Phenotypic and Molecular Heterogeneity in Philadelphia Chromosome–Positive Acute Leukemia

By Cheryl Hirsch-Ginsberg, Craig Childs, Kun-Sang Chang, Miloslav Beran, Ann Cork, James Reuben, Emil J. Freireich, L.C.M. Chang, F.J. Bollum, Jose Trujillo, and Sanford A. Stass

Philadelphia chromosome–positive (Ph') acute leukemia is a heterogeneous subset of acute leukemia with a poor prognosis. We studied five patients to determine the potential for phenotypic and molecular heterogeneity. Cellular characterization studies included light myeloperoxidase (L-MPO), terminal deoxynucleotidyl transferase (TdT), ultrastructural MPO (U-MPO), and immunophenotyping by flow cytometry using T11, T3, T4, T8, Leu 1, B1, Leu 12, HLA-DR (Ia), CALLA (J5), OKM1, My4, My7, My8, My9, and My10. DNA was analyzed for rearrangements of the breakpoint cluster region (bcr), immunoglobulin heavy chain, joining region (JH), immunoglobulin λ light chain constant region (Cλ), and T cell beta receptor (TCRβ). RNA dot blots were hybridized by using molecular probes for MPO and TdT. We found that four of five cases were acute mixed-lineage leukemia (AMLL). One patient had acute unclassifiable leukemia. Of the four patients classified as having AMLL, three showed myeloid and lymphoid features, with one patient showing myeloid, T cell, and B cell features. The last case showed T cell and B cell features only. In one patient MPO/RNA was positive in spite of insufficient L-MPO or U-MPO to diagnose acute myelogenous leukemia (AML), thereby suggesting significant MPO gene expression before the production of sufficient MPO protein to meet the French-American-British criteria for AML. Three of the five patients showed rearrangement of bcr (cases 1, 2, and 5). Studies of these five patients support the concepts of molecular and phenotypic heterogeneity in Ph' acute leukemia, demonstrate a high incidence of AMLL in this subset of acute leukemia, and support the use of lineage-associated molecular probes to define lineage at an earlier stage than previously possible.

© 1988 by Grune & Stratton, Inc.
untreated adult acute leukemia were seen from January 1982 to samples were incubated overnight at 37°C in Ham's Grand Island, NY), supplemented with 10% fetal medium (GIBCO, marrow aspirates from each January 1986. Ten patients were Ph'-positive, and examined with a Jeol I200EX (Jeol USA, Inc. Peabody, MA) at a LKB III ultramicrotome (LKB Instruments, Gaithersberg, MD) clear cell suspensions obtained from peripheral blood by leukaphere-

sions were performed according to institutional guidelines. 

The diagnosis was based on the French-
al deoxynucleotidyl transferase (NSE) esterases, PAS, and acid phosphatase (AcPhos). Termi-

Wright-Giemsa, MPO, chloracetate (NASD), and a-naptyl buty-

Hematologic studies. Bone marrow smears were stained for Wright-Giemsa, MPO, chloracetate (NASD), and a-naptyl buty-

Hemoglobin (Hb) P1t WBC-PBt Karyotype Time (wk) Time

The following fluoroscinc inositoxyanate–conjugated, lymphoid-associated monoclonal antibodies were used: OKT11, OKT3, OKT4, OKT8 (Ortho Diagnostics); Leu 1, J5 (anti-CALLA), Leu 12, HLA-DR (1a) (Becton Dickinson); and B1 (Coulter Immunology, Hialeah, FL). The following purified monoclonal antibodies were used to detect myeloid antigens: My4, My7, My8, My9 (Coulter Immunology); OKM1 (Ortho Diagnostics); and My10 (Becton Dickinson Monoclonal Center). Reactivity of OKT4 was confirmed with purified anti-Leu 3 (Becton Dickinson). The second step reagent was goat antimouse IgG (Tago Diagnostics, Inc. Burlingame, CA). The cellular distribution of reactivity with the monoclonals used are given in Table 1.

Molecular studies. Samples from the five patients with cryopreserved cells were thawed and lysed in 4 mol/L guanidine isothio-

nate and fractionated by ultracentrifugation on a CsCl gradient, as described by Maniatis et al.10

Ten micrograms of DNA was restriction digested to completion with 50 units BamH1, HindIII, EcoR1, Kpnl, Xbal (Bethesda Research Laboratories Life Technologies, Inc, Gaithersburg, MD), or BglII (New England Biolabs, Inc, Beverly, MA), electrophoresed a 0.75% agarose gel, and transferred to Nytran membrane (S&S, Keene, NH) according to the procedure of the manufacturer. These membranes were hybridized with 2 × 106 cpm/mL 32P-oligolabeled DNA probes: bcr (break point cluster region of t(9;22)) 1.2-kb HindIII-BglII fragment on BglII, HindIII, BamH1, EcoR1, Kpnl, and Xbal digests (Oncogene Sciences, Mineola, NY); Ig (*immunoglobulin heavy chain, J region), 6-kb BamH1-HindIII fragment16 (courtesy of Dr Philip Leder, Harvard Medical School, Boston) on HindIII, BamH1, and EcoR1 digests; C, (immunoglobulin κ, light-chain constant region), 2.5-kb EcoR1 fragment17 (courtesy of Dr

bovine serum (Armour Pharmaceutical Co, Kankakee, IL) and Pen-Strep antibiotics (GIBCO). Harvesting and slide preparations were done according to established procedures.49

A minimum of 25 well-spread Giemsa-banded metaphases were analyzed from each of the cultures. Karyotypes were prepared on at least two metaphases of each cell type by using the International System for Human Cytogenic Nomenclature (ISCN 1985).50

Immunophenotyping. Surface marker analysis was performed on cryopreserved, density gradient–separated, peripheral blood leukemic blasts (all samples had >60% blasts) that were incubated with AB serum at 37°C for one-half to one hour before incubation with monoclonal antibody. The cells were then analyzed with an Ortho Spectrum III flow cytometer (Ortho Diagnostics, Westwood, MA) equipped with a 2140 Data Analyzer that allows discrete analysis of the blasts on the basis of forward and right-angle light scatter. Results were recorded as a percentage of positive cells over background; a marker is considered positive if reactive with 20% or more of the cells in the blast region. Nonspecific binding was controlled by mouse type-specific IgG control reagents (Becton Dickinson Monoclonal Center, Mountain View, CA). The following fluorescent isothiocyanate–conjugated, lymphoid-associated monoclonal antibodies were used: OKT11, OKT3, OKT4, OKT8 (Ortho Diagnostics); Leu 1, J5 (anti-CALLA), Leu 12, HLA-DR (1a) (Becton Dickinson); and B1 (Coulter Immunology, Hialeah, FL). The following purified monoclonal antibodies were used to detect myeloid antigens: My4, My7, My8, My9 (Coulter Immunology); OKM1 (Ortho Diagnostics); and My10 (Becton Dickinson Monoclonal Center). Reactivity of OKT4 was confirmed with purified anti-Leu 3 (Becton Dickinson). The second step reagent was goat antimouse IgG (Tago Diagnostics, Inc. Burlingame, CA). The cellular distribution of reactivity with the monoclonals used are given in Table 1.

Molecular studies. Samples from the five patients with cryopreserved cells were thawed and lysed in 4 mol/L guanidine isothiocyanate and fractionated by ultracentrifugation on a CsCl gradient, as described by Maniatis et al.10

Ten micrograms of DNA was restriction digested to completion with 50 units BamH1, HindIII, EcoR1, Kpnl, Xbal (Bethesda Research Laboratories Life Technologies, Inc, Gaithersburg, MD), or BglII (New England Biolabs, Inc, Beverly, MA), electrophoresed a 0.75% agarose gel, and transferred to Nytran membrane (S&S, Keene, NH) according to the procedure of the manufacturer. These membranes were hybridized with 2 × 106 cpm/mL 32P-oligolabeled DNA probes: bcr (break point cluster region of t(9;22)) 1.2-kb HindIII-BglII fragment on BglII, HindIII, BamH1, EcoR1, Kpnl, and Xbal digests (Oncogene Sciences, Mineola, NY); Ig (*immunoglobulin heavy chain, J region), 6-kb BamH1-HindIII fragment16 (courtesy of Dr Philip Leder, Harvard Medical School, Boston) on HindIII, BamH1, and EcoR1 digests; C, (immunoglobulin κ, light-chain constant region), 2.5-kb EcoR1 fragment17 (courtesy of Dr

Table 1. Cellular Distribution of Reactivity With Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cellular Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT11</td>
<td>Sheep E rosette receptor</td>
<td>51, 52</td>
</tr>
<tr>
<td>OKT3</td>
<td>Pan-T lymphocyte (mitogen)</td>
<td>53</td>
</tr>
<tr>
<td>OKT4</td>
<td>Helper/inducer T cells, monocytes, AML</td>
<td>54-56</td>
</tr>
<tr>
<td>OKT8</td>
<td>Suppressor/cytotoxic T cells</td>
<td>54</td>
</tr>
<tr>
<td>Leu 1</td>
<td>Pan-T, B cell</td>
<td>57, 58</td>
</tr>
<tr>
<td>B1</td>
<td>Pre-B cells, B cells</td>
<td>59</td>
</tr>
<tr>
<td>Leu 12 (B4)</td>
<td>Pre-B cells, B cells</td>
<td>60</td>
</tr>
<tr>
<td>J5</td>
<td>Anti-CALLA</td>
<td>61</td>
</tr>
<tr>
<td>la</td>
<td>B cells, monocytes, activated T cells, early myeloid CFU-GEMM</td>
<td>62, 63</td>
</tr>
<tr>
<td>OKM1</td>
<td>Monocytes, promonocytes, myeloid cells (more mature than CFU-C)</td>
<td>62, 64</td>
</tr>
<tr>
<td>My4</td>
<td>Monocytes, promonocytes</td>
<td>65</td>
</tr>
<tr>
<td>My7</td>
<td>Monocytes, promonocytes, CFU-C, myeloid cells</td>
<td>65</td>
</tr>
<tr>
<td>My8</td>
<td>Monocytes, promonocytes, myeloid cells (more mature than CFU-C)</td>
<td>65</td>
</tr>
<tr>
<td>My9</td>
<td>Monocytes, promonocytes, CFU-C, myeloid cells</td>
<td>66, 67</td>
</tr>
<tr>
<td>My10</td>
<td>CFU-GEMM, blasts (not lineage specific)</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 2. Clinical Data and Cytogenetics

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dL)</th>
<th>WBC-B (x 10⁶/μL)</th>
<th>Karyotype Presentation</th>
<th>Remission Time (wk)</th>
<th>Survival Time (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>F</td>
<td>8.2</td>
<td>510</td>
<td>[46,XX,t(9q+;15q-)]</td>
<td>0</td>
<td>2, dead</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>F</td>
<td>8.7</td>
<td>37</td>
<td>[46,XX,t(9q+;15q-);17q-;22q]</td>
<td>0</td>
<td>35, dead</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>M</td>
<td>11</td>
<td>54</td>
<td>[46,XY,t(9;22)]</td>
<td>0</td>
<td>24, dead</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>M</td>
<td>10.3</td>
<td>22</td>
<td>[47,XY,t(9;22),+17]</td>
<td>S/P BM T</td>
<td>104, alive</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>F</td>
<td>6.4</td>
<td>90</td>
<td>[46,XX,-7,t(9q+;15q-);22q]</td>
<td>1</td>
<td>50, dead</td>
</tr>
</tbody>
</table>

*Platelet count in peripheral blood.
†WBC count in peripheral blood.
‡Status after bone marrow transplantation.
Table 3. Light and Ultrastructural Morphology and Cytochemistries of Bone Marrow Blasts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blasts (%)</th>
<th>MPO</th>
<th>NASD</th>
<th>NSE</th>
<th>PAS</th>
<th>AcPhos</th>
<th>FAB Diagnosis</th>
<th>Electron Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.4</td>
<td>-</td>
<td>Weak</td>
<td>Rare</td>
<td>FG+</td>
<td>NA</td>
<td>AUL</td>
<td>MPO+ blasts, rare, with population of lymphoid blasts and smaller population of undifferentiated blasts</td>
</tr>
<tr>
<td>2</td>
<td>79.8</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>AML</td>
<td>MPO+ blasts with populations of lymphoid and undifferentiated blasts</td>
</tr>
<tr>
<td>3</td>
<td>85.6</td>
<td>+2%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ALL</td>
<td>MPO+ blasts, rare, with populations of lymphoid and undifferentiated blasts</td>
</tr>
<tr>
<td>4</td>
<td>51.4</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>FG+</td>
<td>NA</td>
<td>ALL</td>
<td>MPO+ blasts, very rare, with population of lymphoid blasts</td>
</tr>
<tr>
<td>5</td>
<td>83.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>FG+</td>
<td>NA</td>
<td>ALL</td>
<td>Lymphoid</td>
</tr>
</tbody>
</table>

Abbreviations: BL, block positivity; FG, fine granularity; NA, not assessed.

Philip Leder) on HindIII, BamHI, and EcoRI digests; T cell β receptor (JK1 clone) including variable, joining, and constant regions (TcR) 1.3-kb PstI fragment (courtesy of Dr. Tak W. Mak, Ontario Cancer Center, Toronto) on BamHI, HindIII, and EcoRI digests. Hybridization was detected by autoradiography using Kodak X-OMAT AR x-ray film and exposing at −70°C from three to seven days.

Five micrograms of total RNA was applied to nitrocellulose filter by using the Trans-Blot apparatus (Bio-Rad Laboratories, Rockville Centre, NY) and hybridized to the following 32P-oligolabeled DNA probes: MPO probe (PMP503 cDNA clone), 1.8-kb EcoRI fragment; and TdT (pT223 cDNA clone), 1.7-kb BamHI-EcoRI fragment.

RESULTS

Table 2 summarizes the clinical and cytogenetic data of the patients. Their ages range from 34 to 56 and include three females and two males. Only one patient (no. 3) had t(9;22) as the sole cytogenetic abnormality. Two patients demonstrated monosomy of chromosome 7 (patients no. 2 and 5). Patient no. 4 showed trisomy of chromosome 17, and one patient (patient no. 1) had a complex translocation t(9q+;15q−;17q;22q−). Only one patient achieved a brief complete remission with the chemotherapy protocols, but cytogenetic studies were not done at that time (patient no. 5). Patient no. 4 achieved long-term survival with a bone marrow transplant and has maintained a diploid karyotype.

Patients no. 2 to 5 were considered to have acute mixed-lineage leukemia (AMLL). The results will be presented by a discussion of the individual patients on the basis of data summarized in Tables 3 to 5 and Figs 1 to 8.

Patient no. 1 was classified as having acute unclassifiable leukemia (AUL) on the basis of FAB criteria. The blasts failed to demonstrate TdT or MPO by light microscopy, although there were rare MPO-positive blasts at the ultrastructural level. Immunophenotypic analysis demonstrated HLA-DR positivity as well as positivity of lymphoid-associated antigen B1 and myeloid antigens My7 and My9. DNA studies showed rearrangement of bcr after digestion with BamHI, EcoRI, KpnI, and XbaI restriction endonucleases. There were no rearrangements of Jb, Cκ, or TcRα.

RNA studies confirmed the absence of TdT RNA. The presence of MPO RNA was attributed to the residual 10%

Table 4. Immunophenotyping

<table>
<thead>
<tr>
<th>Marker</th>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts (%)*</td>
<td></td>
<td>84</td>
<td>67</td>
<td>80</td>
<td>61</td>
<td>77</td>
</tr>
<tr>
<td>T11</td>
<td>19</td>
<td>8</td>
<td>6</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu 1</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>23</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>27</td>
<td>6</td>
<td>8</td>
<td>17</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Leu 12 (B4)</td>
<td>0</td>
<td>31</td>
<td>25</td>
<td>27</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>JS (CALLA)</td>
<td>0</td>
<td>54</td>
<td>91</td>
<td>51</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>TdT</td>
<td>-</td>
<td>+</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Ia (HLA-DR)</td>
<td>85</td>
<td>61</td>
<td>94</td>
<td>91</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>OKM1</td>
<td>15</td>
<td>8</td>
<td>2</td>
<td>53</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>My4</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>My7</td>
<td>68</td>
<td>45</td>
<td>23</td>
<td>80</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>My8</td>
<td>48</td>
<td>22</td>
<td>8</td>
<td>67</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>My9</td>
<td>21</td>
<td>18</td>
<td>11</td>
<td>53</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>My10</td>
<td>80</td>
<td>33</td>
<td>70</td>
<td>24</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

These data are based on analysis of cryopreserved, Ficoll-hypaque leukapheresis samples. The figures represent the percentage of positive cells over background in the blast region.

*The blast percentages are based on differential counts of cytospin preparation of the samples. Underscored are those that we interpret as expressed by the blasts on the basis of the 20% criterion.
Table 5. Summary Table of Phenotypic and Molecular Studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cytochemistries</th>
<th>Positive Markers</th>
<th>DNA Probes Showing Rearrangements</th>
<th>Positive RNA Probes</th>
<th>FAB Diagnosis</th>
<th>AMKL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UMPO+, rare</td>
<td>IA, B1, My7, My9</td>
<td>bcr</td>
<td>MPO*</td>
<td>AUL</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>L-MPO+</td>
<td>Leu12, J5, IA, My7, My9</td>
<td>bcr, Jp, C (deleted)</td>
<td>MPO, TdT</td>
<td>AML</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>U-MPO+ (rare), TdT+</td>
<td>Leu12, J5, IA, My7</td>
<td>Jp, C, BcR, C, TdT</td>
<td>MPO, TdT</td>
<td>ALL</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>U-MPO+ (rare), TdT+</td>
<td>Leu12, J5, IA, OKM1, My4, My7, MPO, TdT</td>
<td>Jp, TdT</td>
<td>MPO, TdT</td>
<td>ALL</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>TdT+</td>
<td>Leu12, J5</td>
<td>bcr, Jp, C, TdT</td>
<td>MPO, TdT</td>
<td>ALL</td>
<td>+</td>
</tr>
</tbody>
</table>

*MPO + due to residual myeloid cells (10% to 23%).
†B1 commonly found on monocytes.7
‡Ten percent MPO blasts.
§Low percentage MPO + cells (2% MPO + blasts, 4% total MPO + cells).

Fig 1. (A) Light micrograph from the Wright-Geimsa stain of bone marrow in patient 2 (AMLL). Note the two populations of blasts: the small lymphoid blasts with a high (nuclear to cytoplasmic ratio) N/C ratio and coarse chromatin with inconspicuous nucleoli in the larger myeloid appearing blasts having more cytoplasm, finer nuclear chromatin, and distinct nucleoli (original magnification x 40). (B) Electron micrograph of a myeloblast (U-MPO +) from the same patient. Note the MPO staining in the endoplasmic reticulum, the perinuclear membrane, and the membrane-bound granules (original magnification x 7,500; current magnification x 5,625).

Fig 2. (A) Electron micrograph from patient no. 3 (AMLL) demonstrating two different types of blasts: undifferentiated (arrow) and lymphoid (arrow point). Note the difference in chromatin margination (heavy in the lymphoid and absent in the undifferentiated) and the quantity of heterochromatin (more coarse in the lymphoid and almost absent in the undifferentiated). (original magnification x 5,000; current magnification x 3,750). (B) Electron micrograph from patient no. 3 shows a myeloblast with MPO-positive granules. Note the similarities of nuclear morphology with the undifferentiated blast in Fig 3 (original magnification x 8,500; current magnification x 6,375).
Fig 3. Electron micrograph from patient no. 5 (AMLL) showing two lymphoblasts (original magnification ×6,250; current magnification ×4,688).

Fig 4. Southern transfer of DNA restriction digested with XbaI and hybridized with bcr probe (1.2-kb HindIII-BgIll fragment). The 9.7-kb germline band is marked by (•) and the positions of the λ molecular weight markers are marked by (—). Note the rearrangements in patients no. 1, 2, and 5. Lane C is the control from a normal volunteer. The germline bands for patients 4 and 5 appear slightly out of line from the other samples due to slightly retarded mobility from excess DNA.

Fig 5. Southern transfer of DNA restriction digested with HindIII and hybridized to the Jμ probe (6-kb BamHI-HindIII probe). The 11-kb germline position is marked by (•), and the positions of the λ molecular weight markers are marked by (—). Note the rearrangement of at least one allele in patients no. 2 to 5. Lane C is the control from a normal volunteer.

MPO+ cells (<3% MPO+ blasts). Although lymphoid lineage may be suggested by reactivity with anti-B1, a relatively late marker of B cell differentiation, lymphoid lineage commitment is excluded by the absence of Jμ rearrangement. This is consistent with the fact that B1 is normally expressed by monocytes and is therefore limited as a marker of lineage commitment.

Patient no. 2 met the criteria for AML on the basis of ultrastructural MPO positivity (see Fig 1B). However, the bone marrow specimen was TdT+ and had two populations of blasts seen by light microscopy (Fig 1A): large blasts, which were MPO+, and small MPO− blasts. Immunophenotypic analysis demonstrated positivity of HLA-DR, CALLA, and B4, which suggested a pre-B phenotype. There was also positivity of myeloid markers My7 and My8. Molecular studies demonstrated rearrangement of bcr after digestion with EcoRI, KpnI, and XbaI. Lymphoid lineage commitment at the DNA level was suggested by rearrangement of Jμ, deletion of Cμ, Deletion or rearrangement of Cμ alleles precedes the rearrangement of the λ chain DNA. RNA studies confirmed the presence of TdT and MPO RNA (11% MPO+ cells, including 10% MPO+ blasts).

Patients no. 3 and 4 met the standard criteria for ALL. They were MPO-negative by light microscopy, with the usual pre-B phenotype (CALLA+, Ia+, Leu 12+, TdT
Fig 6. (A) Southern transfer of DNA restriction digested with BamHI and hybridized with the Cμ probe (2.5-kb EcoRI fragment). The 12-kb germline position is marked by (-), and the positions of the λ molecular weight markers are marked by (--). Note the rearrangement in patients 3 and 5 and the deletions of both alleles in patient 2. Lanes labeled A, B, and C represent DNA in the germline configuration from t(4;11)(q21;q23) ALL patients. (B) Identical filter hybridized with the Jμ probe. The 16.5-kb germline position is marked by (-), and the positions of the λ molecular weight markers are marked by (--). Note the definite presence of DNA from patient 2 as well as the rearrangements of at least one allele in patients 2 to 5. Lanes labeled A, B, and C represent DNA from t(4;11)(q21;q23) ALL patients. These patients also show rearrangement of Jμ. The faint band appearing in the same position in all of the lanes represents artifact from the plasmid DNA.

Fig 7. Southern transfer of DNA restriction digested with HindIII and hybridized to the TcRγ probe (1.3-kb PstI fragment). The germline constant region positions are marked by (<) and include bands of 8.0, 6.5, and 3.5 kb. The positions of the λ molecular weight markers are marked by (--). Note the rearrangements of the 3.5-kb band in patients 4 and 5. Negative controls include 3 t(4;11)(q21;q23) ALL patients labeled A, B, and C.

positivity). However, they showed myeloid-associated antigen expression (My7+) and had rare blasts showing ultrastructural MPO positivity (see Figs 2A and 2B). Neither patient showed bcr rearrangement. This lack of rearrangement was confirmed in patient no. 3 by Dr Kurzrock et al who failed to demonstrate rearrangement when using a more 5' probe22; however, it is possible that a probe more 5' to ours could reveal a rearrangement in patient no. 4. Both patients demonstrated rearrangement of Jμ, with additional rearrangements of the Cμ in patient no. 3 (indicating lymphoid lineage commitment). In patient 4, the probe for the TcRγ receptor showed a loss of the 23-kb, 11-kb, and 3.5-kb constant-region germline bands when digested with BamHI, EcoRI, and HindIII, respectively. Two rearranged bands were present in the HindIII digest of patient 4 (Fig 7). RNA studies confirmed the presence of TdT and MPO RNA. The low number of light MPO+ (L-MPO+) cells (4%, including 2% MPO+ blasts) in patient no. 3 and only rare ultrastructural MPO+ (U-MPO+) blasts suggests myeloid lineage commitment with message production before the appearance of sufficient MPO protein to diagnose AML by FAB criteria.47 Patient 4 had 23% MPO+ cells (<3% MPO+ blasts), which accounted for the MPO/RNA positivity. Seven patients with ALL (negative for Ph1, myeloid surface anti-
gens, and MPO staining at the light level) failed to show hybridization with the MPO probe (placed on same nitrocellulose filter and hybridized concurrently with material from these five patients) (see Fig 8A).

Patient 5 met the standard criteria for ALL (MPO-negative at the light and ultrastructural level). The blasts were TdT-positive, and the immunophenotype was typical for pre-B cells: CALLA+, HLA-DR+, and B4+. Myeloid surface markers were negative. DNA studies demonstrated rearrangement of bcr after digestion with BglII, BamH1, EcoR1, Kpn1, and Xbal. There were rearrangements of JH, Cα, and Tcrβ receptor genes that were consistent with concurrent T and B cell lineage. RNA studies demonstrated TdT and MPO RNA, with the latter attributed to the 14% MPO+ residual cells (no MPO+ blasts).

**DISCUSSION**

Ph+ acute leukemias are a heterogeneous subset of acute leukemia. With respect to rearrangement of bcr, only two of our patients (no. 1 and 5) showed patterns similar to those seen in CGL, which involves detection of the bcr rearrangement after digestion of the DNA with BamH1 and BglII.16 One additional patient showed rearrangement only after digesting DNA with additional enzymes (EcoR1, Kpn1, and Xbal) that generate segments more 5' in the sequence.18 Two patients (no. 3 and 4) failed to demonstrate any rearrangement of the bcr, although in patient no. 4, a probe more 5' to ours might reveal a rearrangement. Patient no. 3 was recently included in a report by Dr Kurzrock et al.22 They confirmed the lack of bcr rearrangement in patient 3 by using a more 5' bcr probe and, in addition, demonstrated an abnormal 7.4-kb mRNA species and a 190-kd kinase protein. Patient no. 5, also included in their recent report, demonstrated the abnormal 8.4-kb mRNA and 210-kd tyrosine kinase, as seen in cases of CGL.10,13,15,16 These results are consistent with what has been found by other authors indicating that Ph+ positive acute leukemia is heterogeneous with respect to rearrangements of bcr.14,17-23

Our findings indicate that Ph+ acute leukemias demonstrate a high propensity for mixed lineage, as defined by concurrent expression of lymphoid and myeloid associated markers or evidence of T and B lineages in the same leukemia.24,25 AMLL was present in four of the five patients, with patients no. 2 and 3 showing myeloid and lymphoid lineages, patient no. 5 showing T and B cell lineages, and patient 4 showing myeloid, T cell, and B cell lineages. This tendency towards mixed lineage in the Ph+ acute leukemias was suggested as early as 1978 by Janossy et al who noted two patients with Ph+ positive ALL with 15% myeloblasts and 33% lymphoblasts respectively.24 In 1982, Parkin et al reported three of seven patients with lymphoid Ph+ positive lymphoblastic leukemia that had U-MPO, which suggested a myeloid component to what by light microscopy alone appeared to be a purely lymphoid process.26 More recently Nowell et al reported a single case of Ph+ positive acute leukemia in an adult with mixed myeloid and lymphoid morphology.9 In our experience, one of 126 patients with AML had lymphoid antigen expression (CALLA and TdT) (unpublished data). The single patient was Ph+ positive and is reported in this study (patient no. 2). In a review of Greaves et al of 573 patients with AML, 14 had lymphoid-associated markers (CALLA and TdT). Of these 14, two patients were Ph+ positive and were among ten later reclassified as having ALL. Three of the remaining patients showed clear evidence of two populations of blasts.74 At our institution, there is a 30% incidence of mixed myeloid and lymphoid antigen expression in cases morphologically compatible with ALL (unpublished data). This compares favorably with a recent large series where 21 of 76 patients with ALL had dual antigen expression.75 The cytogenetic studies of these patients were not reported. In a review of 30 patients with CGL in blast crisis, only one patient had populations of both myeloid and lymphoid blasts.76

Finally, the RNA dot blot was useful in elucidating lineage heterogeneity in patient no. 3. In this case, by using the MPO probe we were able to detect expression of the MPO gene before the production of sufficient MPO protein to diagnose AML by FAB criteria. Additional evidence to support a myeloid lineage in patient no. 3 was MPO+ positivity. Thus, the use of lineage-associated molecular probes to detect messages will likely enable us to define the lineage at an earlier stage than previously possible.
In summary, we have provided evidence of a high incidence of AMLL in cases of Ph'-positive acute leukemia. We have been able to demonstrate mixed-lineage expression at the molecular level as well as provide evidence to support the expression of lymphoid and myeloid phenotypes by light microscopy, ultrastructure, and immunophenotype. These findings have clinical implications for patients diagnosed as having Ph'-positive acute leukemia due to the possibility of being refractory to therapy targeting a particular phenotypic clone. These findings support the theory of involvement of the pluripotent stem cell in Ph'-positive leukemia and provide evidence for a model in which the stem cell expresses several different phenotypic markers before finally committing itself to a single lineage. Alternative proposals for leukemogenesis could involve derangement of the usual eukaryotic gene expression as a result of chromosomal abnormalities far removed from the sites for transcription of particular genes. Additionally, TdT could play a role in the alteration in gene expression. It is commonly present in AMLL. In this study it was expressed in all of the patients with AMLL. Further studies clarifying the nature of the target cell and regulation of lineage-associated gene expression in cells with the t(9;22) will help in understanding the mechanism of lineage heterogeneity in this subset of acute leukemias.

REFERENCES


52. Mirro J, Antoun GR, Zipf TF, Melvin S, Stass SA: The E-rosette associated antigen of T-cells can be identified on blasts from patients with acute myeloblastic leukemia. Blood 65:363, 1985


57. Royston I, Majda JA, Baird SM. Meserve BL, Griffiths JC: Human T-cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen for T-cells (T65) is also found in chronic lymphocytic leukemia cells bearings surface immunoglobulin. J Immunol 125:725, 1980


64. Breed J, Reinherz EL, Hung PC, Goldstein G, Schlossman


Phenotypic and molecular heterogeneity in Philadelphia chromosome-positive acute leukemia

C Hirsch-Ginsberg, C Childs, KS Chang, M Beran, A Cork, J Reuben, EJ Freireich, LC Chang, FJ Bollum and J Trujillo