Duration of the Preclinical Phase of Chronic Myelogenous Leukemia: A Case Report

By Ellin Berman, Annabel Strife, David Wisniewski, Suvas Desai, Subash Gulati, Suresh Jhanwar, and Bayard D. Clarkson

The molecular events that allow for clonal expansion of the malignant population in chronic myelogenous leukemia (CML) are poorly understood. Recent experiments in transgenic mice suggest a close temporal relationship between expression of the aberrant protein and manifestation of a hematologic neoplasm that resembles CML; tracing the same phenomenon in humans has not been possible. We studied a patient who underwent autologous bone marrow harvest after completion of chemotherapy and radiation therapy for advanced stage Hodgkin's disease. At the time of harvest his peripheral blood counts and bone marrow were morphologically normal. Sixteen months later he developed the clinical manifestations of CML. Detailed molecular evaluation of the harvested marrow showed that a small number of cells contained the Philadelphia chromosome. The time interval required for expansion of the malignant clone, as suggested by this particular patient, was at least 16 months although it is recognized that this figure may be variable. © 1991 by The American Society of Hematology.

It has been well documented that Philadelphia chromosome (Ph) positive chronic myelogenous leukemia (CML) is a clonal disorder arising from a somatically mutated pluripotent hematologic stem cell. The Ph chromosome, a balanced translocation between chromosomes 9 and 22, has been the focus of intense study during the last decade as molecular technology has allowed for characterization of the breakpoint region on each chromosome, the fusion gene product, and the transcribed protein. However, little is known about the temporal relationship between the appearance of the Ph chromosome and the clinical manifestations of the disease. While recent experiments in mice made transgenic with either the p210 or p190 bcr-abl DNA construct show a close relationship between protein expression and clinical evidence of the leukemia, the same phenomenon has not been able to be traced in humans.

We have had the opportunity to study a patient who developed laboratory and cytogenetic evidence of CML 16 months after completing therapy for advanced stage Hodgkin's disease (HD). Re-evaluation of autologous marrow that had been harvested after completion of all HD therapy showed that the Ph chromosome was present at the time, despite a normal complete blood count (CBC) and normocellular marrow. This provided a unique opportunity to obtain data on the duration of the preclinical stage of CML.

CASE REPORT

A 32-year-old man presented in January 1987 with a short history of night sweats, fever, and right supraclavicular adenopathy. Biopsy of the lymph node performed elsewhere showed HD, nodular sclerosing subtype. The patient was subsequently referred to Memorial Hospital.

Initial evaluation here in February 1987 showed a well-appearing young man with right supraclavicular fullness but no other adenopathy or hepatosplenomegaly noted. A CBC including differential was normal (Table 1). Further work-up included a chest x-ray (CXR) demonstrating mediastinal widening. An abdominal/pelvic CAT scan was normal, as was a bone marrow biopsy. The patient underwent a staging laparotomy with splenectomy with no evidence of HD noted in the spleen, liver, or sampled lymph nodes. He was therefore staged as II-B.

In April 1987 he began radiation therapy and received 3,960 cGy to a full mantle with an additional 400 cGy boost to the mediastinum. He also received 3,600 cGy to the para-aortic field. All therapy was completed in June 1987. A CXR at that time showed only minimal mediastinal widening. A repeat CBC was normal (Table 1).

In August 1987, 2 months after completion of radiation therapy, he again developed night sweats and fever. In September 1987, right inguinal adenopathy developed concurrent with the development of hip pain. Table 1 shows the CBC at this time. Biopsy of a right inguinal lymph node in October 1987 again showed HD, nodular sclerosing subtype. A CXR was unchanged; CAT scan of the abdomen and pelvis demonstrated bony destruction of the left anterior iliac crest. A bone marrow biopsy performed in the region of the lytic hip region showed HD present in the bone. The marrow aspirate showed a cellular specimen with a mild increase in eosinophils but was otherwise normal and had no evidence of any Reed-Sternberg cells.

The patient was subsequently begun on combination chemotherapy that included alternate cycles of CAV (CCNU 100 mg/m2; alkeran 6 mg/m2; vinblastine 4 mg/m2), MOPP (nitrogen mustard 6 mg/m2; vincristine 1.4 mg/m2; prednisone 40 mg/m2; procarbazone 100 mg/m2), and ABV (doxorubicin 25 mg/m2; bleomycin 6 U/m2; vinblastine 6 mg/m2). He received a total of nine cycles of chemotherapy, all treatment ending November 1988. Because it was felt that the risk of a second relapse was reasonably high, the patient underwent an autologous bone marrow harvest in December 1988. A CBC before harvest was again normal (Table 1). A bone marrow aspirate was interpreted as poor quality such that the cellularity could not be assessed; a bone marrow biopsy was somewhat hypocellular with all hematopoietic elements seen and no HD evident. Approximately 700 cc of marrow was harvested and stored in dimethyl sulfoxide and hydroxyethyl starch (HES) as previously described.

In January 1990, 14 months after completion of chemotherapy and 30 months after completion of radiation therapy, his white blood cell (WBC) count was noted to be slightly elevated (Table 1). In April 1990 his WBC count was 22,900/μL and a bone marrow aspirate and biopsy showed marked hypercellularity with a myeloid:

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Cytogenetic analysis. Chromosome preparations from the bone marrow aspirate were obtained following methods described earlier. Air-dried metaphase spreads were G-banded and the karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

Materials and Methods
Preparation of frozen marrow for analysis. A sample of the marrow that had been harvested and frozen in 1988 was defrosted. The cells were incubated on ice for 30 minutes with DNase (60 U/mL) (Worthington Biochemical, Freehold, NJ) followed by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) separation to remove nonviable cells. A sample of marrow cells derived from the patient in May 1990 for differential: 13% bands, 49% polymorphonuclear leukocytes, 14% lymphocytes, 1% basophils, 10% myelocytes, 13% metamyelocytes.

Table 1. Serial Peripheral Blood, Bone Marrow, Cytogenetic and Molecular Analysis of Patient Who Developed CML After Therapy for Hodgkin's Disease

<table>
<thead>
<tr>
<th>Blood Counts and Bone Marrow Evaluations</th>
<th>Cytogenetic and Molecular Analysis of Bone Marrow Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status</td>
<td>No. Metaphases</td>
</tr>
<tr>
<td>At time of HD diagnosis</td>
<td>2/87</td>
</tr>
<tr>
<td>(pathologically staged</td>
<td>9,600</td>
</tr>
<tr>
<td>(t(8;14)); before splenectomy</td>
<td>(84P, 6M, 11L)*</td>
</tr>
<tr>
<td>After completion of radiation therapy</td>
<td>6/87</td>
</tr>
<tr>
<td>(mantle, para-aortic)</td>
<td>9,700</td>
</tr>
<tr>
<td>New inguinal adenopathy and</td>
<td>10/87</td>
</tr>
<tr>
<td>B symptoms; lytic lesion left hip</td>
<td>12,600</td>
</tr>
<tr>
<td>After completion of 9 cycles of</td>
<td>12/88</td>
</tr>
<tr>
<td>MOPP/ABV/CAV (see text for details and</td>
<td>6,600</td>
</tr>
<tr>
<td>just before autologous marrow harvest</td>
<td>(58P, 10M, 32L)</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>2/89</td>
</tr>
<tr>
<td>6,100</td>
<td>13.8</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>4/89</td>
</tr>
<tr>
<td>6,900</td>
<td>14.5</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>6/89</td>
</tr>
<tr>
<td>6,000</td>
<td>14.0</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>9/89</td>
</tr>
<tr>
<td>7,600</td>
<td>15.2</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>1/90</td>
</tr>
<tr>
<td>10,900</td>
<td>15.1</td>
</tr>
<tr>
<td>Follow-up visit</td>
<td>4/90</td>
</tr>
<tr>
<td>22,900†</td>
<td>13.1</td>
</tr>
<tr>
<td>Referral to MH</td>
<td>5/90</td>
</tr>
<tr>
<td>27,400</td>
<td>14.9</td>
</tr>
</tbody>
</table>

*Differentiation: P, polymorphonuclear leukocytes; M, monocytes; L, lymphocytes.
†Differentiation: 13% bands, 49% polymorphonuclear leukocytes, 14% lymphocytes, 1% basophils, 10% myelocytes, 13% metamyelocytes.
‡Positive NDN: Quantity of DNA obtained insufficient for BCR analysis.
§Performed on in vitro day 14 marrow CFU-GM-derived colonies grown in semisolid medium.
Though this writing he has fully engrafted and remains disease-free.

Results
Serial blood counts and bone marrows and their relationship to disease status are outlined in Table 1. At the time of the patient's HD diagnosis (February 1987) his CBC, differential, and bone marrow appeared normal. He underwent splenectomy with staging laparotomy followed by radiation therapy to the mantle and para-aortic fields. He developed transient leukocytosis and thrombocytosis with a mild left-shift when HD presented again as new inguinal adenopathy and a lytic lesion in the left hip (October 1987). After completion of chemotherapy, his blood count and

Breakpoint cluster region analysis. BCR analysis was performed on fresh marrow cells obtained from the patient in May 1990 for Southern blotting following methods previously described. The 5.8-kb Phi-3/bcr probe (Oncogene Science, Inc, Manhasset, NY) was labeled to high specific activity with ²⁰P following the method described by Feinberg and Vogelstein. PCR analysis. This method has been previously described in detail elsewhere. Briefly, individual 14-day CFU-GM-derived colonies were removed from the methyl cellulose cultures with a finely drawn Pasteur pipette and total RNA from each colony was isolated according to the method of Chomczynski and Sacchi. First-strand complementary DNA (cDNA) corresponding to all mRNAs in the sample was obtained by using random hexamers in a reverse-transcription reaction. cDNA then served as the substrate in the PCR analysis. To insure the integrity of the RNA extracted from each colony, β-actin and bcr-abl cDNA sequences were amplified in the same reaction vessel. Positive controls included RNA isolated from K562 colonies and CFU-GM derived from a patient with Ph-positive blast crisis. Negative controls included RNA isolated from normal CFU-GM-derived colonies.
differential again normalized; just before bone marrow harvest, a bone marrow biopsy was mildly hypocellular (December 1988). Follow-up blood counts off all therapy were normal for the next 16 months (April 1990) when leukocytosis was again noted, this time with early myeloid cells seen in the differential. No evidence of HD was found either on physical examination or scan. A bone marrow sample showed hypercellularity with no evidence of HD. However, cytogenetics showed the presence of a Ph chromosome. BCR analysis of this sample showed the rearrangement typically seen in CML. 

A total of seven metaphases were available for cytogenetic analysis from the frozen marrow harvested in December 1988 (Table 1). Only one cell was Ph-positive. Although no clonal rearrangement of the BCR gene could be detected because of an inadequate amount of DNA obtained, PCR analysis of day 14 colonies grown from the defrosted sample showed bcr-abl rearrangement in 1 of 40 colonies examined. 

**DISCUSSION**

This patient with HD had the Ph chromosome detected in some hematopoietic precursor cells 18 months after completion of radiation therapy and 1 month after completion of chemotherapy for relapsed disease. However, another 16 months elapsed before the clinical manifestations of the CML appeared. Given the treatment regimens that this patient received, one can question whether these may have played a causative role in the development of CML. While not as commonly reported as secondary myelodysplastic syndromes or acute myelogenous leukemia, CML has been reported after primary treatment for other malignancies with the time interval between initial therapy and the development of CML ranging from 36 to 81 months. Few cases of CML have been reported after treatment for HD. Swaim et al described a patient with HD who developed a CML-like syndrome 7 years after having received a total of 443.2 Gy to various body sites although no cytogenetic data were presented. Verhof et al reported a patient with stage III-B nodular sclerosing HD who received combination chemotherapy with MOPP for a total of 8 months. Pretreatment bone marrow biopsy and karyotype were normal. This patient subsequently developed Ph-positive CML 7 years after completion of chemotherapy.

Our patient had a 38-month interval between the diagnosis of HD and the laboratory evidence of CML. Uniquely, cytogenetic and PCR documentation of rare Ph-positive cells was made 16 months before the development of the clinical manifestations of the disease (Table 1).

Little information is available on the duration of the preclinical phase of CML. Kamada and Uchino constructed a model of disease evolution after studying 16 atomic bomb survivors who had received close hematologic follow-up for 5 to 10 years before the development of CML and 102 patients with CML with or without atomic bomb exposure. Based on these data, it was calculated that the elapsed time from occurrence of a single cell containing the Ph chromosome to a leukemic cell burden of 100,000 cells/µL was 6.3 years. This formulation appeared reasonable when compared with the peak incidence of CML in atomic bomb survivors that occurred about 6 to 8 years after exposure, with younger individuals (<15 years) having a shorter latency period than older individuals (>30 years). The earliest cases of CML were diagnosed 4 to 5 years after the explosion.

Such estimates are crude, but are nonetheless consistent with previous observations which suggest that there may be a period of at least several years between the original cytogenetic transformation and the development of the disease. Long-term cytogenetic follow-up of patients who have undergone allogeneic bone marrow transplantation (BMT) for CML also suggests that there may be a relatively long period between reappearance of Ph-positive cells in the marrow and hematologic relapse. Offit et al studied serial cytogenetics in 64 patients with CML who had undergone T-cell-depleted allogeneic BMT. Sixteen patients had a cytogenetic relapse; 12 of these patients relapsed clinically a median of 6 months (range 0 to 12 months) after reappearance of Ph-positive cells. Three patients with evidence of cytogenetic relapse remained free of clinical disease 6, 12, and 22 months from time of cytogenetic relapse. Similarly, Arthur et al reported that 11 of 48 patients who underwent allogeneic BMT in chronic phase CML had recurrence of the Ph chromosome without hematologic relapse.

In other instances it is apparent that the development of the Ph chromosome and the disease manifestations are more closely linked. CML has been reported in a 3-month-old infant, which suggests the time from cytogenetic transformation to frank disease was, in this case, relatively rapid; however, the mass of leukemia cells is of course significantly smaller in infants.

Chronological study of our patient is of interest because it has defined at least a 16-month interval between the appearance of Ph-positive cells and clinical signs of the disease. Few other similar case studies have been reported. Canellos and Whang-Peng reported a patient who had a normal blood count but had metamyelocytes and myelocytes present in the peripheral smear; the bone marrow had the Ph chromosome present in 22% of metaphases but no leukocytosis developed for 5 years.

There are obviously few opportunities to study the very early phase of CML in humans as most patients present simultaneously with the clinical manifestations of the disease and the Ph chromosome present in the majority of cells. It is important to note that in the current study the earliest specimen available for analysis was the frozen sample obtained 16 months before the full manifestations of the disease and that the PCR analysis was performed on the cryopreserved buffy coat marrow cells that were subsequently thawed and grown in methyl cellulose. If fresh buffy coat marrow cells had been available, it is possible that the fraction of Ph-positive cells would have been higher because the major expansion of the CML population has been shown to occur in the later maturation compartment.
the freezing process preferentially destroys more mature
progenitor cells. Thus, the ratio of CML: normal proge-
nitors determined from the cryopreserved specimen may
not have been an accurate assessment of the size of the CML
progenitor population 16 months before the clinical mani-
festation of the disease.

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