Results of Treatment of Ph' + Chronic Myelogenous Leukemia With an Intensive Treatment Regimen (L-5 Protocol)

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Thirty-seven patients with Philadelphia-chromosome-positive (Ph'+) chronic myelogenous leukemia who were untreated or minimally pretreated were entered on the L-5 protocol. This protocol consisted of sequential treatment with splenic irradiation, splenectomy, arabinosylcytosine and 6-thioguanine, and L-asparaginase. Maintenance therapy was hydroxyurea or a multiple-drug regimen. The median survival of the 37 patients is 50 mo. Twelve patients showed a temporary reduction in the percentage of Ph'+ marrow metaphases to less than one-third of the initial values and in 7 of these patients none were found. The duration of the Ph'+ chromosome reduction ranged from 1 to 43 mo. The median survival of the responders has not yet been reached. It is concluded that whereas overall survival is not appreciably extended, patients who have a reduction in Ph'+ cells in the marrow may survive longer than the average; also, the reduction occurs most frequently in patients who have relatively small spleens at diagnosis. The reduction is difficult to maintain, and it may be reinduced in some patients with intensive chemotherapy.

IN 1960 Nowell and Hungerford1 discovered that dividing cells in the bone marrow of patients with chronic myelogenous leukemia (CML) are characterized by a deletion in the long arm of one of the group G chromosomes. Until the advent of the banding techniques, the identity of this chromosome, the so-called Philadelphia (Ph') chromosome, was not certain. Rowley2,3 has established that the Ph' chromosome is not a deletion but a balanced reciprocal translocation between the long arms of chromosomes 9 and 22. Translocations involving chromosome 22 and chromosomes other than 9 have also been observed in a small number of patients with Ph'-positive CML.3 Because of its presence in erythrocyte, megakaryocyte, granulocyte, and monocyte precursors, leukemic transformation is considered to take place in an ancestral cell common to these myeloid cell types.4,5

There is now strong evidence pointing to a uniclonal origin of the Ph'-positive leukemic clone. Patients with CML who were heterozygous for the enzyme glucose-6-phosphate dehydrogenase6,7 and 2 patients with sex chromosome mosaicism8,9 were found to have the Ph' chromosome restricted to just one of their dual cell lines, and this strongly suggests that the Ph'+ stemline arises from a single cell. Although many investigators have found only Ph'+ cells in colonies growing in semisolid media derived from marrow and peripheral blood of patients with CML,10,11 Chervenick et al.12 found that some patients had both Ph'+ and Ph'—
colonies, but no mixed colonies, thus indicating that populations of both normal and leukemic cells exist in the marrow of at least some patients with CML.

The defect is an acquired one, since the Ph' chromosome is not found in lymphocytes7 nor in fibroblasts of skin13 or marrow,7,14 and monozygous twins of patients with CML do not have Ph'+ cells in their marrow.15-19 Only rare instances of familial occurrence have been reported.20

The Ph' chromosome is found in the marrow of about 85% of patients with CML,21 but not all patients with the Ph' marker have it in 100% of marrow metaphases at diagnosis. Sakurai et al. have suggested that patients with some normal cells at diagnosis may survive longer.22 Two interesting examples are the patients reported by Gatti23 and Canellos,24 both of whom had some normal metaphases at diagnosis of Ph'+ CML. Both were followed for 5 yr before chemotherapy was instituted.

The literature contains isolated reports of patients with reduced populations of Ph'+ cells after therapy of chronic-phase CML, and many of these patients are among the long survivors (7-17 yr) in busulfan-treated series.25-33 Seven patients were reported with reduced Ph'+ cells on examination 3-16 yr after diagnosis, and there are two reports29,35 of complete absence of the marker at 9 and 13 yr. All of these patients were treated with busulfan, some to marrow hypoplasia29,32,33 and others not so intensively.33,34 There have also been a few reports of reduction in Ph'+ cells with treatment other than busulfan, i.e.,32P, 6-mercaptopurine (6-MP), methotrexate (MTX), vincristine (VCR), and prednisone,36-39 including 2 patients who presented in the blastic phase of CML.38,39 One of these latter patients survived 24 mo; the other, who was seen at Memorial Hospital, survived 13 mo, with transient complete loss of the Ph' marker from the marrow.39

In 1924 the median survival of untreated CML patients was reported by Minot et al.40 to be 31 mo from onset of symptoms. Since then, therapeutic attempts to improve survival have been generally disappointing; median survivals from diagnosis of 1 1/4-3 1/2 yr have been reported in several large series.26,30,41-43

The retrospective study by Monfardini et al.44 of all CML patients seen at Memorial Hospital from 1948 through 1967 revealed an overall median survival from diagnosis of 31 mo, with a longer median (47 mo) for the 19 patients who received 32P, splenic irradiation, and chemotherapy. Because conventional treatment had not appreciably extended survival in almost 50 yr, it was decided that a more aggressive therapeutic approach to treating early CML might be beneficial.

In recent years attention has focused on the role of the spleen in CML. Both the Ph' chromosome and additional stemlines that characterize progression of the disease have been noted in the spleen,45,46 and a few reports have suggested that both these markers may be seen in the spleen before the marrow.47-50 Armenta et al.48 reported a patient with a long history of polycythemia vera whose disease changed to CML in blastic transformation; the Ph' chromosome was found in the spleen and lymph nodes but not in the marrow or peripheral blood when examined up to 5 mo later. There are also several reports of more typical cases of CML in which blastic transformation first appears to have occurred in the spleen49-51 or other extramedullary sites.52,53 Loss of aneuploidy as well as a decrease in the percentage of Ph'+ cells have been noted with remission of blastic crisis induced by chemotherapy.37,39,54,56 There has been one report of response to splenectomy that
resulted in loss of hyperdiploidy and reduction of the Ph\(^+\) metaphases from 100% to 23%, with survival of 3 yr after transformation.\(^{51,57}\)

Our therapeutic program, the L-5 protocol, was designed to include early splenectomy (Fig. 1). It was reasoned that splenectomy would remove a large mass of leukemic cells and eliminate a potential site for blastic transformation and, in addition, obviate the late complications of massive splenic enlargement and hypersplenism. Irradiation was administered prior to splenectomy to reduce the size of the spleen and to control the leukocyte and platelet counts, because elevated levels may be associated with hemorrhagic and/or thromboembolic complications during or following surgical procedures.

It was further reasoned that drugs that are effective only against proliferating cells should be selectively lethal to the Ph\(^+\) cells, which have an (undefined) proliferative advantage.\(^{58}\) Arabinosylcytosine (ara-C) and 6-thioguanine (6-TG) were chosen as the primary chemotherapy because this combination had been found to have at least some selectivity of action in acute myelogenous leukemia (AML).\(^{59}\) Three courses were given, because some patients with AML required three courses before achieving remission.\(^{60}\) It had previously been shown that L-asparaginase destroyed leukemic cells in CML with fair regularity and that it had relatively little effect on normal hematopoiesis,\(^{61}\) and thus this drug was added to the regimen to further reduce the leukemic population. The decision regarding further treatment depended on the results of chromosome analysis at the end of the intensive phase of treatment.

**MATERIALS AND METHODS**

**Patient Population**

Between January, 1970, and July, 1976, 37 adults with Ph\(^+\) CML were entered on the L-5 protocol. Patients were excluded if they were more than 60 yr old, if they were considered to be poor operative risks or too unreliable to follow the protocol, or if they elected to receive conventional treatment after being told that the L-5 protocol was experimental. Initially, patients were also excluded if they were thought to be undergoing blastic transformation, but later 2 patients (No. 31 and No. 37) were entered to determine whether there would be any beneficial effect on advanced disease. Previously untreated patients were preferred, but some patients with prior therapy were accepted if there were none of the contraindications listed above. There were 26 males and 11 females ranging in age from 16 to 52 yr; the median age was 33 yr.

Tables 1 and 2 show the hematologic status of each patient at the time of beginning the protocol. Sixteen patients had received no prior treatment, except for short-term leukopheresis in 3 patients. Fifteen patients had had prior busulfan; the duration of treatment was 4 mo or less, except in 2 patients.
Table 1. Hematologic Status at Start of L-5 Protocol of Patients Showing Reduction in Ph' + Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Prior Therapy</th>
<th>Duration</th>
<th>WBC* (× 10^9/liter)</th>
<th>Hgb* (g/dl)</th>
<th>PLT* (× 10^9/liter)</th>
<th>Spleen* (cm)</th>
<th>Percentage Blast At Start of L-5 (%)</th>
<th>Lowest (%)</th>
<th>Ph' Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>M</td>
<td>None</td>
<td>24 mo</td>
<td>12.8</td>
<td>318</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>None</td>
<td>16 mo</td>
<td>14.2</td>
<td>456</td>
<td>3</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>F</td>
<td>None</td>
<td>14 mo</td>
<td>10.3</td>
<td>536</td>
<td>0</td>
<td>1</td>
<td>3.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>F</td>
<td>None</td>
<td>4 mo</td>
<td>12.5</td>
<td>750</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100</td>
<td>6</td>
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<tr>
<td>5</td>
<td>41</td>
<td>F</td>
<td>None</td>
<td>1 mo</td>
<td>13</td>
<td>538</td>
<td>0</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>F</td>
<td>BUS 1 mo (1 yr prior)</td>
<td>53</td>
<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>M</td>
<td>BUS, ARA 4 days</td>
<td>187</td>
<td>12.2</td>
<td>811</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>M</td>
<td>BUS 1 mo</td>
<td>177</td>
<td>16.3</td>
<td>439</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>F</td>
<td>ARA, TG 10 days</td>
<td>475</td>
<td>14.6</td>
<td>278</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>47</td>
<td>M</td>
<td>HU, BUS 3 wk</td>
<td>587</td>
<td>5.0</td>
<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>M</td>
<td>ARA 5 days</td>
<td>98</td>
<td>13.5</td>
<td>523</td>
<td>6</td>
<td>5.5</td>
<td>96</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>M</td>
<td>BUS 2 mo</td>
<td>109</td>
<td>12.2</td>
<td>276</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>96</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values at beginning of L-5 protocol: BUS = busulfan; ARA = arabinosylcytosine; TG = thioguanine; MTX = methotretate; 6-MP = 6-mercaptopurine; HU = hydroxyurea

who had received busulfan for 12 and 24 mo. One patient had received 6-MP, MTX, and splenic irradiation (300 rads). Six patients were given ara-C, either by continuous 24-hr infusion or every 12 hr with TG (1 patient), just prior to starting the protocol because of very high leukocyte counts (160–548 × 10^9/liter) and the clinical necessity of rapidly reducing these counts. Three of the latter patients presented with priapism that resolved after the ara-C infusions.

The numbers of Ph' + cells present in the marrow of each patient before beginning the L-5 protocol are shown in Tables 1 and 2. All patients initially had normal 46 stemlines except patient No. 26, who had both 46 and 47 stemlines (hyperdiploidy has been reported to herald blastic transformation, but this did not occur in her case until 29 mo after this was first noted). All but 3 patients initially showed 75%–100% Ph' + metaphases; the others were patient No. 19, who had 52% and 50% Ph' + cells on two determinations 10 days apart prior to initiation of therapy, patient No. 12, who had 86% initially and 38% after busulfan, and patient No. 21, who had two pretreatment studies, one of which showed 100% and the other 26% (vide infra).

Treatment Program (L-5 Protocol)

The protocol began with irradiation to the spleen (Fig. 1). Radiation was delivered through anterior and posterior portals using cobalt 60 in doses of 50–100 rads three times weekly for a total of 150–1950 rads. The median does was 825 rads given over 24 days. Three patients (Nos. 24, 26, and 12) were not given irradiation, as their peripheral blood counts were already near normal after 4, 8, and 12 wk of busulfan.

After the spleen was reduced in size and the peripheral blood counts were nearing normal levels, irradiation was stopped and the patient was allowed to rest until the blood counts stabilized at satisfactory levels. The amount of time between completing irradiation and surgery depended on the time required for the blood counts to recover and ranged from a few days to 2 mo, with an average of 30 days. At the time of surgery the white blood cell (WBC) and platelet counts were within the normal range in the majority of patients. In 3 patients there was difficulty in controlling the WBC count with splenic irradiation. Patient No. 37, whose disease was accelerating at entry (16% blasts), had progression of disease while receiving 400 rads, with rapid increases in WBC, marrow blasts, and spleen size. He was subsequently treated with chemotherapy and splenectomy without control of his disease. The other 2 patients (Nos. 20 and 22) initially had good responses to 790 and 590 rads, but within 4 wk of stopping irradiation the WBC counts rose rapidly, and it was necessary to reinstitute irradiation.
Blastic transformation occurred in one of these patients (No. 20) during his second course of irradiation and in the other (No. 22) a few weeks after splenectomy.

Splenectomy was performed on 36 patients; the 1 patient who was not operated on was No. 20. There were no complications during surgery and no operative deaths. One patient (No. 15) was found to have extensive paraaortic node involvement at surgery, and shortly thereafter he developed extramedullary blastic transformation in his peripheral lymph nodes. He was withdrawn from the protocol and given other treatment, but his marrow became blastic 6 mo later. Another patient (No. 35) was found to have blastic marrow after recovery from surgery, and she was similarly given other therapy. Complications during the postoperative period included a pulmonary embolism 8 days after surgery in 1 patient (No. 13) when his platelet count was over 1000 $\times 10^9$/liter. One patient developed a wound infection and had two episodes of gastrointestinal bleeding postoperatively; the sites of bleeding were not determined. All recovered completely.

Chemotherapy was begun after recovery from surgery, usually about 1 mo later, in the 32 patients who remained in the "chronic" phase. The protocol was discontinued in the 5 patients who had already recovered completely.
undergone blastic transformation, and they received other chemotherapy. In most cases the WBC counts remained stable postoperatively, but 3 patients (Nos. 13, 18, and 25) required hydroxyurea in the postoperative period to control rapidly rising WBC counts until they had recovered from the operation and could tolerate more intensive chemotherapy. Postoperative thrombocytosis occurred to more than 500 $\times$ 10$^9$/liter in 13 patients and to more than 1000 $\times$ 10$^9$/liter in 9 others. Ara-C (3.0 mg/kg i.v.) was given with TG (2.5 mg/kg p.o.) every 24 hr to the first few patients, and later every 12 hr to the majority of patients. The patients were treated similarly to those with AML on the L-6 protocol, but not so intensively as to cause severe marrow hypoplasia. Patients usually received three courses of ara-C and TG, each time in sufficient dosage to cause moderate marrow hypocellularity, with rest periods of 3–4 wk between courses. Immediately following the last course of ara-C and TG, most of the patients (24 of 32) were given L-asparaginase (E. coli) at 200 IU/kg i.v. three times a week for six doses.

The choice of treatment at the conclusion of the protocol depended on the chromosome response. Several patients who initially showed a reduction of Ph$^+$ cells in the marrow with subsequent return to Ph$^+$ predominance were given additional courses of intensive chemotherapy in an attempt to reinduce a response, either at the end of the protocol or during maintenance. Three of these patients (Nos. 6, 11, and 12) received daunorubicin and ara-C, and two of them (Nos. 6 and 11) were treated for severe marrow hypoplasia. In an attempt to continue suppression of the Ph$^+$ population in the responders, therapy was continued with the maintenance part of the L-6 protocol for AML, which consists of rotating courses of multiple drugs as previously described and as outlined in Fig. 2. In cases in which there had been no significant decrease in the percentage of Ph$^+$ cells or in whom attempts to reinduce a response had failed, hydroxyurea was given, and the dosage was adjusted according to the results of weekly blood counts (usually 20–40 mg/kg/day as a single dose). Maintenance therapy was continued indefinitely until blastic transformation or acceleration of the disease dictated a change to more aggressive treatment. Several patients required additional treatment with thio-TEPA and/or melphalan to reduce excessively high platelet counts that could not be controlled with hydroxyurea.

**Cytogenetic Methods**

Before and after each phase of the protocol, bone marrow was aspirated for evaluation of cellularity and differential count and was analyzed for the presence of the Ph$^+$ chromosome. Marrow cells were incubated for 1–2 hr in a balanced salt solution that also contained Colcemid. Chromosome preparations were made following standard procedures; 0.075-M KCl was used as the hypotonic, and acetic acid/methanol (1:3) was used as the fixative. Slides were stained either in Giemsa or aceto-orcein and by the G-banding procedures. ASG as well as trypsin methods were used for producing G-banding. In unbanded preparations the Ph$^+$ chromosome was identified by the presence in the complement of a group G chromosome with a conspicuously short long arm. In the banded preparations it was identified by its banding pattern.

Whenever possible, 25 to 50 marrow metaphases were counted. Responses were arbitrarily considered significant only if the percentage of Ph$^+$ metaphases decreased to less than one-third of the value obtained just prior to starting the L-5 protocol.

Leukocyte alkaline phosphatase (LAP) scores were not followed serially in most patients and were not used as criteria for response. Only a few patients were studied for terminal deoxynucleotidyl transferase activity at blastic transformation, as this test was not available during the major part of the study.

![Fig. 2. Maintenance part of L-6 protocol](image-url)
RESULTS

Survival

The median survival of the 37 patients since diagnosis is 50 mo (Fig. 3). Fourteen patients are alive, all in the chronic state at 17–107 mo after diagnosis. Twenty-three patients have died, 19 of them after blastic transformation at 5–61 mo after diagnosis. Three of the latter group (Nos. 31, 26, and 32) also developed myelofibrosis; their survivals were 17, 34, and 50 mo. None of the 19 patients with blastic transformation responded satisfactorily to subsequent chemotherapy, and their median survival from transformation was 3½ mo. Two patients (Nos. 18 and 13) died in an accelerated phase of the disease at 12 and 40 mo; they were thus termed because the disease was clearly out of control and was poorly responsive to therapy, although they did not fully meet our criteria for blastic transformation (30% blasts in the blood and 50% in the marrow). The cause of death of patient No. 34 is unknown, as he died in another country. One patient (No. 11) died of lung cancer while his CML was in complete remission 52 mo after diagnosis; his course is described in Fig. 4.

Ph' + Chromosome Response

Of the 37 patients who began the protocol, 5 patients became blastic before chemotherapy was begun, 1 patient failed to follow the prescribed protocol and had protracted intervals between phases of treatment, and 2 other patients were inevaluable for chromosome response because most of their marrow aspirations were inadequate for study due to development of myelofibrosis. Of the remaining 29 patients evaluable for chromosome response, 12 patients were considered to have significant reductions in the percentages of Ph' + cells in their marrow (< one-third of baseline value), and 17 patients did not respond. Transient reduction
Fig. 4. Course of patient No. 11, who had a relatively small spleen at diagnosis and who showed reduction in Ph' + cells after splenic irradiation and after splenectomy and a further reduction after chemotherapy. After December, 1974, the percentage of Ph' + marrow metaphases remained zero to 6% until his death in November, 1977. The last (single) Ph' + metaphase found was in August, 1976. No Ph' + cells were found thereafter among a total of 153 metaphases examined in preparations made at approximately 2-mo intervals during the last year of his life, including one about a week prior to death when 30/30 Ph'-negative cells were found with 46 normal chromosomes. During the last 3 yr he continued treatment on the L-6 maintenance, and except for transient marrow suppression due to the chemotherapy, his hematologic status remained normal. He remained clinically well until July, 1977, when he developed meningeal carcinomatosis, originating from a small peripherally located adenocarcinoma of the lung; no other metastases were detectable. He failed to respond to cranial irradiation, prednisone, and intrathecal and intraventricular chemotherapy, and he died as a result of progression of his meningeal disease and pneumonia due to Pneumocystis carinii.
occurred in some of the nonresponders, but in no case was the minimum value less than 50% of baseline, except in patient No. 21, who will be described later. The lowest percentages of Ph'+ metaphases achieved in each patient are noted in Tables 1 and 2.

The median survival of those patients with reduced populations of Ph'+ cells has not been reached, as only 4 patients have died, but it appears that it will be appreciably longer than the 34-mo median of nonresponders (Fig. 3). Three of the responders (Nos. 8, 10, and 9) died between 3 and 7 mo after undergoing blastic transformation, which occurred at 36, 45, and 59 mo after diagnosis, respectively. One responder (No. 11) died in remission, as noted previously. The other 8 responders continue in the chronic phase at 17–107 mo.

There was considerable variation in the duration of the Ph' chromosome reduction. Reduction lasted less than 2 mo in 3 patients (Nos. 7, 8, and 12) and 2–4 mo in 5 others (Nos. 2, 3, 4, 5, and 9); it was inevaluable in patient No. 10, whose follow-up study was not done for 9 mo. One patient (No. 1) had two periods of reduction of 8 mo each (Fig. 5), and only 2 patients had sustained reduction for more than 3 yr (Nos. 6 and 11).

Reduction was first noted at different points in the protocol: after irradiation in 2 patients (Nos. 7 and 11), after splenectomy in 2 patients (Nos. 4 and 6), and after the first or second course of ara-C and TG in 7 patients (Table 3). One other patient (No. 5) showed an absence of Ph'+ cells after treatment with ara-C and TG, but since no examinations were made after irradiation or splenectomy, it is not possible to determine at which point the reduction began.

Two of the previously mentioned patients who showed reductions in Ph'+ cells before receiving chemotherapy had the longest duration of response. Patient No. 11 showed 2% Ph'+ cells after completion of the intensive chemotherapy, and this remained at zero to 8% for the remaining 42 mo of his life (Fig. 4). Patient No. 6 maintained zero to 2% Ph'+ cells in her marrow for 43 mo while she received first maintenance L-6 therapy and later hydroxyurea. At her request, all therapy was

Table 3. Reduction in Percentage of Ph' + Metaphases As Related to Phase of L-5 Protocol

<table>
<thead>
<tr>
<th>Patient</th>
<th>Prior Therapy</th>
<th>Before L-5</th>
<th>After Splenic Irradiation (RT)</th>
<th>After Splenectomy</th>
<th>After Ara-C + TG Courses:</th>
<th>After L-asparaginase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-5</td>
<td>Irradiation (RT)</td>
<td>Splenectomy</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3/3</td>
<td>89</td>
<td>81</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>23</td>
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</tr>
<tr>
<td>3</td>
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<td>100</td>
<td>98</td>
<td>71, 86</td>
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<td>5</td>
<td>0</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
<td>3/8</td>
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</tr>
<tr>
<td>6</td>
<td>+</td>
<td>82</td>
<td>89</td>
<td>92, 32</td>
<td>13</td>
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</tr>
<tr>
<td>7</td>
<td>+</td>
<td>97</td>
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<td>10</td>
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<td>43</td>
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</table>

*ND = Karyotype not done.
†X = Course not given.
then discontinued; her marrow metaphases were 16% Ph'+' at that time. Subsequently the percentage of Ph'+' cells gradually increased to 82% over a period of 10 mo, and later to 100%. However, she remained clinically well and required no treatment for 31 mo after stopping chemotherapy, at which time her WBC rose from around 30 × 10^9/liter to nearly 100 × 10^9/liter. Hydroxyurea was again initiated, with a satisfactory response of the WBC, but her marrow remains 100% Ph'+' 107 mo after diagnosis.

The responses first noted after ara-C and TG were all of short duration; in 5 of the 7 patients the majority of marrow cells were again Ph'+' by the end of the intensive part of the protocol. In 4 of these patients (Nos. 1, 8, 9, and 12) an attempt was made to reinduce a response with additional intensive treatment. This was successful transiently in 3 patients (Nos. 1, 9, and 12). The best response was in patient No. 1, whose marrow remained consistently negative for over 8 mo after the first course of ara-C and TG (Fig. 5), after which Ph'+' cells reappeared in the marrow while he continued on hydroxyurea. The Ph'+' cells did not diminish on retreatment with ara-C and TG, but they largely disappeared for another 8 mo after institution of the L-6 maintenance regimen.

Response was never noted later than the ara-C and TG phase of the protocol, unless reinduction was attempted. Eleven of the 12 patients who showed reduction received L-asparaginase, but in all cases it was given after the initial decrease had been demonstrated, and in four of these cases the Ph' marker had already reappeared (Table 3). L-asparaginase did not effect reduction in any of the patients. The maintenance therapy did not result in reduction except in the case of patient No. 1, who showed a second reduction after starting the L-6 maintenance, as noted previously.

In addition to the 12 patients who responded, there were 2 "nonresponders" with significant numbers of Ph'-negative cells, but in neither was this attributable to the L-5 protocol. Patient No. 21 had two studies done prior to receiving splenic irradiation; one showed 100% Ph'+' metaphases of 11 counted, and the other showed 26% of 19. He had received no chemotherapy and was receiving only an (unidentified) anticoagulant after a myocardial infarction. After he received a total of 475 rads to the spleen, his marrow showed 10% Ph'+' cells of 19 scored, but after splenectomy the marker was found in 88%, and there was no change thereafter. Another patient (No. 19) who had about 50% Ph'+' cells on repeated studies prior to any treatment continued to show similar proportions of Ph'+' and Ph'− cells throughout the L-5 protocol, and at its completion there were still 36% Ph'+' cells in the marrow. His blood counts remained stable with very little treatment except intermittent hydroxyurea for the next 2½ yr, but the percentage of Ph'+' cells in the marrow gradually increased to nearly 100%. He has remained clinically well with his disease under control on hydroxyurea 43 mo after diagnosis, with nearly all marrow metaphases demonstrating the Ph' marker.

Factors That May Influence Response

Among the variables that might possibly predict a favorable prognosis for survival or loss of the Ph' marker, it appears that the size of the spleen at diagnosis may be important. Measurements of spleen size on palpation at diagnosis were available for 34 patients. Sixteen had relatively small spleens (<7 cm below the left
Fig. 5. Course of patient No. 1, who was asymptomatic and had no palpable spleen at diagnosis and who had two prolonged periods of reduction of Ph' + cells. While on maintenance hydroxyurea, no Ph' + cells were found for 8 mo, when they began to reappear in the marrow in November, 1973, and gradually increased until there were over 50% in May, 1974. There was no significant reduction on reinstitution of additional courses of ara-C and TG, but after the start of the L-6 maintenance, the percentage of Ph' + cells fell to 6% and remained 2% - 7% for the next 8 mo. Beginning in March, 1975, the Ph' + cells again began to increase, and the marrow has shown 99% - 100% Ph' + cells since November, 1975. He remained clinically well with nearly normal blood counts on hydroxyurea until December, 1977. At his request, another attempt was then made to reduce the Ph' + population with daunorubicin and ara-C, but except for a slight reduction this was unsuccessful. He presently remains well, still in the chronic phase of his disease.
costal margin) at diagnosis, whereas the other 18 had larger spleens (>10 cm). The median survivals of the two groups are similar at the present time: 44+ mo in the former group and 37 mo in the latter group. However, 63% of the patients with small spleens are still alive, and the eventual median survival of this group will probably be appreciably longer than that of the larger-spleen group, in which only 22% of patients are alive.

It is noteworthy that 8 of the 12 patients with Ph' chromosome reduction had small spleens at diagnosis (Table I), and their diseases appeared to be slowly progressive. Additionally, in 3 of these patients (Nos. 1, 2, and 6) the WBC and platelet counts remained stable at moderate levels or rose only very gradually for many months before institution of any treatment. This group with small spleens and indolent disease included the 3 patients (Nos. 1, 6, and 11) with the longest chromosome response. Seven of the 8 responders with small spleens are still alive; the only death resulted from an unrelated carcinoma (No. 11). Four of the responders had large spleens at diagnosis (Table 1). The duration of chromosome response was very brief in all of these patients, and in 3 of these patients the disease underwent blastic transformation and the patients died.

At the time of beginning the protocol there was a total of 25 patients with small spleens because some patients who had large spleens when diagnosed had responded to chemotherapy before coming to Memorial Hospital. This group with small spleens had an appreciably better response; their median survival is 45+ mo, with 48% still alive, as compared with 29 mo, with only 16% still alive, for the 12 patients with larger spleens, half of whom had received no prior treatment.

Of the 25 patients with small spleens, 22 were given splenic irradiation (the other 3 were considered to have had adequate control of spleen size and WBC following busulfan). In reviewing this study it became evident that these 22 patients had received a higher dose of irradiation per gram of splenic tissue than the group with larger spleens. The median dose in the former group was 875 rads (range 200-1550 rads), as compared with a median dose of 600 rads (150-1950 rads) for the 12 with larger spleens. The median weight of the 24 spleens in the small group was 318 g, and that of the larger spleens was 795 g (one of the patients in the former group did not have a splenectomy). It is interesting to note that the 4 patients with larger spleens who received higher amounts of irradiation (>875 rads) had smaller spleen weight (525 g versus 930 g) and longer survival than those who received less irradiation (46 versus 25 mo). There was no correlation between pretreatment WBC level and amount of irradiation. The amount of irradiation received depended on the rate at which the WBC count and spleen size decreased and the appraisal of the examining physician.

These findings suggest a correlation of small spleen size at diagnosis with longer survival and greater probability of Ph' chromosome reduction; however, as is evident from examination of Table 2, some patients with small spleens failed to show a reduction in Ph'+ cells. The dose of splenic irradiation may also be important, but this study was not designed to answer that question, and no conclusions can be drawn concerning the significance of irradiation dosage.

Another difference between responders and nonresponders was that marked thrombocytosis (>1000 × 10^9/liter) occurred at some time in the protocol in only 4 patients in the group with the Ph' reduction, whereas it was frequently observed in
the nonresponders (16 of 20). The 4 responders were patients Nos. 4, 7, 8, and 10, and all of these patients had only very transient reduction in Ph'+ cells. Platelet counts between 500 and 1000 $\times 10^9$/liter were observed at some time in most patients in both groups, and no correlation of this moderate thrombocytosis with response can be made.

In only two cases was thrombocytosis a life-threatening problem. The one postoperative pulmonary embolism has already been noted. Another patient (No. 26) experienced an abrupt rise in platelets to 7000 $\times 10^9$/liter while recovering from the last course of ara-C and TG, and she developed multiple pulmonary emboli and cardiac arrest. She was successfully resuscitated, and with chemotherapy her platelet count was reduced to 357 $\times 10^9$/liter. She lived another 2 yr before her death due to myelofibrosis and megakaryocytic leukemia with massive liver involvement.

One other patient (No. 6), who was a responder, had a thrombotic complication while on the protocol, but it was not related to thrombocytosis. About 2 mo after completing the intensive phase of chemotherapy she developed thrombophlebitis of an iliac vein and pulmonary emboli while on the L-6 maintenance arm of the protocol. Her platelet count remained between 180 and 350 $\times 10^9$/liter. She eventually required plication of the inferior vena cava and had no further complications until 6 yr later, when she developed thrombophlebitis of the other iliac vein; on this occasion the platelet count was around 500 $\times 10^9$/liter. The thrombophlebitis responded to conservative therapy.

**DISCUSSION**

Analysis of the L-5 protocol has revealed the following facts: (1) A significant reduction in the Ph'+ population is possible in a certain number of cases (32% in this study). (2) This reduction occasionally can be achieved by splenic irradiation followed by splenectomy as well as by chemotherapeutic treatment. (3) Severe marrow hypoplasia is not required. (4) The reduction is usually transient and difficult to maintain for more than a few months. (5) The reduction may sometimes be reinduced by further intensive treatment with ara-C and TG or daunomycin and ara-C, but not by L-asparaginase or hydroxyurea in conventional dosages. (6) The reduction of the Ph'+ population most often occurs in patients with small spleens and slowly progressive disease, and it is in this group that duration of response is longest. (7) Patients showing a reduction may survive longer than those who do not; it is not known whether or not this longer survival is related to treatment with the L-5 protocol, because it is quite possible that such patients with slowly progressive or highly responsive disease might also live longer with less intensive treatment. (8) Although the 50-mo median survival for all patients treated with the L-5 protocol is longer than that of our historical control series of CML patients (31 mo), it cannot be concluded that the L-5 protocol is responsible for prolonging survival, because the patients entered on this protocol were a selected group in that some patients with early blastic transformation were excluded. Moreover, unlike the situation in our historical series, only Ph'+ patients were included in the L-5 study, and they are known to have longer survival than patients with Ph'-negative CML.
There have been seven other series reported in which splenectomy was performed in CML,\(^6\)\(^{1}\) including more than 150 comparable patients. All were prospective studies except that of Didolkar et al.\(^6\)\(^8\). All or most of the patients in each series were Ph\(^+\), and most of the operations were performed within 1 yr of diagnosis. Median survivals had been reached in three studies: 40 mo,\(^6\)\(^8\) 43 mo,\(^6\)\(^5\) and 44 mo\(^6\)\(^7\) from diagnosis. These median survivals are comparable to the 50-mo median in our L-5 series. The median survival times for the other series have not been established, but two are reported to be ongoing at 30+ mo\(^\dagger\) and 36+ mo.\(^6\)\(^9\) It is thus likely that all studies will show similar median survival times of between 3 and 4 yr, and we can conclude that early splenectomy in CML does not appreciably extend survival. The incidence of blastic transformation was reported for only two of these series:\(^6\)\(^5\)\(^6\)\(^7\) 100% and 76%. The median survivals from transformation were 2 and 4 mo, which are similar to the 3\(1/2\) mo found in our series.

In almost all cases, early splenectomy was relatively free of serious complications and probably beneficial in preventing late complications associated with massive splenomegaly. Operative mortality was reported by three groups, from infection in 17%\(^6\)\(^5\) and 2%\(^6\)\(^6\) of patients and from bleeding in 9%.\(^6\)\(^8\) As in our study, there were no operative deaths in three other series.\(^6\)\(^4\)\(^6\)\(^7\)\(^6\)\(^9\) Operative morbidity varied from 12% to 34% in the three studies in which it was reported,\(^6\)\(^4\)\(^6\)\(^6\)\(^7\)\(^6\)\(^7\) as did the seriousness of the morbidity observed. Postoperative thrombocytosis to levels over 1000 \(\times\) 10\(^9\)/liter was reported in 16%,\(^6\)\(^7\) 33%,\(^6\)\(^5\) 54%,\(^6\)\(^4\) and a “majority”\(^6\)\(^6\) of patients in the series in which platelet counts were recorded. The median spleen weight at operation varied, as did the choice of preoperative chemotherapy. Didolkar et al.\(^6\)\(^8\) reported mean spleen weights in chronic (1408 g) and blastic (1830 g) cases. The chronic cases had received busulfan, 6-MP, or hydroxyurea, and operation was carried out when the disease was out of control. Tura et al.\(^6\)\(^6\) reported a median spleen weight of 906 g in patients who had received hydroxyurea and were not in remission. Ihe et al.\(^6\)\(^7\) found the median spleen weight to be less than 500 g in patients who were in “remission” from busulfan.

Chemotherapeutic regimens varied in the different series of splenectomized patients. Most groups relied on conventional treatment with busulfan, dibromomannitol, or hydroxyurea prior to surgery,\(^6\)\(^4\)\(^6\)\(^9\) and the study by Didolkar et al. included some who had also received splenic irradiation.\(^6\)\(^8\) Three groups employed intensive antimetabolite therapy at various points in their protocols: TG,\(^6\)\(^4\)\(^7\)\(^2\) ara-C,\(^6\)\(^6\) and ara-C and TG.\(^6\)\(^9\) The final results of these studies have not been reported. The preliminary report by Fuscaldo et al.\(^6\)\(^9\) suggests that this approach may improve survival, as the only three deaths in the group of 16 patients were at 5, 5\(1/2\), and 7\(1/2\) yr.

Only three of these reports\(^6\)\(^4\)\(^6\)\(^5\)\(^6\)\(^7\) included evaluation of chromosome response to therapy. Schwarzenberg et al.\(^6\)\(^5\) concluded that the Ph\(^+\) marker “persisted,” and Spiers et al. noted that in a group of 17 patients treated intensively with TG prior to splenectomy, all maintained 100% Ph\(^+\) marrow.\(^7\)\(^2\) However, in the group reported by Fuscaldo et al.\(^6\)\(^9\) and Brodsky\(^7\)\(^0\) at least 2 of 16 patients have shown reductions in Ph\(^+\) cells to date. One had a decrease from 100% to 13% Ph\(^+\) metaphases after splenectomy, and another showed a reduction from 67% to 16% after cell-cycle-specific chemotherapy.\(^7\)\(^0\)
The seven prospective early splenectomy protocols are similar with regard to basic treatment approach and probable ultimate median survival. Of the seven early splenectomy series, our study is one of only two in which Ph'+ population reduction was apparent. The reasons for this are not clear. However, as has been noted, the duration of repopulation with Ph'-negative cells in the marrow was short in some of our patients and might have been missed if frequent serial cytogenetic examinations had not been performed. It seems possible that similar instances of transient repopulations with Ph'-negative cells might have been missed in some of the other series due to failure to conduct cytogenetic examinations sufficiently frequently after treatment. In our series there was, in addition, a relatively low operative morbidity (11%) and a low incidence of postoperative thrombocytosis to levels above $10^9$/$\text{liter}$ (23%), and the median spleen weight (358 g) was smaller than in the other series noting it. The principal difference between our protocol and the others was that splenic irradiation rather than chemotherapy preceded splenectomy. It is possible that this treatment achieved better control of the disease and played a role in the decreases in the Ph'+ cell populations that we observed. The Medical Research Council study concluded that splenic irradiation was not as effective as busulfan in terms of extending survival, but the timing and doses of irradiation were not consistent in the radiation therapy group and the patients received various chemotherapeutic agents in addition. The role of irradiation as primary treatment is currently being investigated by Jacobs et al.

Smalley et al. treated a series of selected patients intensively with ara-C and TG after their diseases had been brought under good control with busulfan; they had nearly normal blood counts and no splenomegaly at the time of beginning ara-C and TG. Splenectomy was not done. Two of the 10 evaluable patients showed a significant reduction in Ph'+ cells. One patient showed complete absence of the Ph' marker on one determination after 3 mo of treatment, with 11% after six courses. She was alive at 6 yr after diagnosis. The other patient showed only 7% Ph'+ cells after the sixth course, which is particularly interesting because his marrow had been consistently 100% Ph'+ through five courses, as was the study following the 7% determination.

Although overall median survival may not have been significantly prolonged by the aggressive approach that all these studies demonstrate, the possibility remains that survival may be longer in those patients who show evidence of decreases in percentages of Ph'+ metaphases in their marrow, as suggested by the occasional reports prior to these current studies, the preliminary reports of Fuscaldo and Smalley, and the present report of our group.

Significance of Study in Relation to Pathophysiology of CML

In CML there is characteristically a greatly enlarged total granulocyte pool and a high granulocyte turnover. The extent of enlargement of the granulocyte pool and the rapidity with which any CML population expands varies, and the level at which any CML population equilibrates so that production and death rates are equal differs among different populations and depends on the cells' degree of escape from normal controls.

In early CML, Ph'+ myeloblasts are able to differentiate almost normally, and the mature cells generally have only minor enzymatic and functional defi-
These deficiencies often appear to be related to the increased cell density that occurs, because the values may return toward normal after the cell concentration has been reduced by therapy (e.g., leukocyte alkaline phosphatase and platelet functional abnormalities). Because Ph'+ cells are capable of maturing and performing most of the essential functions of normal cells, they are able to support life even when no normal hematopoietic cells are present.

During the chronic phase of the disease the percentage of myeloblasts in CML is generally not increased and may even be lower than that found in normal marrow. However, because of the greatly expanded mass of granulopoietic cells in the marrow, spleen, blood, and sometimes other tissues, the absolute number of myeloblasts may be considerably increased. The Ph'+ myeloblasts and later granulocytic precursors in CML generally proliferate more slowly and pass through the maturation compartments more slowly than the corresponding cells in normal marrow. In untreated CML, the Ph'+ myeloblasts in the marrow usually have a mitotic index (MI) of about 1% or less and a pulse ³H-thymidine labeling index (LI) of around 20%, which are roughly half the values generally found for myeloblasts in normal subjects. The mean intermitotic time of Ph'+ myeloblasts is usually around 60 hr, or about twice as long as that of normal myeloblasts. It has also been shown in the chronic phase of CML that there is a markedly increased incidence of colony- and cluster-forming cells in the marrow and blood that show normal in vitro maturation, colony size, and colony/cluster ratio. Using the ³H-thymidine suicide method, it has also been shown that only about 20% of the colony-forming cells (CFC) in CML during the active phase of the disease are in DNA synthesis, as compared with about 40% of the CFC in normal marrow. These values are very close to the pulse ³H-thymidine LIs of myeloblasts found in CML and normal marrow, respectively.

Stryckmans has reported a relationship between WBC count and the ³H-thymidine pulse LI in CML. When the WBC count was greater than 40,000 cells/cu mm the mean myeloblast LI was about 20%, whereas when the WBC count was below 20,000 cells/cu mm during early disease or after treatment, the average myeloblast LI was 46%, or in the same range as that found for myeloblasts in normal marrow. During relapse after discontinuing treatment, the myeloblast LI again decreased. The normal LI values in CML patients with low WBC counts were not due to repopulation of the marrow with normal cells, since all of the marrow metaphases were Ph'+ in this series of patients. Thus the lower proliferative rates reported for CML myeloblasts in the studies referred to earlier may be due to the fact that proliferation was slowed as a result of high cell density rather than because they inherently proliferate more slowly than normal myeloblasts.

The Ph'+ cell population invariably replaces the normal one. The proliferative advantage of the Ph'+ cells presumably depends on their degree of unresponsiveness to normal controls, so that the Ph'+ cells continue to proliferate, after exceeding normal cell density, while the normal cells stop dividing, since they recognize the leukemic cells as normal. Only rarely are normal and Ph'+ populations almost evenly balanced. As noted earlier, patients with CML who have mixed populations of normal and Ph'+ cells in the marrow may live longer than average. Patient No. 19 in the present series had equal numbers of Ph'+ and Ph'-negative cells in his marrow at diagnosis, and he maintained almost this same
proportion during the L-5 protocol. This suggests that in addition to being almost evenly balanced from a proliferative standpoint, the two populations were almost equally sensitive to the therapeutic components of the protocol.

In the patients who have no significant repopulation of their marrow with normal cells following destruction of the majority of Ph' + cells by treatment, it has not been determined if the normal stem cells have been displaced by the Ph' + population or if they are still present in a nonproliferative state. The subendosteal region of the bone marrow is the preferred zone of proliferation of early hematopoietic precursors, and this region may simply be too crowded in advanced CML to permit both stem cell populations to coexist. Because normal and Ph' + precursors have similar proliferative rates at comparable cell densities and because the normal cells may fail in many cases to participate in repopulating the marrow following release from drug-induced marrow suppression, it may be that the normal cells are actually displaced in such cases. We are presently conducting experiments to try to answer this question. In instances where mixed populations do coexist, either early in the disease or after treatment, it is likely that the Ph' + cells have a lesser proliferative advantage over the normal cells, and as a result, the disease may be less rapidly fatal. Our observations in the L-5 series of patients suggest that there may be less tendency to blastic transformation in these latter patients, but a larger series and longer period of observation will be necessary to confirm this impression.

When the Ph' + myeloblasts (or earlier precursors) undergo further malignant change and the disease enters a true blastic phase, the proliferative behavior of the blasts may then resemble that of acute leukemic cells. However, many gradations occur, and the transition from chronic phase to blastic phase may occur slowly or rapidly, depending on the extent of malignant progression and the comparative proliferative properties of the chronic- and acute-phase stem cells. Based on the present observations in the L-5 series, it would appear that chronic-phase Ph' + cells, which have only a minimal proliferative advantage over normal cells, are less liable to malignant progression than are those that have a more complete loss of responsiveness to normal controls.

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