LINEAGE SWITCH AND MULTILINEAGE INVOLVEMENT IN TWO CASES OF Ph CHROMOSOME-POSITIVE ACUTE LEUKEMIA: EVIDENCE FOR A STEM CELL DISEASE

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ABSTRACT

Philadelphia chromosome-positive acute leukemias (Ph+ AL) show variable cytologic features, possibly reflecting heterogeneous stem cell involvement. Morphologic, immunologic and cytogenetic studies were performed in two cases of Ph+ acute lymphoblastic leukemia (ALL) in order to better delineate the clinicobiological features of this cytogenetic subset of AL.

Sequential cytoimmunologic studies in patient 1 documented a lineage switch from pro-B ALL with a minor myeloid component at diagnosis to minimally differentiated acute myeloid leukemia (AML) at relapse. In this patient the major breakpoint cluster region (M-bcr) was in a rearranged configuration and all metaphase cells showed t(9;22)(q34;q11), both at diagnosis and at relapse. In patient 2 a diagnosis of Ph+ early T-cell ALL with minor myeloid component was made. In this patient the M-bcr was in a germline configuration. Cytogenetic studies documented the presence of the Ph chromosome in all metaphases from a lymphoid cell population obtained by fine-needle aspiration of an enlarged lymph node, and from a bone marrow cell fraction enriched in granulocyte precursors. This finding suggests multilineage involvement in this patient.

Lineage switch and multilineage involvement in two patients suggest that a pluripotent stem cell may be affected rather frequently in patients with Ph+ AL. These findings show that biologically Ph+ AL may resemble chronic myelogenous leukemia blast crisis, since it may originate from an undifferentiated stem cell carrying the t(9;22) translocation.

Key words: Ph+ AL, lineage switch, multilineage involvement, stem cell

T he Philadelphia (Ph) chromosome, which results from a reciprocal t(9;22)(q34;q11) translocation, is found in 15-25% of adult patients with acute lymphoblastic leukemia (ALL). Molecular genetic findings in Ph chromosome-positive (Ph+) ALL are variable. In approximately half of the cases the molecular events resemble those of chronic myelogenous leukemia (CML); the breakpoint on chromosome 22 is located within a small 5.8 kb region of the bcr gene, the so-called major breakpoint cluster region (M-bcr), whereas in most of the remaining cases the breakpoint is located in a region of the bcr gene more proximal to the centromere.

Investigation of the lineages involved in Ph+ ALL has demonstrated heterogeneous stem cell involvement. Indeed some patients with Ph+ ALL may show the Ph chromosome in myeloid and erythroid lines when cell separation techniques or single hemopoietic colony studies are...
employed, indicating pluripotent stem cell involvement. Conversely, the Ph chromosome may be restricted to lymphoid cells in other patients, suggesting involvement of a more differentiated lymphoid committed progenitor cell.

The heterogeneity of Ph+ AL is also apparent cytologically: some cases manifest the typical features of acute lymphoblastic leukemia or acute myelogenous leukemia, whereas others have frequently revealed mixed-lineage characteristics at immunologic and electron microscopy studies.

The impact of these observations on the debate surrounding the distinction between de novo Ph+ ALL and chronic myelogenous leukemia presenting in lymphoid blast crisis (BC) is unclear. We studied two patients with Ph+ ALL in whom clinical and biological features indicated pluripotent stem cell involvement. The results of cytoimmunologic, cytogenetic and molecular studies in these patients are presented and discussed insofar as they relate to the diagnosis of this cytogenetic subset of acute leukemia and to the relationship between Ph+ ALL and CML lymphoid BC.

Patients and methods

Patient #1. A 23-year-old previously healthy male was referred to our Institution with fever and splenomegaly (8 cm below the costal margin). Peripheral blood analysis revealed: Hb 12 g/dL, Plts 48×10^9/L, WBC 184×10^9/L with 65% small to medium-sized agranular blast cells, 6% blast cells with myelomonocytic features, some of which had fine azurophilic granules, 19% promyelocytes to neutrophils, 8% monocytes, 2% lymphocytes. Bone marrow contained 70% blast cells, 20% granulocyte precursors and a few erythroid cells. Cytochemical staining showed PAS positivity in 25% of the blast cells and 5% Sudan black-B positivity. The leukocyte alkaline phosphatase (LAP) score was 148 (n.v. 150-330). Blast cell immunophenotype was consistent with a diagnosis of pro-B ALL with a minor myeloid component (Table 1). Chromosome studies revealed 100% cells with the classical t(9;22)(q34;q11). No additional anomalies were seen.

The patient was treated with an antilymphoid protocol that included vincristine, daunorubicin, prednisone and asparaginase. After 1 month, 60% residual blast cells with a pro-B phenotype (Table 1) were detected in an otherwise hypoplastic bone marrow. Teniposide and intermediate doses of cytarabine briefly reduced BM blast cells to 5%.

The patient was subsequently treated with 1 course of mitoxantrone and cytarabine, followed by alternate monthly administration of teniposide and cytarabine or vincristine and idarubicin, which maintained the percentage of BM cells between 10 and 20%.

Four months later overt relapse occurred with a WBC count of 20×10^9/L and 80% undifferentiated blast cells in the peripheral blood. PAS staining and myeloid cytochemistry were negative. Immunologic studies revealed positivity for myeloid-associated markers (CD13, CD33 and CD11c) and negativity for B-cell-associated antigens (CD10 and CD22). Only abnormal metaphases carrying the Ph chromosome and no additional abnormalities were detected at cytogenetic analysis.

Partial short-term remission was obtained with idarubicin and cytarabine, and the patient died 7 months after diagnosis in leukemia progression.

Patient #2. An 85-year-old man was admitted to our Institution with diffuse lymph node enlargement and fever. No splenomegaly was detected at admission. A colorectal adenoma had been surgically removed 10 months earlier; at that time, physical examination, as well as laboratory and hematologic profiles, were unremarkable. Peripheral blood values at admission were: Hb 13.2 g/dL, Plts 233×10^9/L, WBC 52×10^9/L with 41% blast cells, 5% promyelocytes to myelocytes, 39% neutrophils, 5% lymphocytes, and 10% monocytes. Seven days later his WBC count rose to 85×10^9/L and the spleen was palpable 3 cm below the left costal margin.

Bone marrow aspiration showed a 4/1 myeloid/erythroid ratio with dysplastic changes in the granulocytic lineage and 40% blast cell infiltration. The majority of the blast cells were morphologically undifferentiated, although 5% of them contained scattered azurophilic granules.

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PAS and acid phosphatase staining were positive in the majority of blast cells; Sudan black-B positivity was also detected in 3% of them. The immunophenotype showed positivity for early T-cell markers (Table 1), and all metaphase cells
### Table 1. Summary of hematologic, cytologic and cytogenetic data from 2 patients with Ph+ ALL.

<table>
<thead>
<tr>
<th>Stage of disease</th>
<th>morphology/cytochemistry</th>
<th>immunophenotype (*)</th>
<th>BM karyotype (% abnormal) M-bcr (**)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient 1</strong></td>
<td></td>
<td></td>
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<tr>
<td>Diagnosis</td>
<td>ALL-L2 with minor myeloid component PAS 25%+ (coarse granules), SBB 5%+</td>
<td>CD34 82%+, Lymphoid markers: TdT 85%+, CD19 78%+, CD10 72%+, CD22 75%+, Myeloid markers: CD13 15%+, CD33 16%+, CD11b negative</td>
<td>46,XY,t(9;22)(100%) M-bcr R</td>
</tr>
<tr>
<td>post CT</td>
<td>undifferentiated blasts with 10% PAS+ cells</td>
<td>Lymphoid markers: CD19 90%+, CD22 90%+, Myeloid markers: not done.</td>
<td>46,XY/46,XYt(9;22)(55%)</td>
</tr>
<tr>
<td>overt relapse</td>
<td>AML-M0 with undifferentiated blasts PAS-, SBB-, Pox-, MPO+ (rare cells) at EM study</td>
<td>Lymphoid markers: CD10 and CD22 negative. Myeloid markers: CD13 75%+, CD33 73%+, CD11b 30%+</td>
<td>46,XY,t(9;22)(100%) M-bcr R</td>
</tr>
<tr>
<td><strong>Patient 2</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Diagnosis</td>
<td>ALL-L2 with minor myeloid component PAS 90%+ Acp 90%+, SBB 3%+</td>
<td>CD34 54%+, Lymphoid markers: CD7 72%+, CD2 67%+, TdT 80%+, CD5 72%+, CD3 12%+, CD4 and CD8 negative. B-cell markers negative. Myeloid markers: CD13 21%+, CD15 negative</td>
<td>46,XY,t(9;22)(***)(100%) M-bcr G</td>
</tr>
</tbody>
</table>

(*) % positive cells in the blast gate; negative: <5% positive cells (**) R: rearranged, G: germline (***): 10 metaphase cells from an involved lymph node contained the Ph chromosome CT: chemotherapy, PAS: periodic acid-Schiff, SBB: sudan black-B, Pox: peroxidase, AcP: acid phosphatase, MPO: myeloperoxidase, EM: electron microscopy.
from a BM sample presented t(9;22) (q34;q11). Fine-needle aspiration of an enlarged lymph node revealed homogeneous blast cell infiltration; an immature T-cell phenotype was detected by immunocytochemical staining (see below). Progressive leukocytosis and lymphadenopathy prompted cytoductive treatment with mitoxantrone and cytarabine; however, pancytopenia and sepsis developed and the patient died one week later.

**Cytologic studies**

Morphological diagnoses were made on the basis of MGG-stained BM and PB films, supported by cytochemical stainings. The cytochemical, immunological, ultrastructural and cytogenetic techniques employed in our laboratory have been described previously.8,11,12

**Immunological studies**

Bone marrow and peripheral blood cell immunophenotype was determined by cytofluorimetric analysis. Isotype-matched myeloma protein was used as control. Results were reported as percentage of cells in the blast gate showing greater fluorescence than controls.

Expression of the following surface antigens was studied at diagnosis in both patients by means of commercially available monoclonal antibodies: lymphoid markers CD2, CD7, CD3, CD4, CD8, CD19, CD22, CD10; myeloid markers CD33, CD13, CD15, CD14, CD11b. CD34 stem cell antigen expression was investigated with the HPCA-1 monoclonal antibody. Terminal deoxynucleotidyl-transferase (TdT) was assayed using polyclonal rabbit antiserum.

In order to document lineage switch, sequential immunological studies were conducted (at diagnosis, after induction treatment and at overt relapse) in patient #1.

Immunophenotypic studies were carried out in patient #2 using the APAAP method as previously reported 15 on cells obtained by fine-needle aspiration of an enlarged lymph node.

**Cytogenetic and molecular genetic studies**

A synchronization technique14 was employed for chromosome studies on BM and PB cells. The configuration of the M-bcr of the bcr gene was assessed by previously described conventional Southern blot procedures.15

In order to determine whether more than one lineage was involved in patient #2, chromosome studies were performed on both a pure lymphoid cell population obtained by fine-needle aspiration of an enlarged lymph node, and on BM cells following enrichment of granulocyte precursors. Details of these cell separation methods have been reported elsewhere.16 Briefly, a single-cell suspension consisting of 60×10⁷ cells obtained from BM particles was stratified over 2 mL of a Percoll layer with a density of 1.065 mg/mL and centrifuged at 600×g for 20 min. After centrifugation, the cells at the bottom of the tube were collected and washed twice under sterile conditions. Part of these cells were spun in a cytocentrifuge and stained with May-Grunwald-Giemsa for cyto-logic study. The remaining cells were cultured for cytogenetic analysis.

**Results**

Salient cytologic, immunological and cytogenetic findings in the two Ph’ ALL patients are summarized in Table 1.

**Lineage switch in patient #1**

Morphologically, leukemic cells at diagnosis resembled ALL-L2 blasts according to the FAB classification (1976), a finding supported by the presence of 25% cells with coarse granular PAS positivity.

Immunophenotypic data, which showed strong positivity for TdT, CD19, CD22 and CD10, were consistent with an unequivocal pro-B cell phenotype. A minority of blast cells with myelomonocytic features was also documented at electron microscopy study.

According to the FAB criteria,17 these findings are consistent with a diagnosis of minimally differentiated acute myeloid leukemia (AML-M0).

**Multilineage involvement in patient #2**

In patient #2 cytogenetic analysis of a cell population obtained from an involved lymph node revealed the presence of the Ph chromosome in 10 metaphases examined. These cells
were undifferentiated blasts with the following phenotype: CD2+ 95%, CD7+ 90%, TdT+ 90%, CD3+ 20%, CD4 and CD8 negative; myeloid-associated markers were also negative. Because of the suboptimal quality of metaphase spreads in this sample, the presence of additional chromosome anomalies could not be ruled out.

Separation of BM cells yielded a myeloid-enriched cell fraction with a density greater than 1.065 mg/mL consisting of more than 85% granulocyte precursors and less than 10% agranular blast cells. Several mitotic cells with cytoplasmic granules were also observed in cytospin preparations obtained from this fraction. Chromosome studies of this fraction, as well as of unseparated BM samples, revealed 100% cells with t(9;22) (q34;q11).

Discussion

The cytologic and clinical features of these two patients raise some questions concerning:

a) the significance of phenotypic conversion and multilineage involvement;

b) the criteria for diagnosing hybrid acute leukemia in Ph+ ALL patients with a minor myeloid component;

c) the relationship between de novo Ph+ ALL and lymphoid blast crisis in Ph+ CML.

Lineage switch and multilineage involvement

A lineage switch from ALL at diagnosis to minimally differentiated AML at relapse was documented by immunophenotypic data and supported by cytologic and electron microscopy studies in patient #1. Development of a second unrelated leukemia appears to be ruled out by the presence of the same cytogenetic abnormality at diagnosis and at relapse; therefore involvement of a pluripotent stem cell capable of differentiating along multilineage pathways is likely in this patient. Two similar cases, in which Ph+ ALL was followed by a relapse with AML features, have recently been reported, suggesting that lineage switch may not be an uncommon event in Ph+ AL.

Acute leukemia lineage switches were described in 6/100 relapsed patients, none of whom carried the 9;22 translocation. Chromosome findings in this series showed complex karyotypes with frequent involvement of the 11q23-25 chromosome region (3 patients). Monosomy 7 and trisomy 8 were detected in 1 patient each. Trisomy 8, in association with an additional copy of chromosomes 6 and 13, was observed in another patient undergoing lineage switch reported by Neame and co-workers. Finally, Paietta et al. described two cases showing lineage switch during induction treatment, with monosomy 7 as the only clonal abnormality. It is worth noting that rearrangements of 11q23, monosomy 7 and the Ph chromosome are not specifically associated with any cytologic subset of leukemia and have been regarded as chromosome aberrations conferring proliferative advantage to undifferentiated stem cells.

The clinical and cytologic features of patient #2 at presentation afforded an opportunity to analyze directly whether or not the lymphoid and the granulocytic lineage carried the 9;22 translocation. The presence of the Ph chromosome in 100% of the metaphase cells obtained from an involved lymph node and from a BM cell fraction consisting of granulocyte precursors shows that, cytogenetically, both the lymphoid and the granulocytic lineages were involved in this patient.

Thus, lineage switch in patient 1 and multilineage involvement in patient 2 indicate that Ph+ ALL may be a stem cell disease. These two patients add to a series of 9 cases of Ph+ ALL recently reviewed in which evidence of pluripotent stem cell involvement was available.

Interestingly, the clinical outcome in these patients with stem cell Ph+ ALL appears to be better than that of similar cases in which the Ph chromosome was shown to be restricted to the lymphoid lineage. Our two patients, however, suffered a particularly severe outcome. More cases must be studied to confirm or refute these findings.

Ph+ ALL or Ph+ hybrid acute leukemia?

The minor myeloid component documented cytologically and immunologically in our two patients deserves attention. The presence of a minority of blast cells with the features of myeloblasts has already been described in some cases of Ph+ ALL. It is normally necessary to document more than 10% of cells with the features of another lineage in order to identify leukemia with mixed-lineage characteristics. However, a recent study of Ph+ AL showed that it may be clinically important to recognize Ph+ ALL patients with a minor myeloid compo-
nent. This suggests that a figure as low as 5% may be a more appropriate cut-off point for the diagnosis of hybrid AL (HAL) in such patients. Moreover, since uniformly accepted criteria for identifying this form of AL have not been formulated yet, it is difficult to decide whether these Ph+ acute leukemias should be classified as ALL, or if they would be more properly characterized as HAL.

**de novo Ph+ ALL or CML lymphoid blast crisis?**

The relationship between Ph+ acute leukemia and CML blast crisis remains controversial. Lineage switch and multilineage involvement in two cases of Ph+ ALL suggest that biologically **de novo** Ph+ ALL may resemble CML blast crisis, since it may originate from a transformed pluripotent stem cell carrying the t(9;22) translocation. Other clinicobiological features of our patients did not univocally indicate whether the correct diagnosis was **de novo** Ph+ ALL or CML lymphoid blast crisis.

In patient #1 the M-bcr was located in a rearranged configuration found in virtually all cases of CML. Despite the presence of the molecular hallmark of CML, this patient had no other chromosome aberrations in addition to t(9;22); he presented some normal metaphases when partial remission was achieved and never showed a cytologic picture consistent with chronic phase CML following myeloablative chemotherapy.

In patient #2 the presence of a minor myeloid component to the lymphoid process, along with hyperplasia of the granulocytic lineage and 20% granulocyte precursors in the peripheral blood film, suggest that CML blast crisis with undetected chronic phase might have been the correct diagnosis. Yet this patient failed to show M-bcr rearrangement at Southern blot analysis and presented an unequivocally normal hematologic profile 10 months before coming to our attention.

Thus, the hematologic and genetic findings in this and other reports document that no combination of clinical and laboratory parameters provides a clear-cut distinction between **de novo** Ph+ acute leukemia and Ph+ CML presenting in blast crisis. This finding is not surprising if one considers that these two disease entities share a fundamental genetic defect resulting in the production of chimeric proteins with enhanced tyrosine kinase activity and that additional genetic changes are likely to play a role in the genesis of blast crisis and of acute leukemia.

**References**