Conversions of leukemic cell lineage (lymphoid or myeloid) have been reported only rarely. Our review of the cytochemical and immunophenotypic features of 89 cases of childhood leukemia in marrow relapse indicated lineage switch (lymphoid to myeloid or the reverse) in six patients (6.7%). Five patients with acute lymphoblastic leukemia (ALL) at diagnosis had converted to acute nonlymphoblastic leukemia (ANLL), and one had converted from ANLL to ALL. Each child received lineage-specific multiguent chemotherapy when initially diagnosed, and all achieved a complete remission. After conversion, four patients readily achieved second remissions with treatment for the phenotype evident at lineage switch. Two patients with ANLL at conversion failed ALL-directed reinduction, while one of the two responded to high-dose cytarabine but died during bone marrow hypoplasia, emphasizing the importance of prompt recognition of lineage switch and selection of an appropriate plan of retreatment. Cytogenetic studies disclosed evidence of clonal selection in one patient and clonal stability in two. These findings indicate an unexpectedly high frequency of lineage switch in patients who relapse in the bone marrow after intensive chemotherapy. Although specific causative factors could not be identified, our observations suggest at least two general mechanisms for lineage switch in acute leukemia. In one, chemotherapy appears to eradicate the dominant clone present at diagnosis, permitting expansion of a secondary clone with a different phenotype. In the second, drug-induced changes in the original clone may either amplify or suppress differentiation programs so that phenotypic shift is possible.

**MATERIALS AND METHODS**

From April 1979 to August 1981, 216 newly diagnosed and previously untreated children with acute lymphoblastic leukemia (ALL) and 48 with acute nonlymphoblastic leukemia (ANLL) were enrolled in therapeutic studies at St Jude Children’s Research Hospital. Of the 239 patients who attained complete remission, 89 have relapsed in the bone marrow, with six demonstrating lineage switch. The clinical and laboratory findings for the latter group are shown in Table 1.

All patients were enrolled in studies that included a cell profile analysis approved by the institution's clinical trials committee; informed consent was obtained in each instance. Patients with high-risk ALL (No. 1 through 3) received cytarabine, teniposide (VM-26), prednisone, vincristine, and L-asparaginase for remission induction. Continuation therapy consisted of 6-mercaptopurine (6-MP) and methotrexate (MTX) for 30 months, with pulses of cytarabine and VM-26 the first 12 months. Intrathecal MTX and cranial irradiation were given for central nervous system (CNS) prophylaxis. Patients with standard risk ALL were treated with either prednisone, vincristine, and daunorubicin, followed by intrathecal MTX, cranial irradiation, and 6-MP with MTX (patient 4) or prednisone, vincristine, and l-asparaginase, followed by intermittent high-dose MTX and intrathecal MTX imposed on an otherwise appropriate plan of retreatment.
Table 1. Clinical Summary

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis (yr)</th>
<th>Sex</th>
<th>Leukocyte count at diagnosis (x 10^9/L)</th>
<th>Mediastinal mass</th>
<th>Initial therapy</th>
<th>Time to conversion from diagnosis (yr)</th>
<th>No. of antileukemic drugs received before conversion</th>
<th>No. of hematologic relapses at conversion</th>
<th>Receiving chemotherapy at conversion</th>
<th>Achieved remission after conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>M</td>
<td>92</td>
<td>Yes</td>
<td>ALL</td>
<td>1.1</td>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>M</td>
<td>165</td>
<td>Yes</td>
<td>ALL</td>
<td>1.5</td>
<td>0</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>F</td>
<td>246</td>
<td>Yes</td>
<td>ALL</td>
<td>1.2</td>
<td>9</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>F</td>
<td>14</td>
<td>No</td>
<td>ALL</td>
<td>4.0</td>
<td>7</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>M</td>
<td>5</td>
<td>No</td>
<td>ANLL</td>
<td>3.6</td>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>M</td>
<td>86</td>
<td>No</td>
<td>ANLL</td>
<td>1.0</td>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*High-risk protocol.  
†Standard risk protocol. 
‡Standard induction therapy with limited early intensification (X-S).  
§Standard induction therapy followed by intensive maintenance treatment with sequentially administered drug pairs.  
¶Not treated with standard therapy for ANLL.

conventional continuation regimen of 6-MP and MTX (patient 5).  
The single child with ANLL at diagnosis received daunomycin and cytarabine as induction therapy. An intensive two-drug maintenance schedule was used, employing doxorubicin plus cytarabine, etoposide (VP-16) plus 5-azacytidine, and cytarabine plus 6-thioguanine, with monthly prophylactic intrathecal MTX for the first six months.

Morphological and Cytochemical Studies

Bone marrow smears were stained with Wright-Giemsa, periodic acid-Schiff (PAS), myeloperoxidase (MPO), Sudan black B (SBB), chloroacetate esterase (CAE), and alpha-naphthyl butyrate (ANB). Morphological classification followed French-American-British (FAB) conventions.

Immunophenotyping and Terminal Transferase Assay

Bone marrow samples showing > 80% leukemic blasts were studied for rosette formation with sheep erythrocytes incubated at 37°C or pretreated with S-(2-aminoethyl)isothiouronium bromide hydrobromide (AET). Two hundred cells were counted for each patient, and results were considered positive if rosette formation was noted for > 3% (37°C) or > 15% (AET) of blasts. In these studies, cytosin preparations of rosettes were evaluated to assure that the blasts formed rosettes. Further immunophenotyping included surface immunoglobulin (SIg), T antigens (T101, T11, 3A1), the common ALL leukemia antigen (CALLA) as detected with J5, and HLA-DR, performed as previously described. Cells of selected patients were examined for myeloid-associated surface antigens: My-1, MCS-1, and J1-1D1. Any sample in which 35% of cells were reactive by indirect immunofluorescence was considered positive. Terminal deoxynucleotidyl transferase (TdT) was identified by use of indirect immunofluorescence.

Cytogenetics

Chromosome studies were performed by use of a direct bone marrow technique that includes a trypsin-Wright staining to obtain G-banding.

RESULTS

Of the 89 patients with ALL or ANLL who developed bone marrow relapse, six (6.7%) demonstrated lineage switch: five from ALL to ANLL and one from

Table 2. Laboratory Studies of Leukemic Cells at Primary Diagnosis (Dx) and Lineage Switch (LS)

<table>
<thead>
<tr>
<th>Immunologic markers</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>E rosettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T101</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CALLA</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>TdT</td>
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<td></td>
<td></td>
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<tr>
<td>S Ig</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>My-1</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCS-1</td>
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<td></td>
<td></td>
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<tr>
<td>SJ-1D1</td>
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<tr>
<td>Cytochemical tests</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MPO, SBB, CAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAB subtype</td>
<td>L1</td>
<td>L2</td>
<td>M1</td>
<td>L1</td>
<td>M1†</td>
<td>L1</td>
</tr>
</tbody>
</table>

MPO, myeloperoxidase; SBB, Sudan black B; CAE, chloroacetate esterase; ANB, alpha-naphthyl butyrate; CALLA, common acute lymphoblastic leukemia antigen; TdT, terminal deoxynucleotidyl transferase; FAB, French-American-British; ND, not done; (−) negative; (+) positive.

*Unclassifiable (see text).  
†Auer rods present.
ANLL to ALL. Their clinical features are listed in Table 1. Although chemotherapy was being administered to five patients when phenotypic conversion occurred, two had completed their initial treatment and one had not received antileukemic drugs for 13 months. Lineage switch was evident at first hematologic relapse in four patients and at second hematologic relapse in two. The mean time from diagnosis to phenotypic conversion was 2.2 years (range, one to four years).

The mean number of drugs received before lineage switch was 7.8 (range, six to ten); no single agent was administered to all six patients. 2'-Deoxycoformycin (2'-dCF), the only agent previously linked to lineage switch, was being administered to patient 1 when he first showed evidence of a phenotypic change. Patient 1 had the first bone marrow relapse seven months after initial diagnosis and achieved a second remission with standard ALL therapy. A second relapse occurred 1 year after initial diagnosis, at which time the blasts still lacked myeloid features by cytochemical criteria. However, one month later, after receiving 2'-dCF, his blasts were clearly positive for myeloid-associated cytochemistries and surface antigens (Table 2). Patient 2 relapsed one year after diagnosis with morphological and phenotypic ALL. He failed to respond to two reinduction attempts directed to ALL. The first attempt included cyclophosphamide, vincristine, and VP-16, and the second included prednisone, vincristine, and daunomycin. At 17 months after initial diagnosis, after the patient's failure to achieve a second remission, myeloperoxidase-positive blasts were present in his marrow. One week later, after receiving 2'-dCF, the blasts also became SBB- and CAE-positive (Table 2). The patient did not respond to two reinduction attempts directed to ALL. The first attempt included cyclophosphamide, vincristine, and VP-16, and the second included prednisone, vincristine, and daunomycin. At 17 months after initial diagnosis, after the patient's failure to achieve a second remission, myeloperoxidase-positive blasts were present in his marrow. One week later, after receiving 2'-dCF, the blasts also became SBB- and CAE-positive (Table 2). The patient did not respond to 2'-dCF and then received a trial of high-dose cytarabine, but died during bone marrow hypoplasia. No evidence of residual leukemia was found at autopsy.

When initially diagnosed, the five ALL patients who switched to ANLL appeared morphologically to be ALL (Fig 1A) and lacked cytochemical evidence of myeloid or monocytic features (Table 2). Three of the five had clinical characteristics of T cell leukemia, including mediastinal masses and markedly elevated leukocyte counts. A T cell origin for their disease was supported by an immunologic phenotype of either TIO1,HLA-DR (patient 1) or ER,HLA-DR (patients 2 and 3). One patient (No. 4) demonstrated a common ALL phenotype at diagnosis (CALLA',HLA-DR',TdT'). Patient 5 lacked clinical features of T cell leukemia, but his blasts formed rosettes with AET-treated erythrocytes; complete initial phenotyping could not be performed because of inadequate cell numbers. Testing with myeloid monoclonals was not performed on any of the five patients with ALL at diagnosis because of the unmistakable lymphoid characteristics of their leukemic blasts and negative cytochemical tests.

When examined at lineage switch, blasts of the five ALL patients were clearly nonlymphoid, as judged by moderate amounts of cytoplasm and scattered cytoplasmic granules, as well as reactivity with nonlymphoid-associated cytochemical stains, including MPO, SBB, CAE, and/or ANB (Table 2). Cells from patients 3 (Fig 1B) and 5 contained Auer rods and, together with blasts of patient 2, were classified as FAB M1. Patient 4 had myelomonocytic leukemia (FAB M4). The remaining child (patient 1) demonstrated a monomorphic proliferation of immature myeloid cells with granules and moderate cytoplasm that could not be precisely classified by FAB criteria.

The myeloid character of leukemic blasts was confirmed at relapse in four patients by reactivity for myeloid-associated antigens and the absence of lymphoid-associated phenotypic markers. Thus, by morphological, cytochemical, and immunologic criteria, patients 1, 3, 4, and 5 had converted to nonlymphoid leukemias. Patient 2, morphologically and cytochemically, converted to ANLL, but had a phenotype of TIO1',HLA-DR',TdT'. Simultaneous TIO1 and
Table 3. Cytogenetic Results

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Metaphases (Examined/Normal)</th>
<th>Diagnosis</th>
<th>Metaphases (Examined/Normal)</th>
<th>Lineage Switch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14/2</td>
<td>47,XY, + C*</td>
<td>20/1</td>
<td>47,XY, + C, 13p+, del(17)p11/12</td>
</tr>
<tr>
<td>2</td>
<td>34/34</td>
<td>46,XY</td>
<td>22/6</td>
<td>46,XY, del(7)p14, 9p+</td>
</tr>
<tr>
<td>3</td>
<td>19/9</td>
<td>46,XX,del(11;14)(p13;q13)</td>
<td>30/16</td>
<td>46,XX,del(6)q24</td>
</tr>
<tr>
<td>4</td>
<td>24/24</td>
<td>46,XX</td>
<td>12/12</td>
<td>46,XX</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>Insufficient quantity</td>
<td>8/0</td>
<td>47,XY, + 11,i(17q)</td>
</tr>
<tr>
<td>6</td>
<td>22/22</td>
<td>46,XY</td>
<td>13/13</td>
<td>46,XY</td>
</tr>
</tbody>
</table>

*No banding available.
†The two karyotypes listed in the second column for patient 1, and separated by a diagonal line are from the same clone, the second (10% of cells) being a side-line of the first.
‡The two karyotypes listed for patient 3 at diagnosis represent independent clones. The minor stemline (6q deletion) was found in less than 15% of the cells at diagnosis.

Additionally, the HLA-DR antigen was absent, consistent with findings in T cell ALL.23

The cytogenetic features of all patients are shown in Table 3. In patient 3, two stem lines were apparent initially when the diagnosis of ALL was made. The predominant stem line contained an 11;14 translocation, with no other abnormality. The secondary stem line contained only a small 6q deletion. At the time of relapse, when the patient underwent lineage switch to ANLL, only the stem line containing the 6q deletion was found. These findings are consistent with the reported specific association of the 11;14 translocation with E'T cell ALL29 and suggest that a 6q abnormality may be associated with ANLL in this case.

In patient 1, the karyotype at diagnosis was 47,XY+C, but lacked banding studies. At relapse 1 year later, multiple chromosomal abnormalities were evident by banding, including an isochromosome 7q, 9p+, and 17p-. Morphology and phenotype at this time were still indicative of ALL. After lineage switch, some abnormalities persisted (isochromosome 7q, 17p-), others disappeared (9p+), and one new abnormality developed (13p+), indicating clonal evolution (Table 3).

Patients 4 and 6 had a normal karyotype both at diagnosis and after lineage switch, suggesting chromosomal stability within their malignant clones. Both patients had adequate numbers of metaphases (Table 3) with good banding quality, indicating that the karyotype of the leukemic cells was normal. Although patients 2 and 5 lacked complete cytogenetic studies, neither these two nor any other patient showed evidence of a Philadelphia chromosome.

Patient 1 converted only after receiving 2'-dCF and died before receiving additional therapy. Patient 2 failed reinduction therapy directed at relapse ALL and showed early evidence of conversion, with only weak MPO reactivity in 23% of blasts (many of which still...
appeared to be lymphoid morphologically) before receiving 2'-dCF. Further conversion was evident 1 week after 2'-dCF, with strong MPO (= 60%), SBB, and CAE reactivities and a clearly myeloblastic appearance (Table 2). The patient subsequently received high-dose cytarabine with an antileukemic effect documented by the absence of detectable leukemia at autopsy.

The three remaining patients who converted from ALL to ANLL were retreated with a regimen designed for ANLL, including daunorubicin and cytarabine, and all achieved a complete remission. The child with a diagnosis of ANLL who converted to ALL failed further therapy for ANLL but achieved a complete remission when treated with vincristine, prednisone, and L-asparaginase.

DISCUSSION

The phenotypes of blast cells from patients with ALL in relapse most often adhere to the original lineage.20 The consistency of such observations supports the suggestion that differentiation programs of leukemic cells are frozen or blocked, but not necessarily aberrant.21 Nonetheless, we demonstrate phenotypic conversion in six of 89 cases (6.7%) of acute leukemia in marrow relapse, including an apparently unique case of ANLL converting to ALL. A recent review of the literature22 revealed 17 cases of ALL converting to ANLL, with a mean interval from diagnosis of 32 months, which is similar to the mean of 26.4 months for our patients.

In contrast to earlier findings of acute leukemia with mixed expression,21,22 our cases represent a lineage switch. Definite myeloid characteristics22 were uniformly lacking when the five cases were diagnosed as ALL, but were uniformly present when they demonstrated lineage switch. In the single case judged to involve a lineage switch from ANLL to ALL, the clearly myeloid cytochemical pattern of reactivity at diagnosis reverted to negative. Retention of HLA-DR, T101, and E rosettes (AET) by cells of patient 2 could be interpreted as demonstrating a transitional stage, leading to eventual disappearance of lymphoid markers with the development of myeloid markers. Further evidence for this was progressive acquisition of additional myeloid cytochemistries 1 week after the patient received 2'-dCF.

Four patients had T cell-associated characteristics either at diagnosis (patients 1, 2, and 3) or at lineage conversion (patient 6), suggesting that T lymphoblasts may have the potential for myeloid differentiation or that there may be a multipotential progenitor cell capable of both T lymphoblast and myeloblastic differentiation. A previous report would support this hypothesis.19 It may be of interest to note that there were 30 patients with T cell ALL at diagnosis, and ten of the 89 patients who relapsed had T cell ALL. Thus, 30% of T cell ALL patients who relapsed demonstrated lineage switch.

The factors responsible for lineage switch remain uncertain. All patients in the study received at least six antineoplastic drugs, making assessment of specific drug effects extremely difficult. In patient 3, whose karyotype showed two independent clones at diagnosis (Table 3), selection pressure from treatment appears to have eradicated the dominant lymphoid-associated clone, allowing expansion of the remaining, possibly myeloid-associated, clone (clonal selection). The absence of karyotypic changes in patients 4 and 6 at relapse would suggest, though not prove, that a single clone was capable of either lymphoid or myeloid differentiation. This concept is supported by chronic myeloid leukemia with the potential for expressing both lymphoid and myeloid phenotypes in blast crisis.

Rapid lineage switch in patient 1 and progressive conversion in patient 2 during administration of 2'-dCF would support a direct effect of chemotherapy on cell differentiation programs.24,25 The exact mechanism for this effect remains unknown. However, demethylation of DNA, a proposed mechanism for gene activation,33 is one possibility in view of the secondary effects of 2'-dCF.24,25

We do not believe that these cases represent second malignancies because (1) the blasts of two patients contained Auer rods, an unusual finding in second neoplasms,24,25 (2) patients 1 and 2 showed accelerated conversion to nonlymphoid leukemia; (3) lymphoid leukemia as a second malignancy in a patient with ANLL, to our knowledge, has never been reported nor seen at this institution; and (4) the cytogenetic results are generally incompatible with a second malignancy.

Prompt recognition of lineage switch may be helpful in selecting an effective therapeutic regimen. Although more data are necessary, therapy appropriate for the leukemic phenotype at the time of lineage switch may be advisable.

Our study indicates that lineage switch in acute leukemia is more common than might be expected, possibly because of the wider use of intensive chemotherapy. In one case, we have evidence for clonal selection and, in at least two others, the data suggest, but do not prove, that leukemic transformation can occur in a multipotential hematopoietic progenitor cell. Studies are needed to identify the target cells in leukemogenesis, evaluate their spontaneous or drug-induced capacity for lineage deviation and differential...
tion, and elucidate the relation between the genetic and phenotypic characteristics of leukemic cell lineages.

NOTE ADDED IN PROOF
Since acceptance of this manuscript, we have observed two additional patients with T-cell ALL who underwent lineage switch to ANLL.

ACKNOWLEDGMENT
We wish to thank Drs Ritz, Fauci, Minowada, and Civin for kindly supplying the monoclonal antibodies and John Gilbert for his editorial assistance.

REFERENCES

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Lineage switch in acute leukemia

S Stass, J Mirro, S Melvin, CH Pui, SB Murphy and D Williams