Mo AML, Clinical and Biological Features of the Disease Including AML1 Gene Mutations. A report of 59 Cases by the Groupe Français d’Hématologie Cellulaire (GFHC) and the Groupe Français de Cytogénétique Hématologique (GFCH)

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Abstract

Mutations of the AML1 gene are frequent molecular abnormalities in M0 AML, a rare type of AML. In this retrospective multicenter study, morphological, immunophenotypical, cytogenetic and molecular features of 59 de novo M0 AML cases were analysed and correlated to AML1 mutations.

Point mutations of AML1 gene were observed in 16 cases (27%). They were correlated with higher WBC count (p=0.001), greater marrow blast involvement (p=0.03), higher incidence of Ig H/TCR gene rearrangement (p<0.0001) and with a border line significance lower incidence of complex karyotypes.

In the 59 patients FLT3 mutations were the only significant prognostic factor associated with short survival.

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Introduction

Minimally differentiated acute myeloblastic leukemia (AML), now classified as M0 AML, is a rare type of AML associated with poor prognosis \(^1\)-\(^2\). Recently, we and others reported in M0 AML a high frequency of mutations of AML1 gene, a gene which plays a pivotal role in myeloid differentiation \(^3\)-\(^6\). In this multicenter cooperative work, we analysed clinical and biological characteristics of a large series of M0 AML and compared, in particular, patients with and without AML1 mutation.

Patients and methods

Patient population

Fifty nine patients diagnosed as de novo M0 AML between 1993 and 2000 in the Hematology departments of 9 French university hospitals (Besançon, Bordeaux, Dijon, Lille, Marseille, Nantes, Paris, Reims and Toulouse) were included. All cases were centrally reviewed by members of the GFHC for cytology, cytochemistry and immunophenotyping, and members of the GFCH for cytogenetic findings.

Patients with older age and/or poor clinical condition received supportive care only or moderate chemotherapy (table1). Other patients received anthracyclin-AraC induction chemotherapy based on European or French multicenter protocols for AML (ALFA, BGMT, EORTC and GOELAMS cooperative groups) \(^7\)-\(^10\). Patients who achieved CR received consolidation with high dose AraC based chemotherapy, autologous or allogeneic stem cell transplantation depending on age, donor availability and trial design.

Morphological studies

Bone marrow and peripheral blood smears were stained by May-Grünwald-Giemsa. Myeloperoxidase, naphtol AS-D-acetate esterases with and without fluoride inhibition, or naphthyl acetate butyrate esterases cytochemical reactions were performed.

Immunophenotypic studies

Immunophenotyping was performed by flow cytometry. Membrane expression of : CD 2,3,5,7,10,19,20,22,24,79a,34,13,33,117,14,15,41,61,65 and HLADR was tested. For CD 3,13,22 and MPO, intracytoplasmic antigen expression was also tested. Diagnostic criteria of M0 AML
were: (i) < 3% myeloperoxidase positive blasts, (ii) expression of at least one of the following myeloid markers: CD13, CD33 or MPO, (iii) no expression of lymphoid markers except CD4 or CD7 (EGIL criteria)\textsuperscript{11}.

\textit{Cytogenetic and fluorescence in situ hybridization (FISH) studies}

For conventional cytogenetic analysis, chromosomes were identified by RHG and/or GTG banding and abnormalities described according to ISCN nomenclature (1995)\textsuperscript{12}. FISH was performed according to standard methods and manufacturer’s instructions using whole chromosomes or locus specific probe MLL, AML1 (Vysis, Downer-grooves, Ill) and YACs probes for AF10, CALM and ETV6 regions obtained from the CEPH (Paris) (815c7, 814d9, 936e2).

\textit{Molecular studies}

Detection of AML1 mutations was made on DNA and/or cDNA from bone marrow cells, as previously reported\textsuperscript{3,6}. Study of FLT3 duplication, Ig H (FR1, FR3, DJ), TCR gamma (Vg 1\rightarrow Vg 11; J1J2; Jγ\frac{1}{2}; JP\frac{1}{2}; JP) and TCR delta (Vd2Dδ3; Vd1Jδ1) gene rearrangement were performed according to Kiyoi et al., Delabesse et al, Davi et al and Cave et al respectively\textsuperscript{13-17}.

\textbf{Result and Discussion}\n
\textbf{Clinical, biological characteristics and outcome.}

Clinical and hematological findings are summarized in table 2. They confirmed the association between M0 AML and older age, high WBC counts, CD7 expression and high incidence of cytogenetic abnormalities\textsuperscript{18-21}. Median age of the 59 patients was 62 years. No patient was younger than 15 years, confirming the very low incidence of M0 in children. The morphology of blast cells was that of small to medium sized blasts with high nucleocytoplasmic ratio and agranular basophilic cytoplasm and less often that of monocyteid shaped blast, as previously reported\textsuperscript{22}. Morphological and cytochemical data were not sufficient and
immunophenotypical studies were required in all cases for accurate diagnosis of M0 AML.

Abnormal karyotype was found in 24 of 40 available cases and was complex (≥3 abnormalities) in 54% of them (table1). Aberrations involved preferentially chromosome 5, 7, 3 and 11. This high incidence of complex karyotypes with unbalanced changes such as -5/del(5q), -7,del(7q) and 3q abnormalities is usual in AML occurring in elderly patients and could partially explain the poor prognosis observed in our cohort. Abnormalities of chromosome 13 were found in only two cases, contrasting with a previous report which linked those abnormalities to M0 AML.

Molecular and immunophenotypic findings are summarized in table 1 and figure 1. FLT3 duplication was found in 13 patients (22%) as compared to 16% of M0AML in the recent series of Thiede et al. IgH or TCR gene rearrangement were found in 9 (15%) and 5 (8%) respectively of the patients and were not correlated to lymphoid marker expression. One patient had simultaneous IgH and TCR gamma rearrangements.

Three patients, aged 35, 47 and 64 years, died before onset of treatment. Nineteen patients received moderate single agent chemotherapy or supportive care only, due to older age and/or poor clinical condition (18 of them were older than 70). Intensive chemotherapy was administered to the 37 remaining patients, of whom 23 (62%) achieved CR. Median CR duration was 12 months. Median survival of intensively treated patients was 10 months, and 20 months in patients who achieved CR. Median survival of the whole cohort was 1 months (fig 2A). Those results confirmed the poor prognosis of M0 AML, due besides frequent chromosomal rearrangements to the older age of many patients, who could not receive intensive chemotherapy.

The poor prognosis of FLT3 duplication in other AML as a whole is now well documented. Here, in M0 patients treated intensively, FLT3 duplication was the only prognostic factor with a median survival 9 months in patients with FLT3 duplication versus 16 months in patients without FLT3 duplication (p=0.05) (fig 2C).

**AML1 mutation in the M0AML population**

AML1 mutation was found in 16 patients (27%) (table 2), an incidence similar to that observed in previous reports. All mutations, involved the RUNT domain, were missense (n=7)
or stop codon mutation (n=15), were biallelic (except one case) therefore probably inactivating AML1 protein. Those characteristics are similar to our preliminary results and show the strong correlation between lack of AML1 function and M0 FAB subtype26.

In our M0 AML series, no differences were found between patients with or without AML1 mutations for age, sex, platelet count, Hb value, myelodysplastic features, response to chemotherapy and survival (table 2). On the other hand, patients with AML1 mutation showed significantly higher leukocyte counts, and higher marrow blast percentage, suggesting that AML1 mutations are associated with greater cell proliferation, lower incidence of CD33 and higher incidence of HLA DR expression and IgH or TCR genes. There was also a trend for lower incidence of chromosomal abnormalities and complex chromosomal abnormalities in our M0 cases with AML1 mutation. The higher incidence of IgH/TCR gene rearrangement in mutated cases could be related to AML1 loss of function. Indeed AML1 has been reported to act as a transcriptional repressor by recruitment of TLE/Groucho proteins27. One of the target genes of this repression is TCR enhancer28,29. AML1 mutated blast cells did not show expression of lymphoid markers, indicating absence of lymphoid differentiation, which could be linked to abnormal VDJ recombination in myeloid committed cells30. However we cannot exclude that AML1 mutation occurred only in very immature cells not committed to lymphoid or myeloid differentiation, in which TCR or Ig gene recombination would be a normal event.
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<th>Age</th>
<th>Karyotype</th>
<th>wild type</th>
<th>treatment</th>
<th>First allele</th>
<th>Second allele</th>
<th>Treatment regimen</th>
<th>achievement (months)</th>
<th>survival (months)</th>
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<td>1</td>
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<td>45</td>
<td>Not Done</td>
<td>No</td>
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<td>C114ins</td>
<td>F131del</td>
<td>Supportive care</td>
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<td>-</td>
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<td>38</td>
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<td>Germline</td>
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<td>No</td>
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</table>

Note: The table provides information on patients with FLT3 rearrangement AML1 gene alteration. The columns include patient number (No), sex (Sex), age (Age), karyotype (Karyotype), treatment (Treatment), first allele (First allele), second allele (Second allele), treatment regimen (Treatment regimen), achievement (months), and survival (months).
Table 2: Pretreatment characteristics of M0 AML patients according to AML1 gene mutations

<table>
<thead>
<tr>
<th></th>
<th>Overall population</th>
<th>Presence of AML1 gene mutation</th>
<th>Absence of AML1 gene mutation</th>
<th>p value</th>
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<tr>
<td>n</td>
<td>59</td>
<td>16</td>
<td>43</td>
<td></td>
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<tr>
<td><strong>mean Age (range)</strong></td>
<td>62 (15-88)</td>
<td>65 (29-88)</td>
<td>56 (15-87)</td>
<td>0.1146</td>
</tr>
<tr>
<td><strong>Females (%)</strong></td>
<td>34</td>
<td>25</td>
<td>37</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>mean WBC (G/l) (range)</strong></td>
<td>52.7 (1.1-309)</td>
<td>115 (1.4-309)</td>
<td>30 (1.1-208)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>mean Platelet count (G/l) (range)</strong></td>
<td>91.3 (4-287)</td>
<td>83 (8-223)</td>
<td>94 (4-287)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>mean Hb (g/dl) (range)</strong></td>
<td>8.8 (3.4-14.8)</td>
<td>8.46 (3.9-14.8)</td>
<td>9 (3.4-14.7)</td>
<td>0.27</td>
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<tr>
<td><strong>mean Circulating Blasts (%) (range)</strong></td>
<td>67.3 (1-100)</td>
<td>77 (12-100)</td>
<td>63.5 (1-100)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>mean Circulating Blasts (%) (range)</strong></td>
<td>84.4 (36-100)</td>
<td>91.4 (75-100)</td>
<td>82 (36-100)</td>
<td>0.032</td>
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<td><strong>WBC&gt;50G/l (% pts)</strong></td>
<td>35</td>
<td>80</td>
<td>19</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>Dysgranulopoiesis (%) pts</strong></td>
<td>22</td>
<td>30</td>
<td>19</td>
<td>0.437</td>
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<tr>
<td><strong>Dyserythropoiesis (%) pts</strong></td>
<td>16</td>
<td>9</td>
<td>19</td>
<td>0.65</td>
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<tr>
<td><strong>DYSmegakaryopoiesis (%) pts</strong></td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>1</td>
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<tr>
<td><strong>FLT3 duplication (%) pts</strong></td>
<td>22</td>
<td>28</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td><strong>IgH or TCR genes rearrangement (%) pts</strong></td>
<td>22</td>
<td>58</td>
<td>7</td>
<td>&lt;0.0001</td>
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<td><strong>Abnormal Karyotypes (%)</strong></td>
<td>32.5</td>
<td>12.5</td>
<td>37.5</td>
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<td><strong>AML1 mutation (%)</strong></td>
<td>27</td>
<td>/</td>
<td>/</td>
<td>/</td>
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<td><strong>CD13 expression (%)</strong></td>
<td>85</td>
<td>100</td>
<td>77</td>
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<td><strong>CD33 expression (%)</strong></td>
<td>61</td>
<td>41</td>
<td>70</td>
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<td><strong>CD7 expression (%)</strong></td>
<td>35</td>
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<td><strong>CD34 expression (%)</strong></td>
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<td><strong>DR expression (%)</strong></td>
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<td><strong>CR achievement in patients treated intensively (%)</strong></td>
<td>62</td>
<td>68</td>
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<tr>
<td><strong>Median CR duration (Months)</strong></td>
<td>12</td>
<td>10</td>
<td>17.5</td>
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Figure 1: Antigenic expression profile

The percentage of positive patients for the expression for each antigen for the whole series □ is compared with that of patients with AML1 gene mutation ■ and without AML1 gene mutation □ .
Figure 2

A
% survival

B
% survival

C
% survival

P=0.0
Figure 2

A: Kaplan-Meier overall survival of the whole series of patients.

B: Kaplan-Meier survival of patients intensively treated.

C: Kaplan-Meier survival according to FLT3 duplication in patients intensively treated:
   —— patient without FLT3 duplication,  Patient with FLT3 duplication,
(p=0.05 between the two groups)
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