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# Core binding factor acute myeloid leukaemia and c-KIT mutations

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**Abstract.** Core binding factor (CBF) acute myeloid leukaemia (AML) represents 5-8% of all AMLs and has a relatively favourable prognosis. However, activating c-KIT mutations are reported to be associated with higher risk of relapse and shorter survival. To verify the incidence and prognostic value of c-KIT mutations in CBF AML, we retrospectively analysed bone marrow samples of 23 consecutive adult patients with *de novo* CBF AML [14 inv(16) and 9 t(8;21)] treated at a single institution from 2000 to 2011. All patients received standard induction chemotherapy with cytarabine, idarubicin and etoposide; 13 underwent allogeneic stem cell transplantation. c-KIT mutations in exons 8, 9, 10, 11, 13, 14 and 17 were assessed by PCR amplification in combination with direct sequencing. c-KIT mutations (3 in exon 10 and 4 in exon 17) were detected in 7/23 (30.4%) patients, 3 with t(8;21) and 4 with inv(16). No difference in c-KIT mutation status was observed between cases with inv(16) or t(8;21) alone and cases with additional cytogenetic abnormalities. No association between gender, age, white blood cell and platelet count, peripheral blood and bone marrow blast cells at diagnosis, achievement of complete remission, cytogenetic risk groups and Wilms tumour gene 1 (WT1) levels was found. On the contrary, lactate dehydrogenase (LDH) values were higher in mutated than in non-mutated patients (P=0.01). Overall survival (OS) rates were longer in CBF compared to the other types of AML and disease-free survival (DFS) was longer in inv(16) than in t(8;21) AML. OS and DFS were similar in mutated and non-mutated CBF AML patients. Our results confirm a better prognosis for CBF AML than all other AML categories, and for inv(16) than t(8;21) AML. However, no prognostic value for c-KIT mutational status was found in our series. The association between LDH levels and c-KIT mutation would indicate a more active proliferation for mutated CBF AML.

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## Introduction

RUNX1-RUNX1T1 [t(8;21)] or CBFB-MYH11 [inv(16)] fusion transcripts identify the core binding factor (CBF) acute myeloid leukaemia (AML). Both t(8;21) and inv(16) are characterised at the molecular level by disruption of genes encoding different subunits of CBF (1). CBF AML represents 5-8% of all AML (2) and has a relatively favourable prognosis, following treatment with high dose cytarabine in the consolidation phase (3-5). Mutations of c-KIT occur in 20-25% of t(8;21) and in approximately 30% of inv(16) cases (6). In CBF AML, c-KIT mutations occur frequently within exon 17, which encodes the activation loop in the kinase domain, and in exon 8, which encodes the extracellular portion of the KIT receptor (7). Older age, CD56 expression and activating c-KIT mutations are reported to be associated with higher incidence of relapse and lower survival (6,8,9) while inv(16) patients with +22 secondary abnormality have a better prognosis (10,11). However, no significant differences in overall survival (OS) rates according to c-KIT mutation status have been reported in CBF AML patients (12). In the present study, we retrospectively analysed 23 patients with CBF AML in order to investigate the incidence and prognostic value of c-KIT mutations.

## Materials and methods

*Patients*. Two hundred and forty-nine consecutive unselected adult patients with newly diagnosed AML were admitted to the Division of Haematology, Città della Salute e della Scienza, University of Turin, Italy, from 2000 to 2011. Among these, 23 patients (12 female and 11 male) with *de novo* CBF AML were retrospectively examined. The mean age was 42.7 years (range, 19-64). Diagnosis of CBF AML was performed according to the WHO criteria (2). Inv(16) was present in 14 patients (60.8%), 9 with isolated inv(16) and 5 with additional cytogenetic abnormalities. Nine patients (39.2%) showed t(8;21); 7 had isolated t(8;21) and 2 t(8;21)

Table I. Primer sequences for mutation analysis.

| | Sequences |
|---|---|
| FLT3 ITD | F: TGTCGAGCAGTACTCTAAACA<br>R: ATCCTAGTACCTTCCCAAACTC |
| FLT3 D835 | F: CCGCCAGGAACGGCTTG<br>R: GCAGACGGGCATTGCCCC |
| NPM-I11f-FAM | GTGGTAGAATGAAAAATAGAT |
| NPM-E12r | CTTGGCAATAGAACCTGGAC |
| NPM1 | F: TGGTTCTCTTCCCAAAGTGG<br>R: CCTGGACAACATTTATCAAACACG |
| CK9 | F: TCCTAGAGTAAGCCAGGGCTT<br>R: TGGTAGACAGAGCCTAAACATCC |
| CK11 | F: CCAGAGTGCTCTAATGACTG<br>R: AGCCCCTGTTTCATACTGAC |
| CK13 | F: GCTTGACATCAGTTTGCCAG<br>R: AAAGGCAGCTTGGACACGGCTTTA |
| CK17 | F: TGAACATCATTCAAGGCGTACTTTTG<br>R:TTGAAACTAAAAATCCTTTGCAGGAC |
| CK14 | F: TCTCACCTTCTTTCTAACCTTTTC<br>R: AACCCTTATGACCCCATGAA |
| KIT10 | F: TGCCAAAGTTTGTGATTCCA<br>R: GTGGGGAGAAAGGGAAAAAT |
| CKIT8 | F: GCAGCCTCAGGAAGGTTGTA<br>R: AATTGCAGTCCTTCCCCTCT |

F, forward; R, reverse.

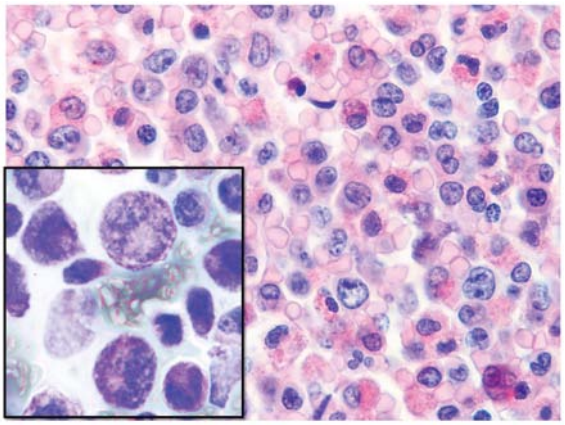

Figure 1. Inv(16) AML (FAB M4 Eo). Bone marrow biopsy (Dominici's staining, x600) showing proliferation of both neutrophil and monocyte precursors, and abnormal eosinophils with basophilic granules. (Inset, Giemsa staining, x1,000).

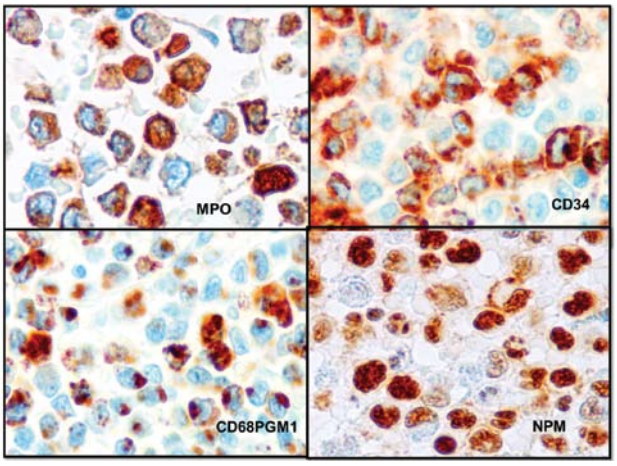


Figure 2. Inv(16) AML. Blasts expressing MPO, CD34, CD68PGM1 and intranuclear NPM. (Bone marrow biopsy; immunoperoxidase staining, x600).

with additional cytogenetic aberrations. All patients received standard induction chemotherapy with cytarabine, idarubicin and etoposide (ICE), followed by consolidation treatment with high-dose cytarabine. Thirteen patients with suitable HLA matched donors (related or unrelated) underwent allogeneic stem cell transplantation in first (10 cases) or second (3 cases) remission. To avoid confounding effect of the transplant procedure, patients were censored at the time of the transplantation. General informed consent was obtained according to the local Ethics Committee guidelines. Samples were numerically identified, maintaining patient anonymity.

*Molecular analysis*. c-KIT mutations in exons 8, 9, 10, 11, 13, 14 and 17 were assessed by polymerase chain reaction (PCR) amplification in combination with direct sequencing from bone marrow (BM) samples.

Amplification of c-KIT exons was performed by PCR with specific oligonucleotide primers (Table I) (13-15), and DNA sequencing was executed using the cDNA from AML BM samples. Sequencing reactions were carried out using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA), and the analysis was performed on an ABI 3130 automated capillary system. FLT3-ITD and D835 mutation status was determined by conventional PCR and direct sequencing (16) and NPM1 mutation status was determined by PCR-capillary electrophoresis methods (17), followed by direct sequencing for positive sample characterization (18) (primers in Table I). The electropherograms were compared to published germ-line sequences using basic local alignment search tool (BLAST) on the Internet. Wilms tumour gene 1 (WT1) expression was quantified using a real-time quantitative PCR (WT1 ELN kit, Nanogen, Buttigliera Alta, Turin, Italy).

*Histology*. Formalin-fixed, paraffin-embedded BM biopsies were stained with H&E, Dominici, Perls, reticulin and immunostained with monoclonal antibodies anti-CD2, CD13, CD33, CD34, CD56 (all from Novocastra, Newcastle, UK), anti-human nucleophosmin, CD68PGM1, and polyclonal antibodies anti-human myeloperoxidase and CD117 (all from Dako, Glostrup, Denmark) (Figs. 1 and 2).

*Statistical analysis*. The association between c-KIT mutation and clinical or haematological parameters was assessed by the one-way analysis of variance (ANOVA) and the Fisher's exact test. Univariate survival analyses were based on Kaplan-Meier

Table II. c-KIT mutations observed in 23 CBF AML cases.

| | No. of cases | c-KIT mutated | Mutation type | c-KIT negative (polymorphism) | Polymorphism |
|---|---|---|---|---|---|
| t(8;21) | 9 | 3 | D816H (exon 17)<br>D816V (exon 17)<br>M541L (exon 10) | 6 (3) | I798I<br>I798I<br>I798I, K546K (simultaneous) |
| inv(16) | 14 | 4 | D816Y (exon 17)<br>D816V (exon 17)<br>M541L (exon 10)<br>M541L (exon 10) | 10 (3) | K546K<br>I798I<br>K546K |
| Total | 23 | | | | |

Table III. Association between c-KIT mutations and t(8;21) AML (N=9).

| | No. of cases | t(8;21) alone | t(8;21) plus additional cytogenetic abnormalities | P-value |
|---|---|---|---|---|
| Mutation | 3 | 2 | 1 | 0.58 |
| No mutation | 6 | 5 | 1 | |
| Total | 9 | 7 | 2 | |

Table IV. Association between c-KIT mutations and inv(16) AML (N=14).

| | No. of cases | inv(16) alone | inv(16) plus additional cytogenetic abnormalities | P-value |
|---|---|---|---|---|
| Mutation | 4 | 2 | 2 | 0.45 |
| No mutation | 10 | 7 | 3 | |
| Total | 14 | 9 | 5 | |

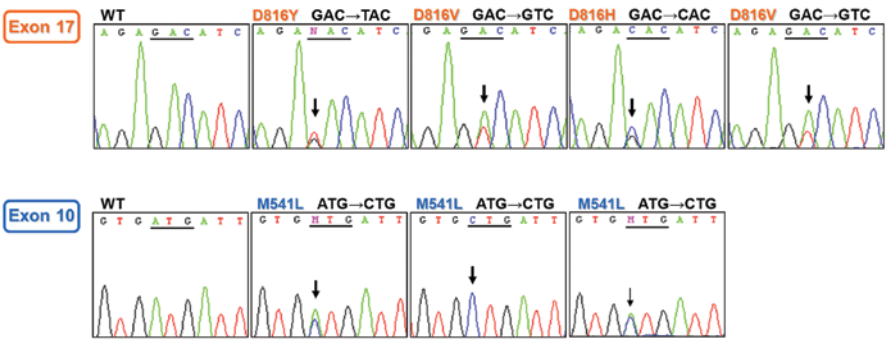


Figure 3. c-KIT mutation electropherograms. Representation of peaks corresponding to wild-type (wt) and mutant c-KIT alleles (indicated by arrows) in chromatograms of cDNA from leukaemic cells of the seven mutated cases in exon 10 or 17. Wt sequences of control cases are also reported.

product-limit estimates of survival distribution, and differences between survival curves were tested using the Cox-Mantel test.

## Results

c-KIT mutations were detected in 7/23 (30.4%) patients. M541L mutation (exon 10) was found in 3 samples and D816V or D816H or D816Y mutation (exon 17) in 4. Two SNPs (K546K and I798I) were detected in 6 AML samples (Table II). c-KIT mutation electropherograms are shown in Fig. 3. FLT3 ITD, FLT3 D835 and NPM1 mutations rarely occurred (data not shown).

*Association between c-KIT mutation and clinical and haematological characteristics*. c-KIT mutations were detected in 3/9 (33.3%) patients with t(8;21) and in 4/14 (28.6%) patients with inv(16). No significant difference in c-KIT mutation was

Table V. Association between c-KIT mutation and clinical and haematological characteristics in CBF AML (N=23).

| | | c-KIT mutated (n=7) | c-KIT non mutated (n=16) | |
|---|---|---|---|---|
| Variables | No. of cases | Mean ± SD | Mean ± SD | P-value |
| Age (years) | 23 | 51±11.2 | 39±13.4 | 0.06 |
| WBC count (x$10^9$/l) | 23 | 34.045±33.9 | 21.702±19.918 | 0.3 |
| Plt count (x$10^9$/l) | 23 | 30.428±31.320 | 46.133±24.023 | 0.2 |
| LDH (UI/l) | 23 | 1,386±629 | 753±312 | 0.01 |
| PB blasts (%) | 23 | 48.43±23.04 | 54.92±19.67 | 0.5 |
| BM blasts (%) | 23 | 48.85±24.88 | 60.64±14.25 | 0.17 |
| WT1 (number of WT1 copies/$10^4$ ABL copies) | 15 | 17,307±22,628 (4) | 15,687±17,780 (11) | 0.8 |
| Gender | | | | |
| Male | 11 | 3/7 | 8/16 | |
| Female | 12 | 4/7 | 8/16 | 0.5 |
| Cytogenic risk | | | | |
| Low | 18 | 6/7 | 12/16 | |
| High | 5 | 1/7 | 4/16 | 0.5 |
| Remission | | | | |
| Complete remission | 21 | 7/7 | 14/16 | |
| No remission | 2 | 0/7 | 2/16 | 0.5 |

WBC, white blood cell; Plt, platelet; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; WT1, Wilms tumour gene.

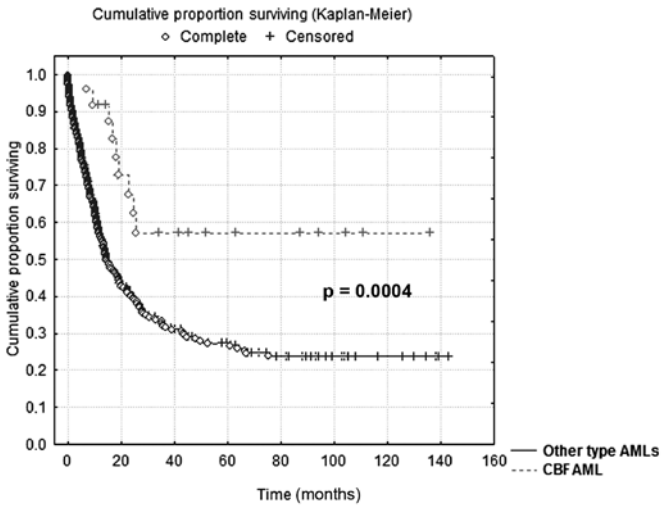


Figure 4. Overall survival of patients with CBF AML and other types of AML.

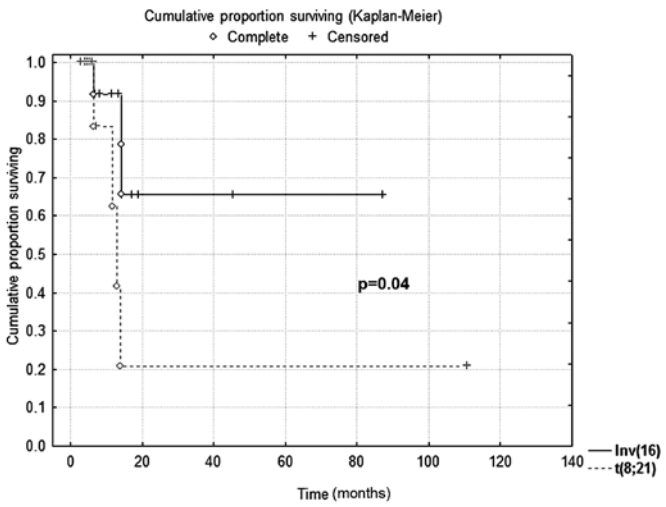


Figure 5. Disease-free survival for patients with inv(16) and t(8;21) AML.

found between cases with t(8;21) or inv(16) alone and cases with additional cytogenetic aberrations (Tables III and IV).

c-KIT mutation status was not associated with gender, age, white blood cell and platelet count, percentage of peripheral blood and bone marrow blasts at diagnosis, cytogenetic risk groups and WT1 levels. Also, no association was found for the achievement of complete remission (CR), although the two patients who did not achieve CR were non-mutated. On the contrary, lactate dehydrogenase (LDH) levels were higher (1386 UI/l) in c-KIT mutated than in non-mutated patients (753 UI/l; P=0.01) (Table V).

*Correlation of c-KIT mutation with overall and disease-free survival*. In the 23 CBF AML patients OS was significantly longer than in the 226 patients with other types of AML treated at the same institution during the same period; at the 10-year follow-up, 57% of CBF AML patients were alive compared to 24% of patients with all other AML categories (P=0.0004) (Fig. 4).

No difference in OS was found between inv(16) and t(8;21) CBF AML; after 88 months, 76% of inv(16) and 60% of t(8;21) patients were alive, respectively (P=0.6). However, DFS for inv(16) AML was significantly longer than that for t(8;21)

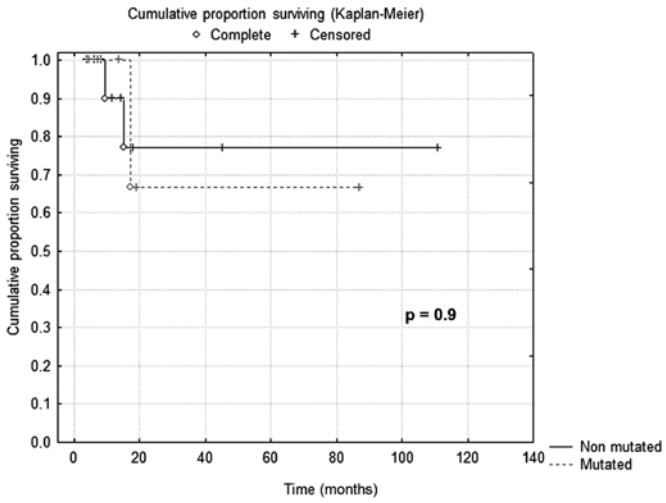


Figure 6. Kaplan-Meier overall survival curves for patients with CBF AML categorised according to c-KIT mutation.

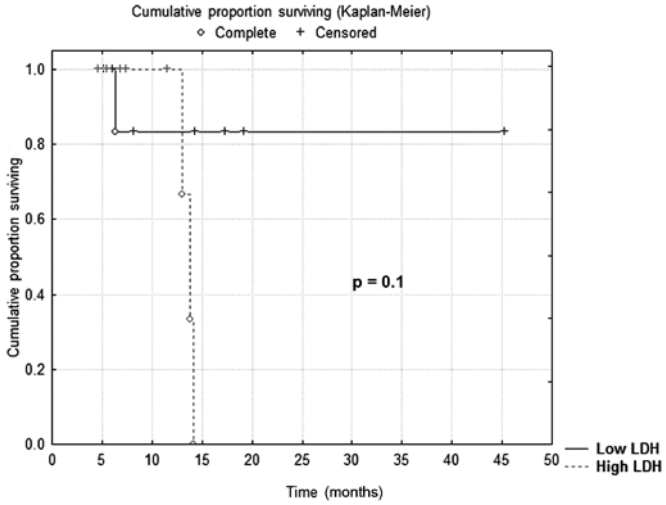


Figure 7. Kaplan-Meier disease-free survival curves for CBF AML patients categorised according to the median LDH value.

cases; after 88 months, 67% of inv(16) patients were free of the disease, vs. 20% of those with t(8;21) (P=0.04) (Fig. 5).

No difference in OS was found when CBF AML patients were categorised according to c-KIT mutation; after 88 months, 78% of c-KIT non-mutated and 68% of mutated patients were alive, respectively (P=0.9) (Fig. 6). DFS was similar in c-KIT mutated and non-mutated CBF AML patients (P=0.6).

## Discussion

Our results showed an overall incidence of c-KIT mutation in 30.4% of cases, as previously reported in adult CBF AML (6), and a better prognosis for CBF AML than for cytogenetically normal or other subtypes of AML, which is in agreement with previous data (19,20). In our study, inv(16) AML had a significantly longer DFS than t(8;21) AML, consistent with previous reports demonstrating that patients with t(8;21) have significantly shorter survival times after relapse than patients with inv(16), possibly related to a lower response to salvage treatment in patients with t(8;21) (10,11,21).

c-KIT mutations in our CBF AMLs were associated with higher LDH levels, suggesting a possible prognostic role. It is well known that high LDH values are associated with a poorer outcome both in AML and myelodysplastic syndromes (MDS) (22-24). This was observed in our study as well; when cases were categorised according to the median LDH value (880 UI/l), all patients with higher LDH values relapsed after 15 months, while 84% of patients with lower LDH values were free of the disease. However, possibly due to the small number of cases, the result is only of borderline significance (P=0.1) (Fig. 7). The association between c-KIT mutation and LDH levels is likely to indicate a more active proliferation in mutated CBF AML.

Contrary to most published studies, no association was found in our CBF AML group between c-KIT mutations and achievement of CR, OS and DFS; this may be due to the small number of cases and to considering CBF AML as a single group. Indeed, previous reports showed a prognostic value of c-KIT mutations in t(8;21) but not in inv(16) CBF AML (7,25). Therefore, t(8;21) and inv(16) AML should be regarded as distinct clinical entities to be stratified and reported separately, as already suggested (11), and possibly treated with a tailored approach (26).

Therefore, further studies are required to clarify the prognostic value of c-KIT mutations in newly diagnosed adult AML.

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