Multipotent Stem Cell Involvement in Megakaryoblastic Leukemia: Cytologic and Cyto genetic Evidence in 15 Patients

By Antonio Cuneo, Cristina Mecucci, Simonetta Kerim, Elisabeth Vandenbergh, Paolo Dal Cin, Angeline Van Orshoven, Jean Rodhain, André Bosly, Jean-Louis Michaux, Philippe Martiat, Marc Boogaerts, Maria G. Carli, Gianluigi Castoldi, and Herman Van Den Bergh

Cytologic and cytogenetic results obtained from patients fulfilling the FAB criteria for the diagnosis of acute non-lymphocytic leukemia (ANLL) of megakaryocytic lineage (ANLL-M7) are reported. Eleven cases were de novo ANLL-M7, of whom three presented with acute myelofibrosis. Four cases were megakaryoblastic transformations of chronic myelogenous leukemia (two cases), refractory anemia with excess of blasts (one case), and polycythemia vera (one case). Four patients showed a minority of granular blasts, with occasional Auer rods in one. Positive myeloperoxidase and/or sudan black-B stainings and CD13 positivity in these cases were consistent with the presence of a myeloid involvement. Morphologic evidence of associated myelodysplastic features was detected in all evaluable patients with de novo ANLL-M7. These cytotologic findings indicate that ANLL-M7 may frequently represent a multilineage proliferation. Cytogenetic studies revealed -7/7q- and +8, alone or in combination with additional aberrations, in three cases each. Rearrangements involving bands 3q21 or 3q26 were seen in two patients and +21, as an additional aberration, in one. Other structural rearrangements all observed in a single patient were inv(16)(p13q22) at megakaryoblastic relapse with bone marrow eosinophilia, t(13:20)(q13or14;q11), del(20)(q11), and der(7)t(7;17)(p14;q22). Most breakpoints of these aberrations are located at bands frequently rearranged in malignant myeloid stem cell disorders. A review of 31 cases of the literature showed a frequent occurrence of -7/7q- and -5/5q- in ANLL-M7. Many of the chromosome aberrations so far described in ANLL-M7 appear to be shared by a spectrum of myeloid neoplasias and may be related to mechanisms conferring proliferative advantage to undifferentiated stem cells.

MYELOID NEOPLASIAS involving the megakaryocytic lineage, presenting as acute leukemia or as transformation of myelodysplastic and myeloproliferative disorders, have been recognized in the past years on the basis of morphologic, cytochemical, and electron microscopy studies.4,8 Later a more specific identification of megakaryocyte precursors was obtained with the demonstration of platelet peroxidase (PPO) on ultrastructural cytochemistry9 and with immunologic studies detecting specific platelet markers.10 The development of these techniques prompted the FAB group to propose standardized criteria for the identification of acute nonlymphocytic leukemia of megakaryocytic lineage (ANLL-M7).6

Since then several reports have described the clinical and immunologic features of this FAB entity.7,8 Because of bone marrow fibrosis and consequent difficulty in obtaining samples, cytogenetic studies have been less frequently performed and most reported cases represent occasional observations.11,12 Sometimes made on megakaryoblastic proliferations not fulfilling the FAB criteria,11,14 Furthermore, the cytologic features of the nonblast cell population in cases of ANLL-M7 have only occasionally been described in some patients.14,15 Whereas evidence is now emerging that acute myeloid leukemia with associated myelodysplasia may represent a distinct clinical entity,16,17 Cytologic and cytogenetic characterization of 11 cases of ANLL-M7 plus four cases of megakaryoblastic transformation of myeloid neoplasias is reported in the present study. Cases previously published fulfilling the FAB criteria, in which banded chromosome studies have been performed, have also been reviewed.

MATERIALS AND METHODS

Nine patients with de novo acute leukemia, here referred to as ANLL-M7, and four megakaryoblastic transformations of myeloid neoplasias could be retrospectively documented among the patients referred to the Genetic Centre, Leuven, Belgium. Two additional ANLL-M7 were observed at the Department of Hematology, Ferrara. Thus, a total of 15 patients with megakaryoblastic leukemia are included in this report. One patient (no. 15) has previously been reported in detail.18 Diagnosis of ANLL-M7 was made when more than 30% blasts were seen on bone marrow films and when at least 20% mononuclear cells expressed platelet markers at immunophenotyping. In patients with scanty dilute aspirates bone biopsies were evaluated to assess the percentage of bone marrow blasts, which had to be greater than 30%. These patients were considered as having ANLL-M7 if at least 10% mononuclear cells from peripheral blood showed unequivocal positivity for antiplatelet monoclonal antibodies. These criteria were also used for the diagnosis of megakaryoblastic transformation of myeloid neoplasia.

Characterization of the blast cell population. Bone marrow (BM) and/or peripheral blood (PB) smears were stained with May-Grunwald Glemsa (MGG) and cytochemical stains including...
myeloperoxidase (MPO), sudan black B (SBB), naphthol AS D-chloroacetate esterase (NASDCAE), alpha-naphthyl acetate esterase (ANAE), alpha naphthyl butyrate esterase (ANBE), periodic acid-Schiff (PAS), acid phosphatase (AcP), and prussian blue.

Bone biopsies were made in all cases except in patients 3, 14, and 15. Immunologic analysis had been routinely performed at presentation by indirect immunofluorescence technique on BM mononuclear cells when aspiration yielded adequate sample. The expression of the following markers using commercially available reagents was tested: (1): platelet and myeloid, protein complexed with Gplb (CDw42), Plt-2 (CDw41), anti-factor VIII related antigen (Dakkopats), MY10 (CD34), My9 (CD33), My7 (CD13), My4 (CD14), LeuM1 (CD15); and (2): lymphoid, J5 (CD10), B4 (CD19), B1 (CD20), OKT11 (CD2), OKT16 (CD7), OKT10 (CD38). Terminal deoxynucleotidyl transferase (Tdt) activity was assayed by indirect immunofluorescence technique using rabbit anticalf Tdt serum (Bethesda Research Laboratories, Bethesda, MD). In patients with BM fibrosis precluding an adequate aspiration, the expression of one of the platelet markers was tested on peripheral blood mononuclear cells.

Cytologic studies. Morphologic features were reviewed in each patient on BM and PB smears. The cases presenting as de novo ANLL-M7 were assessed for the presence of associated myelodysplastic features previously defined by the FAB group.9 Differential counts on 100 cells of each lineage were made to determine the percentage of abnormal erythroblasts and granulocytes and, when possible, at least 20 megakaryocytes were examined. The patients with more than 25% dysplastic erythroblasts and more than 50% abnormal neutrophils and megakaryocytes were classified as de novo ANLL with trilineage myelodysplasia (TMDS) as previously proposed.14 Available cytochemical stains were also reviewed.

Cytogenetic studies. Sequential chromosome investigations were performed in all patients. BM or PB samples were cultured for 24 to 48 hours without mitogens. Synchronization with methotrexate and bromodeoxyuridine was carried out. Metaphases were R banded with acridine orange. G banding with Wright stain was also employed.20 At least ten karyotypes were studied at each cytogenetic investigation. Chromosome aberrations were described according to the ISCN.21

RESULTS

Eleven patients (nos. 1 to 11) were diagnosed as de novo ANLL-M7 and 4 as megakaryoblastic evolution of chronic myeloid leukemia (CML) (two cases), polycythemia vera (PV) and refractory anemia with excess of blasts (RAEB). Their ages ranged from 2 to 79 years (median, 50 years). Three patients (nos. 8, 10, and 11) presented an initial picture fulfilling the criteria previously defined for the recognition of acute myeloblastosis (AMF), ie, BM fibrosis precluding an adequate aspiration, trilineage proliferation, absence of organomegaly, minimal variation in red blood cell morphology and a rapidly fatal course.22 Cytotoxic treatment was given to all patients except patients 10 and 12, and complete remission was achieved in patients 3, 4, and 7. An allogeneic bone marrow transplant (BMT) was performed in patient 2 who was refractory to standard chemotherapy. Patient 7 received an autologous BMT. Clinical data and laboratory findings are reported in Table 1.

Cytology. Morphologically, blast cells showed cytoplasmic blebs in four cases (9, 12, 13, and 15). In six patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age (years)</th>
<th>Toxic Exposure†</th>
<th>Hb (g/dL)</th>
<th>WBC (x 10⁹/L)</th>
<th>Pts (x 10⁹/L)</th>
<th>Treatment/Response</th>
<th>Survival (months)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/24</td>
<td>Solvents</td>
<td>4.2</td>
<td>3/5</td>
<td>72</td>
<td>DNR + Ara-C/PR</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>F/26</td>
<td>NE</td>
<td>10.1</td>
<td>17.2/23</td>
<td>431</td>
<td>Amsa + HD Ara-C/  CR</td>
<td>8 + §</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NR; BMT/CR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F/2</td>
<td>NE</td>
<td>4.8</td>
<td>16/5</td>
<td>17</td>
<td>DNR + Ara-C/CR</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>F/12</td>
<td>NE</td>
<td>8.3</td>
<td>9.7/75</td>
<td>63</td>
<td>DNR + Ara-C/CR</td>
<td>4 +</td>
</tr>
<tr>
<td>5</td>
<td>M/60</td>
<td>Herbicides, varnishes</td>
<td>7.1</td>
<td>6.8/26</td>
<td>96</td>
<td>LD Ara-C/PR</td>
<td>14 +</td>
</tr>
<tr>
<td>6</td>
<td>M/73</td>
<td>Herbicides</td>
<td>9.1</td>
<td>2.7/28</td>
<td>126</td>
<td>LD Ara-C/NR</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>M/33</td>
<td>NE</td>
<td>9.6</td>
<td>11/73</td>
<td>25</td>
<td>DNR + Ara-C/CR;  ABMT</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>M/61</td>
<td>Unknown</td>
<td>10.1</td>
<td>5/11</td>
<td>89</td>
<td>DNR + Ara-C/NR;  Amsa + Ara-C/NR</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>F/54</td>
<td>NE</td>
<td>8.8</td>
<td>3.9/66</td>
<td>35</td>
<td>DNR + Ara-C/NR</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>F/79</td>
<td>Unknown</td>
<td>8.7</td>
<td>3.7/9</td>
<td>103</td>
<td>No therapy</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>M/68</td>
<td>NE</td>
<td>7.1</td>
<td>2.9/7</td>
<td>91</td>
<td>DNR + Ara-C/PR</td>
<td>8 +</td>
</tr>
<tr>
<td>12</td>
<td>F/50</td>
<td>Hydroxyurea</td>
<td>6.6</td>
<td>25/11</td>
<td>1193</td>
<td>No therapy</td>
<td>3 +</td>
</tr>
<tr>
<td>13</td>
<td>M/28</td>
<td>Petroleum products, hydroxyurea</td>
<td>8.9</td>
<td>19.4/26</td>
<td>269</td>
<td>DNR + Ara-C/NR</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>F/15</td>
<td>NE</td>
<td>6.1</td>
<td>18/75</td>
<td>90</td>
<td>DNR + Ara-C/NR</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>M/56§</td>
<td>NE</td>
<td>11</td>
<td>16/24</td>
<td>135</td>
<td>DNR + Ara-C/PR</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; PR, partial response; NR, no response; BMT, allogeneic bone marrow transplant; ABMT, autologous bone marrow transplant; NA, not available; NE, anamnestically not exposed; DNR, daunorubicin; Ara-C, cytarabine; HD Ara-C, high-dose cytarabine; Amsa, m-Amsacrine; LD Ara-C, low-dose cytarabine.

*Patients 1 to 11: ANLL-M7 (nos. 8, 10, 11 with AMF); patient 12: PV 6 years before; patient 14: RAEB 5 months before; patients 13 and 15: CML 4 and 1 year before, respectively.
†Retrospective evaluation according to Mitalman et al.44
‡§ + indicates that the patient is alive.
§Relapse in February 1989.
||Patient previously reported.14
(7, 9, 10, 12, 13, and 15) there was a relevant number of micromegakaryocytes. In the other cases most blast cells were undifferentiated. A minority of granulated blast cells was present in patients 2, 4, 6, and 7 (Fig 1), and Auer rods were occasionally seen in patient 4. Cytochemical staining patterns and immunophenotype are reported in Table 2. Various degrees of myelodysplasia were present in all patients with de novo ANLL-M7 (Fig 1). When evaluable, all three cell lineages were always involved, and three cases (2, 6, and 8) fulfilled the diagnostic criteria of ANLL with

Fig 1. Myeloid involvement and myelodysplastic features in ANLL-M7. (A) Megakaryoblasts with cytoplasmic budding; a granular blast is arrowed. (B) Leukemic picture showing agranular neutrophil (below right) and an abnormal erythroblast with unstained cytoplasmic area (arrowed). (C) PAS-positive cells in ANLL-M7: two dysplastic erythroblasts (upper left) exhibit a diffuse positivity. A weak granular reaction is present in some blasts. More mature megakaryocytelike cell shows coarse granules in the cytoplasmic margin. Megakaryocyte fragments are visible (arrowed).
Table 2. Cytochemistry and Immunophenotype in 15 Cases of Megakaryoblastic Leukemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cytochemical Stains (+/-)</th>
<th>% Blast</th>
<th>Platelet and Myeloid Markers</th>
<th>Lymphoid Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( % positive blasts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PAS+ (60)</td>
<td>NA</td>
<td>CD42+ ‡</td>
<td>ND</td>
</tr>
</tbody>
</table>
| 2        | SBB+ (6), PAS-, ANBE- AcP+ (85) | 67      | CD42+, CD41 + , CD34 + , CD13 + , CD33 + , CD14 - | CD7-, CD2-, Tdt- , CD20-, CD10-, CD19-
| 3        | MPO-, ANAE+ (80), ANBE-, AcP+ (74) | >95     | CD42+, CD41 + , CD33 + , CD14 + /- | CD7-, Tdt-, CD20-
| 4        | SBB+ (5), NASDCAE-, PAS+ (5), ANBE-, AcP+ (51) | 83      | CD41 + , CD33 + , CD13 + /- | CD20-, CD10-, CD7-, CD2-
| 5        | SBB-, NASDCAE-, ANAE+ (90)§ | NA      | CD42 + /- , antifactor VIII + , CD33 - , CD15 - | CD20-, CD19-, CD7-
| 6        | SBB+ (7), NASDCAE+ (3), ANAE+ (82), PAS+ (65), AcP+ (91) | 75      | CD41 + /- , antifactor VIII + , CD34 + , CD33 + , CD13 + , CD14 + /- | CD2-
| 7        | MPO+ (8), SBB+ (7), ANAE+ (61)§ | >95     | CD41+, CD42+, CD34-, CD33+/- , CD14-, CD7+ , Tdt- | CD10-, CD38+ |
| 8        | NA                        | NA      | CD42 + /- ‡                  | ND              |
| 9        | MPO-, NASDCAE-, PAS+ (85), AcP+ (90) | 71      | CD41 + , CD42 + , CD33 -, CD13 + , CD15- | ND              |
| 10       | PAS-                      | NA      | CD42 + /- ‡                  | ND              |
| 11       | NASDCAE-, PAS+ (60), ANAE+ (58) | NA      | CD41 + ‡                    | ND              |
| 12       | MPO-, PAS-, AcP+ (86)     | 76      | CD41+ + , CD42+ + , CD33 - , CD13 + , CD14 + | CD38+/- , CD3 + , CD2 + , CD7 + , CD19+/- , CD20-
| 13       | AcP+ (72)                 | NA      | CD41+ ‡                     | ND              |
| 14       | MPO-                      | 89      | CD41+, CD33+ +, CD13-       | CD38+          |
| 15       | NA                        | NA      | CD41+ + , CD42+/- , CD2+/- , CD7-, CD3- , CD19-, CD10-, CD20- | CD38+          |

Abbreviations: NA, not available; ND, not done.

*+ < 10% positive; +/-, 10-20% positive; +, 20-30% positive; ++, 30-50% positive; ++++, >50% positive mononuclear cells.

†On the mononuclear layer.
‡Slight inhibition by NaF.
§On PB mononuclear cells.

Cytogenetics. Chromosome studies at leukemia presentation were successful in 12 patients, 11 of whom showed clonal aberrations. No mitoses were detected in patients 3, 6, and 11; in the latter two, however, analyzable metaphases were obtained during follow up: patient 3 exhibited chromosome abnormalities at relapse, whereas patient 11 showed a normal karyotype in partial remission phase. Thus, karyotypes of 14 patients were available, of which 12 showed clonal aberration. Detailed results of sequential chromosome studies are reported in Table 4.

Grouping the chromosome aberrations according to the Chicago classification adopted at the Fourth International Workshop on Chromosomes in Leukemia (FIWCL), it results that -7/7q- and +8, alone or in combination with other abnormalities, were observed in three cases each. In patient 2 monosomy 7 was associated with a direct insertion of part of the long arm of chromosome 3 in the long arm of chromosome 12, resulting in two abnormal chromosomes described as follows: 12pter → 12q14-.3q21 → 3q27:: 12q14 → 12qter and 3pter → 3q21:.3q27 → 3qter (Fig 2).

In patient 1 trisomy 21 was associated with trisomy 8 in part of the metaphases. Patients 3, 8, 12, and 15 presented pseudodiploid clones with different rearrangements: inv(16)(p13q22), t(13;20)(q13or14;q11), del(20)(q11), and dir ins (21;3)(q22q13q26), respectively. In patient 13 the karyotype was interpreted as 45, XY, -7, +der(7)(t(7;17)(p14q22), t(9q22)(q34p11) -17. Finally, patient 9 presented a complex karyotype with great variability from cell to cell and with frequent polyploidy of abnormal cells. Recurring associated defects were monosomy 3 and a t(9;?) (p13;?), which were seen in six of 12 abnormal metaphases.

DISCUSSION

A preliminary methodologic problem in this retrospective evaluation of megakaryoblastic leukemia was the selection of...
patients fulfilling the FAB criteria among the cases of acute leukemia with megakaryocytic involvement. It has been shown that in well-documented cases of ANLL-M7, less than 40% of the blast population is generally reactive for specific antiplatelet monoclonal antibodies. In our cases shown that in well-documented cases of ANLL-M7, less than 40% of the blast population is generally reactive for antiplatelet monoclonal antibodies. We diagnosed these cases as megakaryoblastic leukemia; it has not been reported in some series of de novo ANLL-M7. The presence of a concomitant myeloid participation, however, has been suspected on the basis of immunologic findings and has been clearly documented in a previous report where up to 14% SBB positive cells were seen. Taken together, these observations and our findings seem to indicate that megakaryocytic involvement in ANLL-M7 may occur rather frequently, thus demonstrating in such cases that the leukemic event occurred in an undifferentiated stem cell capable of differentiating along different lineages. Interestingly, a direct demonstration of this feature has recently been provided.

The presence of dyserythropoiesis or dysmegakaryocytosis in acute leukemias has recently been emphasized along with its possible clinical correlations. In ANLL-M7, however, megakaryocytic dysplasia may derive from aberrant differentiation of the leukemic clone and, generally, dyserythropoiesis alone may be secondary to BM invasion. Therefore, we looked for the presence of dysmyelopoiesis simultaneously involving all three hematopoietic lineages and found that all cases had some features of myelodysplasia and that three of six evaluable patients could be classified as ANLL with TMDS as previously defined. This illustrates that ANLL-M7 is frequently associated with a myelodysplastic picture. As already pointed out, an attractive interpretation of this finding is that we may be dealing in such cases with a subgroup of acute myeloid leukemias originating from a multipotent stem cell. The observation that three of

Table 3. Bone Marrow Features in 15 Cases of Megakaryoblastic Leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>% BM Blasts</th>
<th>Blast Morphology</th>
<th>BM Fibrosis</th>
<th>Myelodysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>99%</td>
<td>Undiff</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 2</td>
<td>95%</td>
<td>Undiff</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 3</td>
<td>75%</td>
<td>Undiff</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 4</td>
<td>65%</td>
<td>Undiff, 7% granular bls</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 5</td>
<td>72%</td>
<td>MicroMega 3% granular bls</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>Case 6</td>
<td>9%</td>
<td>Undiff</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 7</td>
<td>57%</td>
<td>MicroMega</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>Case 8</td>
<td>18%</td>
<td>MicroMega</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>Case 9</td>
<td>36%</td>
<td>Undiff</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 10</td>
<td>41%</td>
<td>MicroMega</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 11</td>
<td>38%</td>
<td>MicroMega</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>Case 12</td>
<td>98%</td>
<td>MicroMega</td>
<td>+ +</td>
<td>NE</td>
</tr>
<tr>
<td>Case 13</td>
<td>25%</td>
<td>MicroMega</td>
<td>+ +</td>
<td>NE</td>
</tr>
</tbody>
</table>

Abbreviations: EbIs, Erythroblasts; MicroMega, micromegakaryocytes; RS, ringed sideroblasts; DEP, dyserythropoiesis; DGP, dysgranulopoiesis; DMKP, dysmegakaryocytosis; Undiff, undifferentiated; NE, not evaluable.

* - absent; +, moderate; ++, marked.
† - absent; +, 10-25%; ++, 25-50%; ++++, >50% dysplastic cells of each lineage.
‡Scanty dilute aspirate; bone biopsy contained over 30% blasts.
§De novo ANLL with TMDS.
¶Scanty dilute aspirate; diagnosis of megakaryoblastic transformation based on 24% peripheral blasts, rapidly increasing to 60%.
#Few cells.
#On trunhpine biopsy.

From www.bloodjournal.org by guest on November 15, 2017. For personal use only.
our cases were indistinguishable from AMF, as defined by Bearman, et al., and in line with this concept, AMF being considered a trilineage myeloproliferative disorder.

Results of chromosome analysis in 11 patients with de novo ANLL-M7 and in four cases of megakaryoblastic transformation of myeloid neoplasias are reported in this paper. Besides numerical aberrations, such as -7 and +8, commonly seen in other subtypes of acute leukemias and in myelodysplastic syndrome (MDS), some structural rearrangements detected in our patients are interesting.

The dir ins (12;3)(q14;q21q27) in a patient with ANLL-M7 and the dir ins (21;3)(q22;13q26) in a case of megakaryoblastic evolution of CML can be grouped with the different types of 3q21 and/or 3q26 rearrangements described in a spectrum of myeloid neoplasias with disorders megakaryocytopenia, including de novo and secondary myeloid leukemias, and myelofibrosis with myeloid metaplasia (MMM). Seemingly, these chromosome bands may contain loci for genes related with neoplasias involving the megakaryocytic lineage. It is of interest to note that many of the cases of acute leukemia with 3q21 and/or 3q26 rearrangements so far reported, have been classified either as undifferentiated or as M1 according to the FAB group. Because the expression of specific platelet markers has only been tested occasionally, the megakaryoblastic nature of some of these cases can not be excluded.

A t(13;20)(q13or14;q11) and a derivative chromosome 7 resulting from a t(7;17)(p14;q22) have been detected in a case presenting as AMF and in a blast crisis of CML, respectively. What these translocations have in common is the expression of specific platelet markers has only been tested occasionally, the megakaryoblastic nature of some of these cases can not be excluded.

A t(13;20)(q13or14;q11) and a derivative chromosome 7 resulting from a t(7;17)(p14;q22) have been detected in a case presenting as AMF and in a blast crisis of CML, respectively. What these translocations have in common is the expression of specific platelet markers has only been tested occasionally, the megakaryoblastic nature of some of these cases can not be excluded.

A t(13;20)(q13or14;q11) and a derivative chromosome 7 resulting from a t(7;17)(p14;q22) have been detected in a case presenting as AMF and in a blast crisis of CML, respectively. What these translocations have in common is the expression of specific platelet markers has only been tested occasionally, the megakaryoblastic nature of some of these cases can not be excluded.
ANLL-M7 and in eight of nine megakaryoblastic transformations of myeloid neoplasias. Although few cases have been reported, no significant differences seem to emerge by comparing the group of de novo ANLL-M7 with megakaryoblastic transformations, all chromosome changes being found in both groups. Indeed, the involvement of bands 3q21 and/or 3q26, alone or in combination with other abnormalities has been reported in three ANLL-M7 and in three cases of megakaryoblastic evolution of CML (two cases) and of MMM.

Trisomy of chromosomes 8 and 21 has been described in seven and three patients, respectively. Finally, 33% of the cases (12 of 36 de novo ANLL-M7 and 3 of 9 megakaryoblastic transformations) presented either -7/7q-, or -5/5q-, or a combination of any of these in the same abnormal clone. This percentage is higher than in any other FAB subtype reported at the FIWCL except M6, in which these aberrations occurred in 13 of 32 patients. The frequent involvement of chromosome 5 and 7, deletions of which are typically associated with secondary leukemias and environmentally induced myeloid neoplasias, raises the question whether ANLL-M7 is frequently preceded by exposure to leukemogenic agents or not. In our series toxic compounds may have played a role in three patients (1, 5, and 6). In

Fig 2. 44.XX.-7.ins(12;3)(q14;q21q27).-18 karyotype in patient 2. (A) Monosomy 18 is a random loss. (B) Partial karyotypes show enlargement of chromosomes 3 and 12.
Table 5. Karyotypes in Patients With Megakaryoblastic Leukemia Fulfilling the FAB Criteria Reported in the Literature

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/Ag.</th>
<th>Diagnosis</th>
<th>Abnormal Karyotypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/62</td>
<td>ANLL-M7</td>
<td>2q-:5q-†</td>
<td>Huang et al²</td>
</tr>
<tr>
<td>2</td>
<td>F/29</td>
<td>ANLL-M7</td>
<td>-2,-3,-5,-7,-8,-12,+16†</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M/40</td>
<td>PV→ANLL-M7</td>
<td>42,X,-2,3p-,5,-7,14p-,-16,-17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M/40</td>
<td>ANLL-M7</td>
<td>5q-,-7,14q-,-18,19q-†</td>
<td>Chan et al¹¹</td>
</tr>
<tr>
<td>5</td>
<td>F/1.1</td>
<td>ANLL-M7</td>
<td>50,XX,+2,+8,-1,+1q-,+1p-,+1p+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F/1.7</td>
<td>ANLL-M7</td>
<td>49-56,XX,t(11;21),+21</td>
<td>Pinto et al¹⁶</td>
</tr>
<tr>
<td>7</td>
<td>F/57</td>
<td>ANLL-M7</td>
<td>46,XX,inv(3)(q21q26)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M/43</td>
<td>ANLL-M7</td>
<td>46,XY,5q-</td>
<td>Hruban et al²⁰</td>
</tr>
<tr>
<td>9</td>
<td>M/0.6</td>
<td>ANLL-M7</td>
<td>45,XY,-2,-12.dup(7)(q22→q36),inv(9)(p11q13),+der(2)t(2;?)(p27;?)</td>
<td>Cairney et al³</td>
</tr>
<tr>
<td>10</td>
<td>M/25</td>
<td>ANLL-M7</td>
<td>44,X,-5,-7,-16,-18,del(3)(q22),+der(4),+der(7),+der(16),+M</td>
<td>Lichtman et al¹³</td>
</tr>
<tr>
<td>11</td>
<td>M/58</td>
<td>ANLL-M7</td>
<td>44,XY,-5,-15,del(20)(q11)/same with del(7)(q31q32)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F/1.1</td>
<td>ANLL-M7</td>
<td>51,XX,+9,+15,+20,+21,t(10;11)(p11; q12)/46,XX,-8,+9,+20,+21</td>
<td>Sariban et al¹²</td>
</tr>
<tr>
<td>13§</td>
<td>NR</td>
<td>ANLL-M7</td>
<td>45,XX,-7</td>
<td>Berger et al³¹</td>
</tr>
<tr>
<td>14</td>
<td>NR</td>
<td>ANLL-M7</td>
<td>47,XX,+8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>NR</td>
<td>ANLL-M7</td>
<td>45,XY,-5/(50),XY,+8,+14,+19,+20</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F/30</td>
<td>CML→ANLL-M7</td>
<td>48,XX,t(8;22)(q34;q11),t(11;3)(q23;q26),+22q-,+8</td>
<td>Akahoshi et al²²</td>
</tr>
<tr>
<td>17</td>
<td>F/60</td>
<td>MMM→ANLL-M7</td>
<td>46,XX,dir dup(1)(q21→q42),t(3;21) (q26;q22)</td>
<td>Teyssier et al³³</td>
</tr>
<tr>
<td>18</td>
<td>F/30</td>
<td>PNH→ANLL-M7</td>
<td>48,XX,-9, +i(9p),(9p).del(12)(p12)/47,XX,-5,+8,+17</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Ph + ET, Philadelphia chromosome positive essential thrombocythemia; MMM, myelofibrosis with myeloid metaplasia; PNH, paroxysmal nocturnal hemoglobinuria; NR, not reported.

Ten patients with ANLL-M7, seven of whom reported by Berger et al³¹ and three by Huang et al³ had a normal karyotype. One patient with Ph + ET→ANLL-M7 reported by Michiels et al³⁴ did not show additional aberrations.

†Detailed karyotype not given.
‡Constitutional inv(9)(p11q13).
§Three cases, one of whom had additional aberrations.

patients previously reported, occupational hazards were unremarkable in three cases (7, 10, and 11 in Table 5) and were not specified in other adult patients. As previously reported, however, the shortcomings of such retrospective evaluations should be emphasized and, indeed, toxic compounds are difficult to categorize and may not necessarily have been present in the environment at work, the only environment clinical records refer to. Ongoing prospective studies may provide more precise information on the true incidence of toxic exposure in such patients. Perhaps factors other than chemicals may bring about the same cytogenetic imbalances leading to deregulated cell growth, especially in young patients. The young age of some of our patients is really striking.

In conclusion, even though sufficient evaluable cases of megakaryoblastic leukemia have not been identified to draw a cytogenetic profile, the above results show that megakaryoblastic proliferations share cytogenetic features with a wide group of myeloid neoplasias. Most chromosome aberrations so far disclosed may therefore be related to mechanisms conferring a proliferative advantage to undifferentiated stem cells, rather than being specifically associated to the megakaryocytic phenotype. Trisomy 21, the possible relationship of which with ANLL-M7 in patients with Down's syndrome has recently been suggested,²⁶ does not frequently appear in patients with a normal constitutional karyotype. A more direct relationship with megakaryocyte differentiation processes can be claimed for 3q21 and 3q26 rearrangements. As yet, however, caution is required in assigning them a specific role in ANLL-M7, more accurate cytologic characterization being necessary in such cases. Taken together, the cytologic and cytogenetic aspects described in this series provide indirect evidence that ANLL-M7 may frequently derive from a multipotent stem cell capable of differentiating in multilineage pathways. The recent introduction of techniques for the simultaneous identification of immunophenotype and karyotype of dividing cells seems to offer the opportunity to directly demonstrate, in prospective studies, multilineage involvement in megakaryoblastic leukemia. The identification of such leukemias is becoming increasingly important not only on biologic and clinical grounds but also for the therapeutic implications, the type of transformed stem cell being one of the proposed guideline features in the therapeutic strategy for acute myeloid leukemias.

ACKNOWLEDGMENT

We thank the technicians of the Centre for Human Genetics for excellent technical assistance, and R. Logist for secretarial help. We also thank Dr Breton-Gorius for the immunologic study of patient 1.
**REFERENCES**


47. Farrow A, Jacobs A, West RR: Myelodysplasia, chemical exposure, and other environmental factors. Leukemia 3:33, 1989
Multipotent stem cell involvement in megakaryoblastic leukemia: cytologic and cytogenetic evidence in 15 patients

A Cuneo, C Mecucci, S Kerim, E Vandenberghhe, P Dal Cin, A Van Orshoven, J Rodhain, A Bosly, JL Michaux and P Martiat