Molecular Heterogeneity in Acute Leukemia Lineage Switch

By Gregory A. Gagnon, Craig C. Childs, Anne LeMaistre, Michael Keating, Ann Cork, Jose M. Trujillo, Kevin Nellis, Emil Freireich, and Sanford A. Stass

Six cases of acute leukemia that underwent lineage switch from acute lymphocytic leukemia to acute myelogenous leukemia are reported. The mean age of the patients was 24 years, time to conversion was 36 months, and survival after conversion was only 3 months. Of the three cases which showed abnormal metaphases at both diagnosis and conversion, two (cases 2, 5) showed related cytogenetic abnormalities, and the third showed (case 3) independent chromosomal changes. Molecular analysis for immunoglobulin heavy chain and T-cell receptor β chain genes showed that five of the six cases had rearrangement of at least one of these lymphoid associated genes at conversion to acute myelogenous leukemia. The single case (case 3) in which there were no lymphoid gene rearrangements at conversion was also the only case in which independent karyotypic abnormalities at diagnosis and conversion were demonstrated. Our findings suggest that lineage switch can represent either relapse of the original clone with heterogeneity at the molecular level or the emergence of a second new leukemic clone without molecular heterogeneity.

THE CORNERSTONE for diagnosis and lineage assignment in acute leukemia continues to be the light microscopic and cytochemical criteria established by the French-American-British (FAB) cooperative group. Lineage switch is the term that has been used to describe acute leukemias that meet standard FAB criteria for a lineage (lymphoid or myeloid) at initial diagnosis but at relapse meet the criteria for the opposite lineage. Lineage switch is a unique example of the lineage heterogeneity that exists in some acute leukemias. The frequency of lineage switch among patients with acute leukemias that relapse is estimated to be between 6% and 9%. Most reports have been acute lymphocytic leukemia (ALL) to acute myelogenous leukemia (AML) conversions. However, rare cases of AML converting to ALL have been described. Most of the lineage switch cases reported have occurred in children.

Several mechanisms have been suggested to account for lineage conversion in acute leukemia. Leukemic transformation may occur in a multipotential hematologic progenitor cell that has the capacity to differentiate along either lymphoid or myeloid pathways. Exogenous factors such as chemotherapy or endogenous changes such as acquired chromosomal abnormalities could alter the differentiation program of the leukemic cell, leading to a switch in phenotype at relapse. Alternatively, a new leukemogenic event might be the cause of an apparent switch in lineage. The finding of distinctly different cytogenetic abnormalities at diagnosis and lineage switch supports this mechanism. We present six cases of lineage switch from ALL to AML. Striking lineage heterogeneity, especially at the molecular level, was demonstrated. The cytogenetic and molecular findings in these cases suggest that lineage switch can either represent true relapse of the original clone with accompanying lineage heterogeneity at the molecular level or the emergence of a second new malignancy without lineage heterogeneity at the molecular level.

MATERIALS AND METHODS

Patients. One hundred two adults at M.D. Anderson Cancer Center with a diagnosis of acute leukemia relapsed between December 1985 and March 1988. At initial diagnosis, 35 patients had ALL. Five of these patients (14% of adult ALLs that relapsed) and a sixth pediatric patient at relapse met FAB cooperative group criteria for a lineage different from that documented at initial diagnosis. These patients form the basis of this report.

Morphological and cytochemical analysis. Marrow aspirate smears were routinely stained with Wright's-Giemsa. Cytochemical stains included myeloperoxidase (MPO), α-naphthyl butyrate esterase, naphthyl AS-D chloroacetate esterase, and periodic acid-Schiff. An indirect immunofluorescence technique was used to identify terminal deoxynucleotidyl transferase (TdT). Cytochemical and TdT staining was graded as follows: < 5% blasts positive, + 5% to 25% blasts positive, ++ 25% to 75% blasts positive, and +++ > 75% blasts positive.

Ultrastructural examination of leukemic blasts for myeloperoxidase was performed according to standard methods. The sections were analyzed on a Jeol 1200EX electron microscope at 60 KU.

Immunophenotype. The composite surface immunophenotype of the leukemic cells was determined with a battery of monoclonal antibodies according to previously described methods. The following FITC-conjugated monoclonal antibodies were used: OKT11(CD2), OKT3(CD3), OKT4(CD4), OKT8(CD8), OKM1(CD11) (Ortho Diagnostics, Westwood, MA); Common ALL antigen (CALLA, CD10), Leu-1(CD5), Leu-12(CD19), HLA-DR (Becton Dickinson Monoclonal Center, Mountain View, CA); B1(CD20), B4(CD19), MY4(CD14), MY7(CD3), MY8 and MY9(CD33) (Coulter Immunology, Hialeah, FL). The analysis was performed on an Ortho Spectrum III flow cytometer (Ortho Diagnostics) equipped with a 2140 data analyzer which can use forward- and right-angle light-scatter characteristics to gate on the blast population. Reactivity over background in more than 20% of the blasts was considered a positive result.

A direct immunofluorescent technique using FITC-labeled anti-μ heavy chain antibody (Kallestead, Austin, TX) determined the presence of cytoplasmic immunoglobulin. The percentage of blasts with positive cytoplasmic staining was determined by visualization under a fluorescent microscope.

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Cytogenetics. Chromosomal studies were performed on bone marrow cells at diagnosis and relapse. Previously described techniques for culture, harvest and staining were used.26'27,39 Restriction enzyme digested metaphases were analyzed, and karyotypes were reported according to the International System for Human Cytogenetic Nomenclature (ISCN 1985).37 A maximum of 25 metaphases were examined. Karyotypes were considered related when metaphases at diagnosis and relapse showed common rearrangements or patterns of chromosome gain or loss. They were considered unrelated when no similar chromosome abnormalities at diagnosis and relapse were identified.

Molecular analysis. DNA from leukemic samples was extracted by previously described procedures.26 Restriction enzyme digestion with EcoRI, HindIII, or BamHI (Bethesda Research Laboratories Life Technologies, Gaithersburg, MD) was performed on 10 µg DNA. Digests were electrophoresed on a 0.7% agarose gel, transferred to nylon filters, and hybridized with the following radiolabeled probes: Jκ, immunoglobulin heavy chain joining region, 6-kilobase BamHI, HindIII fragment (Oncor, Gaithersburg, MD, cases 2, 4, and 6; and Dr Phillip Leder, cases 1, 3, and 5), and Tcβ, T-cell receptor β gene cDNA hybridizing to constant regions Cβ1 and Cβ2 (Oncor). After hybridization, filters were exposed to Kodak XOMAT-AR x-ray film at -70°C for 1 to 7 days.

Results

Clinical course and outcome. Clinical course and outcome are shown in Table 1. All cases were conversions from ALL to AML. Lineage was determined using standard FAB morphological criteria. The patients at diagnosis were aged 7 to 55 years (mean 24 years). Time to conversion ranged from 8 to 63 months (mean 36 months). In four patients, phenotypic conversion occurred at the first relapse; in two, it occurred at the time of second relapse. All patients at conversion were treated for the new lineage (AML). Survival after conversion was ≤2 months in all but one of the patients. After conversion, patient 3 achieved a 6-month remission but subsequently relapsed and died with disease. Therapy at initial diagnosis and conversion is shown in Table 1. The leukemia at conversion in four of the six patients appeared to be resistant to chemotherapy. The remaining two patients had hypoplastic marrows at the time of death without evidence of disease.

The five adults with lineage switch occurred in an ALL population with a cumulative risk during the study period of 113 patient years, which is an incidence in this population of one lineage switch case every 22.6 patient years at risk.

Morphology and enzymatic analysis. Morphology and enzymatic analysis are shown in Table 2 and Figs 1 and 2. All patients were MPO negative and TdT positive and morphologically appeared to be lymphoid at initial diagnosis, meeting the standard criteria for ALL. Cases 1 through 5 were examined by electron microscopy at initial diagnosis.

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<tr>
<th>Patient</th>
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<th>Blasts (%)</th>
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Abbreviations: NA, not assessed; LY, lymphoid; MY, myeloid; MO, monocytic; ID, initial diagnosis; LS, lineage switch.
Fig 1. Patient 1. Blasts at initial diagnosis showed lymphoid features by light (A) and electron microscopy (B). MPO cytochemical stain was negative. At conversion, the morphology was that of myelomonocytic blasts by light (C) and electron microscopy (E). MPO-positive granules were evident by electron microscopy (arrows). Cytochemical stain for MPO was positive (D).

All displayed lymphoid ultrastructural features and absence of MPO granules. Although case 6 was not examined by electron microscopy, its morphology by light microscopy was clearly that of the L1 subtype of ALL.

At conversion, all cases demonstrated MPO positivity in at least 10% of the blasts. Five cases displayed positive staining in more than 75% of the blasts. TdT remained positive in three cases at conversion. Two cases (cases 1 and 3) displayed blasts with cytochemical or ultrastructural features of monocytes.

Immunophenotype. Immunophenotype of blast cells (Table 3) was determined in five cases at initial diagnosis. Cytoplasmic heavy chain and/or B-cell-associated surface markers were present in two cases (cases 3 and 4), T-cell–associated markers in one case (case 1) and null phenotype (HLA-DR only) in one case (case 6). Case 5 displayed immunologic surface features of lymphoid (CD2, CD10) and myeloid (CD15, CD13, CD33) lineage.

At lineage switch, five of six cases displayed at least one (case 6) and in most instances three or more (cases 1, 3, 4, and 5) myeloid associated surface markers. In addition to myeloid surface markers, four of these cases were also
Fig 2. Patient 2. At initial diagnosis, the blasts were lymphoid (A). MPO granules were ultrastructurally absent in cytoplasm of blasts (B). At conversion, blasts displayed myeloid morphology by light (C) and electron microscopy (E). MPO stain was positive (D).

Positive for either T-cell-associated (cases 1, 3, and 5) or B-cell-associated (case 6) markers. At conversion, case 2 displayed a pre-B-cell phenotype (CD10, 19, 20, clg) in more than 90% of the blast cells and MPO-positive staining in more than 90% of the blasts.

Cytogenetics. Cytogenetics is shown in Table 4. In five of six patients chromosomal abnormalities were identified at either initial diagnosis or conversion. Although most of the karyotypes were complex, three cases did include translocations that involved a break in the long arm of chromosome 11 (cases 3, 4, and 5). All occurred at 11q23-25 but involved three different reciprocal chromosomes, 4, 9, and 10.

Three patients had abnormal metaphases at both initial diagnosis and conversion (cases 2, 3, and 5). In two of these cases (cases 2 and 5), similar karyotypic changes were noted in cells at diagnosis and conversion, suggesting a clonal relationship. Conversely, case 3 showed unrelated karyotypes at initial diagnosis and conversion, suggesting the emergence of a new independent clone at conversion.

Molecular analysis. Result of molecular analysis is
Table 3. Immunophenotype

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Abbreviations: NA, not assessed; + >20% + over background; −<20% +.

Given in Table 5 and Fig 3. All cases were probed with JH and Tcβ at conversion. Five of the six patients showed rearranged bands with JH, and three showed rearranged bands with Tcβ. Cases 5 and 6 demonstrated both JH and Tcβ rearrangements. Case 3, the only case in which independent cytogenetic abnormalities at diagnosis and conversion were demonstrated, was also the only case that had exclusively germline bands at conversion when probed with JH and Tcβ.

DNA from leukemic cells at initial diagnosis and lineage switch were available from patient 5. Both samples showed a faint germline band and an intense single rearrangement with the JH probe. By analyzing the DNA on the same gel in adjacent lanes, we determined the relative mobility of the bands. Based on this analysis, the rearrangements were of identical molecular weight at initial diagnosis and lineage conversion. At diagnosis, the Tcβ gene was in the germline configuration.

DISCUSSION

Standard FAB morphologic and cytochemical criteria were applied to assign the lineage of each case at diagnosis.

Table 4. Cytogenetics

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<th>Initial Diagnosis</th>
<th>Lineage Switch</th>
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<tr>
<td>1</td>
<td>NA</td>
<td>23 Cells: 45 to 47 chromosomes with unrelated clones and cells with additional changes. Clone 1: 45, XY, -7, t(3;7)(q27; q11), t(8;17)(p11;p13) Clone 2: 45, X, -Y, del(1)(p22p34), del(11)(p11p15), t(16;?)q12;?) del(19)(p13), t(21;?)q22;?)</td>
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<tr>
<td>2</td>
<td>5 Abnormal metaphases with 46 to 47 chromosomes</td>
<td>2 Cells: 46,XY 15 Cells: 47, X, +X, +Y, +5, +8, +9, +12, +18, del(9)(p13p22), t(9;12)(p24q13) 1 Cell: 46,XY</td>
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<td>11 Cells: 46,XX 2 Cells: 46,XX, i(7q), t(14;19)(q23;p13)</td>
<td>20 Cells: 46,XX, del(6)(q23p27), t(9;11)(p22q25) 4 Cells: 46,XX, t(9;11)(p22q25) 15 Cells: 46,XX 10 Cells: 103-107 chromosomes with related clones and t(4;11)(q21;q23)</td>
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<tr>
<td>4</td>
<td>Insufficient metaphases</td>
<td>18 Cells: 41 to 47 chromosomes with related clones 7 Cells: 68 to 88 chromosomes with related clones</td>
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<td>5</td>
<td>11 Cells: 46,XX, t(10;11)(l5p15; q23) 6 Cells: 45,XX, -5, -9, t(4;7)(p16; q7), t(10;11)(p15; q23), del(17)(p11p13), +mar 6 Cells: 46,XX</td>
<td>6 Cells: 46,XX 6 Cells: 43 to 45 chromosomes with related clones 5 Cells: Abnormal polyploid metaphases</td>
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<tr>
<td>6</td>
<td>12 Cells: 46,XY</td>
<td>Chromosome abnormalities similar to those reported at diagnosis, i.e., t(10;11)(p15; q23). Insufficient yield of analyzable metaphases</td>
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Abbreviation: NA, not assessed.
and relapse.1-3 All cases were initially diagnosed as ALL and relapsed as AML. This is consistent with most of the previously reported cases of lineage switch, which are also conversions from ALL to AML.3-8 Undifferentiated acute leukemia may not be easily distinguishable from ALL FAB subtype L2 by light microscopy and cytochemistry alone.23,30 At the ultrastructural level, many undifferentiated leukemias display MPO granules and are actually myeloid leukemias. All of our cases examined ultrastructurally at diagnosis displayed typical lymphoid features such as peripheral heterochromatin and few cytoplasmic organelles. In addition, the absence of MPO-stained cytoplasmic granules was confirmed.

TdT is an enzyme present in the majority of lymphoblasts in most cases of ALL.22 However, it is not restricted to ALL, having been demonstrated in up to 10% of AMLs.32,33 All of our lineage-switch cases demonstrated positive TdT staining in more than 75% of the blasts at initial diagnosis. At conversion, three cases continued to display TdT-positive blasts, but in decreased percentages. These cases also had JH and/or TcB gene rearrangements at conversion, whereas the only case that was germline at conversion (case 3) was also TdT negative. This is of interest in view of the association of TdT with gene rearrangements.34,35

Acute mixed-lineage leukemia has been described as acute leukemia whose blasts coexpress differentiation markers of more than one lineage.24 The relationship between lineage switch and acute mixed-lineage leukemia is uncertain.4 However, some cases of lineage switch may arise from leukemias that were of mixed lineage at initial diagnosis, suggesting that lineage switch may be part of the biologic spectrum of mixed leukemia. An example of this is patient 5 who met morphologic and cytochemical criteria at light and ultrastructural levels for the diagnosis of ALL, but an expanded immunophenotype panel including several antimyeloid antibodies showed positive staining with these myeloid-associated markers, demonstrating the mixed lineage nature of this leukemia. At relapse, the blasts of patient 5 retained myeloid surface antigens and CD5. In addition, they now expressed the morphologic and cytochemical features of AML. Although lineage switch can occur in the setting of acute mixed-lineage leukemia, the phenomenon is not necessarily restricted to mixed leukemias. An expanded panel of six myeloid-associated antibodies failed to react to the lymphoid blasts at initial diagnosis in patient 4. In this instance, no evidence for mixed lineage was present at diagnosis, yet lineage conversion occurred at relapse.

Associations between T-cell features in ALL and the occurrence of lineage switch have been reported.5,9,10,37 Three of the patients we studied displayed T-cell-associated phenotypic markers at either initial diagnosis (cases 1 and 5) and/or conversion (cases 3 and 5). In addition, patient 1 at

![Fig 3](https://www.bloodjournal.org/content/processing.png)

**Fig 3.** Molecular analysis of blasts from patients 1, 2, 4, and 6 at the time of conversion to AML. Rearrangement of JH and/or TcB was evident in each case (arrows). Germline bands are shown by a long bar with corresponding molecular weight. Short bars mark crosshybridization artifact.

Table 5. Molecular Analysis for Lymphoid Genes at Conversion

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<tr>
<td>5*</td>
<td>R</td>
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</tr>
<tr>
<td>6</td>
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</tbody>
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Abbreviations: G, germline. R, rearranged.

*Identical JH rearrangement was present at initial diagnosis and conversion (described in the Results section).
initial diagnosis had a mediastinal mass and high white blood cell count, suggesting T-cell immunophenotype. These cases of lineage switch may arise from a common T-cell/myeloid progenitor. Alternatively, they may be the result of altered regulation and expression of lineage-related genes. In-depth studies at the molecular level are necessary to resolve this question.

A striking example of the immunophenotypic heterogeneity that can be noted with lineage switch is illustrated by the immunologic marker results obtained on the blasts of patient 2 at the time of conversion to AML. Even though MPO was positive in more than 90% of the blast cells, a pre-B-cell immunophenotype (CD20,19, 10, and clg) with more than 90% positive blasts for each antibody was found. This unusual case supports the contention that aberrant gene expression might be responsible for the apparent presence of myeloid and lymphoid features in the same leukemic cell.

Certain lymphoid and myeloid leukemias have been associated with specific chromosome abnormalities, many of which appear to have prognostic significance. Cyogenetics can also be used to assess clonal relationships in leukemias. Cases 2 and 5 had related chromosomal abnormalities at diagnosis and relapse, suggesting that lineage switch represented a relapse of the same leukemic clone. Alternatively, the finding of independent karyotypic abnormalities at diagnosis and conversion, as in case 3, suggests that a new clone was responsible for the relapse, perhaps representing a second acute leukemic transformation.

Translocations involving chromosome 1q have been associated with lineage heterogeneity in acute leukemia. We extend this observation in the context of lineage switch. Three of the cases we report displayed translocations involving 1q23 to 25. Once the molecular details of this region are established, its role in lineage commitment and differentiation in acute leukemia may become evident.

Studies of these cases at the time of lineage conversions with the lymphoid-associated gene probes JH and Tcβ revealed unexpected heterogeneity. Four cases had rearrangements of the immunoglobulin gene, and three had rearrangements of the Tcβ gene. Both genes were rearranged in two cases. Rearrangements of JH and Tcβ have been reported in AML. Raghavachar et al report the conversion of an acute undifferentiated leukemia to a monocytic leukemia in which the same JH rearrangement was identified at diagnosis and conversion. Gene rearrangement studies performed on our patient 5 at diagnosis and conversion demonstrated an identical rearrangement. This patient is described in greater detail elsewhere. The presence of the same rearrangements as well as similar cytogenetic findings at diagnosis and conversion support the clonal nature of these cases despite a morphologic switch in lineage.

One patient (patient 3) in our series was germline for JH and Tcβ at conversion. This case was also the only one in which independent abnormal chromosome changes at diagnosis and conversion were found. In addition, patient 3 achieved a remission and survived for 1 year after conversion. None of the other cases in our series achieved remission after conversion and none lived longer than 2 months after conversion. In contrast, in a previous report of six pediatric patients with lineage switch acute leukemia, four achieved remission with chemotherapy directed toward the phenotype at conversion.

Our data suggest that some cases of lineage switch represent a relapse of the original leukemic clone with morphologic conversion. Lineage switch acute leukemias that arise through this mechanism appear to exhibit molecular heterogeneity with lymphoid gene rearrangements persisting at conversion to AML. However, one of our cases and previous reports indicate that in some instances acute leukemia lineage switch may represent emergence of a new leukemic clone characterized by a different morphology from that observed at initial diagnosis without lymphoid gene rearrangements at conversion to AML. Further studies of the mechanisms of lineage commitment and differentiation in acute leukemia will help us understand lineage switch.

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Molecular heterogeneity in acute leukemia lineage switch

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