 9 patients (25%). Only 2 patients with FLT3 ITD had a favorable cytogenetics: t(15;17), and inv(16), respectively. Acute promyelocytic leukemia (APL) with t(15;17) now differs from other AML subtypes thanks to an effective and tailored therapeutic approach, therefore one APL patient with FLT3 ITD has been excluded from our analysis. Although, there are some data that FLT3 ITD could influence the outcome of patients with t(15;17) [15]. Unfavorable cytogenetics has been found in 3 FLT3 ITD positive patients. All patients with FLT3 D835 mutation had a normal karyotype.

Analysis of FLT3 ITD

A modified method published by Kiyoi *et al* [16] has been used for detection of FLT3 gene internal tandem duplication of juxtamembrane domain (exons 14 and 15; formerly called exons 11 and 12). Total RNA was extracted from the samples of bone marrow and peripheral blood using a RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized from each RNA using a random hexamers (Random Hexamers, Applied Biosystems, Foster City, CA, USA) and reverse transcriptase (MuLV Reverse Transcriptase, Applied Biosystems, Foster City, CA, USA). cDNA products were amplified with a PCR reaction. The forward primer R5 (5‘-TGTCGAGCAGTACTCTAAACATG-3‘) and reverse primer 12R (5‘-CTTTCAGCATTTTGACGGCAACC-3‘) were used. The PCR products were then run on a 2% agarose gel, stained with ethidium bromide, and visualized under a UV light.

Analysis of FLT3 D835 mutation

A modified method of Yamamoto *et al* [13] has been used. Exon 17 of the FLT3 gene was amplified by genomic DNA PCR using primers 17F (5‘-CCGCCAGGAACGTGCTTG-3‘) and 17R (5‘-GCAGCCTCACATTGCCCC-3‘). PCR products were digested with EcoRV endonuclease (New England BioLabs, Ipswich, MA, USA) and run on an agarose gel. Undigested PCR products, which carry of a FLT3 D835 mutation, were cut out and extracted from gel (QIAquick Gel Extraction Kit, Qiagen, Hilden, Germany) for direct nucleotide sequencing (Beckman Coulter CEQ8000, Fullerton, CA, USA).

Statistical analysis

Standard Kaplan – Meier survival analysis was applied to quantify survival time-related profiles of patients stratified according to treatment and molecular genetic groups. Stratified survival data were mutually compared by log-rank test. Probability values of less than 0.05 were considered as significant. The overall survival of patients was calculated from the day of diagnosis and censored to the day of last control record.

Results

Response to induction therapy

There was no significant difference in response to standard induction therapy (antracycline plus cytarabine) between patients without FLT3 mutations, and patients with FLT3 mutations. 59% of the patients without FLT3 mutations, and 75% of the patients with FLT3 ITD or FLT3 D835 achieved complete remission (CR) (P=0.08). After induction CR was achieved in 3 of 4 patients with FLT3 D835.

Disease free survival and overall survival

With a median follow-up of 40 months, FLT3 mutation status strongly predicted for remission duration and survival of the AML patients. In patients achieving CR after induction therapy median disease free survival (DFS) was significantly shorter for patients with FLT3 ITD (28.2 weeks) than for those without FLT3 mutation (93.9 weeks) (P=0.013; Fig. 1). Median overall survival (OS) was also significantly shorter for patients with FLT3 ITD (34.8 weeks) than for those without FLT3 mutations (67.7 weeks) (P=0.028; Fig. 2). In the patients achieving CR after induction therapy OS of FLT3 ITD group was 45.3 weeks, and OS of FLT3 mutation nega-

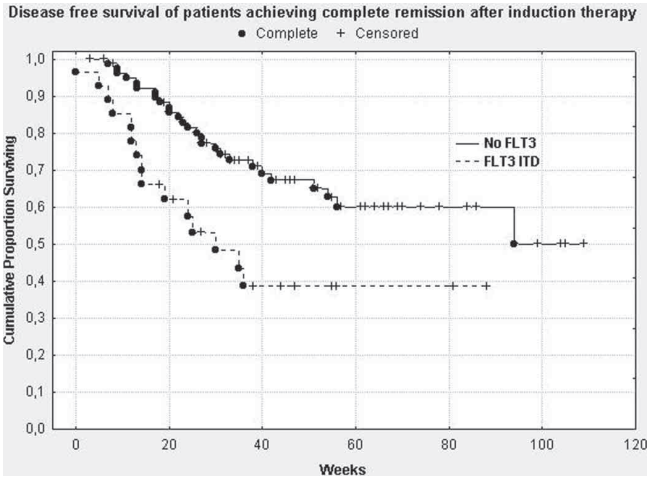

Figure 1. Disease free survival of the patients with complete remission after induction therapy according to FLT3 mutation status (P=0.013). FLT3 ITD – FMS-like tyrosin kinase 3 internal tandem duplication.

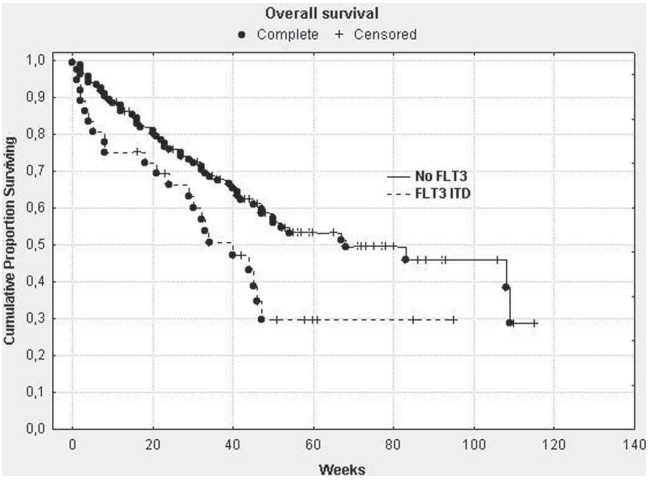

Figure 2. Overall survival of the AML patients according to FLT3 mutation status (P=0.028). FLT3 ITD – FMS-like tyrosin kinase 3 internal tandem duplication.

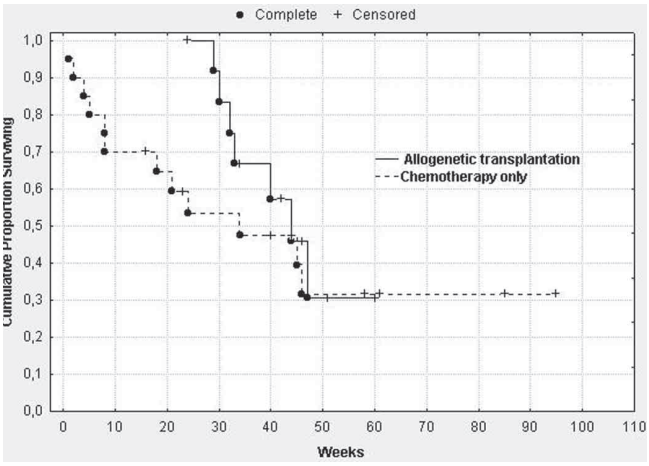

Figure 3. A comparison of the overall survival of FLT3 internal tandem duplication positive patients according to type of treatment (P=0.362).

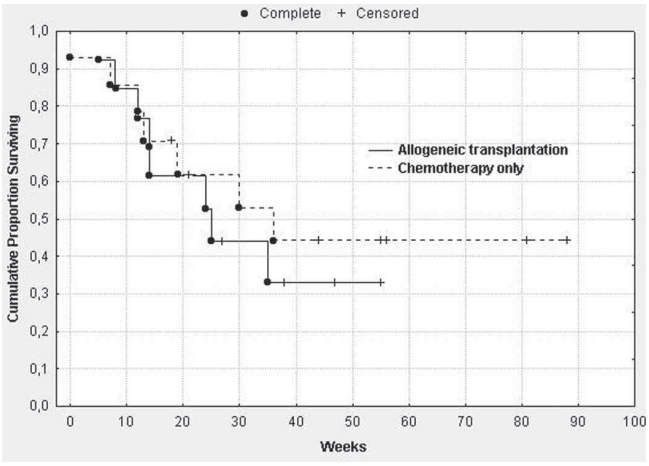

Figure 4. A comparison of the disease free survival of the FLT3 internal tandem duplication positive patients in complete remission after induction therapy according to type of treatment (P=0.609).

tive group was 108.3 weeks (P=0.005). Among patients diagnosed with FLT3 D835 mutations three patients have survived after therapy, two of them have survived disease free. Patients with FLT3 D835 did not undergo transplantation.

The impact of FLT3 status on outcome following transplantation Of the 32 patients with FLT3 ITD, 9 received allogeneic bone marrow or peripheral blood stem cells transplantation from HLA identical siblings, and 4 an allograft from matched unrelated donors. There were no FLT3 ITD positive patients who received an autograft.

Median OS of patients with FLT3 ITD who had allogeneic transplantation was 42.5 weeks; median survival of FLT3 ITD positive patients treated only with chemotherapy was 29.6 weeks (P=0.362; Fig. 3). In the patients achieving CR after induction therapy OS of FLT3 ITD positive group was 42.5 weeks with transplantation, and 45.6 weeks with chemotherapy only (P=0.7). Median DFS of the same patients (in CR after induction) was 24.3 weeks in transplanted patients and 32.1 weeks in patients treated only with chemotherapy (P=0.609; Fig. 4).

There was no significant difference in outcome after allograft between patients without FLT3 mutations, and FLT3 ITD positive patients. After allogeneic transplantation, median OS of patients with FLT3 ITD was 42.5 weeks, and

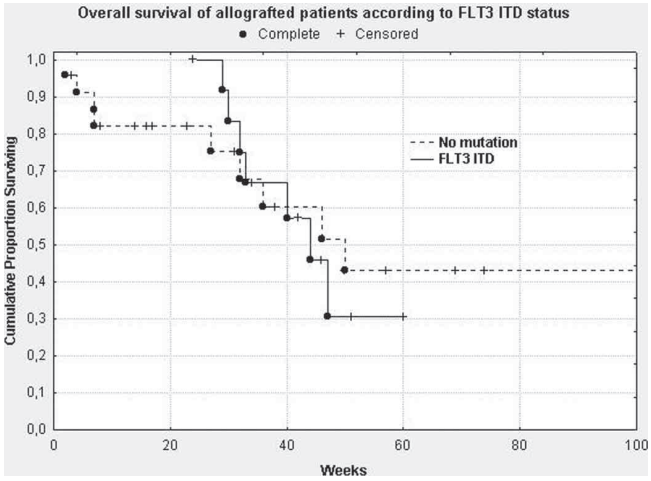

Figure 5. Overall survival according to FLT3 ITD status in patients who received an allogeneic transplant (P=0.443). FLT3 ITD – FMS-like tyrosin kinase 3 internal tandem duplication.

median survival of FLT3 mutations negative patients was 46.7 weeks (P=0.443; Fig. 5).

Discussion

The discovery of FLT3 mutations in AML has important implications for the management of adults with this disease. First, assessment of the FLT3 mutation status in patients with normal cytogenetics allows the identification of a subset of patients who do not benefit from intensive chemotherapy, including postremission therapy with high-dose cytarabine [9]. Second, the high frequency of activating mutations may result in their use as molecular targets for monitoring minimal residual disease [17]. Finally, the perception that activating FLT3 mutation play an important role in leukemogenesis, has led to the development of biologically targeted therapies with class 3 receptor tyrosin kinase inhibitors [5, 18].

70% to 100% of AML cases overexpess the FLT3, but only a fraction of these have activating mutations. However, it is not yet known, what effect FLT3 inhibition might have on AML associated with overexpression of the wild-type FLT3 allele [19, 20].

Our results of FLT3 ITD and FLT3 D835 mutations incidence and prognostic value correspond with the published data [9, 10, 14, 21], although this analysis has limitations. The first problem is that the analysis was based on observational study. Secondly, our data were obtained in patient population that was heterogeneous with regard to treatment regimens. However, the results of any large prospective controlled study regarding FLT3 status and outcome of AML patients has not been published as yet [10].

This study proved statistically significant difference in the survival of patients with FLT3 ITD and without FLT3 mutations. FLT3 ITD presence in the leukemic blasts is a major predictor of poor DFS and, ultimately, of poor OS. Several previous studies have suggested that the presence of FLT3 ITD is associated with poor outcomes in adults with AML [9, 10, 22, 23].

It is noteworthy that within the good-risk cytogenetic group of AML there are distinct biologic entities and they showed marked variation in the incidence of FLT3 mutations. The frequency is high in t(15;17) at 36%, and low in t(8;21) and inv(16) at 9% and 7%, respectively. It is possible that the clinical impact of a FLT3 ITD also varies between these different cytogenetic entities [23]. In our study, only 2 patients with FLT3 ITD have a favorable cytogenetics: t(15;17), and inv(16), respectively.

The optimal treatment for patients with AML is currently unknown. Strategies such as bone marrow or stem cell transplantation require evaluation [5, 24]. Although the benefit of transplantation in standard or poor risk disease remains unclear, there is some indication that it leads to a reduction of relapse rate [24, 25]. Kottaridis *et al* [23] found that autologous bone marrow transplantation did not negate the poor prognostic outcome of a FLT3 ITD. Unfortunately, in the allogeneic group, an unexplained high level of transplant-related mortality in the recipients who had a FLT3 ITD confounded this analysis. However, there was a suggestion that the relapse rate was no higher in FLT3 ITD patients than in patients without FLT3 mutations [23]. Thiede *et al* [11] examined outcome in 175 transplanted patients and found no difference in the OS and DFS between FLT3 ITD-positive and -negative patients in the autologous and unrelated allogeneic groups, but an increased rate of relapse in the FLT3 ITD-positive patients receiving a related donor transplant.

Gale *et al* [14] evaluated outcome according to FLT3 ITD status in 1135 adult patients treated according to United Kingdom Medical Research Council (UK MRC) AML protocols: 141 received an autograft, and 170 received a matched sibling allograft in first complete remission. An FLT3 ITD was detected in 25% of patients and was an independent predictor for relapse. It remained prognostic for increased relapse in patients who received a transplant, with no evidence of a difference in effect between patients who received an autograft and patients who received an allograft or between patients who did or did not receive a transplant. Results of our analysis correspond with these data. There was no significant difference in outcome after allograft between patients without FLT3 mutations and FLT3 ITD positive patients. After allogeneic transplantation, median OS of patients with FLT3 ITD was 42.5 weeks, and median survival of FLT3 mutations negative patients was 46.7 weeks. Median OS of FLT3 ITD positive patients treated only with chemotherapy was 29.6 weeks. In the patients achieving CR after induction therapy OS of FLT3 ITD positive group was 42.5 weeks with transplantation, and 45.6 weeks with chemotherapy only.

Undoubtedly, this issue requires further investigation, as it is important to determine whether allogeneic transplanta-

tion can overcome the poor prognostic significance of a FLT3 ITD.

At present, the clinical data on D835 mutation is limited. The prognostic relevance of D835 mutation is not clear, although most likely it also has a negative prognostic effect on the patients with AML [4, 10]. In our study 3 out of 4 patients with FLT3 D835 achieved CR after induction therapy and 2 of them have not relapsed within a follow-up. These patients did not undergo transplantation.

Conclusion

Our results suggest that at present there is no strong evidence that FLT3 status alone should influence the decision to proceed to allogeneic transplantation in AML patients. Decision to proceed to allogeneic transplantation should not be based on the FLT3 status only, but it should also consider other prognostic factors. However, irrespective of the type of treatment, the FLT3 ITD mutation mean higher risk of relapses, and it significantly shorten the OS and DFS of AML patients.

Supported by the Grant of the Ministry of Health of the Czech Republic No. NR 8080-3/2004.

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