Cytogenetics in acute leukemia

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Summary Cytogenetic analyses in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) have revealed a great number of non-random chromosome abnormalities. In many instances, molecular studies of these abnormalities identified specific genes implicated in the process of leukemogenesis. The more common chromosome aberrations have been associated with specific laboratory and clinical characteristics, and are now being used as diagnostic and prognostic markers guiding the clinician in selecting the most effective therapies. Specific chromosome aberrations and their molecular counterparts have been included in the World Health Organization classification of hematologic malignancies, and together with morphology, immunophenotype and clinical features are used to define distinct disease entities. However, the prognostic importance of less frequent recurrent aberrations in AML and ALL, both primary and secondary, is still to be determined. This review summarizes current views on clinical relevance of major cytogenetic findings in adult AML and ALL.

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KEYWORDS
Acute myeloid leukemia; Acute lymphoblastic leukemia; Chromosome aberrations; Human; Karyotyping; Prognosis

Introduction

The role of cytogenetics in determining the biologic basis of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is now widely recognized. By identifying acquired chromosome aberrations that recur in AML and ALL, and providing precise chromosomal location of breakpoints in leukemia-associated translocations and inversions, cytogenetics aided in cloning of many genes whose activation or fusion with other genes contributes to the neoplastic process.\textsuperscript{1–3} Further characterization of these genes revealed that they are often involved directly or indirectly in the development and homeostasis of normal blood cells, and that abnormal protein products of fusion genes created by specific translocations and inversions can dysregulate proliferation, differentiation or programmed cell death (apoptosis) of blood cell precursors.\textsuperscript{1} This has paved the way to designing novel therapeutic agents targeting specific genetic abnormalities in leukemic blasts, such as imatinib mesylate, the Bcr–Abl tyrosine kinase inhibitor that suppresses proliferation of cells harboring the BCR–ABL fusion gene created by t(9;22) (q34;q11.2), a recurrent chromosome aberration in chronic myelogenous leukemia (CML) and ALL.\textsuperscript{4}

Furthermore, cytogenetic analysis is now routine part of clinical practice, being an important...
tool in diagnosis and prognostication of acute leukemias. Specific chromosome aberrations and their molecular counterparts have been included in the World Health Organization (WHO) classification of hematologic malignancies, and together with morphology, immunophenotype and clinical features are being used to define distinct disease entities. Pretreatment cytogenetic findings have been repeatedly shown to be among the most important, independent prognostic factors in both AML and ALL.20–26 Consequently, cytogenetic analyses are considered mandatory for analyzing outcome of many clinical trials and are currently used to stratify patients for different types of therapy.27–29 In this review, we will summarize the clinical relevance of major cytogenetic findings in adult AML and ALL.

**Acute myeloid leukemia**

Clonal chromosome abnormalities, that is, an identical structural aberration or gain of the same, structurally intact chromosome detected in at least two metaphase cells or the same chromosome missing from a minimum of three cells, are consistently found in the majority of AML patients at diagnosis. However, in contrast to patients diagnosed with CML, who are invariably positive for t(9;22) or its variants, the cytogenetic picture of AML is much more complex. To date, approximately 200 different structural and numerical aberrations such as reciprocal translocations, inversions, insertions, deletions, unbalanced translocations, isochromosomes, isodicentric chromosomes, isolated trisomies and monosomies have been found to be recurring chromosome changes in AML. Many of these aberrations are very rare, being so far detected in a few patients worldwide, whereas others occur more frequently. These more common abnormalities, together with their frequencies among adults and children with AML, are presented in Table 1. A more complete list of specific AML-associated recurrent chromosome aberrations, and genes affected by their formation (if known), can be found in Refs. 27, 30–32.

The incidence of abnormal karyotypes is in general lower in adult than in pediatric de novo AML. While 55% of 4257 adults with AML enrolled onto three large cooperative studies displayed chromosome aberrations (range 53–60%), 76% of 1184 patients included in the four largest series of childhood AML had an abnormal karyotype (range 68–85%; Table 1). The reasons for such a discrepancy are unknown, but may be related to biological differences between pediatric and adult disease. This is likely reflected by varying proportions of specific chromosome aberrations in children and adults with AML. For example, balanced rearrangements, mostly reciprocal translocations, involving band 11q23 and disrupting the MLL gene are on average four times more common in children than in adults. The frequency of 11q23 rearrangements in patients with AML decreases appreciably with age: they are detected in 43–58% of infants aged 12 months or less,33–35 in 39% of children aged 13–24 months,39 in 8–9% of children older than 24 months,35 and in just 4–7% of adults (Table 1), among whom, according to the United Kingdom Medical Research Council (MRC) study,19 the frequency of 11q23 rearrangements goes down from 5% in patients aged 15–34 years to 2% among those aged 35–55 years and 1% in patients older than 55 years. Some other, relatively rare, recurrent aberrations are essentially restricted to young children with AML, not being detected in adult patients thus far. The t(1;22)(p13;q13), a translocation resulting in the OTT-MAL gene fusion and highly correlated with acute megakaryoblastic leukemia, has been to date detected exclusively in children, 96% of whom were younger than 24 months.36–37 Interestingly, almost all infants younger than 6 months had t(1;22) as a sole aberration whereas it was predominantly part of a complex karyotype in the majority of older children (p = 0.00004).36 Another aberration hitherto detected only in infants aged 20 months or younger is t(7;12)(q36;p13). This subtle translocation, involving the ETV6 gene and almost always occurring together with trisomy 19, was often misdiagnosed as del(12p) in the past and only recently has been shown using fluorescence in situ hybridization (FISH) to be a consistent chromosome aberration in infant AML.38,39 In contrast, t(15;17)(q22;q12-21) and t(8;21)(q22;q22), the two most common reciprocal translocations in both adults and older children with AML (Table 1), have never been detected in infants aged less than 12 months.31 However, while the incidence of t(15;17) [and inv(16)(p13q22)/t(16;16)(p13;q22)] is similar in adults and older children, t(8;21) is twice as common in pediatric as in adult AML (Table 1). On the other hand, –5, del(5q) and other unbalanced structural abnormalities resulting in loss of material from 5q are much more frequent in adult than childhood AML. Likewise, both inv(3)(q21q26) and t(3;3)(q21;q26), which are found in 2% of adults, are extremely rare in children and have so far never been detected in a patient with de novo AML younger than 12 years.31
Table 1 Frequencies of the more common cytogenetic abnormalities in adult and childhood AML

<table>
<thead>
<tr>
<th>Cytogenetic abnormality</th>
<th>Cooperative Group Study (No. of patients)</th>
<th>Adults total(^a)</th>
<th>Children(^a,b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CALGB(^c) (n = 1311)</td>
<td>MRC(^d) (n = 2337)</td>
<td>SWOG/ECOG(^e) (n = 609)</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>None (normal karyotype)</td>
<td>582 (44)</td>
<td>1096 (47)</td>
<td>244 (40)</td>
</tr>
<tr>
<td>+8</td>
<td>123 (9)</td>
<td>211 (9)</td>
<td>53 (9)</td>
</tr>
<tr>
<td>-7/7q</td>
<td>95 (7)</td>
<td>209 (9)</td>
<td>52 (9)</td>
</tr>
<tr>
<td>del(7q)</td>
<td>47 (4)</td>
<td>136 (6)</td>
<td>NA</td>
</tr>
<tr>
<td>Loss of (7q)(^f)</td>
<td>19 (1)</td>
<td>73 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>t(15;17)(q22;q21)</td>
<td>88 (7)</td>
<td>210 (9)</td>
<td>27 (4)</td>
</tr>
<tr>
<td>-5/5q</td>
<td>86 (7)</td>
<td>183 (8)</td>
<td>36 (6)</td>
</tr>
<tr>
<td>del(5q)</td>
<td>42 (3)</td>
<td>104 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>Loss of (5q)(^f)</td>
<td>18 (1)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>81 (6)</td>
<td>104 (4)</td>
<td>50 (8)</td>
</tr>
<tr>
<td>inv(16)(p13q22)/t(16;16)(p13;q22)</td>
<td>96 (7)</td>
<td>53 (2)</td>
<td>53 (9)</td>
</tr>
<tr>
<td>-Y</td>
<td>58 (4)</td>
<td>NA</td>
<td>20 (3)</td>
</tr>
<tr>
<td>t/inv(11q23)</td>
<td>54 (4)</td>
<td>45 (2)</td>
<td>42 (7)(^g)</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>27 (2)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>abn(12p)</td>
<td>33 (3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>+21</td>
<td>28 (2)</td>
<td>51 (2)</td>
<td>NA</td>
</tr>
<tr>
<td>abn(17p)</td>
<td>30 (3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>del(9q)</td>
<td>33 (3)</td>
<td>37 (2)</td>
<td>17 (3)</td>
</tr>
<tr>
<td>inv(3)(q21q26)/t(3;3)(q21;q26)</td>
<td>12 (1)</td>
<td>61 (3)(^i)</td>
<td>12 (2)</td>
</tr>
<tr>
<td>del(11q)</td>
<td>12 (1)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>10 (1)</td>
<td>16 (1)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>8 (1)</td>
<td>10 (&lt;1)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Complex karyotype with ≥3 abn</td>
<td>135 (10)</td>
<td>NA</td>
<td>71 (12)</td>
</tr>
<tr>
<td>Complex karyotype with ≥5 abn</td>
<td>99 (8)</td>
<td>222 (9)</td>
<td>53 (9)</td>
</tr>
</tbody>
</table>

Abbreviations: CALGB, Cancer and Leukemia Group B; MRC, United Kingdom Medical Research Council; SWOG/ECOG Southwest Oncology Group/Eastern Cooperative Oncology Group; abn, abnormality; NA, not available.

\(^a\) Percentages for particular abnormalities calculated using only those studies that provided relevant data.

\(^b\) Data from Leverger et al.\(^{166}\) (130 children aged from 2 months to 16 years diagnosed with untreated AML); Raimondi et al.\(^{167}\) (121 children diagnosed with de novo AML); Martinez-Climent et al.\(^{168}\) (115 children and adolescents aged from 0 months to 19.2 years diagnosed with de novo AML); Raimondi et al.\(^{169}\) (478 children and adolescents younger than 21 years diagnosed with de novo AML).

\(^c\) Data from Byrd et al.;\(^{20}\) the study comprised patients aged 15–86 years diagnosed with de novo AML.

\(^d\) Data from Grimwade et al.\(^{16}\) and Grimwade et al.;\(^{19}\) Grimwade et al.\(^{16}\) comprised 1272 patients aged 15–55 years, the majority of whom were diagnosed with de novo AML; up to 9.4% of patients had AML secondary to chemotherapy or radiotherapy or to an antecedent hematologic disorder. Grimwade et al.\(^{19}\) comprised 1065 patients aged 44–91 years, 817 of whom were diagnosed with de novo AML and 248 with AML secondary to chemotherapy or radiotherapy or to an antecedent hematologic disorder.

\(^e\) Data from Slovak et al.;\(^{18}\) the study comprised patients aged 16–55 years diagnosed with untreated AML.

\(^f\) Loss of 7q and Loss of 5q refer to unbalanced structural abnormalities, other than del(7q) and del(5q), that result in loss of material from the, respectively, 7q and 5q chromosome arms (e.g., unbalanced translocations, additions, isochromosome of 7p or 5p, etc.).

\(^g\) Category defined as “abn 11q”, might also include patients with other abnormalities of 11q including del(11q).

\(^h\) Including three cases with i(17)(q10).

\(^i\) Category defined as “abn 3q”, might also include patients with other abnormalities of 3q.
Prognostic significance of cytogenetics in AML

The Fourth International Workshop on Chromosomes in Leukemia (4IWCL) was the first large, prospective, multi-center study that established pretreatment karyotype as an independent prognostic factor in AML.\(^8\) Significant differences in complete remission (CR) rate, CR duration (CRD) and overall survival (OS) were demonstrated when the 716 patients were prioritized first according to the presence of t(8;21); then t(15;17); followed by \(-5\) or del(5q), \(-7\) or del(7q); concurrent occurrence of \(-5\) or del(5q) and \(-7\) or del(7q), and then abnormalities of 11q, +8 and +21. Patients without any of the above-mentioned abnormalities were classified according to the chromosome number [hypodiploid, pseudodiploid, diploid (normal) and hyperdiploid]. Cytogenetic findings so classified were independent prognostic factors for both duration of first CR and overall survival in the subset of 305 patients treated adequately.\(^8\) In the follow-up studies, a group of patients with abnormalities of 16q22 was added, all cases with aberrations of chromosomes 5 and 7 were combined into one category, and patients with +8 and +21 included in the hyperdiploid group.\(^12\)\(^14\)\(^40\) The multivariate analyses performed at the third follow-up of the 4IWCL, which then comprised 628 cases with de novo AML with a median follow-up of 14.7 years for patients alive, confirmed karyotype as an independent predictor of survival for all patients and for those 291 patients who received induction therapy that would be deemed standard by present-day criteria.\(^14\)

Many studies, both smaller, performed in a single institution and large, collaborative multi-institutional ones, have confirmed that pretreatment karyotype constitutes an independent prognostic determinant for attainment of CR, CRD, risk of relapse and survival.\(^9\)\(^-\)\(^11\)\(^13\)\(^15\)\(^16\)\(^18\)\(^20\) Recently, three large collaborative studies proposed prioritization schemata that assign AML patients to one of the three risk groups, favorable, intermediate or adverse, based on pretreatment cytogenetic findings.\(^16\)\(^18\)\(^20\) The three cytogenetic risk systems share many common features, but differ with regard to some aspects (Table 2). In the MRC schema, any abnormality that is not classified as favorable or adverse, and is not accompanied by any additional chromosome aberration classified as favorable or adverse, is categorized in the intermediate-risk group. In contrast, both the Southwest Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG) and Cancer and Leukemia Group B (CALGB) schemata explicitly categorize particular abnormalities into risk groups, and leave as not classified aberrations too infrequent to be analyzed. Moreover, MRC and SWOG/ECOG classify patients with a given abnormality into one of the three risk groups once, whereas CALGB provides risk-group assignment separately for probability of induction success, cumulative incidence of relapse (CIR) and OS. Consequently, in the CALGB schema, the same abnormality [e.g., t(6;9)(p23;q34)] may be categorized in the intermediate-risk group with regard to probability of achieving a CR, in the adverse-risk group with respect to OS and not being classified with regard to CIR, because too few patients attained a CR to be analyzed.

Despite these differences, the inv(16)/t(16;16) and t(8;21), cytogenetic hallmarks of core-binding factor (CBF) AML, have been categorized in the favorable group by all three cytogenetic risk systems. However, SWOG/ECOG classified as favorable also patients with del(16q) whereas MRC and CALGB did not. We believe that patients with true del(16q) [i.e., deletions that do not represent misinterpreted inv(16) or t(16;16) and are usually found in AML with morphology other than that of acute myelomonocytic leukemia with abnormal eosinophils (AMML Eo)] should not be included in the favorable risk group, because del(16q) differs from inv(16)/t(16;16) at the molecular level, and has not been associated with a favorable outcome comparable to that of inv(16)/t(16;16).\(^28\)\(^41\)\(^42\) Consequently, the RT-PCR and/or FISH assays detecting CBF\(/\)MYH11 gene fusion should be performed in all patients with del(16q)(q22) to ensure that they do not harbor a misidentified inv(16)/t(16;16).\(^28\) Moreover, while MRC and CALGB included all patients with t(8;21) in the favorable group, irrespective of whether t(8;21) was the sole aberration or occurred together with one or more secondary abnormalities, SWOG/ECOG classified as favorable only those t(8;21)-positive patients who did not have a complex karyotype with three or more abnormalities or a secondary del(9q). The latter abnormality has been reported as a poor risk indicator in patients with t(8;21) in one study,\(^43\) but this result was not corroborated by the MRC,\(^16\) CALGB,\(^20\) nor by other studies.\(^8\)\(^10\)\(^44\) Indeed, it has been consistently reported that for patients with t(8;21) and inv(16)/t(16;16), neither the presence of secondary abnormalities, including the most frequent in t(8;21)-positive patients \(-Y, -X\) and +8, nor a complex karyotype with three or more abnormalities adversely affects clinical outcome.\(^8\)\(^10\)\(^16\)\(^20\)\(^44\) Instead, adverse prognostic significance among t(8;21)-positive patients has been attributed to such factors as high initial white
as the German AML Study Group,\textsuperscript{49} as three or more abnormalities. A multi-center Italian study defined complex karyotype as "the presence of a clone with more than three cytogenetic abnormalities".\textsuperscript{50} Byrd et al.\textsuperscript{20} compared the outcome of patients with three or four abnormalities [other than t(8;21), inv(16)/t(16;16) or t(9;11)(p22;q23)] with that of patients with five or more abnormalities. Although patients in the former group were younger and had significantly better CRD and 5-year OS than patients with five or more abnormalities, the CR rate and OS of patients with three or four abnormalities were significantly lower and the CR significantly higher than those of patients included in the cytogenetically normal group. Only one patient in the group with three or four abnormalities remained in remission at 5 years. Thus, the authors concluded that these data justify combining patients with three or four abnormalities with patients who have five or more abnormalities into one complex karyotype category defined by the presence of three or more abnormalities.\textsuperscript{20}

It has been striking in the CALGB series that all or almost all patients with −5, del(7q), −17/17p−, −18, and −20 had a complex karyotype, thus precluding assessment of the prognostic significance of these abnormalities independently from complex karyotype. In the MRC study, the outcome of patients with del(7q) that was not part of a complex karyotype with ≥5 abnormalities and was not accompanied by −5/del(5q) or abn(3q) did not differ significantly from outcome of patients with a normal karyotype.\textsuperscript{16} Their findings are consistent with earlier observations suggesting that patients with del(7q) without coexisting aberrations of chromosome 5 may have prolonged survival.\textsuperscript{40,51} In the CALGB series, most patients with del(5q) had a complex karyotype and very poor prognosis. However, a relatively small group of patients with del(5q) in a non-complex karyotype was classified as having intermediate risk with regard to CR rate and OS.\textsuperscript{20}

Patients with various balanced abnormalities involving band 11q23 have been grouped into one cytogenetic category in both the SWOG/ECOG and MRC study, and classified, respectively, as having adverse and intermediate prognosis (Table 2). However, mounting evidence suggests that outcome of patients with translocations involving band 11q23 depends on the partner chromosome involved, with t(9;11)(p22;q23)-positive patients having a more favorable prognosis that places them in the intermediate-risk group.\textsuperscript{20,52} Remarkably, a recent study of 298 infants and children with de novo AML found that t(9;11) was the most important, favorable prognostic factor for patients treated on protocols used at St. Jude Children’s Research Hospital.\textsuperscript{53} Zwaan et al.\textsuperscript{54} suggested that the superior outcome of the t(9;11)-positive patients may be explained by enhanced sensitivity of their leukemic blasts to several chemotherapeutic drugs. In this study, bone marrow or blood samples from children with AML and t(9;11) were significantly more sensitive in vitro to cytarabine, etoposide, the anthracyclines and 2-chlorodeoxyadenosine than samples from patients with other chromosome aberrations. When compared with a subgroup of childhood AML patients harboring other 11q23 translocations, including t(6;11)(q27;q23), t(9;11)-positive samples were significantly more sensitive for cytarabine and doxorubicin, and borderline more sensitive for etoposide.\textsuperscript{54} The survival of adults with t(6;11) and t(11;19)(q23;p13.1) studied by CALGB was significantly shorter than that of the cytogenetically normal group, and, consequently, t(6;11) and t(11;19)(q23;p13.1) were assigned to the adverse-risk group for OS. For patients with other, less frequent 11q23 translocations, the definitive assignment of risk category will be possible only once enough patients are analyzed in large prospective studies.\textsuperscript{20}

The most common trisomies in de novo AML are, in decreasing order of frequency, +8, +22, +13, +21 and +11.\textsuperscript{20} Trisomy 22 is a non-random secondary aberration accompanying inv(16)/t(16;16) and is rarely seen as the only chromosome abnormality.\textsuperscript{31} Although each of the remaining trisomies can be found as a secondary aberration, +8, +13, +11 and +21 are also detected recurrently as the only (isolated) karyotypic changes at diagnosis, with a frequency among adults with de novo AML of 4% for sole +8, 1% each for sole +13 and +11, and 0.4% for sole +21.\textsuperscript{55} With regard to the impact of recurrent trisomies on clinical outcome, most data have been gathered for trisomy 8, but results have been somewhat inconsistent. CR rates of patients with +8 have differed widely, from 29%\textsuperscript{11} to 91%,\textsuperscript{51} as have CRD and survival among studies,\textsuperscript{32} and consequently patients with +8 have been classified either in the intermediate or adverse-risk category. In some reports, this cytogenetic group included both patients with isolated +8 and those who in addition to +8 had other aberrations that may have affected response to treatment and outcome. It has been repeatedly shown that prognosis of AML patients with +8 indeed depends on whether +8 occurs as an isolated abnormality or is accompanying other aberrations.\textsuperscript{16,20,56–58} In the latter situation, +8 does not appear to adversely affect the favorable outcome of patients with t(15;17), inv(16)/t(16;16) and t(8;21).\textsuperscript{16,20,56,57} In contrast,
During the last 30 years, cytogenetic analyses of patients with AML and ALL have discovered a great number of recurrent chromosome abnormalities. Several of the more common abnormalities have been associated with specific laboratory and clinical characteristics, and are being used as diagnostic and prognostic markers that can guide the clinician in selecting the most effective treatment regimens. However, the prognostic importance of less frequent recurrent aberrations, both primary and secondary, is still unknown. Continuing cytogenetic studies are thus necessary to accrue enough patients with these rarer abnormalities to define conclusively their impact on CR rates, remission duration and survival, and to resolve discrepancies in prognostic categorization of some of the more frequent aberrations that currently exist among the major cytogenetic risk-assignment systems. Such studies will likely uncover new recurrent aberrations as they are still being identified by both classical cytogenetic methods and, increasingly, by molecular-cytogenetic techniques such as FISH, spectral karyotyping (SKY), multiplex-FISH (M-FISH) and multiplex FISH telomere assay (M-Tel). Moreover, it is well known that prognostic factors depend on the type of therapy used. A cytogenetic or molecular genetic abnormality conferring an adverse prognosis with one therapeutic regimen may lose its unfavorable prognostic impact when another treatment is used. Therefore there is a constant need for large prospective studies correlating karyotype with selected molecular genetic markers, gene expression profiles, immunophenotype, other biologic parameters and clinical outcome in patients treated with both current therapies and those receiving novel therapeutic agents.

### Table 5 Breakpoints and genes recurrently rearranged with T-cell receptor genes in T-ALL

<table>
<thead>
<tr>
<th>Chromosome breakpoint</th>
<th>Gene involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p32</td>
<td>SCL (TAL-1 or TCL-5)</td>
</tr>
<tr>
<td>1p35-p34.3</td>
<td>LCK</td>
</tr>
<tr>
<td>8q24</td>
<td>PTET1 and cMYC</td>
</tr>
<tr>
<td>9q34.3</td>
<td>TAN-1</td>
</tr>
<tr>
<td>10q24</td>
<td>HOX11</td>
</tr>
<tr>
<td>11p13</td>
<td>RB1T2N2 (TTG2)</td>
</tr>
<tr>
<td>11p15.5</td>
<td>RB1T1N1 (TTG1)</td>
</tr>
<tr>
<td>19p13.2-p13.1</td>
<td>LYL1</td>
</tr>
</tbody>
</table>

**Practice points**

- Cytogenetic analysis should be performed in all newly diagnosed patients with AML or ALL.
- Patients may be stratified to different therapies based on results of standard cytogenetic analysis, FISH and/or molecular genetic investigations (RT-PCR).

**Research agenda**

- Continuing correlation of recurrent chromosome aberrations with response rates, response duration, survival and cure in groups of AML and ALL patients treated with current and novel induction and post-induction regimens.
- Ascertainment of prognostic significance of the less common recurrent abnormalities in AML and ALL that are currently arbitrarily assigned to the intermediate-risk group or not categorized at all.
During the last 30 years, cytogenetic analyses of patients with AML and ALL have discovered a great number of recurrent chromosome abnormalities. Several of the more common abnormalities have been associated with specific laboratory and clinical characteristics, and are being used as diagnostic and prognostic markers that can guide the clinician in selecting the most effective treatment regimens. However, the prognostic importance of less frequent recurrent aberrations, both primary and secondary, is still unknown. Continuing cytogenetic studies are thus necessary to accrue enough patients with these rarer abnormalities to define conclusively their impact on CR rates, remission duration and survival, and to resolve discrepancies in prognostic categorization of some of the more frequent aberrations that currently exist among the major cytogenetic risk-assignment systems. Such studies will likely uncover new recurrent aberrations as they are still being identified by both classical cytogenetic methods and, increasingly, by molecular-cytogenetic techniques such as FISH, spectral karyotyping (SKY), multiplex-FISH (M-FISH) and multiplex FISH telomere assay (MTEL). Moreover, it is well known that prognostic factors depend on the type of therapy used. A cytogenetic or molecular genetic abnormality conferring an adverse prognosis with one therapeutic regimen may lose its unfavorable prognostic impact when another treatment is used. Therefore there is a constant need for large prospective studies correlating karyotype with selected molecular genetic markers, gene expression profiles, immunophenotype, other biologic parameters and clinical outcome in patients treated with both current therapies and those receiving novel therapeutic agents.

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<td>11p13</td>
<td>RBTN2 (TTG2)</td>
</tr>
<tr>
<td>11p15.5</td>
<td>RBTN1 (TTG1)</td>
</tr>
<tr>
<td>19p13.2-p13.1</td>
<td>LYL1</td>
</tr>
</tbody>
</table>

Concluding remarks

During the last 30 years, cytogenetic analyses of patients with AML and ALL have discovered a great number of recurrent chromosome abnormalities. Several of the more common abnormalities have...
Acute Myeloid Leukemia

Session Chair: Hillard M. Lazarus, MD
Speakers: Clara D. Bloomfield, MD; Donald Small, MD, PhD; and Wendy Stock, MD

Hematology 2006, pp. 169-177

Chromosome Aberrations, Gene Mutations and Expression Changes, and Prognosis in Adult Acute Myeloid Leukemia

Krzysztof Mrózek and Clara D. Bloomfield

Pretreatment clinical features and prognosis of patients with acute myeloid leukemia (AML) are strongly influenced by acquired genetic alterations in leukemic cells, which include microscopically detectable chromosome aberrations and, increasingly, submicroscopic gene mutations and changes in gene expression. Cytogenetic findings separate AML patients into three broad prognostic categories: favorable, intermediate and adverse. The cytogenetic-risk classifications differ somewhat for younger adult patients and those aged 60 years or older. In many instances, patients with specific cytogenetic findings, e.g., those with a normal karyotype or those with either t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) [collectively referred to as core-binding factor (CBF) AML] can be further subdivided into prognostic categories based on the presence or absence of particular gene mutations or changes in gene expression. Importantly, many of these molecular genetic alterations constitute potential targets for risk-adapted therapies. In this article, we briefly review major cytogenetic prognostic categories and discuss molecular genetic findings of prognostic significance in two of the largest cytogenetic groups of patients with AML, namely AML with a normal karyotype and CBF AML.

Adult acute myeloid leukemia (AML) is a very heterogeneous disease with regard to clinical features and acquired genetic alterations, both those detectable microscopically as structural and numerical chromosome aberrations, and those detected as submicroscopic gene mutations and changes in gene expression. At present, cytogenetic aberrations detected at the time of AML diagnosis constitute the most common basis for predicting clinical outcome. However, molecular genetic alterations are increasingly being used to refine prognosis further. In this article we briefly review major cytogenetic-risk prognostic categories and then focus on how molecular genetic findings, many of which are or likely will become therapeutic targets, contribute to prognostication of patients belonging to two of the larger cytogenetic groups: AML with a normal karyotype and core-binding factor (CBF) AML.

Prognostic Groups Based on Cytogenetic Findings

Acquired clonal chromosome abnormalities, i.e., a structural aberration or a trisomy observed in at least 2 metaphase cells, are detected in the pretreatment marrow of 50% to 60% of adults with de novo AML. In 10% to 20% of patients, the abnormal karyotype is complex, i.e., contains at least 3 chromosome aberrations, whereas in 40% to 50% of patients no cytogenetic abnormality can be discerned using standard banding methods. Recent large collaborative studies have proposed...
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most important prognostic factors, cytogenetic analysis of
BM is now mandatory in the diagnostic workup of newly
diagnosed patients with AML. Results of this analysis are
also used for selecting therapy. The presence of fusion
genes characteristic of CBF AML, acute promyelocytic leukemia
with t(15;17) and AML with rearrangements of band 11q23
can also be detected by RT-PCR and fluorescence in situ
hybridization (FISH). These methods are especially valu-
able in patients with variants of typical cytogenetic aber-
rations and in patients suspected of carrying cryptic rear-
rangements, e.g., those with FAB M4Eo, M3 or M3v mar-
row morphology but without, respectively, inv(16)t(16;16)
and t(15;17) on standard cytogenetic examination. Cyto-
genetic, FISH and molecular analyses for the more com-
mon recurring translocations are readily available.48

The clinical significance of some of the molecular ge-
test results discussed in this review, e.g., microarray gene-
expression profiling and testing for ERG gene expression,
is not yet firmly established and requires further study be-
fore such testing can be translated into clinical practice.
Moreover, although testing for FLT3 mutations in younger
patients with de novo AML is now recommended by the
Practice Guidelines in Oncology,47 testing for FLT3-ITD
and for the other molecular markers is available mostly at
the large university centers and performed as part of clini-
cial trials. Because two or more genetic alterations can be

Table 4. Prognostic significance of mutations in the KIT gene in CBF AML patients.

<table>
<thead>
<tr>
<th>Chromosome Aberration</th>
<th>Age, Range (Median) Years</th>
<th>No. Patients</th>
<th>Total</th>
<th>Exon 8</th>
<th>Exon 17</th>
<th>Other</th>
<th>Prognostic Significance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)</td>
<td>16-76 (40.5)</td>
<td>36</td>
<td>17 (47%)</td>
<td>5 (14%)</td>
<td>12 (33%)</td>
<td>2 (6%)</td>
<td>OS: Significantly shorter for pts with any KIT mutation (24 mo OS rates: 42% vs 77%, P = 0.017) and for pts with exon 17 mutation (24 mo OS rates: 25% vs 77%, P = 0.006) compared with pts without KIT mutations.</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>15 (44%)</td>
<td>5 (15%)</td>
<td>10 (29%)</td>
<td>2 (6%)</td>
<td>RI: Significantly higher for pts with any KIT mutation (24 mo RI rates: 77% vs 35%, P = 0.005) and for pts with exon 17 mutation (24 mo OS rates: 90% vs 35%, P = 0.002) compared with pts without KIT mutations.</td>
<td></td>
</tr>
<tr>
<td>t(8;21)</td>
<td>15-90</td>
<td>64</td>
<td>8 (13%)</td>
<td>NA</td>
<td>8 (13%)</td>
<td>NA</td>
<td>OS: Significantly shorter for pts with exon 17 KIT mutation (median: 304 d vs 1836 d, P &lt; 0.001). EFS: Significantly shorter for pts with exon 17 KIT mutation (median: 244 d vs 744 d, P = 0.003).</td>
<td>43</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>1-75 (33)‡</td>
<td>50</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>NA</td>
<td>OS: Significantly shorter for pts with any KIT mutation (P = 0.03). EFS: Significantly shorter for pts with any KIT mutation (P = 0.006). RFS: Significantly shorter for pts with any KIT mutation (P = 0.005).</td>
<td>44</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>18-71 (37)</td>
<td>49</td>
<td>11 (22%)</td>
<td>2 (4%)</td>
<td>9 (18%)</td>
<td>NA</td>
<td>OS: No significant difference between pts with or without KIT mutations. CIR: Significantly higher for patients with any KIT mutation (5-year CIR 70% vs 36%, P = 0.017) compared with those without KIT mutations.</td>
<td>45</td>
</tr>
<tr>
<td>inv(16)</td>
<td>15-74 (44)‡</td>
<td>63</td>
<td>20 (32%)</td>
<td>15 (24%)</td>
<td>5 (8%)</td>
<td>NA</td>
<td>OS: No significant difference between pts with and without exon 8 mutations. Mutations in exon 17 not analyzed. RR: Significantly higher for pts with exon 8 mutations compared with pts without exon 8 mutations. Mutations in exon 17 not analyzed.</td>
<td>46</td>
</tr>
<tr>
<td>inv(16)/t(16;16)</td>
<td>17-88 (51)</td>
<td>17</td>
<td>8 (47%)</td>
<td>NA</td>
<td>7 (41%)</td>
<td>NA</td>
<td>OS: No significant differences between pts with any KIT mutation or with exon 17 mutation and those without KIT mutations. RI: No significant difference between pts with any KIT mutation or with exon 17 mutation and those without KIT mutations.</td>
<td>42</td>
</tr>
<tr>
<td>inv(16)/t(16;16)</td>
<td>1-75 (33)‡</td>
<td>46</td>
<td>10 (22%)</td>
<td>9 (20%)</td>
<td>1 (2%)</td>
<td>NA</td>
<td>OS: No significant difference between pts with any KIT mutation and those without KIT mutations.</td>
<td>44</td>
</tr>
<tr>
<td>inv(16)/t(16;16)</td>
<td>19-57 (40)</td>
<td>61</td>
<td>18 (30%)</td>
<td>8 (13%)</td>
<td>10 (16%)</td>
<td>NA</td>
<td>OS: No significant differences between pts with any KIT mutation and those with any KIT mutation, with exon 17 mutation and exon 8 sole mutation. In multivariable analyses, any KIT mutation predicted worse overall survival (OS) after adjusting for sex. CIR: Significantly higher for patients with any KIT mutation (5-year CIR 80% vs 29%, P = 0.005) and pts with exon 17 mutation (5-year CIR 80% vs 29%, P = 0.002) compared with those without KIT mutations. No significant difference between pts with exon 8 sole mutation and those without KIT mutations.</td>
<td>45</td>
</tr>
</tbody>
</table>

Abbreviations: KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; pts, patients; OS, overall survival; RI, relapse incidence; EFS, event-free survival; RFS, relapse-free survival; CIR, cumulative incidence of relapse; RR, relapse rate; NA, not analyzed; NR, not reported.

* Numbers of patients for whom clinical data were available.
† Median age and range of all 1940 patients analyzed for KIT mutations.
‡ Age range and median provided for all patients with CBF AML, including 56 with t(8;21) and 47 with inv(16)t(16;16).
§ Mean age.
| One patient with inv(16) whose clinical outcome was analyzed had a non-exon 17 mutation, but it is unclear if this was an exon 8 or other KIT mutation. |     |
Review in translational hematology

(Blood, 2007; 109: 431-448)

Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification?

Krzysztof Mrozek, Guido Marcucci, Peter Paschka, Susan P. Whitman, and Clara D. Bloomfield

Division of Hematology and Oncology, Department of Internal Medicine, and Division of Human Cancer Genetics, Department of Microbiology, Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio

Recent molecular analyses of leukemic blasts from pretreatment marrow or blood of patients with acute myeloid leukemia (AML) and a normal karyotype, the largest cytogenetic subset (ie, 40%-49%) of AML, have revealed a striking heterogeneity with regard to the presence of acquired gene mutations and changes in gene expression. Multiple submicroscopic genetic alterations with prognostic significance have been discovered, including internal tandem duplication of the FLT3 gene, mutations in the NPM1 gene, partial tandem duplication of the MLL gene, high expression of the BAAALC gene, and mutations in the CEBPA gene. Application of gene-expression profiling has also identified a gene-expression signature that appears to separate cytogenetically normal AML patients into prognostic subgroups, although gene-expression signature-based classifiers predicting outcome for individual patients with greater accuracy are needed. These and similar future findings are likely to have a major impact on the clinical management of cytogenetically normal AML not only in prognostication but also in selection of appropriate treatment, since many of the identified genetic alterations already constitute or will potentially become targets for specific therapeutic intervention. In this report, we review prognostic genetic findings in karyotypically normal AML and discuss their clinical implications. (Blood. 2007;109:431-448)

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Introduction

The development of acute myeloid leukemia (AML) is associated with accumulation of acquired genetic alterations and epigenetic changes in hematopoietic progenitor cells that alter normal mechanisms of cell growth, proliferation, and differentiation. At diagnosis, most patients with AML harbor at least 1 chromosome aberration in their marrow blasts. Numerous recurrent structural and numeric cytogenetic aberrations have been identified and many of them not only are diagnostic markers for specific AML subtypes but also constitute independent prognostic factors for attainment of complete remission (CR), relapse risk, and overall survival (OS). However, in a sizable group of AML patients, 40% to 49% of adults and 25% of children, no microscopically detectable chromosome abnormality can be found on standard cytogenetic analysis.

These cytogenetically normal (CN) patients have usually been classified in an intermediate-risk prognostic category because their CR rate, relapse risk, and survival are worse than those of adequately treated patients with such favorable aberrations as t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22), or t(15;17)(q22;q21) but better than those of patients with unfavorable cytogenetic findings [eg, −7, inv(3)(q21q26)t(3;3)(q21q26), balanced translocations involving 11q23 other than (9;11)(p22;q23), or a complex karyotype]. The outcome of CN patients has varied among studies, with 5-year survival rates between 24% and 42% reported. A recent Cancer and Leukemia Group B (CALGB) study found the relapse risk and disease-free survival (DFS) of these patients was improved by postremission treatment including 4 cycles of high-dose cytarabine (HDAC) or intermediate-dose cytarabine or 1 cycle of HDAC/etoposide followed by autologous stem-cell transplantation (ASCT) as opposed to regimens that include fewer cytarabine cycles or no ASCT. However, these therapeutic approaches do not improve outcome for all CN patients, likely because this cytogenetic subset is very heterogeneous at the molecular level. Indeed, during the last 12 years, several gene mutations and changes in gene expression have been discovered that strongly affect clinical outcome of CN AML patients. In this review, we will first discuss what constitutes a normal karyotype in AML and then those genetic alterations that are clinically relevant as both prognostic markers and potential targets for risk-adapted therapies in CN AML patients. We will begin with the internal tandem duplication (ITD) of the FLT3 gene, shown by many studies to be the most important prognostic factor, and follow with other genetic alterations discussed in order of the number of studies reporting their prognostic significance in CN AML.

The importance of ensuring that the patient’s karyotype is truly normal

The proportion of adults with de novo CN AML has varied between 40% and 49% in the largest cytogenetic studies, although both lower and higher percentages have been reported. The differences among studies could relate to several factors including the number of older patients studied, since the proportion of CN cases increases with age, and differences in cytogenetic methodologies and the criteria used to consider a karyotype normal.

Leukemic blasts carrying AML-associated chromosome aberrations can constitute only a fraction of cells dividing in vitro. Occasionally, the cytogenetically abnormal clone is detectable in cells cultured in vitro for 24 to 48 hours but not in a direct
Prognostic impact of genetic characterization in the GIMEMA LAM99P multicenter study for newly diagnosed acute myeloid leukemia

Francesco Lo-Coco, Antonio Cuneo, Fabrizio Pane, Daniela Cilloni, Daniela Diverio, Marco Mancini, Nicoletta Testoni, Antonella Bardi, Barbara Izzo, Niccolò Bolli, Roberta La Starza, Paola Fazi, Simona Iacobelli, Alfonso Picoci, Marco Vignetti, Sergio Amadori, Franco Mandelli, Pier Giuseppe Pelicci, Cristina Mexucci, Brunangelo Falini, and Giuseppe Saglio for the Acute Leukemia Working Party of the GIMEMA group

ABSTRACT

Background
Recent advances in genetic characterization of acute myeloid leukemia indicate that combined cytogenetic and molecular analyses provide better definition of prognostic groups. The aim of this study was to verify this prospectively in a large group of patients.

Design and Methods
Genetic characterization was prospectively carried out in 397 patients with acute myeloid leukemia (median age, 46 years) receiving uniform treatment according to the LAM99P protocol of the Italian GIMEMA group. The impact of genetic markers on response to therapy and outcome was assessed by univariate and multivariate analyses.

Results
For induction response, conventional karyotyping identified three groups with complete remission rates of 92%, 67% and 39% (p<0.0001). Complete remission rates in NPM1 mutated (NPM1+) and wild-type (NPM1-) groups were 76% and 60%, respectively, for the whole population and 81% and 61% in the group with normal karyotype (p=0.026, respectively). Multivariate analysis indicated that low risk karyotype and NPM1+ were independent factors favorably affecting complete remission. Multivariate analysis of overall and disease-free survival among 269 patients who achieved complete remission showed a significant impact of karyotype on both estimates and of FLT3 status on disease free-survival (FLT3-ITD vs. FLT3 wild-type, p=0.0001). NPM1 status did not significantly influence disease free-survival in either the whole population or in the patients with a normal karyotype in this series, probably due to the low number of cases analyzed.

Conclusions
These results reiterate the prognostic relevance of combining cytogenetic and mutational analysis in the diagnostic work up of patients with acute myeloid leukemia.


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Introduction

Acquired genetic lesions in acute myeloid leukemia (AML) are being increasingly recognized as relevant markers whose identification improves diagnostic refinement, classification and prognostic assessment in this heterogeneous disease. In fact, discrete AML entities requiring specific therapeutic approaches and/or showing different responses to therapy and outcome are better identified based on the detection of these alterations. As a consequence, genetic characterization of all AML patients at presentation is nowadays regarded as mandatory to determine treatment choices and should always integrate first level diagnostic studies based on morphology, cytochemistry and immunophenotype.

The genetic alterations in AML include chromosome abnormalities detectable at the karyotypic level, i.e. translocations and numerical abnormalities, as well as subtle gene alterations that are identified by molecular techniques such as small duplications/insertions and point mutations. Among the latter group, FLT3 and NPM1 aberrations have been reported as the most frequent genetic lesions, are consistently associated with normal karyotype, and show apparently opposite prognostic significance. FLT3 mutations being correlated with poor outcome and NPM1 mutations being associated with a more favorable response to therapy. Other aberrations, which have not yet been well defined at the gene level (e.g. numerical abnormalities such as -7, -5, +8, and others) are detectable by karyotypic or fluorescence in situ hybridization (FISH) analysis only, and are equally important in the clinic because of their association with specific entities (e.g. therapy-related AML) and with unfavorable outcomes.

Based on the above considerations, modern genetic characterization of AML should combine conventional karyotyping and molecular methods – FISH, reverse transcriptase polymerase chain reaction (RT-PCR), sequencing – with the aim of analyzing all major types of clinically relevant alterations. Besides detecting submicroscopic alterations, the routine use of RT-PCR for the analysis of chromosome translocations may unravel cryptic rearrangements and provide invaluable information in the case of failed karyotyping. While such an approach might be carried out routinely in experienced centers, due to logistic and standardization problems it might be more difficult in the context of large multi-institutional clinical trials.

To evaluate the prognostic relevance of an integrated genetic characterization of AML, in 1999 the Italian co-operative group GIMEMA started a clinical trial that included standard induction and consolidation therapy in all cases. Sample centralization, cell banking and standardized cytogenetic and molecular tests were planned to maximize methodological homogeneity and to establish the prognostic role of major genetic lesions in a uniform clinical context.

We report here the results of this study.

Design and Methods

Patients and treatment

Between 1999 and 2003, 509 patients with FAB non-M3 AML (median age 46 years; range, 15-60) were enrolled and started induction therapy in the multicenter LAM99P study of the Italian GIMEMA group. To evaluate the prognostic impact of genetic characterization all patients received a uniform induction and consolidation protocol and diagnostic samples were sent to a central laboratory for cytogenetic and molecular studies. Therapy consisted of a pre-treatment phase with hydroxyurea (2 g/m² for 5 days) followed by induction with daunorubicin (50 mg/m² days 1, 3, and 5), cytarabine (100 mg/m² days 1-10) and etoposide (100 mg/m² days 1-5) and consolidation with cytarabine (500 mg/m²/12 hours days 1-6) and daunorubicin (50 mg/m² days 4-6). After consolidation therapy, eligible patients with an identical HLA donor were planned to receive an allogeneic stem cell transplant whereas the remaining were addressed to peripheral blood autologous stem cell transplantation.

Logistics and organization of the network for centralized sample analysis and biological studies

A scheme illustrating the organization of the central sample analysis and the laboratory network for biological studies is shown in Figure 1. Anticoagulated bone marrow and peripheral blood samples were collected after informed consent at local hospitals participating in the GIMEMA LAM99P trial and sent by overnight courier to a central laboratory at the Department of Cellular Biotechnology and Hematology of La Sapienza University in Rome. While patients were rapidly started on cytoreductive therapy, clinicians were allowed to collect the samples during the 5 days of hydroxyurea pretreatment. This in turn facilitated the collection, shipment and delivery of samples in all cases from Monday through Thursdays thereby avoiding the week-ends. A network of six GIMEMA laboratories contributed, on a rotational basis, to carrying out the cytogenetic and RT-PCR studies. In addition to the central laboratory in Rome, the other five laboratories of the network were located at: the Department of Clinical and Biological Sciences, S. L. Gonzaga Hospital, Orbassano, University of Turin; the Hematology and Bone Marrow Transplantation Unit, University of Perugia; the CEINGE and Department of Biochemistry and Medical Biotechnologies, Federico II University of Naples; the Department of Biomedical Science, Hematology Unit, University of Ferrara; and the Seràgnoli Department of Hematology of the University of Bologna. The central laboratory was responsible for: i) blood sample processing to collect mononuclear cells, preparation and storage of material for genetic studies (fixed nuclei for karyotyping, dry pellets for DNA, and cells in guanidium thiocyanate for RNA); ii) shipment of material for genetic studies to the other laboratories; and iii) participation in performing genetic characterization studies on a rotational basis. For the cytogenetic studies, fixed nuclei from heparinized vials were

| 1018 | haematologica | 2008; 93(7) |
Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study

Marilyn L. Slovak, Kenneth J. Kopecky, Peter A. Cassileth, David H. Harrington, Karl S. Theil, Anwar Mohamed, Elizabeth Paietta, Cheryl L. Willman, David R. Head, Jacob M. Rowe, Stephen J. Forman, and Frederick R. Appelbaum, for the Southwest Oncology Group and the Eastern Cooperative Oncology Group

The associations of cytogenetics with complete remission (CR) rates, overall survival (OS), and outcomes after CR were studied in 609 previously untreated AML patients younger than 56 years old in a clinical trial comparing 3 intensive postremission therapies: intensive chemotherapy, autologous transplantation (ABMT), or allogeneic bone marrow transplantation (alloBMT) from matched related donors. Patients were categorized into favorable, intermediate, unfavorable, and unknown cytogenetic risk groups based on pretreatment karyotypes. CR rates varied significantly (P < .0001) among the 4 groups: favorable, 84% (95% confidence interval [CI], 77%-90%); intermediate, 76% (CI, 71%-81%); unfavorable, 55% (CI, 48%-63%); and unknown, 54% (CI, 33%-74%). There was similar significant heterogeneity of OS (P < .0001), with the estimated relative risk of death from any cause being 1.50 (CI, 1.10-2.05), 3.33 (CI, 2.43-4.55), and 2.66 (CI, 1.59-4.45) for the intermediate, unfavorable, and unknown risk groups, respectively, compared with the favorable group. In multivariate analyses, the effects of cytogenetic risk status on CR rate and OS could not be explained by other patient or disease characteristics. Among postremission patients, survival from CR varied significantly among favorable, intermediate, and unfavorable groups (P = .0003), with significant evidence of interaction (P = .017) between the effects of treatment and cytogenetic risk status on survival. Patients with favorable cytogenetics did significantly better following ABMT and alloBMT than with chemotherapy alone, whereas patients with unfavorable cytogenetics did better with alloBMT. Cytogenetic risk status is a significant factor in predicting response of AML patients to therapy; however, to tighten treatment correlates within genetically defined AML subsets, a significantly larger leukemia cytogenetic database is warranted. (Blood. 2000; 96:4075-4083)
examine the effect of cytogenetic risk groups within the 3 postremission arms. To allow comparison with the 10th United Kingdom Medical Research Council AML trial (MRC AML 10 trial), the cytogenetic data were also coded and analyzed according to published MRC criteria.10

Patients, materials, and methods

Patients and protocol

This study was based on patients registered by the Southwest Oncology Group (SWOG) or Eastern Cooperative Oncology Group (ECOG) to a single phase III intergroup study, E3489/S9034, a comparison of 3 intensive postremission therapies for adult patients (age 16-55) with previously untreated AML. The treatment regimens and clinical results for this trial have been described previously.9 Briefly, all patients received 1 or 2 courses of induction therapy consisting of idarubicin, 12 mg/m² intravenous push daily for 3 days, and intravenous cytarabine, 25 mg/m², followed by 100 mg/m² per day continuously infused for 7 days. Patients achieving a CR received 2 and 5 days of idarubicin and cytarabine, respectively, at the induction doses and were then assigned or randomized to one of 3 postremission therapies. Patients with a histocompatible sibling donor were assigned to allogeneic bone marrow transplantation (alloBMT). Those without matched donors were randomized to either intensive consolidation chemotherapy or autologous bone marrow transplantation (ABMT). FAB morphologic classification was based on morphology and cytochemistry. Flow cytometry was used to confirm FAB M0 cases. Cytogenetic results were not known to morphologists and were not considered in assigning FAB morphologic type. The randomization was stratified by age (16-45 vs 46-55), FAB classification, whether 1 or 2 induction courses were required to achieve CR, and non–peer-reviewed karyotypic categorization (favorable vs intermediate vs unfavorable) as described.8

Cytogenetic analyses

Cytogenetic studies on bone marrow or unstimulated peripheral blood samples obtained prior to induction therapy were performed using standard G-banding with trypsin-Giemsa or trypsin-Wright’s staining in ECOG- or SWOG-approved cytogenetics laboratories. Karyotypes were interpreted using International System for Cytogenetic Nomenclature (1995) criteria.11 Studies were considered normal diploid if no clonal abnormalities were detected in a minimum of 20 mitotic cells examined. Central review of karyotypes for SWOG patients was performed by at least 3 members of the SWOG cytogenetics committee. Similarly, ECOG cytogenetic studies were performed and evaluated according to ECOG standards. In contrast to the data presented in the original report,9 the cytogenetic data reported here were centrally reviewed to assess quality, processing, and final karyotypic interpretation. For this analysis, all studies deemed incomplete or inadequate were excluded.

Cytogenetic abnormalities were grouped according to published criteria adopted by SWOG.2,3,10,12-16 Four cytogenetic categories were defined (Table 1). The favorable risk category included patients with abnormalities (abn) of inv(16)(p13q22), t(16;16)(q22q22), or t(15;17)(q22q21) with any additional abnormalities, or t(8;21) without either a del(9q) or being part of a complex karyotype. The presence of a del(9q) in patients with t(8;21) leukemia has been reported as a poor risk indicator requiring more aggressive treatment.17 The intermediate risk category included patients characterized by +8, +11, +Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of +5del(5q), +7del(7q), -5, -7/del(7q), del(5q)/-5, -7, del(9q), t(6;9), t(9;22) abn, 17p, and complex karyotype defined as 3 or more abnormalities. Twenty-six patients had cytogenetic aberrations considered to have unknown prognostic significance because of their low frequency in AML.

To allow comparison with the MRC AML 10 trial, the cytogenetic data were also coded and analyzed according to published MRC criteria.10 The major differences between these 2 systems are the definition of complex karyotypes (=3 unrelated karyotypic differences in SWOG vs ≥5 aberrations by MRC); the classification by MRC of all t(8;21) studies as unfavorable, despite the presence of del(9q) or complex karyotypes, and the classification of 11q aberrations as intermediate by MRC but unfavorable by SWOG. In addition, all SWOG karyotypes of unknown prognostic significance are designated as intermediate risk by MRC.

Criteria for treatment outcomes

Complete response and relapse were defined according to standard criteria.17 OS was measured from the day of registration on study until death from any cause, censored for patients known to be alive at last contact. Survival from CR was defined similarly but from the date CR was achieved. Disease-free survival (DFS) was measured from the date of CR until either relapse or death from any cause, censored at last contact for patients last known to be alive without report of relapse.

Statistical analysis

Collection and quality control of patient pretreatment and outcome data were performed according to standard ECOG procedures. Analyses involving postremission therapy were based on intention to treat, with all patients analyzed according to their postremission treatment arms irrespective of whether they actually received the designated treatment. Distributions of OS and DFS were estimated by the method of Kaplan and Meier.18 prognostic significance of cytogenetic categories, treatment assignments, and other pretreatment factors (age, sex, performance status, FAB classification, marrow and peripheral counts, disease signs and symptoms, and extramedullary involvement) were investigated in logistic regression models for CR and proportional hazards regression models for OS, survival from CR, and DFS. Quantitative factors such as age, blood or marrow cell counts, or percentages were treated as continuous variables in these regression models. The prognostic effects of the SWOG and MRC cytogenetic classification systems were compared indirectly, as follows. In regression models, collapsing categories of a qualitative predictor variable restricts the model’s ability to fit the data. Measures of the resulting “loss of fit” provide formal statistical tests of whether the collapsed categorization

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Risk status</td>
</tr>
<tr>
<td>FAVORABLE</td>
</tr>
<tr>
<td>INTERMEDIATE</td>
</tr>
<tr>
<td>UNFAVORABLE</td>
</tr>
<tr>
<td>UNKNOWN</td>
</tr>
</tbody>
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SWOG indicates Southwest Oncology Group; MRC, Medical Research Council (United Kingdom); abn, abnormality.

*The intermediate group contains 244 patients with normal karyotypes.

†Risk status for t(6;9) or t(9;22) is not defined by MRC criteria, presumably due to a lack of these low-frequency aberrations in their cohort.
examine the effect of cytogenetic risk groups within the 3 postremission arms. To allow comparison with the 10th United Kingdom Medical Research Council AML trial (MRC AML 10 trial), the cytogenetic data were also coded and analyzed according to published MRC criteria.10

Patients, materials, and methods

Patients and protocol

This study was based on patients registered by the Southwest Oncology Group (SWOG) or Eastern Cooperative Oncology Group (ECOG) to a single phase III intergroup study, E3489/S9034, a comparison of 3 intensive postremission therapies for adult patients (age 16-55) with previously untreated AML. The treatment regimens and clinical results for this trial have been described previously.4 Briefly, all patients received 1 or 2 courses of induction therapy consisting of idarubicin, 12 mg/m² intravenous push daily for 3 days, and intravenous cytarabine, 25 mg/m²; followed by 100 mg/m² per day continuously infused for 7 days. Patients achieving a CR received 2 and 5 days of idarubicin and cytarabine, respectively, at the induction doses and were then randomized or assigned to one of 3 postremission therapies. Patients with a histocompatible sibling donor were assigned to allogeneic bone marrow transplantation (alloBMT). Those without matched donors were randomized to either intensive consolidation chemotherapy or autologous bone marrow transplantation (ABMT). FAB morphologic classification was based on morphology and cytochemistry. Flow cytometry was used to confirm FAB M0 cases. Cytogenetic results were not known to morphologists and were not considered in assigning FAB morphologic type. The randomization was stratified by age (16-45 vs 46-55), FAB classification, whether 1 or 2 induction courses were required to achieve CR, and non–peer-reviewed karyotypic categorization (favorable vs intermediate vs unfavorable) as described.9

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<tr>
<td>Favorable</td>
<td>inv(16)(p13.1;16)(q22.1)</td>
<td>121 (20%)</td>
<td>inv(16)(p13.1;16)(q22.1)</td>
<td>130 (21%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal, +8, +16, −Y, del(12p)</td>
<td>278 (46%)</td>
<td>Normal, +8, del(16q), del(7q), −21, −22, +21</td>
<td>375 (62%)</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>del(3q)−2, −Y, +mar, complex karyotypes (≥ 3 unrelated abn)</td>
<td>184 (30%)</td>
<td>del(3q)−2, −7, +mar, complex karyotypes (≥ 3 unrelated abn)</td>
<td>104 (17%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>All other abnormalities</td>
<td>26 (4%)</td>
<td>Category not recognized</td>
<td>—</td>
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Table notes:
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CENTRALIZED CYTOGENETIC ANALYSIS OF PEDIATRIC ACUTE LEUKEMIA: RESULTS OF AN ITALIAN COLLABORATIVE EXPERIENCE

LAURA SAINATI, * ANNA LESZL, MARIA CATERINA PUTTI, FRANCESCO PASQUALI, EMANUELA MASERATI, EMILIO DONTI, GIOVANNA VENTI, PAOLO SIMI, CECILIA GIULIANI, ADRIANO ANGIONI, MARIO STELLA, ANNA MONTALDI, MARIO SSSAREGO, LUIGI ZANESCO, ANDREA BIONDI, GIUSEPPE BASSO

*Dipartimento di Pediatria, Centro Leucemie Infantili, Università di Padova; *Biologia Generale e Genetica Medica, Università di Pavia; Centro di Genetica Clinica, Università di Sassari; Istituto di Medicina Interna e Scienze Oncologiche, Università di Perugia; Laboratorio di Genetica, Istituto di Clinica Pediatrica, Università di Pisa; Servizio Trasfusionale, Ospedale Bambino Gesù, Rome; Servizio di Genetica Umana e Immutotrasfusionale, Ospedale S. Bortolo, Vicenza; *Dipartimento di Medicina Interna,Università di Genova; Ospedale S. Gerardo, Monza; Dipartimento di Pediatria, Università di Torino; Italy

ABSTRACT

Background and Objective. Cytogenetic analysis of acute leukemia yields important information which has been demonstrated to be correlated to patient survival. A reference laboratory was created in order to perform karyotype analysis on all cases of acute leukemia enrolled in the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica) protocols.

Methods. From January 1990 to December 1995, 1115 samples of children with ALL or AML were sent in for cytogenetic analysis. The results of cell cultures were screened in the Reference Laboratory and then the fixed metaphases were sent to one of the six cytogenetic laboratories for analysis.

Results. The leukemic karyotypes of 556 patients were successfully analyzed. An abnormal clone was detected in 49% of cases of ALL and in 66% of AML. In ALL the most frequent abnormality was 9p rearrangement. Other recurrent abnormalities were t(9;22), t(4;11) and t(1;19). In AML t(8;21), t(15;17) and 11q23 rearrangement were the most frequent structural abnormalities. These findings are similar to the results obtained in other multicenter studies using a similar approach.

Interpretation and Conclusions. Our data confirm the feasibility of performing cytogenetic analysis in a centralized laboratory on mailed samples of bone marrow and/or peripheral blood; this is very important considering that cytogenetic analysis of neoplastic tissue requires a special laboratory and expert staff.

Key words: children, acute leukemias, cytogenetic, reference laboratory, chromosomes, prognosis

In recent years numerous collaborative studies have been conducted all over the world on the treatment of pediatric malignant neoplasms. The aim of these studies was to collect data on clinical and biological features in order to identify prognostic factors and different subgroups of disease with different clinical relevance to improve the treatment. Such collaborative studies have led pediatric oncology to achieve important goals in our understanding of the pathogenesis, diagnosis and treatment of neoplastic diseases. An essential premise for multicentric studies is that all cases, or at least the majority of them, can be adequately analyzed using current evaluation techniques.

The cytogenetic analysis of leukemic blasts in childhood acute lymphoblastic leukemia (ALL) has identified chromosome abnormalities in about 50-80% of cases. The presence of well-defined struc-
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This paper presents an overview of the results of
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this approach with other collaborative studies.

**Materials and Methods**

**Patients**

In 1988 a tissue bank for the storage of bone
marrow and peripheral blood samples of pediatric
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AIEOP was created in Padua at the Onco-Hematol-
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sample to the central laboratory, together with a
sample for the bone marrow bank, thus avoiding
any extra cost of mailing the sample for cytogenetic
analysis alone.

Between January 1990 and December 1995, 1309
samples of bone marrow and/or peripheral blood
from children with suspected acute leukemia were
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these were ALL or AML at diagnosis or relapse. The
other 193 samples belonged to patients with non-
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from the study. The 1115 leukemia patients were
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sent was obtained. There were 667 males and 448
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All the cases were classified according to the
French-American-British (FAB) classification8,9 and
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**Cytogenetic analysis**

Heparinized bone marrow and/or peripheral
blood samples were collected in syringes or test
tubes and mailed to the laboratory at room tem-
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obtained, and cell vitality was checked; cultures
were prepared using 1 × 10⁶ cells/mL of medium
(RPMI 1640, 20% FCS, L-glutamine and 50 µg/mL
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(for direct harvest, overnight exposure to colchemid
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days with a supernatant of a cell line with growth
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with fewer than 5 × 10⁶, or with EDTA used as an
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using routine methods. From 1 to 3 slides were
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results were resubmitted to the reference labo-
rary, where all data were recorded and sent back
to the center to which the patient belonged.

**Results**

The 1115 samples of acute leukemia analyzed
included: 951 cases at diagnosis (802 ALL and 149
AML) and 164 at relapse (134 ALL and 30 AML).
All the patients joined the AIEOP protocols for
childhood ALL or AML. The number of AIEOP cen-
ters requesting cytogenetic analysis has progressively
increased over the years, from 12 in 1990 to 27 in
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Of the 1115 samples received, 391 (35%) were
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Feasibility of centralized cytogenetic analysis of acute leukemia

655
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Since 1990, the Onco-Hematology Laboratory of the Pediatric Department at Padua University has performed cytogenetic analysis on acute leukemia samples, together with diagnostic and biological studies, on request for any institution following the AIEOP protocols.

This paper presents an overview of the results of this collaborative cytogenetic study which involved several AIEOP centers and a few cytogenetic laboratories between 1990 and 1995. The aim of this paper was to establish the feasibility of centralizing cytogenetic studies and to compare the outcome of this approach with other collaborative studies.

**Materials and Methods**

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A karyotype was not definite in 15% of the cases because a very low mitotic index or poor-quality metaphases were obtained from the cell culture (168 cell culture failures).

The proportion of suitable samples did not vary consistently over the years; this proportion derives from the inadequacy of the samples delivered in some instances.

In ALL, unsuitable samples were the main reason for the failure of the cytogenetic analysis (40%) (Table 1a), whereas in the AML they amounted to 15% (Table 1b).

We successfully analyzed the leukemic karyotype of 556 patients, identifying 296 cases with an abnormal clone. In ALL these results were almost all obtained from direct harvesting and overnight cultures, the latter being the most successful. In AML, the results were obtained from overnight cultures and from cultures with added growth factors, as described in the methods.

In the ALL group, the percentage of cases with an abnormal clone varied over the years between 35% and 60% (Table 2). In the AML group, an abnormal clone was observed in 53% and 77% of the cases (Table 3). In the last three years, an abnormal clone was always detected in more than 50% of ALL.

Table 4 shows the distribution based on the immunophenotype of the recurrent structural abnormalities found in ALL at diagnosis. The most frequent abnormality in our series was the rearrangement of the short arm of chromosome 9, which was found in 14 cases with either the B or the T phenotype. Twelve cases presented t(9;22), 8 of these were C-ALL, 1 prepreB, 2 preB and 1 had a hybrid phenotype; t(1;19) was detected in 8 cases, all of which were preB ALL; t(4;11) was detected in 6 cases, 4 of which were C-ALL, 1 was a pre B ALL and 1 presented a hybrid phenotype.

Table 5 shows the ploidy distribution by immunophenotype in ALL at diagnosis in our series.

The most frequent group in the cases with abnormal karyotypes is the pseudodiploid (70 cases, 21%); a hyperdiploid karyotype with more than 50 chromosomes was found in 31 cases (11%), and with 47 to 50 chromosomes in 30 cases (9%). A hypodiploid karyotype was observed in 19 cases (5%).

Tables 6 and 7 show the distributions of recurrent structural abnormalities and ploidy according to FAB subgroups in AML at diagnosis. The most frequent structural rearrangements were the reciprocal translocations t(8;21) in the M1/M2 (11 cases) and t(15;17) in the M3 (12 cases) FAB sub-

**Table 1. Results of centralized cytogenetic analysis.**

<table>
<thead>
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<tbody>
<tr>
<td>Unsuitable samples</td>
<td>21 (33%)</td>
<td>46 (49%)</td>
<td>45 (38%)</td>
<td>72 (42%)</td>
<td>90 (40%)</td>
<td>91 (40%)</td>
<td>365</td>
</tr>
<tr>
<td>Cell culture failure</td>
<td>13 (21%)</td>
<td>8 (9%)</td>
<td>29 (21%)</td>
<td>26 (13%)</td>
<td>40 (19%)</td>
<td>33 (15%)</td>
<td>149 (16%)</td>
</tr>
<tr>
<td>Suitable cases</td>
<td>29 (47%)</td>
<td>39 (42%)</td>
<td>65 (47%)</td>
<td>104 (51%)</td>
<td>103 (45%)</td>
<td>423</td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>63 (100%)</td>
<td>93 (100%)</td>
<td>139 (100%)</td>
<td>202 (100%)</td>
<td>213 (100%)</td>
<td>227 (100%)</td>
<td>937 (100%)</td>
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</tbody>
</table>

**Table 2. Proportion of ALL cases with normal and abnormal karyotypes per year.**

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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18 (62%)</td>
<td>23 (65%)</td>
<td>42 (40%)</td>
<td>42 (50%)</td>
<td>48 (47%)</td>
<td>215 (51%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>11 (53%)</td>
<td>16 (41%)</td>
<td>23 (35%)</td>
<td>41 (60%)</td>
<td>55 (53%)</td>
<td>208 (49%)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (100%)</td>
<td>39 (100%)</td>
<td>65 (100%)</td>
<td>83 (100%)</td>
<td>103 (100%)</td>
<td>423 (100%)</td>
</tr>
</tbody>
</table>

**Table 3. Proportion of AML cases with normal and abnormal karyotypes per year.**

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8 (47%)</td>
<td>3 (23%)</td>
<td>6 (33%)</td>
<td>10 (38%)</td>
<td>6 (27%)</td>
<td>12 (34%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>9 (53%)</td>
<td>10 (27%)</td>
<td>12 (62%)</td>
<td>16 (73%)</td>
<td>25 (68%)</td>
<td>88 (66%)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100%)</td>
<td>13 (100%)</td>
<td>18 (100%)</td>
<td>26 (100%)</td>
<td>22 (100%)</td>
<td>37 (100%)</td>
</tr>
</tbody>
</table>

Cases were classified as "unsuitable" or as "cell culture failures" according to the criteria mentioned in the "Materials and Methods" section.
A karyotype was not definite in 15% of the cases because a very low mitotic index or poor-quality metaphases were obtained from the cell culture (168 cell culture failures).

The proportion of suitable samples did not vary consistently over the years; this proportion derives from the inadequacy of the samples delivered in some instances.

In ALL, unsuitable samples were the main reason for the failure of the cytogenetic analysis (40%) (Table 1a), whereas in the AML they amounted to 15% (Table 1b).

We successfully analyzed the leukemic karyotype of 556 patients, identifying 296 cases with an abnormal clone. In ALL these results were almost all obtained from direct harvesting and overnight cultures, the latter being the most successful. In AML, the results were obtained from overnight cultures and from cultures with added growth factors, as described in the methods.

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Table 1 shows the distribution based on the immunophenotype of the recurrent structural abnormalities found in ALL at diagnosis. The most frequent abnormality in our series was the rearrangement of the short arm of chromosome 9, which was found in 14 cases with either the B or the T phenotype. Twelve cases presented t(9;22), 8 of these were C-ALL, 1 prepreB, 2 preB and 1 had a hybrid phenotype; t(1;19) was detected in 8 cases, all of which were preB ALL; t(4;11) was detected in 6 cases, 4 of which were C-ALL, 1 was a pre pre B ALL and 1 presented a hybrid phenotype.

Table 5 shows the ploidy distribution by immunophenotype in ALL at diagnosis in our series.

The most frequent group in the cases with abnormal karyotypes is the pseudodiploid (70 cases, 21%); a hyperdiploid karyotype with more than 50 chromosomes was found in 37 cases (11%), and with 47 to 50 chromosomes in 30 cases (9%). A hypodiploid karyotype was observed in 19 cases (5%).

Tables 6 and 7 show the distributions of recurrent structural abnormalities and ploidy according to FAB subgroups in AML at diagnosis. The most frequent structural rearrangements were the reciprocal translocations t(8;21) in the M1/M2 (11 cases) and t(15;17) in the M3 (12 cases) FAB subgroups.

---

**Table 1. Results of centralized cytogenetic analysis.**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>40 (19%)</td>
<td>33 (15%)</td>
<td>149 (16%)</td>
</tr>
<tr>
<td>Suitable cases</td>
<td>29 (47%)</td>
<td>39 (42%)</td>
<td>65 (47%)</td>
<td>104 (51%)</td>
<td>83 (39%)</td>
<td>103 (45%)</td>
<td>423 (44%)</td>
</tr>
<tr>
<td>Total cases</td>
<td>63 (100%)</td>
<td>93 (100%)</td>
<td>139 (100%)</td>
<td>202 (100%)</td>
<td>213 (100%)</td>
<td>227 (100%)</td>
<td>937 (100%)</td>
</tr>
</tbody>
</table>

Cases were classified as "unsuitable" or as "cell culture failures" according to the criteria mentioned in the "Materials and Methods" section.

---

**Table 2. Proportion of ALL cases with normal and abnormal karyotypes per year.**

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18 (62%)</td>
<td>23 (59%)</td>
<td>42 (65%)</td>
<td>42 (40%)</td>
<td>42 (50%)</td>
<td>48 (47%)</td>
<td>215 (51%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>11 (38%)</td>
<td>16 (41%)</td>
<td>23 (35%)</td>
<td>41 (60%)</td>
<td>55 (53%)</td>
<td>208 (49%)</td>
<td>423 (50%)</td>
</tr>
</tbody>
</table>

Total | 29 (100%) | 39 (100%) | 65 (100%) | 104 (100%) | 83 (100%) | 103 (100%) | 423 (100%) |

---

**Table 3. Proportion of AML cases with normal and abnormal karyotypes per year.**

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8 (47%)</td>
<td>3 (23%)</td>
<td>6 (33%)</td>
<td>10 (38%)</td>
<td>6 (27%)</td>
<td>12 (32%)</td>
<td>45 (34%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>9 (53%)</td>
<td>10 (77%)</td>
<td>12 (67%)</td>
<td>16 (62%)</td>
<td>16 (73%)</td>
<td>25 (68%)</td>
<td>88 (66%)</td>
</tr>
</tbody>
</table>

Total | 17 (100%) | 13 (100%) | 18 (100%) | 26 (100%) | 22 (100%) | 37 (100%) | 133 (100%) |
Feasibility of centralized cytogenetic analysis of acute leukemia

groups. A rearrangement with a breakpoint in 11q23 was detected in 7 cases; 1 case showed a rearrangement of chromosome 11 at q21. Isolated trisomy 8 and 21 were documented in 10 and 6 cases, respectively. Monosomy 7 was found in 4 cases. On the whole, our results demonstrate that the most frequent clonal karyotype alteration in AML was pseudodiploidy, detected in 45% of cases, while metaphases with a number of chromosomes between 47 and 50 were found in 15% of cases; few cases presented a hypodiploid (3%) or hyperdiploid (2%) karyotype with more than 50 chromosomes.

Discussion
Since several multicenter studies on childhood acute leukemia have been conducted all over the world, we now have a considerable number of equally-studied and homogeneously-treated cases. Most of our understanding of the biology and treatment of leukemias comes from such cooperative studies in children.

Since 1990, the reference laboratory for all the AIEOP centers has performed cytogenetic analysis and all other diagnostic and biological procedures on request.

Collecting many samples at a single laboratory reduces the costs and enables hospitals with no cytogenetic laboratory of their own to have this analysis done inexpensively. The addition of the cytogenetic analysis facility to a pre-existing biological bank has meant a further reduction in costs and an easier procedure for the doctor responsible for the patients and who requests the analysis. Over

<table>
<thead>
<tr>
<th>No. of pre-cases</th>
<th>preB</th>
<th>B</th>
<th>C-ALL</th>
<th>T</th>
<th>Hybrid</th>
<th>nd</th>
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<tbody>
<tr>
<td>t(1;19)(q23;p13)</td>
<td>8</td>
<td>8</td>
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<td>8</td>
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<tr>
<td>t(9;22)(q34;q11)</td>
<td>12</td>
<td>2</td>
<td>6</td>
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<td>6</td>
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<tr>
<td>t(4;11)(q21;q23)</td>
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<td>1</td>
<td>45</td>
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<td>1</td>
</tr>
<tr>
<td>der(11)(q23)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>t(8;14)(q24;q32)</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>der(14)(q11-q13)</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<td>t(7;7)(p15;q36)</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>der(6)(q21-q23)</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>del(9)(p21)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>-20/20q</td>
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<td>4</td>
<td>4</td>
<td>4</td>
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</tr>
<tr>
<td>21</td>
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</table>

Table 4. Recurrent cytogenetic abnormalities by immunophenotype in ALL at diagnosis 1990-95.

Table 6. Recurrent cytogenetic abnormalities by FAB groups in AML at diagnosis 1990-95.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>M0</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>11</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>t(15;17)(q22;q12)</td>
<td>12</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>der(11)(q23)</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>t(11;19)(q23;p13)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>t(8;16)(p11;p13)</td>
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<td>2</td>
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<tr>
<td>t(10;11)(p13;q21)</td>
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<td>1</td>
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<td>inv(16)(p13;q22)</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
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<td>der(9)(q)</td>
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<td>2</td>
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</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
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<td>2</td>
</tr>
<tr>
<td>t(14;11)(q21;q23)</td>
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<td>1</td>
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<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>del(11)(q22)</td>
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<td>2</td>
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<td>2</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>+21*</td>
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<td>Others</td>
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</table>

Table 5. Distribution of immunophenotype by ploidy in ALL at diagnosis 1990-95.

<table>
<thead>
<tr>
<th>No. of pre-cases</th>
<th>preB</th>
<th>B</th>
<th>C-ALL</th>
<th>T</th>
<th>Hybrid</th>
<th>nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid (&gt;50)</td>
<td>37 (11%)</td>
<td>7</td>
<td>24</td>
<td>7</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>Hyperdiploid (47-50)</td>
<td>30 (9%)</td>
<td>1</td>
<td>94</td>
<td>1</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>Diploid (normal)</td>
<td>188 (54%)</td>
<td>1</td>
<td>54</td>
<td>71</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Pseudodiploid</td>
<td>70 (21%)</td>
<td>2</td>
<td>16</td>
<td>30</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>70 (19%)</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>344</td>
<td>4</td>
<td>89</td>
<td>159</td>
<td>3</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 7. Distribution of FAB groups by ploidy in ALL at diagnosis 1990-95.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>FAB groups cases(%)</th>
<th>M0</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid (&gt;50)</td>
<td>3 (2%)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hyperdiploid (47-50)</td>
<td>17 (15%)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Diploid (normal)</td>
<td>38 (35%)</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Pseudodiploid</td>
<td>49 (45%)</td>
<td>3</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>4 (3%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>4</td>
<td>22</td>
<td>16</td>
<td>15</td>
<td>24</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Distribution of FAB groups by ploidy in ALL at diagnosis 1990-95.
Feasibility of centralized cytogenetic analysis of acute leukemia

A rearrangement with a breakpoint in 11q23 was detected in 7 cases; 1 case showed a rearrangement of chromosome 11 at q21. Isolated trisomy 8 and 21 were documented in 10 and 6 cases, respectively. Monosomy 7 was found in 4 cases. On the whole, our results demonstrate that the most frequent clonal karyotype alteration in AML was pseudodiploidy, detected in 45% of cases, while metaphases with a number of chromosomes between 47 and 50 were found in 15% of cases; few cases presented a hypodiploid (3%) or hyperdiploid (2%) karyotype with more than 50 chromosomes.

Discussion

Since several multicenter studies on childhood acute leukemia have been conducted all over the world, we now have a considerable number of equally-studied and homogeneously-treated cases. Most of our understanding of the biology and treatment of leukemias comes from such cooperative studies in children.

Since 1990, the reference laboratory for all the AIEOP centers has performed cytogenetic analysis and all other diagnostic and biological procedures on request.

Collecting many samples at a single laboratory reduces the costs and enables hospitals with no cytogenetic laboratory of their own to have this analysis done inexpensively. The addition of the cytogenetic analysis facility to a pre-existing biological bank has meant a further reduction in costs and an easier procedure for the doctor responsible for the patients and who requests the analysis. Over
the years, the number of samples sent to our laboratory has increased progressively, demonstrating the value of this organization in Italy at this time. The slides with chromosome preparations were split between different cytogenetic laboratories in order to reduce the response time.

A considerable number of samples were lost due to incorrect sampling and preserving methods. The most frequent causes were: faulty cell harvesting methods at bone marrow aspiration; the use of EDTA as an anticoagulant; coagulation of the sample; and contamination of the blood. The percentage of unsuitable samples is higher in the ALL group, probably due to the difficulty in retrieving a large number of blasts by bone marrow aspiration at diagnosis in a consistent number of these leukemia cases, and to problems specific to ALL samples. The percentage of unsuitable samples has not improved with time, possibly because the number of centers sending the samples is increasing, so new centers taking part in the study every year may be responsible for our failure to contain this waste of material. On the other hand, given the large number of suitable samples submitted for cytogenetic analysis, our experience confirms the feasibility of performing cytogenetic analysis on mailed samples of leukemic cases, as reported in the BFM experience.5 The percentage of successful cultures with correct sampling and preserving methods ranged from 68% to 83% for the ALL cases (a total of 672 cases) and from 79% to 95% for the AML cases (a total of 152 cases).

An abnormal karyotype was detected in 208 cases of ALL (49%) and in 88 cases of AML (66%). These results are similar to the findings of a BFM multicenter study conducted on 1843 children with acute leukemia.2 Lampert reports a cytogenetic success in 55-60% of ALL and in 70% of AML, with a proportion of abnormal karyotypes of 60% in ALL and 68% in AML. The percentage of samples with no metaphases, or with a normal karyotype, is reportedly lower among most of the single-center groups with extensive experience than it is in our series, though it is not very dissimilar from other reports.14 In the French cooperative study on karyotypes in childhood leukemia, clonal abnormality was found in a higher percentage of ALL (71%); but this collaboration was organized differently from ours, i.e. in the French cooperative study, karyotype analysis was performed by each hospital and then the karyotypes were reviewed by all those taking part in the group.15 On the other hand, the existence of a central laboratory gives everyone the opportunity to have cytogenetic investigations performed, and thus retrieves many samples that would otherwise be lost for the purposes of cytogenetic analysis. It is also worth noting the fact that our efficiency in detecting abnormal clones has improved in the latter years of our work (Tables 2 and 3), both in ALL and in AML, and a more careful handling of the samples should enable us to improve on the standard of results.

The distribution of the clonal abnormalities detected in ALL at diagnosis (Table 5) is similar to the one of the German cooperative study.1

At this stage, we cannot discuss the leukemic karyotype in detail, since the aim of this study was to verify the feasibility of a cytogenetic cooperative study for analyzing childhood acute leukemia.

**Our results indicate that** overnight exposure to colchicine gives the best chances of success in ALL, in terms of metaphases; whereas in AML, evaluable metaphases are obtained from the three short-term cultures, as the addition of growth factors, is useful.

In conclusion, we believe that our data confirm the feasibility of using a centralized laboratory to carry out cytogenetic analysis on mailed samples of bone marrow and/or peripheral blood of acute leukemia. This approach is not only an easy, inexpensive solution for gaining complete information in multicenter studies, but it also creates easily-accessible storage of material and information available for further studies, being the base of future investigations. Some cytogenetic abnormalities with recognized clinical significance can now be screened using a molecular biology method.16,17 But others with variant or as of yet unknown structures cannot be identified by molecular biological methods. It is also very important to continue looking for other abnormalities that might be relevant in the diagnosis, treatment and follow-up of leukemia,18,19 confirming the relevance of cytogenetic analysis in acute leukemia.

Our data confirm the feasibility of cytogenetic analysis performed in a centralized laboratory on mailed samples of bone marrow and/or peripheral blood of acute leukemias; this could be a very helpful approach in multicenter studies, overcoming the bias of the limited number of laboratories available for cytogenetic analysis. In fact, the cytogenetic analysis of the neoplastic tissue requires a special laboratory with an expert staff.

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References

Patients with de novo acute myeloid leukaemia and complex karyotype aberrations show a poor prognosis despite intensive treatment: a study of 90 patients

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Summary. The clinical significance of complex chromosome aberrations for adults with acute myeloid leukaemia (AML) was assessed in 920 patients with de novo AML who were karyotyped and treated within the German AML Cooperative Group (AMLCG) trials. Complex chromosome aberrations were defined as three or more numerical and/or structural chromosome aberrations excluding translocations t(8;21)(q22;q22), t(15;17)(q22;q11±q12) and inv(16)(p13q22). Complex chromosome anomalies were detected in 10% of all cases with a significantly higher incidence in patients > 60 years of age (17.8% vs. 7.8%, P < 0.0001). Clinical follow-up data were available for 90 patients. Forty-five patients were < 60 years of age and were randomly assigned to double induction therapy with either TAD-TAD [thioguanine, daunorubicin, cytosine arabinoside (AraC)] or TAD-HAM (high-dose AraC, mitoxantrone). Twenty-one patients achieved complete remission (CR) (47%), 20 patients (44%) were non-responders and 9% of patients died during aplasia (early death). The median overall survival (OS) was 7 months and the OS rate at 3 years was 12%. Patients receiving TAD-HAM showed a significantly higher CR rate than patients receiving TAD-TAD (56% vs. 23%, P = 0.04). Median event-free survival was less than 1 month in the TAD-TAD group and 2 months in the TAD-HAM group, respectively (P = 0.04), with a median OS of 4.5 months vs. 7.6 months (P = 0.13) and an OS after 3 years of 7.6% vs. 19.6%. Forty-five patients were > 60 years of age: 28 of these patients were treated for induction using one or two TAD courses and 17 cases received TAD-HAM with an age-adjusted reduction of the AraC dose. The CR rate was 44%, 38% were non-responders and 18% experienced early death. The median OS was 8 months and the OS rate at 3 years was 6%. In conclusion, complex chromosome aberrations in de novo AML predicted a dismal outcome, even when patients were treated with intensive chemotherapy. Patients under the age of 60 years with complex aberrant karyotypes may benefit from HAM treatment during induction. However, long-term survival rates are low and alternative treatment strategies for remission induction and consolidation are urgently needed.

Keywords: acute myeloid leukaemia, cytogenetics, complex aberrant karyotype, prognosis, clinical trial.
HAM and TAD consolidation, and were randomly assigned consolidation and subsequent monthly maintenance for remission induction. This was followed by TAD AraC and mitoxantrone as a second cycle (TAD-HAM). Guanine, cytosine arabinoside (AraC) and daunorubicin went initial randomization between two courses of thioguanine. Patients aged 60 years and over received a second course of TAD or HAM with a reduced dose of AraC of 1·0 g/m² only when they had an inadequate response to the first cycle. TAD consisted of cytarabine 100 mg/m² by continuous intravenous infusion daily on days 1 and 2, and by 30 min intravenous infusion every 12 h on days 3–8, with daunorubicin 60 mg/m² by 30 min intravenous infusion on days 3, 4 and 5, and 6-thioguanine 100 mg/m² orally every 12 h on days 3–9. HAM consisted of cytarabine 3 g/m² by 3 h intravenous infusion every 12 h on days 1–3, with mitoxantrone 10 mg/m² by 30 min intravenous infusion on days 3, 4 and 5 (Büchner et al. 1985, 1991; Hiddemann et al. 1987).

Prior to therapy, all patients gave their informed consent after having been advised about the purpose and investigational nature of the study, as well as the potential risks. The study is in accordance with the modified declaration of Helsinki and the protocols received approval from the ethics boards of the participating institutions.

Cytogenetic studies. Chromosome analyses were carried out in four central cytogenetic laboratories performing 75% of cytogenetic studies and in six local laboratories. Pretreatment bone marrow or blood were analysed cytogenetically. Chromosome analyses were performed using short-term cultures according to standard protocols using G- or R-banding. The chromosomes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

The failure rate was 3% to 10% in the central laboratories and higher in the local ones, leading to an overall failure rate of 13%. While cytogenetic analysis was successful in only 81% of cases in the trial performed from 1986 to 1992, the success rate increased to 93% in the trial started in 1992. After introduction of all-trans retinoic acid (ATRA) into therapy for acute promyelocytic leukaemia, patients with AML M3/t(15;17) were entered into a different trial. Cytogenetic results were available for 920 patients. Aberrant karyotypes were detected in 463 patients (49·8%).

Complex karyotype was defined by the presence of at least three clonal cytogenetic abnormalities. Patients with favourable chromosome aberrations [t(8;21)(q22;q22), t(15;17)(q22;q11–12) and inv(16)(p13q22)] and additional chromosome abnormalities were excluded because the biology of these subgroups of AML appears different.

Complex Karyotype Aberrations in AML

Patients and Methods

Patients. Patients aged > 18 years diagnosed with de novo AML who were admitted to the participating institutions of the German AML Cooperative Group between 1986 and 1996 were eligible. The diagnosis of AML was based on FAB criteria (Bennett et al. 1976, 1985). Patients with a history of myelodysplasia or other antecedent haematological disorder, cytotoxic therapy or radiotherapy were excluded.

Treatment. Patients were treated according to the protocols of the German AML Cooperative Group (AMLCG). These comprise double induction therapy in patients < 60 years of age. In the AMLCG-86 trial, patients underwent initial randomization between two courses of thioguanine, cytosine arabinoside (AraC) and daunorubicin (TAD-TAD) or one course of TAD, followed by high-dose AraC and mitoxantrone as a second cycle (TAD-HAM) for remission induction. This was followed by TAD consolidation and subsequent monthly maintenance for 3 years. In the AMLCG-92 trial, all patients received TAD-HAM and TAD consolidation, and were randomly assigned to long-term maintenance vs. a second consolidation using a sequentially modified version of HAM. Patients aged 60 years and over received a second course of TAD or HAM with a reduced dose of AraC of 1·0 g/m² only when they had an inadequate response to the first cycle. TAD consisted of cytarabine 100 mg/m² by continuous intravenous infusion daily on days 1 and 2, and by 30 min intravenous infusion every 12 h on days 3–8, with daunorubicin 60 mg/m² by 30 min intravenous infusion on days 3, 4 and 5, and 6-thioguanine 100 mg/m² orally every 12 h on days 3–9. HAM consisted of cytarabine 3 g/m² by 3 h intravenous infusion every 12 h on days 1–3, with mitoxantrone 10 mg/m² by 30 min intravenous infusion on days 3, 4 and 5 (Büchner et al. 1985, 1991; Hiddemann et al. 1987).

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The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial

By David Grimwade, Helen Walker, Fiona Oliver, Keith Wheatley, Christine Harrison, Georgina Harrison, John Rees, Ian Hann, Richard Stevens, Alan Burnett, and Anthony Goldstone on behalf of the Medical Research Council Adult and Children's Leukaemia Working Parties

Cytogenetics is considered one of the most valuable prognostic determinants in acute myeloid leukemia (AML). However, many studies on which this assertion is based were limited by relatively small sample sizes or varying treatment approaches, leading to conflicting data regarding the prognostic implications of specific cytogenetic abnormalities. The Medical Research Council (MRC) AML 10 trial, which included children and adults up to 55 years of age, not only affords the opportunity to determine the independent prognostic significance of pretreatment cytogenetics in the context of large patient groups receiving comparable therapy, but also to address their impact on the outcome of subsequent transplantation procedures performed in first complete remission (CR). On the basis of response to induction treatment, relapse risk, and overall survival, three prognostic groups could be defined by cytogenetic abnormalities detected at presentation in comparison with the outcome of patients with normal karyotype. AML associated with t(8;21), t(15;17) or inv(16) predicted a relatively favorable outcome. Whereas in patients lacking these favorable changes, the presence of a complex karyotype, −5, del(5q), −7, or abnormalities of 3q defined a group with relatively poor prognosis. The remaining group of patients including those with 11q23 abnormalities, +8, +21, +22, del(9q), del(7q) or other miscellaneous structural or numerical defects not encompassed by the favorable or adverse risk groups were found to have an intermediate prognosis. The presence of additional cytogenetic abnormalities did not modify the outcome of patients with favorable cytogenetics. Subgroup analysis demonstrated that the three cytogenetically defined prognostic groups retained their predictive value in the context of secondary as well as de novo AML, within the pediatric age group and furthermore were found to be a key determinant of outcome from autologous or allogeneic bone marrow transplantation (BMT) in first CR. This study highlights the importance of diagnostic cytogenetics as an independent prognostic factor in AML, providing the framework for a stratified treatment approach of this disease, which has been adopted in the current MRC AML 12 trial.

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**MATERIALS AND METHODS**

**Patients.** The MRC AML 10 trial began in May 1988 and closed in April 1995, having accrued 1,966 patients, including 364 children (<15 years) and 1,602 adults, mostly up to 55 years of age. A total of 1,797 were registered as having de novo AML (337 children, 1,460 adults), 141 cases of secondary AML were entered (22 children, 119 adults), while the remaining 28 trial patients were excluded from further analysis, as they were subsequently found not to have AML. Cases of AML were classified as secondary on the basis of a history of previous exposure to chemotherapy or radiotherapy or of an antecedent hematologic condition including myelodysplasia and myeloproliferative disorders.

**Therapy.** The trial, which sought to determine the relative efficacy of two different induction protocols and also to establish whether there is a role for allogeneic or autologous BMT in the treatment of patients in first CR, has been fully described previously. Briefly, patients were randomized to receive induction therapy with two courses of DAT (daunorubicin, Ara-C, 6-thioguanine: course 1, DAT 3 + 10; course 2, DAT 3 + 8) or ADE (Ara-C, daunorubicin, etoposide: course 1, ADE 10 + 3 + 5; course 2, ADE 8 + 3 + 5). From January 1993, those with a clinical diagnosis of acute promyelocytic leukemia (APL) were eligible for the MRC ATRA trial whereby 75 patients were randomized to receive either short or extended courses of all-trans retinoic acid (ATRA), in addition to the AML 10 chemotherapy protocol, as previously described. A further six APL patients also received ATRA.
and the difference did not reach statistical significance, it is in accordance with previous reports concerning the prognostic significance of 11q23 abnormalities in children and adults.

Recently, there has been increasing interest to determine whether the presence of additional cytogenetic abnormalities, particularly in the context of the favorable prognosis group, influences outcome. Previous smaller studies have provided conflicting data as to the significance of additional changes in the presence of the t(15;17), while a study that included seven patients with del(9q) advocated that this additional abnormality predicts a poor prognosis in patients with t(8;21). The MRC AML 10 trial affords the opportunity to address these issues in much larger groups of patients. Additional cytogenetic abnormalities, including those associated with the adverse-risk group were found to have no significant effect on CR rates, RR, or OS in patients with t(15;17), t(8;21), or inv(16); indeed the group with t(8;21),del(9q) exhibited the most favorable survival (Table 3). While the number of patients with t(8;21), del(9q) in the present study was too small to confidently attach prognostic significance to this specific abnormality, this result renders the previous suggestion that this karyotype is associated with poor risk somewhat questionable. Furthermore, in our study, the presence of adverse risk abnormalities in patients with intermediate risk changes was found to have a deleterious effect on outcome (Fig 4). On this basis, a hierarchical system of karyotype classification was developed (Table 4) and has been used in the subsequent AML 12 trial to define prognostic groups and determine treatment approach. This classification was evaluated in a variety of clinical contexts in AML 10 and found to retain its predictive value in all age groups examined, in both

Table 4. Cytogenetic Risk Groups

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Abnormality</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(15;17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inv(16)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>del(7q)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>del(9q)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abnormal 11q23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All other structural/numerical abnormalities</td>
<td></td>
</tr>
<tr>
<td>Adverse</td>
<td>-5</td>
<td>Whether alone or in conjunction with intermediate-risk or other adverse-risk abnormalities.</td>
</tr>
<tr>
<td></td>
<td>-7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>del(5q)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abnormal 3q</td>
<td></td>
</tr>
<tr>
<td>Complex</td>
<td></td>
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</tr>
</tbody>
</table>

Hierarchical prognostic classification, derived taking into consideration the influence of additional cytogenetic abnormalities on outcome, and used for directing treatment approach in the current MRC AML 12 trial.

Table 5. CR Rates, Reasons for Failure, Relapse Risk, and Survival by Hierarchical Cytogenetic Risk Group

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>CR (%)</th>
<th>ID (%)</th>
<th>RD (%)</th>
<th>Relapse Risk at 5 yr</th>
<th>Survival at 5 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>377</td>
<td>91*</td>
<td>8</td>
<td>1*</td>
<td>35 (2.8)*</td>
<td>65 (2.5)*</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1,072</td>
<td>86</td>
<td>6</td>
<td>8</td>
<td>51 (1.8)</td>
<td>43 (1.5)</td>
</tr>
<tr>
<td>Adverse</td>
<td>163</td>
<td>63</td>
<td>14</td>
<td>23</td>
<td>76 (4.5)</td>
<td>14 (2.8)</td>
</tr>
</tbody>
</table>

*P < .001, P values are for Mantel-Haenszel (CR and reasons for failure) or log rank (relapse risk and overall survival) test for trend.
Incidence of chromosome abnormalities in the Sultanate of Oman

Mallana T. Goud, PhD, Salma M. Al-Harassi, MSc, Shafiya A. Al-Khalili, BSc, Kamla K. Al-Salmani, Diploma in MLS, Suleiman M. Al-Busaidy, BVMS, PhD, Anna Rajab, MD (Paed), PhD.

ABSTRACT

Objectives: To evaluate the cytogenetic findings in Omani children referred for suspected chromosomal anomalies that caused a variety of clinical disorders. Secondly, to study the frequency of chromosomal abnormalities in these patients and to compare our results with those reported elsewhere.

Methods: We performed chromosomal analysis on 1800 consecutive pediatric patients referred to the Cytogenetics section between June 1999 and May 2004 at Central Public Health Laboratories, Sultanate of Oman. Indication for referrals for exclusion of chromosomal rearrangements was multiple congenital anomalies, dysmorphic features, unclassified mental retardation, developmental delay, growth, and endocrine disorders. We carried out the lymphocyte culture according to standard methods.

Results: We found various types of chromosomal abnormalities in 510 (28.3%) children and showed abnormal karyotypes in the form of trisomy 21 (391; 21.7%), trisomy 18 (32; 1.8%), trisomy 13 (20; 1.1%), sex chromosome aberrations (50; 2.8%) and other types of abnormalities (17; 0.95%). There was a considerable phenotypic-cytogenetic heterogeneity. We found a high rate of chromosomal abnormalities in the present study, and we observed variations in the frequency of chromosomal aberrations reported by different investigators.

Conclusion: The higher incidence of the chromosomal abnormalities demonstrates the importance of cytogenetic evaluation in patients with dysmorphic features and congenital anomalies. Our findings suggest that chromosome analysis is a useful tool in the investigation of children with genetic disorders of unknown origin for confirmation of clinical diagnosis and proper medical care followed by genetic counseling and management.
Incidence of chromosome abnormalities in the Sultanate of Oman

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1. INTRODUCTION

Research over the last two decades has demonstrated that acute leukemias, originally subdivided by morphologic and cytochemical criteria into myeloid and lymphoblastic varieties, represent highly heterogeneous groups of malignancies that for the most appropriate tailoring of therapy, require an extensive routine diagnostic workup, including immunophenotyping as well as cytogenetic and molecular genetic study. This more detailed characterization of leukemic blasts has provided information complementary to the classical morphology- and cytochemistry-based diagnosis and classification of acute leukemias in terms of our understanding of biologically and clinically relevant subsets; more recently, it has been successfully applied to the detection of minimal residual disease (MRD).

Through the use of conventional and molecular cytogenetic analyses, acute leukemias have been recognized as a genetic disease, resulting from a series of acquired or inherited mutations in the structure of certain genes (1). These mutations are passed from the original transformed progenitor cell to its clonal descendants. Most of the genetic aberrations fall into generic classes of functional dysregulation that subvert normal hematopoietic developmental programs by circumvention of cell cycle controls, inhibition of differentiation, and resistance to therapeutic apoptosis in leukemic blasts (2).

The morphologic, cytochemical, and immunophenotypic criteria defined by the French–American–British (FAB) classification (3–6) still represent the gold standard for subclassification of acute myeloid leukemia (AML). On the basis of lineage commitment and the degree of blast cell differentiation, these criteria have led to the recognition of eight major AML subgroups (AML M0–M7). Given the distinct clinical behavior and response to treatment of the FAB categories, however, the identification of specific entities, defined according to a combination of morphologic, immunophenotypic and genetic features and clinical syndromes, has become a desirable goal. This objective, originally addressed by the so-called morphologic, immunologic, and cytogenetic (MIC) working classification of AML (7), has been partly reached by the routine application of cytogenetics and molecular genetics to the initial characterization of AML. It has provided valuable insights into
and APL (124) but not in AML with 11q23 translocations (130) are in line with this statement. Moreover, in future studies, expression of surface antigens should also be interpreted in the context of other cell biologic features, including differentiation stage and functional characteristics reflecting cellular resistance mechanisms to cytotoxic drugs (e.g., multidrug-resistance phenotype, expression of apoptosis-regulating proteins) (157,158,161–163). Our own results in a large series of untreated children and adults de novo AML enrolled in the German AML-Berlin-Frankfurt-Münster (BFM) and AMLCG studies do not show any influence of the expression of individual myeloid-, lymphoid-, and progenitor-cell-associated antigens on prognosis (87,156,164,165) and thus do not indicate that immunophenotyping alone can be applied in risk stratification in AML at diagnosis. These findings are in line with other recent studies in children (148,149) and adults (85) with AML.

7. GENETIC ABNORMALITIES IN AML

Cytogenetic analysis is the most important diagnostic tool for determining prognosis in AML (166–169). Cytogenetic studies have revealed that acquired clonal chromosome aberrations can be observed in most patients with AML (35). Numerous recurrent karyotypic abnormalities have been discovered in AML (Table 3) (37,170). Chromosome analysis has paved the way for molecular studies for those who have identified genes involved in the process of leukemogenesis (171). Furthermore, the identification of specific genomic abnormalities and their correlation with cytomorphic features, immunophenotype, and clinical outcome have led to a new understanding of AML as a heterogeneous group of distinct biologic entities. The importance of cytogenetic findings in AML for classification and for the understanding of pathogenetic mechanisms is increasingly appreciated in a clinical context and also in the new WHO classification, which uses these chromosomal abnormalities as a major criterion (20).

The incidence of abnormal karyotypes in AML has been reported to be 55–78% in adults and 77–85% in children (35,172–178). However, a substantial proportion of patients with AML have no chromosome abnormalities. Although it is possible that normal karyotypes may be attributed in some cases to the existence of nonmalignant cells dividing preferentially in vitro, the fact that in many patients the normal karyotype observed at diagnosis remains normal at relapse suggests that the absence of cytogenetic aberrations is a real phenomenon rather than a failure to detect aberrations (179,180). Recent data indicate that a proportion of cytogenetically normal patients displays submicroscopic gene alterations that can only be detected by molecular methods. For instance, approx 6% of adult AML patients with a normal karyotype display a partial tandem duplication within the MLL gene (181,182).

Attempts to classify cytogenetic data in AML have led to recognition of two distinct karyotypic patterns. One is characterized by balanced rearrangements leading to specific genomic rearrangements, whereas in the other, unbalanced aberrations result in large-scale genomic imbalances. According to the hypothesis of Johansson et al. (36), there are no unbalanced primary aberrations. An unbalanced “primary” abnormality is secondary to a submicroscopic, truly primary change. Therefore, in cases without a balanced primary abnormality, molecular analysis might reveal the underlying primary defect. Especially in patients with complex aberrant karyotypes, who show a variety of different unbalanced aberrations, submicroscopic abnormalities (such as mutations in DNA repair genes) leading to genetic instability must be suspected.

7.1. Primary Chromosome Abnormalities and Their Molecular Correlates

Primary chromosomal aberrations are frequently found as the sole karyotypic abnormality and are often specifically associated with a particular AML subtype. On average, 55% of AML patients with karyotypic abnormalities have only one rearrangement (15–20% have gain or loss of a single chromosome) (35). Primary chromosomal abnormalities are frequently found as the sole karyotypic abnormality and are often specifically associated with a particular AML subtype. On average, 55% of AML patients with karyotypic abnormalities have only one rearrangement (15–20% have gain or loss of a single chromosome) (35).

7.1.1. t(8;21)(q22;q22)/AML1-ETO

A t(8;21)(q22;q22) was first identified in 1973 (183). This is the most frequent abnormality in AML in children (incidence, 10–15%) and occurs in approx 7–10% of patients with AML in Europe and the United States (35,184). However, its frequency varies, and it is reported to be particularly common in Japan (37.5%) and in South Africa (62.5%) (35). The 8;21-translocation is more frequent in the young and is rare beyond the age of 50. In >90% of patients, it is associated with a FAB-M2 subtype (around 10% show a M1-subtype) (55,185). The translocation breakpoints have recently been cloned (186,187). The breakpoints in 21q22 cluster to a limited region of the AML1 gene, which is an important transcription factor in hematopoietic cells (188,189). The 8q22 breaks cluster to the locus of a gene with putative zinc finger DNA binding motifs called ETO or MTG8. The translocation leads to a consistent hybrid gene encoding a novel message that can be consistently detected by reverse transcriptase RT-PCR (190). Data on the detection of MRD with RT-PCR demonstrate that even in patients in long-term clinical remission, AML1-ETO fusion transcripts are still detectable (191). New methods allowing the quantification of transcripts may be more helpful for treatment decisions because the kinetics of the amount of transcripts may be more important than the observation that AML1-ETO transcripts are still detectable (192).
aberrations fulfill this criterion as well but belong to completely different biologic entities. The “real” complex aberrant karyotype shows unbalanced karyotypic abnormalities. The incidence of complex aberrant karyotypes is age-dependent. The incidence in patients younger than 60 yr is <10%, while complex aberrant karyotypes are found in up to 20% of patients older than 60 years. Prognosis is equally poor in all age groups, with less than 10% of patients surviving longer than 1 yr (238).

### 7.2. Secondary Chromosomal Abnormalities

Secondary chromosomal aberrations are rarely or never found alone, rather, they develop in cells already carrying a primary abnormality. Although less specific than the primary changes, secondary aberrations nevertheless demonstrate non-random features with distribution patterns that appear to depend on the primary abnormality and to a lesser degree on the type of leukemia (AML or ALL) (239). In contrast to primary aberrations, which are often balanced rearrangements, such as translocations or inversions, common secondary aberrations almost exclusively lead to genomic imbalances (gains and losses of whole chromosomes, deletions, or unbalanced translocations).

The biologic and clinical significance of particular secondary aberrations associated with specific primary changes in AML is largely unexplored. Published data on the prognostic impact of secondary aberrations are conflicting. Although no influence on prognosis of secondary abnormalities in patients with t(8;21)(q22), inv(16)(p13q22), or t(15;17)(q22;q11-12) was reported in the large Medical Research Council 10 (MRC10) trial and some smaller studies (229,240–244), a negative prognostic impact of additional abnormalities was noted for patients with t(8;21) and t(15;17) in one study each (241,245). The European 11q23 Workshop analyzed 125 patients with t(8;21)(q22), inv(16)(p13q22), or t(15;17)(q22;q11-12). Chromosomal aberrations with an unfavorable clinical course include −5/del(5q), −7/del(7q), inv(3)/t(3;3), and a complex aberrant karyotype. The remainder are assigned to an intermediate prognostic group. This group is highly heterogeneous because it includes patients with a normal karyotype and rare chromosome aberrations with a yet unknown prognostic impact. This group will need further subdivision in the future.

There is as yet no consensus concerning the final details among large clinical study groups on how to classify AML patients according to karyotype and prognosis. Different groups assign cytogenetic categories to different prognostic subgroups according to their experience (Table 4) (168,169,246–248). It has to be kept in mind that treatment itself influences the impact of prognostic parameters. The major objective for the future is to find the best therapy for each biologic entity. To reach this objective, the biologic entities have to be clearly defined, and large well-designed prospective trials are needed to allow a randomized comparison of different treatment strategies even in small subgroups. For APL, this goal has already been achieved. There is worldwide agreement to treat this subgroup of patients within separate trials, implementing ATRA.

Data from Bloomfield et al. (246) suggest that patients with t(8;21) or inv(16) benefit from treatment with high-dose cytarabine. Compared with other cytogenetic risk groups, patients with t(8;21) or inv(16) had the best outcome overall and demonstrated the greatest benefit from increasing doses of cytarabine (246). These data also stress that different treatment strategies can influence the prognosis of distinct cytogenetic subgroups. One important finding concerning cytogenetic

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### Table 4

<table>
<thead>
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<th>Author and reference</th>
<th>Favorable</th>
<th>Intermediate</th>
<th>Unfavorable</th>
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<td>AMLCG, 1999 (169)</td>
<td>t(8;21), t(15;17), inv(16), t(16;16)</td>
<td>Normal, other abnormalities</td>
<td>−5/5q−, −7/7q−, inv(3), 11q23, 12p,17p, complex</td>
</tr>
<tr>
<td>CALGB, 1998 (246)</td>
<td>t(8;21), inv(16), t(16;16),del(16)</td>
<td>Normal</td>
<td>Other abnormalities</td>
</tr>
<tr>
<td>Döhner, 1998a</td>
<td>t(8;21), t(15;17)</td>
<td>inv(16), 11q23, all other abnormalities</td>
<td>−5/5q−, −7/7q−, inv(3), 12p,17p</td>
</tr>
<tr>
<td>Gale et al., 1995 (247)</td>
<td>t(8;21),inv(16), t(16;16), del(16), t(15;17)</td>
<td>+8, +21, t(6;9), other abnormalities</td>
<td>t(9;22),−5,−7, del(11)</td>
</tr>
<tr>
<td>SWOG, 1997 (248)</td>
<td>t(8;21),inv(16), t(16;16),+14</td>
<td>Normal, other abnormalities</td>
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</tr>
<tr>
<td>EORTC</td>
<td>t(8;21), inv(16)</td>
<td>Normal,+8,+21, +22,del(7q), del(9q), abn(11q23), all other numeric or structural abnormalities</td>
<td>−5/5q−, −7, abn 3q, complex</td>
</tr>
<tr>
<td>MRC, 1998 (168)</td>
<td>t(8;21), t(15;17), inv(16)</td>
<td>Normal, +8,+21,+22,del(7q), del(9q), abn(11q23), all other numeric or structural abnormalities</td>
<td></td>
</tr>
</tbody>
</table>

*Personal communication or protocol of the study group.*
abnormalities and prognosis was that the incidence of distinct chromosome abnormalities varies with age, whereas the prognosis of defined cytogenetic aberrations is independent of age.

8. FAB CLASSIFICATION OF ALL

The lineage assignment, subclassification of precursor B- or T-cell leukemia, and stratification of treatment according to cell-biologic risk groups in ALL are in the domain of immunophenotyping and cyto-/molecular genetic analyses. The morphologic categories L1–L3, originally proposed by the FAB group, no longer have clinical importance, with the exception of the L3 subtype. Even in cases that display the L3 morphology (relatively uniform blasts with intensively basophilic cytoplasm and sharply defined, fat-containing vacuoles) the results of cytogenetic analysis and immunophenotyping should be considered before a definite diagnosis of L3-type Burkitt cell leukemia is made. The morphologic appearance of L3 can be imitated by the AML subtypes M0, M1, or M5 or even by several undifferentiated solid tumors. Also, in rare cases of L1- and L2-ALL, vacuolation can be seen.

In some cases of ALL, >40% of the lymphoblasts have a hand-mirror shape, but this feature has also been described in rare instances of AML. At present, this morphologic finding merely seems to identify morphologic variants without distinguishing clinical correlations.

It has been suggested that a number of BCR-ABL-positive ALL cases show a unique morphologic appearance: in addition to the dominant lymphoid blast cell population, there are larger blasts with myeloid characteristics, some of the latter even showing a positive MPO reaction (mostly <3%). This may be confused in some cases with the diagnosis of AML M0 or even AML M1 with the Philadelphia translocation. Conversely, some AML are Philadelphia chromosome-positive.

9. IMMUNOPHENOTYPING OF ALL

Since the demonstration by Borella and Sen that in some children with ALL, leukemic lymphoblasts are of thymic origin, immunophenotyping has become essential in the diagnosis of ALL and has substantially contributed to a more precise and biologically oriented classification of the disease (reviewed in refs. 8, 22, 23, 26, and 253–255). During the last two decades, immunophenotyping in ALL, initially performed with polyclonal antisera and subsequently with a rapidly expanding panel of MAbs, has mainly been applied to distinguishing ALL from AML, lineage assignment of leukemic blasts, phenotypic characterization of pathologic cell subsets, and examining the role of membrane antigen expression in predicting treatment response (reviewed in refs. 8, 9, 23, 25, 254). Additionally, based on observations that leukemic blasts frequently show aberrant or asynchronous antigen expression compared with normal hematopoietic cell differentiation, leukemia-associated phenotypic features have been routinely used to detect MRD in ALL (reviewed in ref. 27). More recently, immunophenotyping in conjunction with cytogenetic and molecular genetic studies has identified biologically and clinically distinct subsets within the major diagnostic subgroups of precursor B- and T-cell ALL and has become decisive in monitoring risk groups in therapeutic studies (reviewed in refs. 8 and 254–256).

Current procedures for the diagnosis, lineage affiliation, and characterization of maturational stages of ALL are outlined in Fig. 1. It should be emphasized that both the lineage affiliation and the definition of maturational stage in ALL are based on patterns of antigen expression demonstrated by an appropriate selection of CD MAbs rather than on the presence or absence of a single antigen. In addition, it is noteworthy that the dominant phenotype of a leukemic cell population reflects the degree of maturation achieved by a leukemic clone and may not correspond to the initial target cell of the disease, mostly a more immature progenitor cell.

The following sections briefly discuss significant associations between immunophenotypic features and numeric and/or structural chromosomal abnormalities that have recently contributed to a refined ALL classification, especially in precursor B-cell ALL. It should be noted that accurate phenotypic predictions of specific translocations in precursor B-cell ALL could not be obtained by simply classifying antigen expression as either positive or negative but that they require more complex descriptions of patterns of expression or combinations of antigens.

9.1. B-Cell Precursor ALL

9.1.1. t(4;11)(q21;q23)

The t(4;11)(q21;q23) chromosomal abnormality occurs in about 2–6% of both children and adults with ALL and has been associated with characteristic immunophenotypic and clinical features (e.g., high leukocyte counts, predominance of females in infants, frequent organ enlargement, and increased incidence of central nervous system (CNS) leukemia at diagnosis); reviewed in refs. 257 and 258). Previous reports, mainly in infant ALL, have suggested that t(4;11)-associated acute leukemias mostly originate in multipotent or very early CD10-negative B-progenitor cells with a high frequency of myeloid-antigen positivity (259, 260). Recent studies have analyzed the immunophenotypic and genotypic features of this subgroup in greater detail. In the vast majority of ALL cases with t(4;11), leukemic blasts show a typical antigenic profile (e.g., CD19+, CD10−, CD24− or weakly +, cyIgM − or +, CD15 and/or CD65s+) indicative of an immature pro-B phenotype with frequent coexpression of particular myeloid antigens (i.e., CD15, CD65s). This clear-cut association of immunophenotypic features with t(4;11), initially described in infant ALL (261–263), has also been recently found in adult patients (264–270). Southern blot analysis revealed Ig heavy-chain gene rearrangements in virtually all cases as well as oligoclonal disease in some of them (261), thus underlining the early B-cell commitment of blast cells with this cytogenetic abnormality. Based on our experience in a large series of childhood and adult ALL patients with 11q23 rearrangements (261, 268, 271, 272), these features, especially the missing or weak expression of CD24 compared with CD19, and the coexpression of CD65s, usually associated with negativity of other panmyeloid antigens (e.g., CD13, CD33), are highly predictive for the cytogenetic and/or molecular demonstration of MLL rearrangements, mostly owing to a t(4;11), or more rarely, other 11q23 aberrations. More recently, the 7.1 MAb, which recognizes a specific an-
the lack of consistent criteria for the diagnosis of My+ ALL or Ly+ AML and for defining positive results, the utilization of various panels of MAbS, the lack of lineage specificity of most of the MAbS used, and several technical factors (e.g., distinct sensitivities of fluorochromes and flow cytometers, inconsistent gating strategies) (88,282,412,419). In view of previous studies pointing to myeloid-antigen expression as a predictor of poor prognosis in both childhood and adult ALL (423,424), considerable interest has focused on the cell biologic features and clinical significance of this subgroup of acute leukemias. Several recent studies, including more than 4000 pediatric patients with ALL (425-427) and our own data in almost 5000 children treated within the ALL-BFM 86, 90, and 95 trials (320,327,428), have failed to demonstrate an association of My+ ALL with poor outcome. In some of these studies, myeloid-associated antigen expression was clearly associated with certain genetic features of leukemic cells, particularly MLL and ETV6-AML1 rearrangements (293,427-429). In contrast to childhood My+ ALL, the clinical importance of myeloid-associated antigen expression in adult ALL is still unknown. The presence of myeloid-associated antigens has been associated with a poor outcome in some (423,430) but not all studies (270,431,432). Most of these studies, however, included only a relatively small number of patients, have not always carefully excluded minimally differentiated AML (AML-M0) (423), and, most important, have not adequately taken into account the prognostic importance of specific genetic abnormalities frequently found in adult patients with My+ ALL, such as Ph positivity or 11q23 rearrangements (268,270,282,433). Further prospective studies, consistently based on well-defined diagnostic criteria, are urgently needed to elucidate more accurately the biologic heterogeneity of My+ ALL and to establish its clinical relevance in adult patients.

A critical review of data published in the literature revealed that most retrospective and prospective studies failed to demonstrate any prognostic significance for Ly+ AML, except for CD7+ AML (88). The latter subgroup has been associated with more frequent expression of progenitor-associated markers (e.g., CD34, CD117, HLA-DR, TdT), concomitant rearrangements of Ig and/or TCR gene rearrangements, and poor prognosis in most (but not all) studies in both childhood and adult AML (86,87,162,434-437). It should be noted that immature CD7+ AML and pro-/pre-T ALL occasionally show biologic similarities, such as reactivity with MAbS recognizing antigens expressed on both immature T-cell ALL and AML (438), responsiveness to several growth factors (437), expression of c-kit at the mRNA and protein levels (164,165,340,439), expression of the multidrug resistance phenotype (440), and similar TCRb gene rearrangements (441), suggesting that in at least some CD7+ acute leukemias, malignant transformation has arisen in a pluripotent progenitor cell with variable differentiation potential along both myeloid and T-lymphoid lineages (429).

Given the significant associations between expression of several lymphoid-associated antigens by AML and specific genetic abnormalities, such as CD19 in AML with t(8;21) and CD2 in AML with t(15;17), as well as AML with inv(16) or t(16;16), cyogenetic and molecular data have to be incorporated into the classification of Ly+ AML, and future studies evaluating the prognostic significance of Ly+ AML have to take into consideration its genetic background.

12.6. Which Conventional and Molecular Cytogenetic Techniques are Necessary to Identify Cytogenetic Alterations and to Provide Cytogenetic Information that Has Clinical Relevance?

In general, classical cytogenetics using banding techniques is still the gold standard for the genetic classification of acute leukemias. These techniques should be performed in each patient with acute leukemia at diagnosis as well as at relapse. New techniques such as FISH, Southern blot, and PCR analyses have added important information to the more sophisticated subgrouping of acute leukemia. These techniques should be used on demand in cases in which these investigations can give information that cannot be obtained by banding analysis. Screening with these techniques for each detectable genetic aberration is very expensive and labor-intensive and thus not cost-effective. In childhood B-cell precursor ALL, screening with RT-PCR or FISH for the detection ETV6-AML1 rearrangement seems mandatory, because t(12;21) is mostly not detectable with conventional cytogenetics. Furthermore, in cases of T-cell precursor ALL, checking for TALI rearrangements with Southern blotting or RT-PCR should be performed. If no banding analysis is available, screening for the most important abnormalities regarding prognosis is recommended: in B-cell precursor ALL: BCR-ABL rearrangement, E2A-PBX1 rearrangement, MLL rearrangements, hyperdiploidy, 9p deletions; in mature B-ALL: translocations involving MYC; in precursor T-cell ALL: TALI rearrangement and in AML: rearrangements of AML1-ETO, PML-RAR, CBFl-MYH11, MLL rearrangements, monosomies 5/7, and deletions 5q/7q and 17p.

12.7. Do We Need an International Cytogenetic Classification for Acute Leukemias and Which Aspects are Important?

An international cytogenetic classification is urgently needed to allow a comparison among different studies. On the one hand, a hierarchical classification according to primary chromosome aberrations is needed; on the other hand, for clinical use a prognostic grouping for distinct cytogenetic abnormalities is required. This is problematic because the prognosis of cytogenetic subgroups is influenced by therapy. Therefore, a biologically orientated classification is necessary that will allow analysis of the impact of certain treatments on a cyogenetically defined subgroup of patients. In AML, the favorable cytogenetic subgroup is well defined, and nearly all study groups agree that patients with t(15;17), t(8;21) or inv(16)/t(16;16) belong to this subgroup. The impact of additional abnormalities on favorable aberrations has to be determined in metaanalyses. For the intermediate and unfavorable subgroups, discrepancies occur. The intermediate subgroup is a mixture of patients with normal karyotypes, karyotypic abnormalities with proven intermediate prognosis, and karyotypic alternation of unknown prognostic significance owing to low frequency of these aberrations. For future analysis, more informative results can be obtained if the intermediate group is analyzed in three subgroups, as mentioned above. In patients
Detailed mapping of a congenital heart disease gene in chromosome 3p25

Elaine K Green, Matthew D Priestley, Jonathan Waters, Chris Maliszewska, Farida Latif, Eamonn R Maher

Abstract
Distal deletion of chromosome 3p25-pter (3− syndrome) produces a distinct clinical syndrome characterised by low birth weight, mental retardation, telecanthus, ptosis, and micrognathia. Congenital heart disease (CHD), typically atrioventricular septal defect (AVSD), occurs in about a third of patients. In total, approximately 25 cases of 3− syndrome have been reported worldwide. We previously analysed five cases and showed that (1) the 3p25-pter deletions were variable and (2) the presence of CHD correlated with the proximal extent of the deletion, mapping a CHD gene centromeric to D3S18. To define the molecular pathology of the 3− syndrome further, we have now proceeded to analyse the deletion region in a total of 10 patients (five with CHD), using a combination of FISH analysis and polymorphic markers, for up to 21 loci from 3p25-p26. These additional investigations further supported the location of an AVSD locus within 3p25 and refined its localisation. Thus, the critical region was reduced to an interval between D3S1263 and D3S3594. Candidate 3p25 CHD genes, such as PMCA2 (ATP2B2), fibulin 2, TMP4, and Sec13R, were shown to map outside the target interval. Additionally, the critical region for the phenotypic features of the 3− phenotype was mapped to D3S1317 to D3S17 (19-21 cM).

These findings will accelerate the identification of the 3p25 CHD susceptibility locus and facilitate investigations of the role of this locus in non-syndromic AVSDs, which are a common form of familial and isolated CHD.

Keywords: congenital heart disease; chromosome 3p25
Materials and methods

PATIENTS AND SAMPLES

Lymphoblastoid cell lines were available for nine patients: P1, P2 (CUMG3.1), P3 (GM10922), P4, P5 (CUMG3.4), P6, P7, P8 (GM10985), and P9, but not P10 (CUMG3.10). DNA was collected from the affected child and both parents in seven families (P1, P2, P5, P6, P7, P9, and P10), from the affected child and mother in one case (P4), and only the affected child for P3 (GM 10922) and P8 (GM 10995).

P1 ARTIFICIAL CHROMOSOME (PAC) ISOLATION AND MAINTENANCE

P1 clones were isolated by PCR based screening using polymorphic marker primers (3p25-26) from the UK HGMP resource centre RPCI1 library.12 P1 clones were maintained in LB media supplemented with 25 µg/ml Kanamycin and PAC DNA was isolated from the host strains by column purification using the Qiagen Maxi kit (Qiagen). The manufacturer's recommended protocol adaptations for optimal recovery of PAC DNA were used. The P1 clones isolated are shown in table 1.

ISOLATION OF COSMIDS

Cosmids were isolated for the markers D3S3088, D3S3714, and D3S3589 from the chromosome 3 library LLO3NCO1 obtained from the UK HGMP resource centre. Hybridisation probes were prepared from the amplified PCR products from YAC 753F7 and 70D6 DNA, purified by Qiagen PCR product kit and radiolabelled by random priming ~25 ng using the Rediprime kit (Amersham). Repetitive sequences were blocked by preannealing with Cot-1 DNA (Gibco BRL) and hybridising under standard conditions12 at 65°C. The identified clones (table 1) were obtained from the UK HGMP resource centre then maintained in LB media supplemented with ampicillin. Cosmid DNA was isolated using the Qiagen Midi kit.

FLUORESCENCE IN SITU HYBRIDISATION (FISH)

Metaphase chromosome spreads were prepared from EBV transformed cell lines using standard methods. Before chromosome harvest, cells were blocked in metaphase by the addition of colcemid to a concentration of 10 µg/ml. Probes (PACs, cosmids, and YACs) were biotinylated by nick translation with biotin-11dUTP (Bionick labelling system, Gibco BRL). Chromosomal in situ suppression (CISS) hybridisation was performed to improve the specificity of the hybridisation, with Cot-1 DNA (Gibco BRL) added to the biotin labelled probe at a ratio of 50:1. For all hybridisations, a biotin labelled chromosome 3 alpha satellite centromere probe (Oncor) was used as a control to identify chromosome 3. Hybridisation signals were visualised by a three layer avidin-fluorescein isothiocyanate (FITC), biotinylate anti-avidin detection system. Analysis was carried out using a BX50 Olympus microscope and images captured with an automated image analysis system (Cytovision, Applied Imaging). At least 20 metaphases were analysed for each probe.

Table 1  Details of FISH probes and microsatellite markers used in the study. The probes marked by * are PAC clones derived from RPCI1 library, and those probes starting with AC are cosmids probes isolated from the library LLO3NCO1, both obtained from the UK HGMP resource centre

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<thead>
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<th>FISH probe</th>
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<tr>
<td>D3S1304</td>
<td>—</td>
<td>Microsatellite</td>
</tr>
<tr>
<td>D3S18</td>
<td>cLib 1</td>
<td>Microsatellite</td>
</tr>
<tr>
<td>D3S597</td>
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Table 2 3p– deletion mapping results

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| + = locus retained. − = locus deleted. NI = non-informative. ND = not done.