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ARTICLE

# Molecular Cytogenetic Aspects of Hematological Malignancies: Clinical Implications

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The field of molecular cytogenetics has had a great impact on many aspects of medical and basic sciences. During the past 30 years, the application of molecular cytogenetic methodologies has resulted in remarkable advances in the field of cancer genetics and cytogenetics. These advances have led to the establishment of chromosome patterns as diagnostic and prognostic indexes in an array of acute and chronic leukemias and lymphomas, as key information in BMT, and as guides for the localization of oncogenes and tumor suppressor genes that are apparently responsible for the development of neoplastic states. With such information, the physician is in a more favorable position to devise therapy, appraise diagnosis, and plan follow-up. © 2002 Wiley-Liss, Inc.

**KEY WORDS:** molecular cytogenetics; chromosomal abnormalities; hematological malignancies

## INTRODUCTION

The provision of information related to the more accurate and specific diagnosis of disease states has been a major contribution of molecular cytogenetics. With such information the physician is in a more favorable position to devise therapy, appraise prognosis and plan follow-up. Chromosomal changes have been established in a wide spectrum of cancers, ranging from the various leukemias to lymphomas and solid tumors. In addition to the clinical application of these cytogenetic changes, the establishment of specific chromosomes or chromosomal bands affected by these changes has led molecular biologists to recognize, characterize, and isolate genes that are affected by and possibly responsible for the conditions studied. Such information not only has been utilized diagnostically but also has led to the development of molecular tests for cancer predisposition and ultimately will form the basis for devising approaches to gene or other forms of therapy.

Evidence has accumulated indicating that tumor formation often is associated with the occurrence of nonrandom chromosomal abnormalities. Generally, carcinomas and some types of lymphoma and multiple myeloma are associated with numerous karyotypic changes compatible with a stepwise process of orchestrated genetic changes necessary for the cell to progress from proliferation to malignant transformation to invasiveness and metastases. In most leukemias and sarcomas, however, relatively simple karyotypes are found, typically including translocations and, less commonly, inversions or insertions, which can be diagnostic of the form of leukemia or sarcoma. Chromosomal deletions are common occurrences in epithelial adenocarcinomas, such as those of the large bowel, lung, breast, and prostate, in which a number of these deletions are often present and may be part of a stepwise process of genetic changes leading to malignant transformation. As new and additional chromosome changes develop in any condition, the disease tends to progress and requires evaluation of therapeutic approaches [Sandberg, 1990; Heim and Mitelman, 1995].

## NONRANDOM CHROMOSOMAL ABNORMALITIES IN HEMATOLOGIC MALIGNANCIES

### Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder arising from neoplastic transformation of a pluripotent stem cell. It is characterized clinically by marked overproduction of granulocytic cells and cytogenetically by the Philadelphia (Ph) chromosome. The Ph chromosome was the first consistent abnormality noted in a human cancer, arising from a reciprocal translocation, t(9;22)(q34;q11.2), and molecularly by the fusion of the proto-oncogene *ABL*, located on the long arm of chromosome 9, with the *BCR* gene of chromosome 22, known as the breakpoint cluster region (bcr) [Rowley, 1973; Groffen et al., 1984]. The diagnosis of CML usually is based on

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morphologic examination of the bone marrow aspirate and its chromosome analysis, which often plays a key role in confirming the diagnosis.

***The Ph chromosome was the first consistent abnormality noted in a human cancer, arising from a reciprocal translocation, t(9;22)(q34;q11.2), and molecularly by the fusion of the proto-oncogene ABL, located on the long arm of chromosome 9, with the BCR gene of chromosome 22, known as the breakpoint cluster region (bcr).***

Historically, more than 85% of patients in whom CML is diagnosed are found to have the Ph chromosome [Sandberg, 1990; Heim and Mitelman, 1995]. In 25% of cases, the Ph chromosome is the only change noted throughout the course of the disease. At the time of diagnosis of CML, all the cells in the marrow usually are Ph-positive (Ph+); this is true even during remission, unlike the situation in acute leukemia, in which the Ph+ cells usually are admixed with cytogenetically normal cells but may disappear from the marrow in complete remission. Intensive chemoradiotherapy, followed by allogeneic bone marrow transplantation (BMT), has been successful in eradicating Ph+ cells and restoring normal hematopoiesis of donor origin. Serial monitoring of residual disease after BMT, by estimating the number of *BCR/ABL* transcripts, provides more information than conventional cytogenetics or nonquantitative polymerase chain reaction (PCR) and may identify patients in need of therapeutic intervention before the onset of overt relapse [Radich et al., 1995].

CML should be defined by the presence of the Ph chromosome. Patients with CML-related features but without the Ph chromosome should be considered to have a different chronic myeloproliferative disorder (MPD) or some type of myelodysplastic syndrome (MDS), such as chronic myelomonocytic leukemia or refractory anemia with excess blasts [Sandberg, 1990]. Variant forms of Ph translocations are seen in 5–10% of CML cases, including simple variant translocations (i.e., the Ph translocation involving another chromosome in addition to 22 and 9) and complex variant translocations (i.e., involving chromosomes 9, 22, and others). In general, no correlation has been found either for the genomic breakpoint site or the *BCR/ABL* RNA transcript in terms of duration of the chronic phase or survival [Shepherd et al., 1995]. All chromosomes except Y have been involved in variant Ph translocations. In a rare patient with CML, the Ph chromosome may be masked by the Ph translocation and hence is not recognizable microscopically (cytogenetically). In these cases, the presence of the Ph translocation can be established by molecular means, that is, by Southern blot analysis or fluorescence in situ hybridization (FISH).

When CML progresses, chromosome aberrations besides the Ph chromosome are noted in 75–80% of cases. These aberrations may precede hematologic progression by 2–6 months or occur at the time of blast crisis, and they are therefore important prognostic markers. The most common additional chromosome changes are trisomy 8, additional Ph, i(17q), trisomy 19, trisomy 21, and loss of a sex chromosome. The additional Ph may precede a lymphoid blast crisis, whereas all changes may precede myeloid blast crisis. Molecularly, the *TP53* gene appears to be affected in about 30% of cases of myeloid blast transformation [Cline, 1994]. It is unlikely that other tumor suppressor genes, such as *RB, WT1, DCC,* and *NF2*, are involved in the progression of CML to blast crisis in the majority of patients [Silly et al., 1994]. Methylation of the *ABL* promoter indicates disease of long standing, most likely associated with a higher probability of imminent blast transformation [Ben-Yehuda et al., 1997]. In addition, loss of imprinting of the insulinlike growth factor type II gene (*IGF2*) [Randhawa et al., 1998] and telomere length shortening [Boultwood et al., 2000] have been reported to be associated with disease progression in CML.

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Recent studies have indicated that up to 29% of CML patients may have large deletions at the t(9;22) breakpoints [Sinclair et al., 2000; Huntly et al., 2001]. Small deletions adjacent to the Ph chromosome have no pathophysiological importance, but deletions on the derivative chromosome 9 may be several Mb in size; *ASS* and *N43E1* are the two most common loci deleted. These deletions appear to occur at the time of formation of the Ph translocation and are associated with shorter chronic phase and overall survival time. The fact that a higher incidence of concomitant deletions is seen in variant Ph translocations than the standard Ph translocation may explain some of the varying results obtained in evaluation of the clinical effect of variant Ph translocations in CML. Similar deletions also have been noted in leukemia patients with inv(16) and 11q23 rearrangements, indicating that deletions are not disease-specific and happen in regions with high

Alu sequence repeats [Kolomietz et al., 2001].

### Polycythemia Vera

Polycythemia vera (PCV) is characterized by bone marrow hyperplasia, an increase in blood volume and the number of red blood cells. The disease may progress to a terminal phase, which can involve transformation to myelofibrosis or overt leukemia. The frequency of clonal chromosomal abnormalities in PCV is about 15% at diagnosis and somewhat higher, perhaps 40%, among treated patients at later disease stages. Of the patients with cytogenetic abnormalities, about half of those who experience disease progression already have karyotypic changes before the development of myelofibrosis. Almost all patients in whom acute leukemia develops in late stages of the disease have bone marrow chromosome abnormalities [Sandberg, 1990; Heim and Mitelman, 1995]. The five most common aberrations, in decreasing order of frequency, are 20q−, +8, +9, 13q−, and structural abnormalities of chromosome 1 resulting in partial trisomy 1q. Both +8 and +9 may persist without further clonal evolution or leukemia development for up to 15 years [Sandberg, 1990]. Generally, −7 or 5q−, in combination with other changes, signal the terminal phase of PCV. Most recently, our findings strongly indicate that an additional i(9)(p10) is a new and recurrent primary chromosome anomaly in PCV. Considering that trisomy 9 is one of the most common anomalies in PCV, gain of a gene(s) on 9p, but not on 9q, may play a crucial role in the pathogenesis of PCV [Chen et al., 1998].

### Essential Thrombocythemia

The main clinical manifestations of essential thrombocytopenia, a rare MPD subgroup, are thromboembolic and hemorrhagic phenomena. Cytogenetically, only about 5% of patients have a definite chromosomal abnormality [Sandberg, 1990; Heim and Mitelman, 1995]. Moreover, no recurring abnormality is present in these patients.

### Myelofibrosis With Myeloid Metaplasia

Myelofibrosis with myeloid metaplasia is characterized by varying degrees of bone marrow fibrosis and extramedullary hematopoiesis with concomitant anemia and circulating immature granulocytes and erythroblasts. Cytogenetic analysis has shown the presence of clonal abnormalities in 35% of these patients. In general, these abnormalities are similar to those noted in other malignant myeloid disorders, most commonly including +8, −7/7q−, 11q−, 20q−, and 13q changes [Sandberg, 1990; Heim and Mitelman, 1995]. A change in the karyotype in myelofibrosis with myeloid metaplasia may signal evolution to acute leukemia.

### Myelodysplastic Syndromes

MDS are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by dysplastic and ineffective hematopoiesis as a result of progressive impairment of differentiation due to a defect at the level of multipotent or pluripotent stem cells. This condition carries a high risk of transformation to acute non-lymphocytic leukemia (ANLL). Etiologically, MDS take the form of both primary disorders (p-MDS) and disorders stemming from cytotoxic chemotherapy or radiotherapy (t-MDS). The incidence of MDS appears to be increasing in the aging population (> 50 years) and already presents a perplexing and serious epidemiological problem. To confirm the diagnosis of MDS, it is necessary to perform morphologic examination of bone marrow aspirate and marrow chromosome analysis. Moreover, in general, the chromosome findings have been shown to be an independent prognostic indicator, second only to the FAB subtype as a predictor of progression to leukemia and survival.

An international scoring system for evaluating prognosis in MDS also has been established, primarily based on cytogenetics [Greenberg et al., 1997]. This system indicates that the major variables having an impact on disease outcome or evolution to acute myeloid leukemia are the presence of cytogenetic abnormalities, the percentage of bone marrow myeloblasts, and the extent of cytopenias. For survival, in addition to the foregoing factors, variables include age and gender. Thus, chromosome studies can help indicate those patients with MDS who are likely to survive long enough to be evaluated adequately with long-term therapy and those patients who must be treated rapidly and aggressively based on chromosomal patterns associated with clinical prognosis. During therapy the cytogenetic findings can be used to monitor the size of the neoplastic clone in the marrow as an indicator of response.

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### p-MDS

Clonal chromosomal abnormalities can be detected in bone marrow cells in 40–70% of patients with p-MDS at diagnosis [Sandberg, 1990; Heim and Mitelman, 1995]. This contrasts with a 70–95% incidence of cytogenetic abnormalities in patients with de novo ANLL. Although both disorders commonly have trisomy 8 and loss of chromosome 5 or 7 or a deletion of 5q or 7q, the specific structural rearrangements that are closely associated with distinct morphologic subsets of de novo ANLL, such as t(15;17) in ANLL-M3, are almost never seen in p-MDS.

Patients with MDS may have single or multiple chromosome changes. Occasionally, several unrelated abnormal clones may be detected; the frequency of such unrelated clones may be higher than that observed in de novo ANLL. Additional aberrations may evolve during the course of MDS, or an abnormal clone may emerge in a patient with a previously normal karyotype; these changes appear to portend transformation to leukemia. With several exceptions, such as the 5q- syndrome and monosomy 7 (−7) syndrome, chromosome changes in p-MDS have not correlated with specific clinical or morphologic subsets using the criteria of the FAB group. The deletion of 20q involving the chromosomal segment 20q11.2-q12 has been shown to carry a poor prognosis in myeloid disorders, with a high rate of transformation of MDS to ANLL. Another nonrandom abnormality, t(5;12)(q31-33;p12-13), has been identified to fuse the *TEL* (12p) and *PDGFRβ* (5q) genes [Golub et al., 1994]. This anomaly is found in one subtype of MDS, namely, chronic myelomonocytic leukemia, and in MPD.

The 5q− syndrome is a distinct hematologic disorder that affects primarily elderly women with refractory macrocytic anemia and normal or elevated platelet counts; monolobulated micromegakaryocytes are a constant feature in this subgroup of MDS. Patients with 5q− as the sole abnormality have long survival times. So far, three types of interstitial deletion have been identified: del(5)(q12-13q31-33)(90%), del(5) (q12q23) and del(5) (q23q32).

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Monosomy 7 (−7) syndrome characterizes a spectrum of childhood hematologic disorders that may have initial features of pre-leukemia or leukemia. Children with −7 and preleukemia usually have no history of exposure to carcinogenic substances, and commonly have refractory anemia, leukocytosis, thrombocytopenia, and recurrent infections; evolution to ANLL is common. The −7 syndrome in children also may be related to a genetic predisposition or familial involvement, suggesting the necessity of chromosome analysis of all siblings of patients with −7.

The most common molecular abnormality in MDS is activation of the *RAS* proto-oncogene, which is found in approximately 3−40% of patients. Although the gene mutation appears at early as well as late stages of leukemic progression, it is thought to be predictive of future malignant transformation. In addition, *TP53* mutations are detected in 10−15% of advanced MDS and are preferentially associated with 17p− [Mitani et al., 1997]. Other findings include extensive apoptosis, resulting in ineffective hematopoiesis and deficient bone marrow functioning [Parker and Mufti, 1998], as well as methylation of the *p15INK4b* gene (an inhibitor of cyclin-dependent kinase CDK4 and CDK6) [Quesnel et al., 1998] and the *FLT3* gene (the human flt3 receptor gene) internal random duplication [Horiike et al., 1997] and increased *BCL-2* expression [Davis and Greenberg, 1998] in association with disease evolution.

MDS represents a small fraction of all hematological malignancies in children (< 10%). More than 80% of childhood MDS are in the advanced stages at diagnosis. Childhood MDS can occur idiopathically after chemotherapy/radiotherapy or in the context of predisposing conditions, such as constitutional chromosomal disorders (Down syndrome), neurofibromatosis, Schwachman syndrome, Kostmann neutropenia, and Fanconi anemia. Familial occurrence of MDS with −7 also has been reported [Sandberg, 1990; Heim and Mitelman, 1995]. The diagnosis and classification of childhood MDS based on cell morphologic features and cytochemical staining are somewhat complex and confusing. Cytogenetic analysis is essential to the evaluation of a child with proven or suspected MDS.

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About 64% of childhood MDS cases have an abnormal karyotype at diagnosis, a higher incidence than in adult MDS [Hasle, 1994; Martinez-Climent, 1997]. Monosomy 7 or deletion of the long arm of chromosome 7—del(7q)—the most frequently observed abnormality, often appears as the sole anomaly rather than as part of a complex of karyotypes, as is seen commonly in adult MDS. Other typical anomalies include +8, +9, +19, del(12p), and del(17p). Certain cytogenetic features have prognostic significance; for example, a normal karyotype is associated with a better outcome than an abnormal karyotype, and −7 alone has a survival advantage compared with −7 with additional anomalies.

## ACUTE LEUKEMIA

The acute leukemias, classified as either lymphoblastic (ALL) or non-lymphocytic (ANLL), result from neoplastic transformation of uncommitted or partially committed hematopoietic stem cells. The common chromosome changes seen in ALL and ANLL are shown in Tables I− III. A large array of structural and numerical chromosomal changes have been described in ANLL and ALL. Some of these changes occur much more frequently than do others. In ANLL, t(8;21), t(15;17), and inv(16) are the most common, followed by del(5q), +8, and del/t(11q23). In ALL, the most common changes are t(9;22), t(4;11),

**TABLE I. Common Chromosome Changes in ANLL★**

| | |
|---|---|
| der(1;7) (q10;p10)[a] | t(9;22)(q34;q11) M1 (M2) (Ph) |
| t(1;22)(q13;q13) M7 | t(11;V)(q23;V)[b] M5(M4) |
| ins(3;3)(q26;q21q26)[a,c] M1 (M7) | del(11)(q23)M5 (M4) |
| inv(3)(q21q26)[a,c] M1 (M7) | +11 |
| t(3;3)(q21;q26)[a,c] M1 (M7) | del(12)(p11p13)[a] M1, M2, M4-M6 |
| t(3;5)(q25.1;q34) | |
| t(3;21)(q26;q22)[a] | +13[a] |
| +4 M2, M4 | +14[a] |
| −5 or del(5)(q12-13 or q31-35)[a] M1-M4 | t(15;17)(q22;q21) M3 |
| +6 | del(16)(q22)[d] M4Eo |
| t(6;9)(p23;q34)[a] M2 (M4) (basophilia) | inv(16)(p13;q22)[d] M4Eo |
| −7 or del (7)(q22)[a] M1-M5 | t(10;16)(p13;q22)[d] M4Eo |
| t(7;11)(p15;p15) | |
| +8[a] | t(16;21)(p11;q22) |
| t(8;16)(p11;p13) M5b (erythrophagocytosis) | I(17q)[a] |
| t(8;21)(q22;q22) M2 (Auer rods)[a] | +19 |
| +9 | del(20)(q11-13)[a] |
| del(9)(q22) | +21 idic(X)(q13)[a] |

★Where appropriate, the type of acute non-lymphocytic leukemia (ANLL) or other information associated with a particular chromosome change also is shown.
[a]Change also seen in MDS.
[b]V, chromosomes 6, 9, 17, and 19.
[c]Associated with platelet and/or megakaryocytic anomalies.
[d]Associated with marrow eosinophilia.

**TABLE III. Structural Chromosome Changes in T-cell Lymphomas and Acute Lymphoblastic Leukemia**

| |
|---|
| t(7;7)(p15;q11) |
| t(7;V)(q34;V)[a] |
| inv(7)(p14q35) |
| del(9)(p13-22) |
| t(9;17)(q34;q23) |
| t(14;V)(q11;V)[b] |
| inv(14)(q14q32) |

[a]V, 1p32-34, 9p24 or q32, 10q24, 11p13, 14q11, and 19p13.
[b]V, 1p32-34, 7q34, 8q24, 10q24, and 11p13-15.

and del(6q), followed by t(8;14), t(1;19), and del(9p). Numerous other changes of much lower incidence occur in both ANLL and ALL, but the incidence may vary in different clinics, because the changes shown may be related to age (pediatric vs. adult cases), geographical location, and the nature of the leukemias examined (primary vs. secondary) [Sandberg, 1990; Heim and Mitelman, 1995].

Determination of the chromosome changes in acute leukemia serves a number of practical purposes, for example, establishing the exact diagnosis, predicting prognosis, and monitoring phases of therapy or BMT. It also serves some basic purposes, such as supplying the molecular biologist with information on the possible location or nature of the genes affected by translocations.

**TABLE II. Common Chromosome Changes in B-Lineage Acute Lymphoblastic Leukemia**

| | |
|---|---|
| t(1;9)(q23;p13) | Pre-B-cell |
| t(2;8)(p12;q24) | L3 (B-cell) |
| t(4;11)(q21;q23) | Biphenotypic, early pre-B-cell |
| t(5;14)(q31;q32) | |
| del(6)(q13-14) or q21-q27) | |
| t(8;14)(q24;q32) | L3 (B-cell) |
| t(8;22)(q24;q11) | L3 (B-cell) |
| del(9)(p13-22) | |
| t(9;22)(q34;q11) | Pre-B-cell, $Ph^+$ |
| del(11)(q14-23) | |
| t(11;19)(q23;p13) | Mixed, early pre-B-cell |
| t(12;V)(p12;V)[a] | B-lineage |
| t(12;21)(p13;q22) | Pre-B-cell |
| t(14;19)(q32;q13) | |
| t(14;22)(q32;q11) | |

[a]V, chromosomes 7, 9, and 17.

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## ANLL

At least two-thirds of ANLL patients have demonstrable clonal chromosome abnormalities at diagnosis. Cytogenetic analysis finds clonal abnormalities in 80–85% of pediatric patients [Martinez-Climent, 1997]. On average, about half of patients with cytogenetic anomalies have only one karyotypic rearrangement. Numerical aberrations are seen in 15–20% of the cytogenetically abnormal cases. Particularly common are trisomy 8 and monosomy 7. Translocations are the most common structural chromosome changes, followed by deletions and inversions. Some of the following changes are diagnostic of particular types of ANLL (Table I).

*t(8;21)(q22;q22)*

This is the most common structural rearrangement reported in ANLL; it is seen in about 15% of all the acute myeloblastic leukemias with differentiation (M2) and some cases of acute myelomonocytic leukemia (M4). The t(8;21) characterizes a morphologically and clinically distinct subset, for example, blasts with indented nuclei and basophilic cytoplasm, often containing Auer bodies. Patients with the t(8;21) appear to have a favorable prognosis, with a uniformly high complete remission rate.

*t(15;17)(q22;q21)*

This translocation is detected in at least 70% of cases of acute promyelocytic leukemia (M3). Some variant translocations involving 17q, but not 15q, have been seen in some cases that are hematologically indistinguishable from the ordinary t(15;17)-associated M3 disease. The t(15;17) is highly specific to ANLL-M3 and has not been found in patients with any other type of leukemia or solid tumor.

*inv(16)(p13q22)*

The inv(16) characterizes a distinct subset of patients with ANLL-M4 associated with quantitative morphologic and cytochemical abnormalities of bone marrow eosinophils (M4Eo). Identical morphologic abnormalities also have been seen in patients with the t(16;16)(p13;q22) or the del(16)(q22). Patients with inv(16) and t(16;16) normally have a good response to intensive chemotherapy and thus have a favorable prognosis.

*Rearrangements of the long arm of chromosome 11*

Several different rearrangements involving band 11q23 have been associated with ANLL: most commonly t(9;11)(p22;q23) and less often t(10;11)(p11–15;q23), t(11;17)(q23;q25), and t(11;19)(q23;p13). Translocations involving 11q23 occur in 5–10% of adult leukemia cases and approximately 60% of infant acute leukemia cases [Sandberg, 1990; Heim and Mitelman, 1995; Martinez-Climent, 1997]. The many different 11q23-related balanced translocations affect the *MLL* gene located at 11q23. Partial tandem duplication (PTD) of the *MLL* gene has been found in 11 of 98 ANLL patients with normal cytogenetic complements [Caligiuri et al., 1998]. Furthermore, patients with *MLL* rearrangements had significantly shorter durations of complete remission compared with patients without *MLL* rearrangements.

*inv(3)(q21q26)*

The inv(3) characterizes a distinct subset of patients with ANLL associated with increased micromegakaryocytes and abnormal thrombocytosis. Identical hematologic abnormalities also have been seen in patients with t(3;3) (q21;q26) or ins(5;3)(q14;q21q26). These changes often are associated with a poor prognosis.

*t(6;9)(p23;q34)*

Patients with t(6;9) account for about 2% of those with ANLL; this translocation frequently is associated with bone marrow basophilia and a poor response to intensive remission-induction therapy.

*t(3;5)(q25.1;q34)*

This is an uncommon karyotypic aberration in ANLL. With the exception of M3, t(3;5) has been reported in association with every other subtype of ANLL, most frequently with ANLL-M6 (acute erythroleukemia).

*Acute megakaryoblastic leukemia*

Acute megakaryoblastic leukemia, or ANLL-M7, accounts for 3–5% of all childhood cases of ANLL and about 20% of infant leukemias. Cytogenetically it can be classified into four different subgroups: M7/Down syndrome (DS), M7/acquired trisomy 21, M7/t(1;22) (p13;q13), M7/abnormalties of bands 3q21 and 3q26, and monosomy 7 or del (7q) [Martinez-Climent, 1997]. In general, patients with M7/DS have a markedly more favorable outcome compared with those without DS, and children with M7/t(1;22) or M7/other abnormalities have a poor prognosis [Martinez-Climent, 1997].

*Therapy-related MDS and ANLL*

Therapy-related MDS (t-MDS) and ANLL (t-ANLL) have been recognized increasingly as serious complications of successful treatment of primary malignant diseases using chemotherapy or radiation or both in both adults and children. Most cases of t-ANLL pass through a myelodysplastic stage. Generally, t-MDS/t-ANLL are much more aggressive clinically than de novo p-MDS/ANLL, and these biological characteristics are reflected in the karyotypes. At least 80% of patients with t-MDS/t-ANLL have chromosomal abnormalities, and the vast majority of cases have several cytogenetic abnormalities. These changes differ in their type and frequency from those noted in primary MDS/ANLL. Examples include −7/7q− and −5/5q−, which are detected in about 90% of cases of t-ANLL compared with 16% of de novo ANLL. In addition to −7/7q− and −5/5q−, other common chromosomal abnormalities in t-MDS/t-ANLL include del(12p), t(1;7)(q10;p10), t(3;21) (q26.2;q22.1), 3p21-related anomalies, and rearrangements at chromosome band 11q23. Based on chromosome banding analysis, two critical regions have been identified in 7q−: one in band 7q22 and a second

in bands 7q32-35. Ring chromosomes, dicentrics and acentric fragments also have been reported in t-MDS/t-ANLL. Any combination of these cytogenetic changes strongly suggests previous exposure of the patient to clastogenic agents and indicates a poor prognosis and generally short survival span.

*Normal karyotypes at diagnosis of ANLL*

The infrequent limitations of conventional cytogenetics are illustrated cogently by the finding in ANLL—with FISH or reverse transcription (RT)-PCR—of genetic changes associated with normal karyotypes. Thus, study of a group of patients with normal karyotypes showed the presence of PTD of the *MLL* gene, as mentioned previously. Southern analysis of the *MLL* gene in pretreatment samples from 98 ANLL patients with normal cytogenetic features found that 11% of these patients' samples had a PTD of the *MLL* gene [Caligiuri et al., 1998]. In another study, samples of 387 consecutive cases of ANLL were examined by one-step RT-PCR and confirmed by genomic long-range PCR testing and Southern blot analysis. PTD of *MLL* were found in 5.7% of cases with a cytogenetically normal karyotype [Schnittger et al., 2000]. In both reports, patients harboring PTD of *MLL* clearly had inferior outcomes compared with age-matched controls with normal karyotypes. It would appear that the group of patients with normal karyotypes at diagnosis is very heterogeneous at the molecular level and that many aberrations occur below the level of detection of conventional cytogenetic analysis, as mentioned earlier herein.

***Southern analysis of the MLL gene in pretreatment samples from 98 ANLL patients with normal cytogenetic features found that 11% of these patients' samples had a PTD of the MLL gene.***

Another example is the presence of an internal tandem duplication (ITD) of *FLT3* in patients with ANLL and a normal karyotype [Nakao et al., 1996; Horiike et al., 1997]. ITDs of *FLT3* were seen in 23% of 201 ANLL patients and patients with normal karyotypes; 28% harbored ITDs of *FLT3* [Nakao et al., 1996; Horiike et al., 1997]. In patients younger than 60 years, the presence of this *FLT3* mutation was the strongest poor prognostic factor of overall survival. The presence of ITDs of *FLT3* and the absence of the wild-type *FLT3* allele were shown to indicate significantly inferior disease-free survival and overall survival rates compared with ITDs of *FLT3* and the wild-type *FLT3* gene or no ITDs of *FLT3* [Whitman et al., 2001]. Another study of patients with ANLL and normal karyotypes identified dominant-negative mutations of the tumor suppressor gene *C/EBPa* in 5% of patients with normal karyotypes [Pabst et al., 2001]. The clinical significance of these findings is yet to be established [Cataland et al., 2001]. Thus, the significance of a normal karyotype in patients with ANLL at diagnosis must be held in abeyance until this area is intensively explored at the molecular level.

## ALL

The karyotype is an important independent prognostic factor in ALL. At least two-thirds of cases of ALL show clonal chromosomal anomalies. Up to 90% of children with ALL have karyotypic abnormalities [Martinez-Climent, 1997]. Hyperdiploidy, which is present in 30%, is more common in ALL than in ANLL, and hypodiploidy is present in 10%. Several cytogenetic anomalies are associated with distinct immunologic phenotypes in ALL.

*t(8;14)(q24;q32)*

This translocation has been detected in 75–85% of cases of Burkitt lymphoma. The identical t(8;14) has been seen in patients with B-cell ALL of the L3 type, indicating a possible commonality of ALL-L3 and Burkitt lymphoma. This group of L3 patients often has a high incidence of central nervous system involvement and a poor prognosis. In 15–25% of cases of Burkitt lymphoma and some cases of ALL-L3, one of two variant translocations is found: t(8;22)(q24;q11) or t(2;8)(p12;q24). The former translocation is seen twice as frequently as the latter.

*t(4;11)(q21;q23)*

This translocation often is detected in patients with ALL-L1 or ALL-L2 and less often in those with biphenotypic leukemia. It also is seen in congenital leukemia.

*t(9;22)(q34;q11)*

There are two major forms of Ph+ leukemia: CML and ALL. The t(9;22) is found in 6% of childhood cases and 17% of adult cases of ALL associated with chromosome abnormalities, representing the most frequent rearrangement in adult ALL. On the molecular level, there are two distinct subgroups of Ph+ ALL. In the first group the molecular rearrangement is identical to that seen in CML. In the second group the breakpoint occurs upstream (5′) of the bcr but still within the *BCR* gene, giving rise to an abnormal fusion mRNA (6.5–7.4 kb) and an abnormal protein (185–190 kd) [Clark et al., 1987; Kurzrock et al., 1987]. A Ph translocation similar to that in ALL also is seen in some cases of ANLL. Molecular *BCR* analysis is the approach of choice in clarifying the nature of the Ph chromosome in these leukemias. Generally speaking, Ph+ ALL is of pre-B-cell lineage and has an unfavorable prognosis.

*t(12;21)(p13;q22)*

Only recently has this translocation been shown to be the most frequent, but cytogenetically largely undetected chromosomal anomaly in childhood ALL (25%). This translocation defines a distinct entity of childhood pre-B ALL with a good prognosis [Shurtleff et al., 1995]. The t(12;21) results in the fusion of two genes: *TEL* on 12p and *AML1* on 21q. Only the *TEL-AML1* may play a key role in leukemogenesis.

**t(1;19)(q23;p13)**

This translocation distinguishes a subgroup of patients with pre-B-cell ALL who have a poor prognosis.

*Hyperdiploidy with 50–60 chromosomes*

Both childhood and adult cases of ALL characterized by numerical abnormalities of 50 or more chromosomes appear to have a favorable prognosis. Such ALL is usually of the L1 or L2 type and of non-T, non-B origin. This type of anomaly is found in 25–30% of childhood cases of ALL [Martinez-Climent, 1997].

***Both childhood and adult cases of ALL characterized by numerical abnormalities of 50 or more chromosomes appear to have a favorable prognosis.***

*Near-haploid ALL*

The chromosome numbers of the near-haploid clones in ALL range from 26 to 36. Although it is extremely rare, this subgroup appears to characterize a subset of ALL with distinct features—they are of a common ALL phenotype, are more frequent in adolescent girls, and carry a poor prognosis.

*T-cell ALL*

T-cell ALL is relatively less common than the B-cell type. Chromosomal patterns often affect regions involving T-cell receptor (TCR) loci, such as 14q11 (*TCRA*), 7q32-36 (*TCRB*), and 7p13 (*TCRG*). Several nonrandom translocations have been noted. These T-cell-specific abnormalities also have been reported in lymphomas of T-cell origin (Table III).

## PROGNOSTIC VALUE OF CYTOGENETICS IN ACUTE LEUKEMIA

### ANLL

Generally, the karyotype is an important prognostic factor in de novo ANLL [Sandberg, 1990; Heim and Mitelman, 1995; Sandberg and Chen, 1995]. Patients with ANLL with major karyotypic anomalies have a less favorable course than patients with minor karyotypic changes, and patients whose marrow consists of only abnormal cells have a poorer prognosis than patients with only normal karyotypes or a mixture of normal and abnormal karyotypes. Specific chromosomal anomalies in ANLL also have been shown to correlate with response to chemotherapy. Patients with t(8;21), inv(16), t(16;16), or 11q changes have high rates of complete response (CR) after initial therapy (60–100%), whereas those with −7/7q− or −5/5q− have low rates (0–36%) of CR. In general, the former patients experience long disease-free survival times, whereas the latter patients have short remissions, even if they eventually achieve CR with further therapy (Table IV).

The prognostic aspects of ANLL may be related to the age of patients and possibly to the cytogenetic changes predominant in elderly patients versus those in younger patients. Although the incidence of abnormal karyotypes appears to be about equal in both groups, the changes associated with a CR rate of more than 80% and a favorable prognosis, that is t(15;17), t(8;21) or inv(16), are seen much more frequently in younger age groups than among elderly patients, who often have −5/5q−, −7/7q−, and +8 as karyotypic changes, associated with a CR rate of less than

**TABLE IV. Prognostic Aspects of Acute Non-lymphocytic Leukemia and Childhood Acute Lymphocytic Leukemia★**

| | Favorable | Unfavorable |
|---|---|---|
| ANLL | | |
| Cytogenetics[a] | t(15;17), t(8;21), inv(16)/del(16q), NN, MIKA | −7, del(7q),−5, 3q or 11q23 involvement, t(9;22), +8, 20q−, AA, MAKA |
| Age | < 45 yr | Infants, >60 yr |
| WBC count | $< 25{,}000/mm^3$ | $>100{,}000/mm^3$ |
| Morphologic features | Auer rods present, eosinophilia, M2, M3, M4 | M0, M1, M5, M6, M7 |
| Markers | HLA-DR negative, TDT−, CD2+, or CD19+ | CD34+, HLA-DR positive, biphenotypic |
| ALL | | |
| Cytogenetics[a] | Hyperdiploid (numerical changes only), t(12;21) | t(9;22), t(4;11), t(8;14), t(2;8), t(8;22) |
| Age | 1–9 yr | < 1 or >10 yr |
| WBC count | $< 50{,}000/mm^3$ | $>50{,}000/mm^3$ |
| Morphologic features | L1, L2 | L3 |
| Immunophenotype | c-ALL T-cell ALL, myeloid antigen negative | Pre-pre-B-ALL, pre B-ALL, myeloid antigen positive (biphenotypic) |

★ANLL, acute non-lymphocytic leukemia; WBC, white blood cell; ALL, acute lymphoblastic leukemia; NN, normal karyotype; MIKA, minor karyotypic change; TdT, terminal deoxynucleotidyl transferase; AA, abnormal cells; MAKA; major karyotypic anomalies.
[a]Cytogenetics is often an independent prognostic factor. See text for details.

40%. Thus, the results of pretreatment cytogenetic evaluation represent a significant prognostic factor in determining response to induction therapy.

More importantly, the relative effectiveness of different postremission therapies may vary according to the cytogenetic risk group. Patients with favorable results of cytogenetic evaluation have done significantly better after autologous BMT and allogeneic BMT than those who underwent chemotherapy alone, whereas patients with unfavorable cytogenetic results have done better with allogeneic BMT [Slovak et al., 2000]. It also has been reported that an *FLT3* ITD in ANLL adds important prognostic information to the cytogenetic risk group. It is the most important factor predicting relapse and disease-free survival and is an independent risk factor of event-free survival and overall survival [Kottaridis et al., 2001]. Thus, detection of an *FLT3* ITD should be a routine test at diagnosis to assist in the evaluation of optimal therapeutic management.

### ALL

Cytogenetic studies in ALL have independent prognostic value even when age, white blood cell count, FAB type, and immunophenotype are considered [Sandberg, 1990; Heim and Mitelman, 1995; Sandberg and Chen, 1995]. Hyperdiploid stem lines with more than 50 chromosomes are seen in 30% of children with ALL, a subset that has proved to have the most favorable prognosis. Hyperdiploidy in adult ALL cases likewise confers the most favorable prognosis, although the rate of treatment failure is higher than has been seen in children. Generally speaking, a pseudodiploid karyotype confers the poorest response to therapy. Normal diploid and near-diploid cases have an intermediate prognosis, but less favorable than that for the hyperdiploid group with 50 or more chromosomes. Near-haploid ALL cases are rare in adults and children and appear to have a very poor prognosis. Cases with multiple leukemic stem lines pose a problem for prognostic classification. In general, the correct prognostic designation can be based on the leukemic line of lowest ploidy.

It is now clear that chromosomal translocations, in general, are indicative of an unfavorable prognosis, whether they are found in a pseudodiploid or a near-diploid karyotype. Examples include B-ALL with t(8;14), t(2;8), or t(8;22), which carry a very unfavorable prognosis (median survival less than 6 months in both adults and children), and Ph+ ALL or ALL with the t(4;11), both conferring an unfavorable prognosis. The t(12;21), however, defines a distinct group of childhood pre-B ALL cases with a favorable prognosis [Shurtleff et al., 1995] (Table IV).

## MALIGNANT LYMPHOPROLIFERATIVE DISORDERS

Burkitt lymphoma is characterized in the majority of cases (80%) by a translocation, t(8;14)(q24;q32). In a lesser number of cases (15%), the translocation is between chromosomes 8 and 22, that is, t(8;22)(q24;q11). In about 5% of Burkitt lymphoma cases, the translocation involves chromosomes 2 and 8, that is, t(2;8)(p12;q24). In each of these instances, the translocation results in the abnormal juxtaposition of immunoglobulin gene sequences with those of the *MYC* gene, leading to the creation of a chimeric gene and thus abnormal protein products [Sandberg, 1990; Heim and Mitelman, 1995]. The latter play a key role in the genesis of the lymphoma.

***Burkitt lymphoma is characterized in the majority of cases (80%) by a translocation, t(8;14)(q24;q32).***

More than 90% of cases of non-Hodgkin lymphoma have been reported to have clonal chromosomal changes. In non-Burkitt non-Hodgkin lymphoma, a 14q+ marker is seen in about 50% of cases. More important, many of the nonrandom anomalies correlate with histology and immunologic phenotype, the most common being t(14;18)(q32;q21) in follicular (nodular) B-cell neoplasms, del(6q) in large-cell lymphomas, t(8;14)(q24;q32) in either small noncleaved cell or diffuse large-cell lymphomas, t(2;5)(p23;q35) in Ki-1-positive lymphomas, and rearrangements of 14q32 in B-cell-type neoplasms [Sandberg, 1990; Heim and Mitelman, 1995]. Structural anomalies of chromosome 6 are common in lymphoma, with at least three regions of minimal cytogenetic deletion (RCD) being reported: RCD at 6q23 associated with low-grade lymphoma (small lymphocytic), RCD at 6q25-27 associated with intermediate-grade lymphoma, and RCD at 6q21 associated with high-grade lymphoma, particularly of the immunoblastic type [Offit et al., 1993].

On the other hand, T-cell neoplasms are characterized by rearrangements of 14q11, 7q34-36, and 7p15. A T-cell lymphoblastic lymphoma (T-LBL) with eosinophilia and myeloid hyperplasia has been reported in association with a characteristic cytogenetic translocation, t(8;13)(p11;q11) [Inhorn et al., 1995]. Furthermore, two variant translocations, t(6;8)(q27;p11) and t(8;9)(p11;q34), also have been reported in T-LBL [Aguiar et al., 1995]. It therefore has been speculated that the gene(s) located at 8p11 play an important role in the pathogenesis of T-LBL with eosinophilia and myeloid hyperplasia.

Approximately 50% of chronic lymphocytic leukemia (CLL) cases show chromosome abnormalities, the most common of which are trisomy 12, 14q+, 13q and 11q abnormalities [Han et al., 1987; Sandberg, 1990; Heim and Mitelman, 1995]. With FISH, Döhner et al. [2000] used a set of DNA probes targeting 3q26, 6q21, 8q24, 11q22-23, 12q13, 13q14, 14q32, and 17p13 and found that 268 of 325 (82%) cases of CLL had chromosomal aberrations, with a frequency of 55% for a deletion in 13q, 18% in 11q, 16% for trisomy 12q, 7% for a deletion in 17p, and 6% in 6q [Döhner et al., 2000]. Five prognostic categories were defined: a median survival of 32 months for patients with 17p−, 79 months for 11q−, 114 months for 12q trisomy , 111 months for normal karyo-

types, and 133 months for 13q− [Döhner et al., 2000]. The presence or absence of a chromosomal abnormality thus yields significant prognostic information.

***Approximately 50% of chronic lymphocytic leukemia (CLL) cases show chromosome abnormalities, the most common of which are trisomy 12, 14q+, 13q and 11q abnormalities.***

Atypical CLL tends to have a relatively high incidence of chromosome abnormalities, frequently involving chromosomes 12, 13q14, 6q21-q23, 11q, and possibly 4q. The appearance of complex karyotypes with concomitant anomalies of 13q, +12, 6q, and 11q may account in part for the relatively aggressive clinical course in these cases [Bigoni et al., 1997]. The exact molecular defect in CLL, however, is largely unknown, and this area remains to be investigated further.

The cytogenetic results in multiple myeloma (MM) have been heterogeneous, ranging from translocations seen in B-cell lymphoid disease, for example, t(11;14)(q13;q32) with associated involvement of the *BCL-1* and *IgH* genes, to complex karyotypes inconsistent in their changes [Sandberg, 1990; Heim and Mitelman, 1995]. Thus, it is possible that MM consists of a number of subentities, at least cytogenetically. Recent data indicate that up to 86% of patients with MM have 13q deletions, as detected by FISH [Kaufmann et al., 2001]. It is particularly noteworthy that deletions of 13q remain an independent adverse prognostic factor after conventional-dose and high-dose therapy [Kaufmann et al., 2001].

**TABLE V. Common Cytogenetic and Molecular Genetic Changes in Leukemias and Lymphomas**

| Malignancy | Chromosomal abnormality | Molecular alterations (genes involved) |
|---|---|---|
| CML | t(9;22)(q34;q11) | *BCR-ABL* |
| ANLL | t(8;21)(q22;q22) | *ETO-AML1* |
| | t(15;17)(q22;q21) | *PML-RARA* |
| | inv(16)(p13q22) | *CBFB-MYH1* |
| | t(11q23) | *MLL*—various |
| | t(6;9)(p23;q34) | *DEK-CAN* |
| | t(3;3)(q21;q26), inv(3)(q21q26) | *EV11* |
| | t(3;5)(q25.1;q34) | *NPM-MLF1* |
| | t(7;11)(p15;p15) | *NUP98-HOXA9* |
| | t(16;21)(p11;q22) | *FUS-FRG* |
| ALL | | |
| B-cell lineage | t(9;22)(q34;q11) | *BCR-ABL* |
| | t(12;21)(p13;q22) | *TEL-AML1* |
| | t(1;19)(q23;p13) | *PBX-E2A (TCF3)* |
| | t(5;14)(q31;q32) | *IL3-IGH* |
| | t(8;14)(q24;q32) | *IgH-MYC* |
| | t(4;11)(q21;q23), t(11;19)(q23;p13) | *MLL*—various |
| | t(17;19)(q22;p13) | *HLF-E2A* |
| T-cell lineage | t(1;14)(p32;q11), t(1;7)(p32;q35) | *TAL-1-TCRD, TCRB* |
| | t(7;9)(q35;q34), t(7;10)(q35;q24) | *TCRB-TCL4, HOX-11 (TCL3)* |
| | t(7;11)(q35;p13), t(7;19)(q35;p23) | *RBTN2, LYL1* |
| | t(10;14)(q24;q11) | *TCL3-TCRD* |
| | t(11;14)(p13;q11), t(11;14)(p15;q11) | *RBTN2, RBTN1* |
| Lymphoma | t(14;18)(q32;q21) | *BCL-2-IgH* |
| | t(8;14)(q24;q32), t(2;8)(p12;q24) | *MYC-IgH, IgK, IgL* |
| | t(8;22)(q24;q11) | |
| | t(11;14)(q13;q32) | *BCL-1-IgH* |
| | t(2;3)(p13;q27) | *IgK-BCL-6* |
| | t(2;5)(p23;q35) | *NPM-ALK* |
| | t(3;14)(q27;q32), t(3;22)(q27;q11) | *BCL-6-IgH,IgL* |

CML, Chronic myelogenous leukemia; ANLL, acute non-lymphocytic leukemia; ALL, acute lymphoblastic leukemia.

## SUMMARY

As described herein, cancer cytogenetics not only provides key information in the care of patients with leukemia and various cancers but also acts as a guide to the identification of genes apparently responsible for the development of these neoplastic states. In general, a gain of a chromosome essentially results in gene amplification. Loss of a whole chromosome or part of a chromosome may result in loss of heterozygosity. The consequence of a translocation, in which two gene sequences located on different chromosomes are juxtaposed, may be an alteration in the transcriptional regulation of an oncogene or the creation of a chimeric gene by the fusion of coding sequences of the two involved genes.

Detailed analyses of the DNA sequences located at the chromosomal breakpoints have allowed investigators to identify at least two major categories of genes, namely, proto-oncogenes and tumor suppressor genes. Examples are listed in Table V.

The cytogenetic events may exceed in number the known molecular events and thus call for further investigation of the cytogenetic/molecular relationships. In other cases, the molecular events may exceed in number the known cytogenetic events, thus indicating that the number of genetic events associated with neoplasia may not be reflected in the number of cytogenetic changes. This applies to some leukemias and soft-tissue tumors, especially conditions in which no chromosomal changes are found. Regardless of the specificity and nature of molecular changes seen in various neoplastic conditions, the cytogenetic changes continue to offer information to the clinician that is useful in the diagnosis of disease and the prognosis and care of patients.

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