A limited understanding of leukemogenesis and the neoplastic phenotype of transformed lymphoid progenitors has been a major obstacle to the development of safe, uniformly effective therapy for children with acute lymphoblastic leukemia (ALL). Studies of isoenzymes, immunologic markers, and other expressions of cell phenotype provide valuable clues to ALL etiology, but ultimately they fail to divulge mechanisms for leukemic transformation or maintenance of the malignant state. A more fruitful approach has been to classify ALL by its cytogenetic features, both numeric and structural. Admittedly, a proportion of the chromosomal changes reported to date have proved to be little more than genetic epiphenomena with vague links to pathogenesis and treatment outcome. This fact notwithstanding, the presence of specific cytogenetic abnormalities in leukemic cells has stimulated molecular study of genes near the chromosomal breakpoints and characterization of their protein products. The picture emerging from these investigations is varied but has a consistent theme: genes disrupted by recurrent translocations or other chromosomal alterations tend to participate in cell regulatory pathways that control cell growth and development. Thus, cytogenetic classification of childhood ALL has the potential to suggest causative mechanisms and thereby to provide consistent guidelines for treatment. This review updates progress in the cytogenetic study of ALL and attempts to relate new findings to prognosis.

**Numeric Classification**

ALL can be classified into five subtypes based on the modal number of chromosomes: hyperdiploid with 47 to 50 chromosomes; hyperdiploid with more than 50 chromosomes; pseudodiploid (46 chromosomes with structural or numeric abnormalities); diploid (normal 46 chromosomes); and hypodiploid (<46 chromosomes). Recognition of ploidy as a distinctive cytogenetic feature in ALL has greatly enhanced the ability to predict clinical outcome and devise risk-specific therapy, as will be discussed in the following sections. The frequency distribution of the major ploidy groups and the 4-year event-free survival (EFS) associated with each are presented in Table 1.

<table>
<thead>
<tr>
<th>Numeric Classification</th>
<th>Percentage of Cases</th>
<th>EFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid 47-50</td>
<td>~25%</td>
<td></td>
</tr>
<tr>
<td>Hyperdiploid &gt; 50</td>
<td>~25%</td>
<td></td>
</tr>
<tr>
<td>Pseudodiploid</td>
<td>~20%</td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>~15%</td>
<td></td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>~10%</td>
<td></td>
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Hyperdiploidy > 50, found in 25% to 30% of cases of childhood ALL, has long been recognized as conferring the most durable responses to treatment. Patients with this feature can be rapidly identified by flow cytometric analysis of the leukemic cell DNA content, although flow cytometry does not provide information about structural chromosomal abnormalities and therefore must be used with conventional cytogenetic techniques. The karyotypes of patients with more than 50 chromosomes have certain features in common. One often finds extra copies of the X, Y, 4, 6, 10, 14, 17, 18, 20, and 21 chromosomes, with duplication of 1q and isochromosome 17q observed in occasional cases. In our recent review of 134 cases with >50 chromosomes, the frequency distribution of modal numbers peaked at 55 chromosomes, with a range of 51 to 65. Structural chromosome abnormalities were found in 62% of these cases, including 20% with translocations. The ability to detect such alterations, including the 17q isochromosome is crucial to optimal risk-group assignment, as patients with numeric changes only have a more favorable prognosis than those with added structural abnormalities. Favorable presenting features commonly associated with hyperdiploidy > 50 ALL include an early pre-B immunophenotype, lower leukocyte counts, lower serum lactate dehydrogenase levels, white race, and age between 2 and 10 years. The absence of T-cell features and cases in infants are notable. Investigators of the Pediatric Oncology Group recently showed that among patients with B-progenitor cell ALL, a DNA index > 1.16 (>53 chromosomes) was the strongest predictor of a favorable response to chemotherapy, regardless of the white blood cell (WBC) count. They identified a subset of patients within this group who would likely fare well on antimetabolite-based treatment, excluding genotoxic agents such as cyclophosphamide, doxorubicin, and the epipodophyllotoxins.

Near-tetraploid ALL (range of modal numbers, 82 to 94) comprises fewer than 1% of reported cases in children. Some of these cases may originate from an endoreduplication because in a few instances two copies of the abnormal chromosomes have been observed in the near tetraploid line. Alternatively, a duplication of a structural chromosome aberration may occur, providing two copies of a given abnormality. Near-tetraploid ALL is more likely to be associated with L2 morphology (30% of cases), a T-cell immunophenotype (47%) and older age (median 8.6 years), as compared with other ploidy groups in childhood ALL.

**Hyperdiploidy 47-50**

This group, accounting for 10% to 15% of cases of childhood ALL, was initially recognized on the strength of studies indicating that 47 to 50 chromosomes confer an intermediate prognosis by comparison with all other ploidy groups. Gains of almost every chromosome have been observed in such cases, with chromosomes 8, 10, and 21 added more...
Table 1. Ploidy Groups in a Recent Trial of Intensive Chemotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (%) of Patients</th>
<th>4-yr EFS ± SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid &gt;50</td>
<td>99 (28)</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>Hyperdiploid 47-50</td>
<td>46 (13)</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>Diploid</td>
<td>31 (9)</td>
<td>87 ± 10</td>
</tr>
<tr>
<td>Pseudodiploid</td>
<td>138 (38)</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>Hypodiploid &lt;45</td>
<td>26 (7)</td>
<td>46 ± 19</td>
</tr>
<tr>
<td>Unknown</td>
<td>18 (5)</td>
<td>73 ± 17</td>
</tr>
</tbody>
</table>

Treatment consisted of remission reinforcement therapy (temiposide, cytarabine, high-dose methotrexate) added to a standard four-drug induction regimen. Continuation therapy was either conventional or consisted of four pairs of drugs rotated weekly or every 6 weeks. Median follow-up was 40 months (range, 19-37 months). The 4-year EFS for all patients in this trial was 73% ± 4% SE. Adapted and reprinted with permission from Rivera et al.29

Diploidy

The incidence of cases that lack apparent cytogenetic abnormalities in ALL varies widely, in part because of the indistinct chromosome morphology that is often encountered. It is not unusual for 10% to 15% of cases in large series to lack detectable chromosomal abnormalities by standard cytogenetic evaluation; the incidence of T-cell cases with normal karyotypes may be as high as 30%.30,31 To ensure adequate cytogenetic analysis, one should study at least 20 banded metaphases per case. Whether so-called cytogenetically normal cases represent mitotically inactive clones or clones with submicroscopic genetic changes is uncertain. The prognosis for this group has been intermediate in most clinical trials.11

Hypodiploidy/Near-Haploidy

Hypodiploidy (<46 chromosomes) is found in 7% to 8% of cases of childhood ALL.6,14 Most hypodiploid cases (80%) have a modal number of 45 and arise from the loss of a whole chromosome, unbalanced translocations, or the formation of dicentric chromosomes. Investigators representing the Third International Workshop on Chromosomes in Leukemia (TIWCL) studied 330 cases of ALL, of which 20 were hypodiploid (two near-haploid).5 Among 409 patients with newly diagnosed ALL studied at St Jude Children’s Research Hospital from November 1978 to October 1986, 31 (7.6%) had a hypodiploid karyotype.32 The distribution of modal numbers was 45 in 26 cases, 28 in 2 cases, and 26, 36, and 43 in 1 case each. Hypodiploidy resulted from whole chromosome loss in 20 patients, unbalanced translocations in 7, and dicentric chromosomes in 4. Chromosome 20 was lost most frequently (9 cases); in 3 cases of common ALL, its absence was the only abnormality, similar to the finding reported by Betts et al.33 Despite relatively favorable presenting features (median leukocyte count, 12.7 × 10^9/L; median age, 5 years), patients with hypodiploidy fared significantly worse than those with chromosomes > 50 with earlier treatment protocols at our institution.33 Furthermore, in our recent trial (Total Therapy Study XI; February 1984 to September 1988) that was effective for other ploidy groups, hypodiploidy had a poor outcome (4-year EFS, 46% ± 19% SE, Table 1),32 suggesting that alternative therapy should be considered for this subtype of ALL.

Although very rare (<1%), near-haploidy cases have been extensively studied.34,35,37,39,42 The main clone contains at least one copy of each chromosome, with a second copy of the sex chromosomes and chromosome 21 found in most cases (90%). Other chromosomes found in two copies include: 18 (65%), 10 (45%), and 14 (45%).35,39,41,42 In general, the morphologic features of these cases are poorly defined, and few or no structural abnormalities can be discerned. In many near-haploid cases, there is a second abnormal line with a hypodiploid karyotype that represents a doubling of the chromosomes in the near-haploid line. This hypodiploid line usually contains exactly two copies of all chromosomes in the near-haploid line. Several of the reported cases have included structural chromosome abnormalities in both cell lines, suggesting a common precursor cell with a near-haploid karyotype.37,40 Near-haploid leukemia has been associated
with a poor prognosis (median survival, 10 months), even though a complete remission was achieved in most cases.\(^{35,39,42}\)

Recently, three subdivisions of the hypodiploid category were recognized: 41-45, 30-40, and <30 (near-haploid) chromosomes.\(^{39,41}\) Because of the limited number of cases reported in each of these categories, further study will be needed to determine the unique prognostic implications of these chromosomal loss syndromes in ALL.

### STRUCTURAL CLASSIFICATION

Describing ALL by the types of structural abnormalities found in chromosomes of the leukemic clone has led to impressive advances in our understanding of leukemia pathobiology and in formulating risk-specific therapy. Molecular analysis of genes adjacent to the breakpoints of these structural anomalies and studies on the functions of their protein products have helped to clarify the complex interactions that promote leukemogenesis and perpetuate the leukemic cell phenotype (Table 2). In this section, we focus on the more prominent structural abnormalities found in leukemic cells and their likely contribution to the disease process.

#### B-Lineage ALL

\(t(8;14), t(8;22), \text{ and } t(2;8)/\text{B-cell ALL.}\) The \(t(8;14)\) (q24;q32) abnormality, followed by the \(t(2;8)(p12;q24)\) and \(t(8;22)(q24;q11)\) variants, was the first immunophenotype-specific translocation to be identified in surface Ig-positive (sIg\(^+\)) B-cell neoplasias, mainly Burkitt’s lymphoma.\(^{4,43}\) This translocation or, infrequently, one of its variants, occurs in 85% to 90% of cases of sIg\(^+\), B-cell ALL.\(^{4,44}\) When associated with French-American-British (FAB)-L3 cellular morphology, these leukemias are considered a disseminated phase of Burkitt’s lymphoma.\(^{45}\) Some cases of L3 B-cell leukemia have been reported to contain only 1q and 6q abnormalities.\(^{35,39,42}\) Thus, the development of lymphoblastic leukemia with Burkitt-like morphology may not absolutely require a microscopically detectable lesion in a chromosome involved in Ig synthesis.\(^{4,44}\) Leukemias with one of these translocations often present with central nervous system (CNS) involvement and/or abdominal tumors.\(^{48}\) Until recently, the complete remission and median survival rates were poor by comparison with results in other groups of patients classified on the basis of leukemic cell karyotypes. Although only 25% of patients with B-cell ALL were curable in earlier trials, use of newer

### Table 2. Recurrent Structural Chromosome Abnormalities in Childhood ALL

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>ALL Overall</th>
<th>Specific Immunophenotype</th>
<th>Chromosome Bands/Genes Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(t(8;14)(q24;q32))</td>
<td>3</td>
<td>B-cell, 90</td>
<td>8q24/CMYC</td>
</tr>
<tr>
<td>(t(2;8)(q12;q24))</td>
<td>&lt;1</td>
<td>B-cell, 4-5</td>
<td>8q24/CMYC</td>
</tr>
<tr>
<td>(t(8;22)(q24;q11))</td>
<td>&lt;1</td>
<td>B-cell, 6-10</td>
<td>8q24/CMYC</td>
</tr>
<tr>
<td>(t(11p12;q32,p13))</td>
<td>5-6</td>
<td>Pre-B, 90</td>
<td>1q23/1PBX1</td>
</tr>
<tr>
<td>(t(9;22)(q34;q11))</td>
<td>2-5</td>
<td>Early pre-B, 75</td>
<td>9q34/ABL</td>
</tr>
<tr>
<td>(t(4;11)(q21;23))</td>
<td>2</td>
<td>Early pre-B, 80</td>
<td>4q21/?</td>
</tr>
<tr>
<td>(t(11p12;q32,p23))</td>
<td>&lt;1</td>
<td>Early pre-B, ?</td>
<td>1p32/?</td>
</tr>
<tr>
<td>(t(11p12;q23,p13))</td>
<td>&lt;1</td>
<td>Early pre-B, ?</td>
<td>19p13/3</td>
</tr>
<tr>
<td>(t(5;14)(q31;q32))</td>
<td>&lt;1</td>
<td>Eosinophilia, ?</td>
<td>5q31/IL3</td>
</tr>
<tr>
<td>(t(17p12;q22,p13))</td>
<td>&lt;1</td>
<td>Early pre-B, ?</td>
<td>17q22/HLF</td>
</tr>
<tr>
<td><strong>T-lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(t(11;14)(p13;q11))</td>
<td>1</td>
<td>T-cell, 7</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(11;14)(p15,q11))</td>
<td>&lt;1</td>
<td>T-cell, 1</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(10;14)(q24;q11))</td>
<td>1</td>
<td>T-cell, 5-10</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(8;14)(q24;q11))</td>
<td>&lt;1</td>
<td>T-cell, 2</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(11p12;q32,p13))</td>
<td>&lt;1</td>
<td>T-cell, 3*</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(11p12;q32,p13))</td>
<td>&lt;1</td>
<td>?</td>
<td>14q11/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;9)(q34;q34))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;9)(q34;q32))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;9)(q34;q34))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;9)(q34;q32))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;10)(q35;q24))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;11)(q35;p13))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;14)(q35))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td>(t(7;19)(q38,p13))</td>
<td>&lt;1</td>
<td>?</td>
<td>7q32-q36/TCR(\beta)</td>
</tr>
<tr>
<td><strong>Nonspecific lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(6q)</td>
<td>4-13</td>
<td></td>
<td>11p13/RBTN2/TTG2</td>
</tr>
<tr>
<td>t(del(8p))</td>
<td>7-12</td>
<td></td>
<td>11p15/RBTN1/TTG1</td>
</tr>
<tr>
<td>t(del(11q))</td>
<td>3-5</td>
<td></td>
<td>10q24/HOX1</td>
</tr>
<tr>
<td>t(del(12p))</td>
<td>10-12</td>
<td></td>
<td>8q24/CMYC</td>
</tr>
</tbody>
</table>

* TAL1 submicroscopic deletion in 15% to 26% of T cells.
regimens has improved this outcome to approximately 60% to 70%.49

Molecular studies have shown that the critical event in all three of these translocations is the juxtaposition of the CMYC (8q24) proto-oncogene locus with the Igμ (14q32), the Igκ (2p12), or the Igλ (22q11) locus.50 These rearrangements bring the CMYC gene under the influence of transcription-stimulating sequences in each of the active Ig loci, leading to dysregulation of CMYC, increased expression, and, presumably, neoplastic growth.51 MYC is a transcription factor that is involved in the control of cell proliferation, and its binding to DNA and activation is enhanced by the MAX and MAZ proteins.52,53

(t(1;19)(q23;p13)/pre-B-cell ALL). The t(1;19)(q23;p13) is the most common translocation in childhood ALL, with an overall incidence of 5% to 6%.54-59 It is closely associated with the pre-B (cIg+) immunophenotype, but may occasionally be found in early pre-B (cIg−) and T-cell cases.56,59,60 This rearrangement occurs in either a balanced, t(1;19)(q23;p13) (25%), or an unbalanced, −1,+der(19)t(1;19)(q23;p13) (75%) form. In the unbalanced form, two normal chromosomes 1 are always present and represent a duplication of the normal homolog. Some leukemic cells contain both forms, implying that the der(1) can be lost through clonal evolution, without loss of the transformed phenotype.

It has been argued that the t(1;19) accounts for the majority of treatment failures in pre-B ALL.57,61 To address this point, we evaluated 112 cases of pre-B leukemia with banded karyotypes, identifying the t(1;19) in 25%.46 This finding was not limited to cIg+ cases; rather, the translocation was also detected in two hyperdiploid cases that had tested negative for cIg expression. The t(1;19) or der(19)t(1;19) was the sole chromosomal abnormality in 8 of the 28 cIg+ cases. Apart from a higher incidence of translocations overall (69% v 50%), the lack of hyperdiploidy > 50 and a lower incidence of normal karyotypes (3.5% v 8% to 10%), the cytogenetic findings in our pre-B group did not differ appreciably from those reported for ALL in general. The presence of the t(1;19) correlated significantly with several recognized adverse prognostic features: higher leukocyte counts, higher serum lactate dehydrogenase levels, nonwhite race, and DNA indexes of less than 1.16. As expected, the t(1;19) was associated with an inferior outcome in patients treated on minimally or moderately intensified protocols. In a more recent study testing reinforced early treatment and rotational combination chemotherapy, the 4-year EFS for the subgroup with t(1;19) rearrangements was comparable with that for the entire cohort (73% ± 4% SE). This improvement illustrates that effective treatment can nullify the negative impact of chromosomal rearrangements in cases of childhood pre-B ALL.52,62 Furthermore, a recent retrospective collaborative group study, for patients of all ages, reported a significantly better outcome for patients diagnosed with the balanced t(1;19) translocation when compared with the unbalanced form.62 Thus, the outcome of these subgroups should be analyzed separately.

The t(1;19) produces a critical fusion of the E2A and PBX1 genes. E2A encodes the Ig enhancer binding factors E12 and E47, and has been mapped to 19p13.43 while PBX1, a homeobox gene of unknown function, maps to 1q23.64,65 The hybrid gene possesses 5' sequences that encode a transcriptional activator domain of E2A, joined to 3' sequences that encode part of a DNA-binding homebox domain of the PBX1 gene. The structure of E2A-PBX1 proteins suggests that they activate gene expression via the E2A trans-activation domain, thus contributing to malignant transformation of either pre-B cells or their progenitors. At least two forms of the chimeric protein induce malignancy when introduced into murine fibroblasts.66 Molecular detection of the t(1;19) is now feasible by RNA-based polymerase chain reaction (PCR) analysis in patients with pre-B ALL.67,68 This technology has made it possible to distinguish cIg+ cases with typical E2A-PBX1 transcripts from cIg− cases in which the t(1;19) does not yield similar transcripts,69 even though it appears identical to the more prevalent translocation by standard cytogenetic criteria. PCR analysis of the t(1;19) breakpoint may also be useful in cIg− cases with normal cytogenetic results, perhaps because of a paucity of dividing cells with the abnormality. Such distinctions may be important in devising risk-specific therapy for patients with B-lineage ALL.

(t(17;19)(q22;p13)/early pre-B-cell ALL). Other nonrandom chromosomal translocations affecting band p13 of chromosome 19 may also involve the E2A gene.69,70 We have molecularly evaluated a t(17;19)(q22;p13) associated with early B-lineage leukemia and disseminated intravascular coagulation.71 This translocation results in a chimeric transcript that contains sequences of the E2A gene fused with those of the recently discovered hepatic leukemia factor gene (HLF) on chromosome 17, which encodes a protein analogous to previously identified leucine zipper-containing transcription factors that regulate developmental stage-specific gene expression.71 Disruption of the HLF gene by the t(17;19) and fusion of its basic region leucine zipper domain with the transactivation domain of the E2A gene appears to be the event giving rise to leukemia in these cases. The prognostic relevance of the t(17;19) remains uncertain because of the limited number of cases identified to date.71

(t(9;22)(q32;q11)/B-cell precursor ALL). The Philadelphia chromosome (Ph), or t(9;22)(q32;q11), occurs in 2% to 5% of children with ALL.6,72,74 Clinically, Ph+ cases tend to be characterized by older age, higher leukocyte counts, larger percentages of circulating blasts, a higher frequency of FAB-L2 morphology and more prevalent pseudodiploid karyotypes than are found in Ph− cases. Most Ph+ blasts have a B-lineage immunophenotype, although isolated cases with a T-cell or mixed phenotype have been reported. Among 2,519 banded cases of childhood ALL, 58 (2.3%) were Ph+.73 Children with this karyotypic feature had a median leukocyte count of 33 × 10⁹/L and a median age of 9.6 years; most had FAB-L2 cytology. The immunologic marker distribution was similar to that in the Ph− cases: early pre-B ALL, 75%; pre-B-cell, 16%; and T-cell, 9%. Cytogenetic evaluation identified the classic t(9;22)(q34;q11) in 85% of the 58 cases; the remainder had a complex translocation involving an additional chromosome or a chromosome 22 translocation without microscopically visible involvement of 9q34 in the formation of the t(9;22). The Philadelphia chromosome was found as a single abnormality in approximately 50% of the cases.
Pseudodiploidy was the most frequent modal number (n = 34), followed in decreasing order by hypodiploidy (n = 15), hyperdiploidy 47-51 (n = 5), and hyperdiploidy > 51 (n = 4). A significantly lower proportion of Ph+ patients entered complete remission on a protocol of intensive chemotherapy compared to the result for patients without this feature (78% vs 96%). Moreover, by 4 years of follow-up, approximately two-thirds of Ph+ responders had relapsed. The consistent lack of success in treating this form of ALL has prompted most investigators to consider bone marrow transplantation (BMT) in first remission as a therapeutic option.

Russo et al found partial or complete monosomy 7 in approximately 25% of 57 children with Ph+ ALL. This observation is notable because the loss of one copy of chromosome 7 generally characterizes myeloproliferative disorders that progress to acute myeloid leukemia. This subgroup of children with Ph+/−7 ALL comprised mainly older males with early B-lineage ALL, whose induction failure rate (31%) was much higher than that among Ph− cases. These findings suggest that leukemic transformation in such patients is a multistep process involving the interaction of a dominant oncogene (Ph; BCR-ABL) with a tumor suppressor gene (−7).

Cytogenetically, the Ph chromosome in ALL is identical to that seen in chronic myeloid leukemia (CML), where the marker is retained throughout the course of the disease. By contrast, when children with Ph+ ALL achieve complete remission, the Philadelphia chromosome is no longer apparent on cytogenetic analysis. At the molecular level, the two translocations are different. In cases of CML with the t(9;22), the ABL proto-oncogene on chromosome 9 at q32 joins to a 5.8-kb region on chromosome 22 (q11) known as the breakpoint cluster region (bcr), which lies within a larger gene, BCR. This results in a unique 8.5-kb mRNA that encodes a 210-Kd protein, p210. By contrast, in most cases of ALL, the ABL proto-oncogene fuses to a region 5′ of the bcr within the BCR gene, resulting in a smaller mRNA transcript and a 190-Kd protein, p190. Both proteins are tyrosine phosphokinases and are thought to play a critical role in the control of cell proliferation. Cloning the t(9;22) translocation has provided the DNA probes for the molecular evaluation of its involvement, young age at presentation, and a poor prognosis.

The frequency of this translocation in childhood leukemia has been uncertain because the few published series have been small. Most reported cases have occurred in children younger than 1 year, commonly newborns. By many criteria—morphologic, cytochemical, ultramicroscopic, immunologic and molecular—blast cells with the t(4;11) have been shown to have mixed lymphoid and myeloid features. Most cases of ALL with this translocation have a B-cell precursor phenotype (pre-B or early pre-B), with the blast cells containing rearranged Ig heavy-chain genes and expressing HLA-DR but lacking sheep erythrocyte receptors or surface Ig. Moreover, after treatment with the differentiation-inducing agent 12-O-tetradecanoyl-phorbol-13-acetate (TPA), blasts containing the t(4;11) acquire ultrastructural, cytochemical, and immunologic properties of monocytes. The finding of monocytic features in cells with characteristics of B-cell precursors has suggested that leukemias with the t(4;11) arise in a stem cell with the potential to differentiate in both the lymphoid and myeloid pathways.

In a collaborative study, the t(4;11)(q21;q23) was identified in 40 (2%) of 1986 children with newly diagnosed ALL. This translocation was associated with female sex (60%), age less than 1 year (60%), hyperleukocytosis (median leukocyte count, 156.5 × 109/L), an early B-cell immunophenotype (79%), and myeloid-related antigen (CD15) expression (63%). Leukemic cell L2 morphology was found in 13% of the cases. Of the 32 cases in which myeloid-associated antigen expression was analyzed, 20 had significant CD15 positivity. Seventy-three percent of the 40 cases had the t(4;11) as the sole chromosome abnormality. The modal chromosome numbers of the leukemic cell lines were 46 (n = 36), 47 (n = 2), 48 (n = 1), and 82 (n = 1). Notably, none of the 40 cases had hyperdiploidy > 50 but less than 80, a favorable cytogenetic feature. Analysis of clinical presenting features by age < 1 year (n = 24), 1 to 9 years (n = 8), and ≥ 10 years (n = 8) showed that infants were significantly more likely to have initial CNS leukemia and less likely to have pre-B-cell ALL. Despite contemporary intensive therapy, complete remission was maintained in only 7 of 24 infants and in only 2 of 8 adolescents, in contrast to 7 of 8 children in the intermediate age group (8 of the 16 cases remaining in remission have been followed for < 1 year). These results suggest that BMT or novel methods of chemotherapy should be considered for infants or adolescents with the t(4;11).

11q23 abnormalities/early pre-B-cell ALL. The q23 region of chromosome 11 is a relatively frequent site of structural rearrangements in ALL. As observed for the t(4;11), patients with 11q23 abnormalities are usually younger, and have higher leukocyte counts than do subgroups lacking this alteration. Although isolated cases have a T-cell immunophenotype, there is a strong concordance between 11q23 abnormalities and the early B-lymphoid, CD10+ immunophenotype. Including cases with the t(4;11), 11q23 abnormalities have been mainly associated with early pre-B-cell ALL, appearing in the blast cells of 4.5% to 5.7% of patients. A disturbing trend has been the development of secondary leukemia with 11q23 alterations in patients, mainly adults, who received genotoxic agents for neuroblastoma, Hodgkin's disease, osteogenic sarcoma, or breast cancer. In children, use of one or both of the epipodophyllotoxins (VM-26 and VP-16) has been implicated in the induction of secondary myeloid leukemias after treatment for ALL; in most instances, the secondary leukemia is of the monocytic or myelomonocytic subtype with characteristic 11q23 abnormalities.

Most cases of translocations involving the 11q23 region result from exchanges with chromosome 4; however, chromosomes 1, 10, and 19 can also contribute to this process.
as exemplified by the t(1;11)(p32;q23), t(10;11)(p14-p15;q22-q23) and t(11;19)(q23;p13).99 Other random and non-
random translocations involving the 1q23 band [eg, the t(9;11)(p22;q23), t(6;11)(q27;q23)] are found more often in acute myelomonocytic and monocytic leukemias,100 although the t(9;11)(p22;q23) can be found in rare cases of ALL.91,101 A recurrent constitutional t(11;22)(q23;q11), not associated with disease, has also been localized to this region of chromosome 11.102 The diverse chromosomal abnormalities involving band 1q23 are indistinguishable by light microscopy; translocations affecting this chromosomal region probably map to the same locus and may play an important role in malignant transformation.103

Several genes that encode proteins active in cell signaling have been localized to this region: eg, THY1, NCAM, genes encoding the γ, δ, and ε subunits of the T3 moiety of the T-cell receptor (TCR) complex, and the ETS1 and CBL2 proto-oncogenes.99 Preliminary mapping of the 11q23 region indicates orientation of these genes: as centromere, NCAM, CD3, THY1, CBL2, and ETS1.104-106 The ataxia telangiectasia locus (AT) has also been mapped to the 11q23 region, presumably centromeric to the THY1 locus.107 Results of recent molecular investigations have identified an additional gene that may be associated with this breakpoint. Designated MLL (myeloid, lymphoid leukemia or mixed lineage leukemia), it spans the 1q23 region in the t(4;11), t(6;11), t(9;11) and t(11;19) translocations and is between the CD3 gene cluster and PBGD, which is distally located to CD3.103,108,109 The MML gene appears identical to the ALL1 locus reported by Cimino et al.109-111 and may be responsible for the unique presenting features of leukemias characterized by alterations in the 11q23 region. Although most 11q23 translocation breakpoints cluster between genes CD3G and PBGD, a few reports indicate a diverse location, implicating additional unknown proto-oncogenes in this region.104,12,113

ALL in infants shows preferential involvement of the 11q23 region, not limited to the t(4;11).114-116 In a recent study of the Pediatric Oncology Group, 35 of 52 infants less than 1 year old had an abnormal karyotype, which included rearrangement of the 11q23 region in 21 cases.117 Results of this and other studies substantiate the impression that 11q23 alterations may preferentially affect stem cells capable of development in either the myeloid or lymphoid pathway. Further molecular investigation of the diverse breakpoints at 11q23 is needed to establish the relationship between cytogenetic alterations in this region and the leukemic transformation of blast cells.

T-Cell Leukemia-Lymphoma

T-cell childhood leukemia, accounting for about 15% of cases of ALL, has a relatively poor prognosis that can be attributed in part to hyperleukocytosis, frequent involvement of the CNS, mediastinal enlargement, and the lack of hypodiploidy > 50 chromosomes. Parasodically, a lower percentage of patients have cytogenetically detectable abnormal clones compared with findings in other immunophenotypes of ALL.24,32,34,118-121 Approximately 30% to 40% of the abnormal karyotypes in T-cell leukemia have nonrandom breakpoints within the 14q11, 7q34-q36, or 7p15 region, which contain the TCRα/β, TCRγ, and TCRδ genes, respectively.122 The four identified loci undergoing structural rearrangement during T-cell differentiation are analogous to the Ig genes in B-cell development. Results of earlier studies with in situ hybridization and somatic cell genetic techniques suggested that inversions and translocations disrupt the α-TCR genes in some T-cell malignancies.123-125 Recent molecular studies have confirmed these observations in numerous chromosomal rearrangements, mainly involving the 14q11 and 7q34-q36 regions (Table 2). The prominent recurring chromosomal abnormalities in T-cell ALL are discussed in the following sections.

14q11(TCRα/β). The most frequent nonrandom abnormality in childhood T-cell ALL is the t(11;14)(p13;q11), detected in 7% of cases.34,136 At the molecular level, the breakpoint is within the α/β locus of the TCR gene on chromosome 14.124,128,129 The breakpoints in 11p13 are clustered within a region of less than 25 kb, termed T-ALLN, suggesting that a specific gene is altered by the translocation.130-133

The t(11;14)(p13;q11), found in 1% of T-cell neoplasias,127,134 also disrupts the α/β locus of the TCR gene on chromosome 14.135 On chromosome 11 the break occurs in a gene named TTG1 (T-cell translocation gene 1) or rhombotin 1 (RBTN1), which encodes a nuclear protein whose structure has been highly conserved among diverse species, suggesting an important functional role in ontogeny (eg, brain development).130-137 Interestingly, another rhombotin-related gene (RBTN2) was found near the previously described T-ALLN region or chromosome 11p13, TTG2,137 a newly recognized gene encoding a cysteine-rich protein with a LIM motif, is overexpressed in T-cell ALL defined by the t(11;14)(p13;q11).138 Hence, members of the rhombotin gene family may be a class of transcription factors with particular relevance for T-cell neoplasia.

Reported in 5% to 10% of patients with T-cell ALL and lymphomas,139 the t(10;14)(q24;q11) occurs in the TCRβ locus on chromosome 14 and in a breakpoint cluster region on chromosome 10.140,141 The tight clustering of t(10;14) breakpoints on chromosome 10 at q24 suggests that the translocation may result in dysregulation of a newly identified homeobox gene, termed HOX111, via illegitimate physiologic recombination with TCRβ.143-145 The HOX11 gene shares homology with other homeobox-containing genes that normally code for sequence-specific DNA binding proteins and when abnormally expressed may contribute to T-cell leukemogenesis. Because of the tight clustering of chromosomal breakpoints, the t(10;14) can be detected by PCR analysis.146

The t(8;14)(q24;q11) is commonly observed in T-cell leukemias (2%) but is not restricted to this lineage.34,147,148 At the molecular level, the TCRα gene is rearranged with the CMYC oncogene, resulting in its transcriptional dysregulation.149,150 In this translocation, the breakpoint occurs between the variable and constant regions of the TCR, resulting in translocation of the constant region distal to the CMYC gene, which corresponds to the mechanisms of CMYC activation in the variant B-cell translocations.152-154

A t(1;14)(p32-p34;q11) has been observed in 3% of T-cell ALL patients.155 Molecularly, TAL1 (SCL1/TCL5) is juxtaposed with the TCRα/β chain locus on chromosome
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The amino acid translation of TAL1 or SCL includes sequences containing a helix-loop-helix motif, a DNA-binding domain present in a family of proteins involved in the control of cell growth and differentiation. Subsequently, a site-specific chromosomal deletion was identified that involved SCL and a locus called SIL (for SCL-interrupting locus). The deletion, which occurs in 15% to 26% of patients with T-cell ALL, cannot be observed by karyotypic analysis but is easily detected by Southern blotting or PCR analysis. Some investigators have speculated that the SIL/SCL deletion contributes to malignant transformation in a manner similar to that of the t(14;16), which also disrupts the 5' regulatory region of SCL.

Hence, alteration of TAL1, either by translocation or other rearrangement, represents the most common genetic lesion associated with T-lineage leukemia. Most importantly, the TAL1 interstitial deletion is the first known instance whereby two genes (neither of which is an antigen receptor gene) are joined through the action of the V(DJ) recombinase system.

7q25 (TCRB). The 7qter region containing the TCRβ gene is affected by chromosomal aberrations less often than the α/β region of chromosome 14.

Molecular evaluation of T-cell lines with a t(7;9)(q34;q34) showed that the breakpoint on chromosome 7 was within the TCRβ gene but no alteration of the ABL oncogene was found on chromosome 9. Another study of a similar case demonstrated consistent breakage between consensus recombinase heptamers of chromosome 9. It has also been shown that the affected region on chromosome 9 contains a gene (TANI) that is highly homologous to the Drosophila gene Notch. TANI is broken by the t(7;9)(q34;q34), producing a truncated version of TANI mRNA. Recently, a helix-loop-helix TAL2 gene was identified on chromosome 9 at q32 and is transcriptionally activated by a t(7;9)(q34;q32).

A t(7;19)(q34;p13) in a T-cell line established from a case of T-cell ALL affects the TCRβ gene and truncates the LYLI gene, which is quite homologous to the TAL1 gene, and whose protein product contains a potential helix-loop-helix DNA-binding motif and may therefore contribute to leukemogenesis.

Similarly, in two cases of T-cell ALL, a t(1;7)(p34;q34) impinged on the TCRβ locus and interrupted the LCK locus on chromosome 1p34. The LCK gene encodes a protein kinase, p56, which is normally restricted to lymphoid cells and is considered to play a crucial role in the transduction of mitogenic signals. Thus, the alteration in the promoter region of the LCK locus may play a role in human cancer. We evaluated molecularly a cytogenetically different t(1;7)(p32;q35) that placed the TAL1 locus near the TCRβ enhancer, which may overtake the TAL1 gene product and thus contribute to leukemogenesis. A t(7;11)(q35;p13) and a t(7;10)(q35;q24), variants of the more frequently observed t(11;14)(p13;q11) and t(10;14)(q24;q11), respectively, also activate DNA-binding transcription factors (RBTN2 and HOX11).

Non-specific Lineage

6q abnormality. Deletion of the long arm of chromosome 6 is a relatively common finding in ALL, reported in 4% to 13% of cases, most of which have the breakpoints localized to 6q15 and 6q21. In a review of 412 consecutive cases of ALL with fully banded karyotypes, we identified 45 (11%) with a 6q abnormality. Eighty-five percent of the alterations were deletions, with other types of structural rearrangements accounting for the remainder. The presenting features and EFS of children with a 6q abnormality were not demonstrably different from those of patients lacking this feature. There was no indication of immunophenotype specificity for these changes, suggesting that the gene or genes affected by 6q abnormalities are broadly active in the multistep process of lymphoid leukemogenesis, or perhaps arise as molecular epiphenomena of more specific lesions. No conclusive molecular evidence for loss of heterozygosity has been reported for 6q deletions.

9p abnormality. Abnormalities of the short arm of chromosome 9 (9p) have been observed in 7% to 12% of cases of childhood ALL. Earlier reports indicated that 9p abnormalities were usually associated with a “lymphomatous” type of ALL, often characterized by a T-cell immunophenotype with prominent lymphadenopathy, mediastinal enlargement, and splenomegaly. Subsequent reports describe 9p abnormalities in a significant number of B-lineage cases. In our own large series of consecutively banded cases of ALL, there was an 10% incidence of 9p abnormalities (26 deletions, 9 unbalanced and 5 balanced translocations). Compared with patients lacking this change, the affected children were older and had higher leukocyte counts, a greater frequency of splenomegaly and the T-cell immunophenotype (25%), and an increased rate of extramedullary relapse. We initially concluded that despite their association with high-risk clinical features, 9p abnormalities did not predict any major adverse events in the clinical course, except for an apparently increased hazard of involvement of the CNS (eight of 17 failures). More recently, in a clinical trial of intensified chemotherapy, patients with 9p abnormalities, whether translocations or deletions, had an inferior outcome (4-year EFS of 50% vs 73% for the study overall). The “critical” region involved in 9p abnormalities is p21-p22, which encompasses the interferon-α gene cluster (IFN4) and the interferon-β gene (IFNB1), both of which are included within the segments known to be deleted in some ALL cases with or without cytogenetic abnormalities of 9p. Conceivably, these deletions lead to loss of a tumor suppressor gene located in the 9p region. A deficiency of the enzyme methylthioadenosine phosphorylase (MTAP), mapped to 9p, was found in many cases of IFN gene deletion, suggesting a link between these genes.

12p abnormality. Abnormalities of the p arm of chromosome 12 appear in approximately 10% of cases of ALL. A breakpoint at band p12 is reported most often, although breaks at both p11 and p13 are seen as well. Most cases with a 12p abnormality have a B-lineage immunophenotype, although some T-cell cases have also been reported. Among 632 banded cases of ALL, the 12p abnormalities consisted of 20 deletions, 22 balanced translocations, 16 unbalanced translocations, 14 dicentric chromosomes, and 4 inversions for an overall incidence of 12%. Abnormalities seen in more than one case were the t(1;12)(q22;p12), n =
2; t(6;12)(q21;p12), n = 2; t(12;13)(p13;q14), n = 2; dic(9;12)(p11;p12), n = 5; dic(7;12)(p11;p12), n = 5; dic(12;17)(p11;p11), n = 2; del(12)(p11), n = 7; del(12)(p12), n = 13; and inv(12)(p13q22), n = 3. A 12p change was the sole chromosomal abnormality in only 30% of the 74 cases; the modal numbers were 45 (17.6%), 46 deletions or dicentric formations.

One of the most frequent recurrent 12p abnormalities, dicentric (9;12)(pl1), n = 2; t(6;12)(q21;p12), n = 2; t(12;13)(p13;q14), t(17;19)(q22;p13), simultaneously were unbalanced with the formation of a dicentric chromosome [dic(7;9)-r(pl3);pl1), dic(7;12)(p11;p12), and dic(12;17)(p11;p11;p12)]. The small size of these subgroups prevented definitive correlations with clinical presenting features or immunophenotype, except for the t(17;19), as indicated previously. Nonetheless, rare translocations in ALL may prove biologically important. For example, a distinct subtype of B-lineage ALL is associated with peripheral eosinophilia and a t(5;14)(q31;q32). It has been shown that the IL3 and IGμ genes are joined by this translocation, resulting in activation of the former.

FISH provides only limited information about the karyotype of leukemic cells, but when used in conjunction with conventional cytogenetic techniques, it has specific advantages that make it useful in both the clinical management of patients and the study of leukemia pathobiology. For example, it can be applied to the study of nondividing or slowly dividing cells, making it useful in assessing terminally differentiated blood cells or cells that are difficult to grow in culture. In one of the many applications of FISH, it may be possible to screen large numbers of cells for residual disease in patients in “complete remission” from acute leukemia with known numeric chromosomal abnormalities, or in hypoplastic or mismatched sex marrows of patients after a bone marrow transplant.

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