Prognostic impact of c-KIT mutations in Core Binding Factor Leukemias. An Italian retrospective study.

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Abstract

Distinct forms of tyrosine kinase domain (TKD), juxtamembrane domain, exon 8, and internal tandem duplication (ITD) mutations of c-KIT, were observed in about 46% of core binding factor leukemia (CBFL) patients. To evaluate their prognostic significance, 67 adult CBFL patients were analysed to ascertain the c-KIT mutation status. In AML with t(8;21), the presence of c-KIT TKD mutation at codon 816 (TKD^{816}) was associated with a high white blood cell count at diagnosis (median 29.60x10^9/L) and a higher incidence (33%) of extramedullary leukaemia (EML) during the course of the disease. Data also showed that the TKD^{816} mutation patients (n = 12) had a significantly higher incidence of relapse and a lower overall survival (OS) at 24 months, compared with the 17 c-KIT unmutated (c-KIT–) patients (90% vs 35.3%, p = 0.002; 25% vs 76.5%, p = 0.006, respectively). No difference in relapse incidence (p = 0.126) and OS (p = 0.474) was observed between the c-KIT mutated other than TKD^{816} (n = 7) and the c-KIT– patients. These findings indicate that c-KIT TKD^{816} mutation has a negative impact on the outcome of AML with t(8;21).
Introduction

A more comprehensive prognostic evaluation of specific markers is important for the management of therapeutic interventions in acute myeloid leukemia (AML), as treatments can be optimized if the estimation of outcome is more accurate. Cytogenetic and molecular genetic aberrations provide important insights into the biology and pathogenesis of AML. Chromosomal abnormalities targeting the core binding factor include t(8;21)(q22;q22) and inv(16)(p13;q22). These abnormalities form a distinct subgroup, with common clinical features and outcomes, defined as core binding factor leukemia (CBFL) (1,2).

t(8;21)(q22;q22) occurs in about 8% of patients with de novo AML. In most cases, this disorder is morphologically associated with the French-American-British AML–M2 subtype, showing a granulocytic maturation along the neutrophil pathway and, in a minority of cases, bone marrow eosinophilia or mastocytosis (3-5). Not infrequently, AML carrying t(8;21)(q22;q22) may develop extramedullary leukemia (EML), involving either orbital or head and neck sites in pediatric series or, more frequently, paraspinal sites in adults (6,7). The French Intergroup study has recently confirmed a high WBC count at diagnosis to be the most important predictor of an inferior outcome (8).

AML carrying inv(16)/t(16;16)(p13;q22) is frequently associated with monocytic and eosinophilic differentiation (AML-M4Eo) and, in some patients, with EML (9). A high WBC count at presentation and age were identified as the main risk factors for relapse, disease-free survival (DFS) and overall survival (OS) (10,11).

Clinically, both inv(16)/t(16;16) and t(8;21)(q22;q22) AML show a high rate of complete remission (CR) and prolonged CR duration, especially following consolidation chemotherapy with high dose cytarabine (HD-ARAC), and are thought to have a better prognosis than patients with normal karyotype or other chromosomal aberrations (1,2,12-16).

Recent advances in the understanding of molecular events leading to AML genesis include the description of activating mutations involving gene coding for class III tyrosine kinase receptors (RTK), such as c-KIT, H-Ras and c-Fms (17-20). Activating mutations of the c-KIT gene have been reported in 12,8-46,1% of adult CBFL and in 12% of pediatric non-APL AML (21-23). Mutations may affect either the Juxtamembrance domain proposed to regulate an otherwise normal enzymatic site of the c-KIT receptor, such as ITD of exon 11 or insertion/deletion of exon 8 of the c-KIT gene, or may involve the structure of the TKD mutation such as the substitution of a single amino acid at codon 816 (TKD^{816}).

Recent results suggest that RTK mutations in patients with AML have an adverse effect on the outcome, even though prognostic implications of c-KIT mutations are still unclear (21,24,25).
In this study, we evaluated the clinical features and the prognostic significance of c-KIT mutations in 67 CBFL adult patients. We confirmed the prevalence of these mutations in this subset and the profile of associated clinical features and found that there is a greater relapse incidence (RI) and a worse OS in patients with t(8;21) carrying the c-KIT TKD816 mutation.

**Materials and methods**

**Patients and data collection**

All patients with untreated AML with either inv(16)/t(16;16) or t(8;21)(q22;q22), diagnosed in six Italian Centres, were included in this analysis. Bone marrow samples from 67 patients were collected and cryopreserved at diagnosis. In blocks, the samples were then analysed at the Department of Biology and Genetics for Medical Sciences, University of Milan, Italy. Each patient gave their informed consent to collect, cryopreserve BM samples and perform DNA analysis for scientific purposes, in accordance with institutional guidelines. A questionnaire containing a predefined set of data including hematologic presentation, cytogenetics, presence of EML, treatment and outcome was filled out for each patient. In January 2005, the referring hematologists from each centre reported any new observations (i.e. date of relapse, type of relapse, presence of EML, hematologic data at relapse, cytogenetics at relapse, treatment administered and outcome). The databases of the six centres were carefully checked for data accuracy by the respective referring doctors and then reviewed centrally for consistency and completeness. A unique database was subsequently created and submitted for the statistical analysis.

**Screening of mutations in the entire coding region of c-KIT gene**

All bone marrow samples cryopreserved and stored at diagnosis were centrally analyzed. Exon 17 mutations in the c-KIT gene were detected using sequencing and various other more sensitive assays such as: HinfI assay for Asp816Val (18,22), Tsp509I assay for Asn822Lys (20) and ARMS (amplification refractory mutation system) PCR for Asp816Tyr and Asp816His (22). Direct sequencing of DNA and cDNA products was performed using Thermo Sequence Dye Terminator sequencing reaction and ABI Prism 3100 sequencing analyser (Applied Biosystems).

**Treatment protocols**

53 out of 67 CBFL patients (aged ≤ 60 years) were enrolled in intensive chemotherapy protocols. As induction therapy, 35 patients received idarubicin 12 mg/m² for three days (days 1, 3, 5) and a 7 days continuous infusion (days 1-7) of cytarabine 100 mg/m²/day. Postremission therapy consisted of three consolidation courses. The first was a course of idarubicin 10 mg/m²/day for two days (days 1 and 3) and cytarabine 3 g/m²/12 h for three consecutive days. The second and the third were courses of cytarabine 3 g/m²/12 h for three consecutive days (patients over 50 years-old received a reduced dose of cytarabine at 2 g/m²/12 h on days 1-3). 18 patients received induction therapy with ICE (idarubicin 10 mg/m² days 1, 3, 5; continuous infusion on days 1-7 of cytarabine
Postremission chemotherapy consisted of one course of NOVIA (mitoxantron 12 mg/m² on days 1-4 and cytarabine 500 mg/m²/12h on days 1-6) and two courses of cytarabine (3 g/m²/12 h for three consecutive days) (26). Patients without an HLA-matched family donor received G-CSF, 300µg s.c. one day after having completed the second consolidation course and underwent leukaphereses to collect PBSC with a target of >3x10⁶ CD34⁺ cells/kg (27). Eight patients underwent autologous stem cell transplantation (ASCT) instead of the third consolidation course. No patients received allogeneic stem cell transplantation (allo-SCT) in 1st CR. Nine transplants (2 ASCT and 7 allo-SCT) were performed in patients experiencing relapse after attempting to achieve a 2nd or subsequent haematological response. The preparative treatment for both allo or auto-SCT was cyclophosphamide 120 mg/kg over 2 days and total-body irradiation in six fractions of 200 cGy (1200 cGy) or busulfan 16 mg/kg over 4 days and cyclophosphamide 50 mg/kg over 4 days.

**Criteria for response and definitions**

CR was defined as less than 5% blasts in normocellular bone marrow with a peripheral neutrophil count greater than 1500/µL and platelet count greater than 100 000/µL. EML was defined as any leukemic collection outside the bone marrow. The diagnosis of EML could be established by either biopsy, clinical, or CSF criteria. Paraspinal masses that were symptomatic and documented by a Computed Tomography or Magnetic Resonance Imaging were accepted as EML. All patients with clinically diagnosed EML had to show no evidence of an active infection at the potential site of EML (6). Relapse was defined as at least 5% leukemic blasts in a bone marrow aspirate or new extramedullary leukaemia in patients with a previously documented CR (12). Overall survival (OS) was calculated from the date of diagnosis until death and all living patients, in CR or not, were censored at the time of last contact. The duration of CR was calculated from the date of the first CR until the date of the first relapse. DFS was calculated from the date of the first CR until the date of relapse or death in first CR.

**Statistical analysis**

Before inference, all data underwent descriptive analysis and was investigated by means of the Shapiro-Wilk and Kolmogorov-Smirnov tests to evaluate its distribution pattern. The normally distributed variables were summarized with mean and standard deviations, whilst the not normal variables were summarized with median and range. Suitable parametric tests were used to compare the normally distributed variables. Nonparametric Pearson’s χ² (with the Yate’s correction for continuity when applicable) or Fisher’s exact test and Mann-Whitney U test were used for contingency tables and for not normal variables respectively. When applicable, parametric tests were also used for these latter variables, following suitable non-linear transformation (logarithmic, or box-cox) or adjusting for non-homogeneous variances between groups (Welch test). To assess
the survival analysis, the cumulative risk \( H(t) \) was calculated using the Nelson-Aalen estimator, then analysis was carried out using the Kaplan-Meier method, followed by the logrank test, in order to evaluate the differences between the survivor function \( S(t) = \exp(-H(t)) \). Statistical analysis was carried out using Stata SE 9.0 (StataCorp LP, College Station, TX).

**Results**

**Frequency of c-KIT mutations in adults with CBFL**

There were 42 cases with \( t(8;21) \) and 25 with \( \text{inv}(16) \). Between the two groups of patients, there were no differences in age (Mann-Whitney \( U \) test: \( p = 0.097 \)), sex (Pearson’s \( \chi^2 \) test: \( p = 0.855 \)) and EML at presentation (Fisher’s exact test: \( p = 0.061 \)). However, the WBC count was considerably higher in patients with \( \text{inv}(16) \) (Mann-Whitney \( U \) test: \( p = 0.009 \)).

Mutational screening reported c-KIT mutations in 31 of 67 patients (46.2%): 20/67 patients (29.9%) showed a \( D816 \) missense mutation (TKD\(^{816}\)), 8/67 (11.9%) an exon 8 in-frame deletion plus insertion mutations, 1 patient had a transmembrane mutation (1.5%) and 2 patients had an ITD mutation within exon 11 (3.0%). According to cytogenetics, 19 out of 42 patients (45.2%) with \( t(8;21) \) and 12 out of 25 patients (48.0%) with \( \text{inv}(16) \) fell into the “c-KIT mutated group” (c-KIT +). Among the c-KIT+ patients, we detected 12 TKD\(^{816}\)mutations in \( t(8;21) \) and 8 in \( \text{inv}(16) \) (Pearson’s \( \chi^2 \) test: \( p = 0.767 \)). The 36 patients who showed no mutations were classified as the c-KIT “negative group” (c-KIT–) (Table 1).

**Correlation between the c-KIT mutational status and WBC count**

The median WBC of the 67 patients was \( 15.95 \times 10^9 \) / L (range: 3.5-277.5) vs \( 10.90 \times 10^9 \) / L (range: 2.1-130.0; Mann-Whitney \( U \) test: \( p = 0.039 \)) for c-KIT + and c-KIT– respectively.

The result was more significant for the 42 patients with \( t(8;21) \); analysis revealed a median WBC count of \( 18.85 \times 10^9 \) / L (range: 3.5-165.0) vs \( 7.50 \times 10^9 \) / L (range 2.1-70.6; Mann-Whitney \( U \) test: \( p = 0.027 \)) for the 19 c-KIT+ and the 23 c-KIT– patients, respectively. Furthermore, the presence of TKD\(^{816}\) mutation resulted highly significant to identify patients with an elevated WBC count at diagnosis (median WBC count \( 29.60 \times 10^9 \) / L vs. \( 7.50 \times 10^9 \) / L; Mann-Whitney \( U \) test: \( p = 0.013 \)). Paralleling the results of WBC, in the subgroup of patients with \( t(8;21) \), the value of WBC-Index (WBC-I), expressed as \( \text{WBC} \times (\% \text{ of marrow blasts}/100) \), was remarkably different (Mann-Whitney \( U \) test: \( p = 0.047 \)) in the c-KIT+ group and, more specifically, in patients carrying the TKD\(^{816}\) mutation (Mann-Whitney \( U \) test: \( p = 0.005 \)). (Table 2).

In the group of patients with \( \text{inv}(16) \) there was no significant difference in WBC count (Welch test: \( p = 0.285 \)) and WBC-I (Mann-Whitney \( U \) test: \( p = 0.828 \)) between the c-KIT+ and c-KIT– patients and between patients with or without TKD\(^{816}\) mutation.
EML and c-KIT mutational status.

Five patients with t(8;21) and 5 with inv(16) out of the 67 cases included in this study, had EML at presentation and/or during the course of AML. In 8 cases the EML manifested as granulocytic sarcoma. According to cytogenetics, 0 out of 23 c-KIT- patients and 5 out of 19 (23.3%) c-KIT+ patients with t(8;21) had EML (Fisher’s exact test: \( p = 0.014 \)), at diagnosis (1 case with ITD exon 8) or at relapse (4/12 cases with TKD\(^{816}\)). The group of patients carrying the c-KIT mutation at TKD\(^{816}\) showed a high risk of developing EML (Fisher’s exact test: \( p = 0.009 \) for TKD\(^{816}\) vs c-KIT–; \( p = 0.021 \) for TKD\(^{816}\) vs c-KIT– in patients aged \( \leq 60 \) years). (Table 3). The association between the c-KIT mutational status and EML was not significant in patients with inv(16) (Fisher’s exact test: \( p = 0.645 \)).

Overall results of treatments

36 patients with t(8;21) and 17 patients with inv(16), aged \( \leq 60 \) years-old (median 40.5 years, range: 16-60), underwent intensive chemotherapy and were assessed for response. 51/53 patients (96.2%) achieved CR and 58.5% (31 patients) were alive after a median follow-up of 34 months (range: 1-111). There was no difference in RI (55.9% vs 66.7%; logrank test: \( p = 0.435 \)) and OS (logrank test: \( p = 0.976 \)) between the patients with t(8;21) and inv(16). (Figure 1).

Treatment outcome by c-KIT mutational status

Of the 53 patients who received intensive chemotherapy, 19/36 with t(8;21) and 8/17 with inv(16) resulted c-KIT+ upon mutational screening. Of these 27 c-KIT+ patients, we recorded 12 cases of TKD\(^{816}\) in t(8;21) and 7 in inv(16) AML. CR was reached in 92.6% (25/27) of c-KIT + patients and in 100% of the remaining 26 c-KIT– cases (Fisher’s exact test: \( p = 0.491 \)). Resistant disease and one toxic death accounted for the two TKD\(^{816}\) patients with t(8;21) who did not achieve CR.

Relapse Incidence

The impact of c-KIT mutations on RI was different in AML with t(8;21) or inv(16).

Among the 34 patients with t(8;21) achieving CR, with a median follow-up of 15 months (range 3-82), we recorded a significantly higher incidence of relapse in c-KIT + (13/17 patients, 76.5%) or TKD\(^{816}\) cases (9/10 patients, 90.0%) compared with c-KIT– (6/17 patients, 35.3%). (Figure 2, Table 3). Data showed no difference in RI between the c-KIT+ other than TKD\(^{816}\) (7 patients) and the c-KIT– cases (4/7 patients, 57.1% vs 35.3%; logrank test: \( p = 0.126 \)). In AML with inv(16), we were not able to demonstrate any difference in RI according to c-KIT mutational status (logrank test: \( p = 0.902 \) for c-KIT+ vs c-KIT–; \( p = 0.974 \) for TKD\(^{816}\) vs c-KIT–).

Overall survival

19/26 of c-KIT- patients (73.1%) and 12/27 (44.4%) of the c-KIT + CBFL patients are still alive (logrank test: \( p = 0.014 \)). However, the impact of c-KIT mutation was only significant for the 36 patients with t(8;21). In this setting, with a median follow-up of 24 months (range: 1-84), we
recorded a lower OS in the c-KIT + group (8/19 patients; 42.1%) and more specifically, in cases carrying the TKD^{816} mutation (3/12 patients, 25%) than in the c-KIT – group (13/17 patients, 76.5%) (Figure 3, Table 3). In contrast, the OS between c-KIT+ with mutations other than TKD^{816} (5/7 patients, 71.4%) vs the c-KIT– patients proved not significant (logrank test: \( p = 0.474 \)). In AML with inv(16), c-KIT mutational status appeared not to influence survival (logrank test: \( p = 0.317 \) for c-KIT+ vs c-KIT–; \( p = 0.289 \) for TKD^{816} vs c-KIT–).

**Discussion**

No large study evaluating c-KIT mutations in CBFL patients is available, even if it has been reported that only a minority of patients have c-KIT mutations in this subset (21). In this study, we confirmed an incidence of c-KIT mutations of 46.2%, as recently reported by our group (22). These mutations were equally distributed among the t(8;21) and inv(16) patients and in most cases were represented by single amino acid substitution at TKD^{816} (Table 1).

Although a recent study suggested that RTK mutations in patients with t(8;21) AMLs are associated with a high cumulative incidence of relapse and poor relapse-free survival, the prognostic significance of c-KIT mutations remains unclear (25). Care et al, reported that inv(16) AML with c-KIT exon 8 mutation have a greater probability of relapse following complete remission (21). In a preliminary report on 18 patients with CBFL, our group demonstrated a weak statistical correlation between the presence of mutation and DFS (28). The present study, on a larger number of patients, shows that the presence of c-KIT mutation is associated with a significantly higher incidence of relapse and a worse survival in AML with t(8;21).

In a population of 53 patients with CBFL, aged between 16 to 60 years, we did not observe differences in complete remission rate, incidence of relapse and overall survival between the 36 patients with t(8;21) and the 17 with inv(16). However, we are able to show that, within these groups, c-KIT mutations have a different impact on outcome.

The c-KIT positive patients with t(8;21) had a higher RI (\( p = 0.005 \)) and a lower OS (\( p = 0.017 \)) than the c-KIT– population. Furthermore, these differences resulted highly significant among the 12 patients carrying mutations at codon 816 (\( p = 0.002 \) for RI and \( p = 0.006 \) for OS). (Figure 2 and 3). In fact, of the 12 TKD^{816} patients, 10 achieved the 1\(^{st}\) CR and 9 subsequently relapsed. The outcome after relapse was very poor in this subset: seven patients died for resistant disease and only 2 achieved and maintained the 2\(^{nd}\) CR (Table 3).

Among the 7 patients with mutations other than TKD^{816} (5 with an exon 8 in-frame deletion plus insertion mutations and 2 with an ITD mutation within exon 11), the RI was 57% and the OS 71%, percentages not different from those observed in the c-KIT- patients.
The reasons why leukaemia with kinase domain mutation has a lower DFS and is less responsive to salvage chemotherapy might be explained, on clinical grounds, by the analysis of WBC count and by the incidence of EML during the course of the disease.

It is worth noting that, in AML with t(8;21) we observed a significant difference in WBC count and WBC-I between the c-KIT– and the c-KIT+ cases at presentation and more specifically, we recorded the highest WBC count and WBC-I in the group of patients carrying the TKD816 mutation (Table 2). The prognostic significance of high blood count (either WBC and ANC) has already been reported in patients with t(8;21) in small series (29,30). Furthermore, in a recent French survey the WBC-I, calculated as WBC count × (% of marrow blasts/100), was found to be the main prognostic factor for DFS, CR duration and OS (8).

As an unexpected clinical feature, we noted an increased incidence of EML in the group of c-KIT+ patients with t(8;21); it is important to note that none of the c-KIT– cases developed EML and that 33.3% of patients with mutation at TKD816 had EML at relapse (Table 3). Existing literature suggests that the presence of EML at presentation and during the course of the disease is associated with a poor outcome in patients with t(8;21). However, authors substantially fail to prove significant differences in age, WBC count, percentage of marrow or peripheral blood blasts, FAB distribution, presence of del(9)(q22) and CD56 expression between the patients with and without EML (6,31,32). Although the association with EML and c-KIT mutational status in t(8;21) is suggestive, we are continuing the recruitment of patients to further confirm the link.

In AML with inv(16), our data indicates that c-KIT mutation does not affect the outcome, WBC count (p = 0.285), WBC-I (p = 0.828) and EML (p = 0.645).

In conclusion, this study shows that t(8;21) AMLs with TKD816 mutations are associated with a higher incidence of relapse following CR, a poor chance of salvage after relapse and an inferior survival. Molecular characterization of t(8;21) may be a useful tool to identify a subset of CBFL patients with higher risk disease.
References


9. Le Beau MM, Larson RA, Bitter MA, Vardiman JW, Golomb HM, Rowley JD. Association of an inversion of chromosome 16 with abnormal marrow eosinophils (AML M4eo) and


<table>
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<th></th>
<th>t(8;21)</th>
<th>Inv(16)</th>
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<td>Patients n°</td>
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<td>25</td>
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<tr>
<td>Median age, years (range)</td>
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<td>51 (17-88)</td>
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<td>18/7</td>
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<td>14.6 (7.6-277)</td>
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Table 1. Clinical and genetic characteristics at presentation of 67 patients with CBF AML

^ LOS: losses of a sexual chromosome
* two patients also had LOS; § two patients also had +22
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<td>Mean age, years ± SD</td>
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<td>Median WBC-Index (range)</td>
<td>4.0 (1.2-42.3)</td>
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Table 2.
Influence of c-KIT mutations at codon 816 on WBC at diagnosis in AML with t(8;21)
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<th>Cytogenetics°</th>
<th>WBC° (10⁹/L)</th>
<th>EML*</th>
<th>Outcome§</th>
<th>OS (months)</th>
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<td>D816Y</td>
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<td>Paraspinal mass (rel)</td>
<td>D/1st res rel</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>40/F</td>
<td>D816V</td>
<td>46,XX,t(8;21)(q22;q22)</td>
<td>21</td>
<td>Paraspinal mass (rel), Sternal mass (rel)</td>
<td>D/2nd res rel</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>40/M</td>
<td>D816V</td>
<td>46,XY/46,XY,t(8;21)(q22;q22)</td>
<td>6.8</td>
<td>Paraspinal mass (rel)</td>
<td>D/2nd res rel</td>
<td>16</td>
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<tr>
<td>4</td>
<td>54/M</td>
<td>D816Y</td>
<td>46,XX,t(8;21)(q22;q22)</td>
<td>36</td>
<td>Skin (rel)</td>
<td>D/3rd res rel</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>40/M</td>
<td>D816V</td>
<td>46,XY,t(2;8;21)(q24;q22;q22)</td>
<td>72.5</td>
<td>Absent</td>
<td>D/prim ref</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>49/M</td>
<td>D816V</td>
<td>45,X , -Y,t(8;21)(q22;q22)</td>
<td>23.2</td>
<td>Absent</td>
<td>A/2nd CR</td>
<td>68</td>
</tr>
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<td>7</td>
<td>31/F</td>
<td>D816V</td>
<td>46,XX,t(8;21)(q22;q22)</td>
<td>15.2</td>
<td>Absent</td>
<td>D/1st res rel</td>
<td>14</td>
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<td>8</td>
<td>16/M</td>
<td>D816V</td>
<td>46,XY,t(8;21)(q22;q22)/ 47,XY,t(8;21)(q22;q22),+13/ 46,XY</td>
<td>39.2</td>
<td>Absent</td>
<td>A/1st CR</td>
<td>82</td>
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<td>9</td>
<td>50/M</td>
<td>D816V</td>
<td>46,XY,t(8;21)(q22;q22)</td>
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<td>Absent</td>
<td>A/2nd CR</td>
<td>26</td>
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<td>10</td>
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<td>D816V</td>
<td>46,XX,t(8;21)(q22;q22)</td>
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<td>Absent</td>
<td>D/1st res rel</td>
<td>57</td>
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<tr>
<td>11</td>
<td>26/M</td>
<td>D816Y</td>
<td>46,XY,t(8;21)(q22;q22)</td>
<td>165</td>
<td>Absent</td>
<td>D/1st res rel</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>57/F</td>
<td>D816Y</td>
<td>46,XY,t(8;21)(q22;q22)</td>
<td>3.5</td>
<td>Absent</td>
<td>D/early death</td>
<td>1</td>
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</tbody>
</table>

**Table 3.**

Clinical characteristics and outcome of patients with t(8;21) and TKD⁸¹⁶ c-KIT mutations

° At diagnosis

* Site and time of onset of extramedullary leukemia (EML); rel: at relapse

§ D: died; A: alive; res rel: resistant relapse; prim ref: primary refractory
<table>
<thead>
<tr>
<th></th>
<th>n° of patients</th>
<th>time at risk (months)</th>
<th>survival (months)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>25%</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>36</td>
<td>1144</td>
<td>14</td>
</tr>
<tr>
<td>inv(16)</td>
<td>17</td>
<td>581</td>
<td>28</td>
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<tr>
<td>total</td>
<td>53</td>
<td>1725</td>
<td>16</td>
</tr>
</tbody>
</table>

n.r. = not reached

Figure 1.
Kaplan-Meier plot showing overall survival of patients with CBFL aged ≤ 60 years
Figure 2.
Kaplan-Maier plots showing relapse incidence of c-KIT−, c-KIT+ (A) and TKD<sup>816</sup> (B) patients with t(8;21)
Figure 3.
Kaplan-Maier plots showing overall survival of c-KIT−, c-KIT+ (A) and TKD816 (B) patients with t(8;21)
Prognostic impact of c-KIT mutations in core binding factor leukemias. an Italian retrospective study

Roberto Cairoli, Alessandro Beghini, Giovanni Grillo, Gianpaolo Nadali, Francesca Elice, Carla B Ripamonti, Patrizia Colapietro, Michele Michelatti, Laura Pezzetti, Monia Lunghi, Antonio Cuneo, Assunta Viola, Felicetto Ferrara, Mario Lazzarino, Francesco Rodeghiero, Giovanni Pizzolo, Lidia Larizza and Enrica Morra