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# Chronic Myeloid Leukemia With Permanent Disappearance of the Ph[1] Chromosome and Development of New Clonal Subpopulations

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**A 6.5-yr follow-up study is reported on a case of chronic myeloid leukemia (CML) diagnosed in 1971 in a man of age of 19 yr. At that time the Ph[1] chromosome was found in all of his bone marrow cells. After 4 yr of intermittent treatment his blood and bone marrow still showed a florid CML picture, but the karyotypes of bone marrow, blood, and spleen cells were normal. Blastic transformation occurred after 5.5 yr and was accompanied by a hyperdiploid karyotype (49,XY,+9,+10,+12) without reappearance of the Ph[1] chromosome. After successful chemotherapy the karyotype again became normal. In 1977 cells with a new clonal aberration (14p+) gradually became the dominating bone marrow cell population, while the peripheral blood was compatible with CML. The 14p+ clone subsequently decreased at the expense of the 49,XY,+9,+10,+12 clone, which had reappeared in the majority of the dividing blood cells. Also, a new clonal subpopulation 45,XY,t(5p; 17q) was found in 7% of the metaphases. This coincided with the occurrence of a second blast transformation, which was treated with chemotherapy. The karyotypes of all dividing cells during the recovery from the induced severe bone marrow hypoplasia still showed the t(5p; 17q) abnormality and chromosome numbers varying from 45 to 51. In some cells additional trisomies were seen as an indication of a clonal evolution. One month before the patient's death a fourth abnormal cell line was seen in 10% of the dividing blood cells.**

THE PHILADELPHIA CHROMOSOME (Ph[1]), described by Nowell and Hungerford in 1960,[1] is a specific acquired anomaly in the bone marrow (BM) karyotype of almost 90% of patients with chronic myeloid leukemia (CML). Banding techniques have identified the Ph[1] anomaly as a translocation of the larger part of the long arm of chromosome 22 onto another chromosome, usually the long arm of chromosome 9: t(9q+,22q−).[2]

Recent reviews of CML patients have stressed the correlation between karyotype and prognosis:[3-6]

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**Table 1. Hematologic Data**

| Date | Clinical Phase | Hb (g/dl) | Thrombocytes ($\times 10^9$/liter) | WBC ($\times 10^9$/liter) | Blood | | | Bone Marrow | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Blast Cells + Promyelocytes (%) | Myelocytes + Metamyelocytes (%) | Normoblasts (per 100 WBC) | Cellularity | M/E Ratio | Blast Cells + Promyelocytes (%) |
| June 1, 1971* | Diagnosis | 8.9 | 127 | 212 | 11 | 15 | 1 | High | 26.2 | 21 |
| 1972 | Chronic | 9.4–14.7 | 40–268 | 5–120 | 1–4 | 0–26 | 0–1 | ND | ND | ND |
| 1973 | Chronic | 8–13.1 | 86–640 | 10–166 | 0–7 | 4–31 | 1–6 | ND | ND | ND |
| 1974 | Chronic | 9.4–12.8 | 69–352 | 6.2–75.2 | 0–2 | 4–26 | 0–1 | ND | ND | ND |
| Nov. 3, 1975* | Splenectomy | 8.2 | 329 | 98 | 2 | 23 | 0 | High | 8.5 | 3.2 |
| Dec. 22, 1976* | Blastic | 5.9 | 73 | 150 | 26 | 9 | 1.5 | Very high | 30 | 29 |
| Jan. 17, 1977* | Hypoplastic | 5.8 | 14 | 0.4 | 0 | 0 | 0 | Low | ND† | ND† |
| Jan. 31, 1977* | Hypoplastic | 9.3 | 99 | 3.3 | 0 | 0 | 3 | Low | 0.8 | 1.8 |
| Mar. 30, 1977 | Remission | 11.4 | 223 | 4.9 | 0 | 1.5 | 4.5 | Normal | 0.5 | 2.2 |
| May 16, 1977* | Chronic | 11.4 | 262 | 8.9 | 0.5 | 2.6 | 14 | Normal | 0.8 | 4.8 |
| Aug. 16, 1977* | Chronic | 10.8 | 506 | 60 | 4.5 | 9.5 | 7 | High | 3.3 | 8.6 |
| Nov. 15, 1977* | Transition | 12.3 | 64 | 128 | 14 | 12.5 | 33 | ND‡ | | |
| Dec. 19, 1977* | Blastic | 8.8 | 78 | 170 | 26 | 11.5 | 9.5 | ND‡ | | |
| Feb. 27, 1978* | Hypoplastic | 10.2 | 18 | 1.8 | 2 | 6 | 3 | Normal | 3.8 | 10 |
| Mar. 13, 1978* | Chronic | 10.1 | 19 | 11.9 | 9 | 38 | 1 | Normal | 2.2 | ND† |
| Mar. 23, 1978* | Chronic | 10.1 | 21 | 28 | 7 | 61 | 5.5 | ND | ND | ND |
| Apr. 13, 1978 | Terminal | 8.8 | 13 | 90 | 17 | 59 | 0 | ND | ND | ND |

ND, not done.

*Chromosome analysis done on the same samples.

†Not enough material.

‡Dry taps.

(1) Ph[1]-negative CML patients have a poorer prognosis as compared with Ph[1]-positive patients, i.e., shorter survival, poorer response to therapy, and earlier acceleration of the disease.[4] (2) The natural history of Ph[1]-positive CML is such that after a chronic phase of 2–4 yr drug resistance, myelofibrosis, or (in 80% of cases) an acute leukemia occurs. Additional chromosomal aberrations in the Ph[1]-positive BM cells usually herald the development of acute leukemia. (3) Very rare cases in which only a minority of the cells are Ph[1]-positive (either on the basis of mosaicism at diagnosis or as a therapeutically induced cytogenetic conversion) have shown long remission and survival for up to 17 yr.[5 12]

The present paper describes a case of CML in a young adult. His BM cells were Ph[1]-positive at diagnosis, but complete conversion to Ph[1]-negative cells occurred during the chronic phase of the disease. A first blast crisis was marked by an abnormal karyotype, but the Ph[1] chromosome did not reappear. The patient responded well to chemotherapy, but after a temporary erythroid hyperplasia another blast transformation developed. These different clinical appearances coincided with specific karyotypic changes.

## CASE REPORT

The patient was a male born in January 1952. Chronic myeloid leukemia was diagnosed in 1971 (age 19 yr) when the patient presented with general complaints, splenomegaly, typical blood and bone marrow findings, and the presence of a Ph[1] chromosome in his bone marrow cells. During almost 5.5 yr, from June 1971 until December 1976, he was maintained in a variable clinical remission of his disease with different therapeutic regimens: June and July 1971 and March through May 1972, busulfan; November 1972, spleen irradiation; March and April 1973, dibromomannitol; September 1973 through October 1975, hydroxyurea; November 1975, splenectomy; November 1975 through May 1976, hydroxyurea; May through December 1976, busulfan.

In December 1976 a blastic phase developed and was treated with doxorubicin, vincristine, and cytosine arabinoside according to a protocol of the European Organization for Research on Treatment of Cancer (EORTC). An identical course was given in February 1977, and subsequently a consolidation treatment with doxorubicin and cytosine arabinoside was given in April 1977. The disease remitted again to a chronic phase, during which the patient was again treated with hydroxyurea for 3 mo followed by busulfan for 5 mo. A second blast phase in December 1977 was treated with cytosine arabinoside, thioguanine, and daunomycin. This resulted in a prolonged pancytopenia due to bone marrow depression. Fever, infections, intestinal bleeding, and cachexy followed. In April 1978 an abdominal tumor was found to be causing intestinal obstruction, and the patient died later that month. Pathologic examination showed generalized infiltration of leukemic cells in lymph nodes, bone marrow, liver, lungs, kidneys, and the small and large intestines. The mesenteric lymph nodes were particularly enlarged, forming a tumor of 10×10×5cm and a paraaortic tumor of 10×6×5cm. A summary of the hematologic findings during the different episodes is given in Table 1.

From 1971 through 1976 the WBC counts remained rather elevated and blood and BM morphology were consistent with a chronic phase of CML. During these years no episode of BM hypoplasia was observed. Blast crisis was defined as the presence of at least 20% blasts and promyelocytes in peripheral blood and 25% blasts in bone marrow. Frequent controls carried out since December 1976 sequentially showed the therapeutically induced hypoplastic phase, the return to a chronic CML with an erythroid overshoot, and the second blast crisis followed by hypoplasia. Leukocyte alkaline phosphatase (LAP) values were repeatedly low or absent. Terminal deoxynucleotidyl transferase tested in December 1977 gave the low value of 0.5 U/$10^8$ cells (as determined in the laboratory of Dr. D. Catovsky, Royal Postgraduate Medical School, London), which is consistent with a myeloid type of transformation.

## MATERIALS AND METHODS

Routinely, BM was aspirated from the patient's posterior superior iliac crest.

Morphology was examined on crush smears stained with May-Grünwald-Giemsa, the PAS technique, and Sudan black. LAP was scored according to the method of Kaplow.[13]

Chromosome analyses were done on BM cells immediately suspended in Ham's F10 medium containing colchicine (0.25 μg/ml). Short cultures of BM were also performed in partly conditioned medium made of 3 vol fresh Ham's F10 medium supplemented with 15% fetal calf serum and 2 vol similar medium conditioned on a fibroblast culture. Whole blood was cultured for 24

**Table 2. Results of Serial Chromosome Analyses and Colony Growth Assays**

| Date | Clinical Phase | Tissue* | No. of Mitoses Analyzed† | Karyotype | CFU-C (per $10^5$ Nucleated Cells) |
|---|---|---|---|---|---|
| 6/1/71 | Diagnosis | BM | 50 | 46,XY,Gq− | ND |
| 11/3/75 | Chronic | BM | 27 | 46,XY | 59 |
| | | Blood | 6 | 46,XY | ND |
| | | Spleen | 31 | 46,XY | 0 |
| 12/15/76 | Blastic | BM | ND | ND | 3 |
| 12/22/76 | | Blood | 23 (22) | 49,XY,+9,+10,+12 | ND |
| | | | (1) | 46,XY | |
| 1/17/77 | Hypoplastic | BM | 6 (5) | 46,XY | 0 |
| | | | (1) | 49,XY,+9,+10,†12 | |
| 1/31/77 | Remission | BM | 26 (21) | 46,XY | 0 |
| | | | (5) | 49,XY,+9,+10,†12 | |
| 5/16/77 | Chronic | BM | 27 | 46,XY | 9 |
| | | Blood | 6 | 46,XY | ND |
| 8/16/77 | Chronic | BM | 34 (16) | 46,XY | 16 |
| | | | (18) | 46,XY,14p+ | |
| 11/12/77 | Blastic | BM | 32 (1) | 46,XY | 9 |
| | | | (26) | 46,XY,14p+ | |
| | | | (5) | 49,XY,+9,+10,+12 | |
| | | Blood | 22 (4) | 46,XY | 9 |
| | | | (16) | 46,XY,14p+ | |
| | | | (2) | 49,XY,+9,+10,+12 | |
| 12/19/77 | Blastic | Blood | 40 (3) | 46,XY,14p+ | 14‡ |
| | | | (34) | 49,XY,+9,+10,+12 | |
| | | | (3) | 45,XY,t(5p;17q) | |
| 2/8/78 | Hypoplastic | Blood§ | 23 (8) | 46,XY | ND |
| | | | (3) | 45,XY,t(5p;17q) | |
| | | | (12) | 49→51 (Table 3) | |
| 2/27/78 | Recovery | BM§ | 45 (12) | 45,XY,t(5p;17q) | ND |
| | | | (33) | 46→51 (Table 3) | |
| 3/13/78 | Chronic | Blood§ | 45 (41) | 49 (Table 3) | 44¶ |
| | | | (4) | 44,XY,+8,t(11;12),17p+,−18,−22 | |
| 3/23/78 | Chronic | Blood§ | 35 (3) | 45,XY,t(5p;17q) | ND |
| | | | (30) | 49 (Table 3) | |
| | | | (2) | 44,XY,+8,t(11;12),17p+,−18,−22 | |

*The blood cultures are unstimulated (no PHA).

†Total number of mitoses analyzed and their repartition according to the observed karyotype.

‡Cytogenetic analysis of 21 colony cells was performed on day 14 of the culture with the following results: 46,XY (2 cells); 49,XY,+9,+10,+12 (5 cells); and 45,XY,t(5p;17q) (14 cells).

§25% of the cells showed random breakage of chromosomes.

¶Cytogenetic analysis of only four colony cells was possible and showed in all four karyotypes 44,XY,+8,t(11q12q),17p+,−18,−22.

and 48 hr without phytohemagglutinin (PHA). A 48- or 72-hr lymphocyte culture (peripheral blood, stimulated with PHA) was used as control.

In all cultures except those prepared in 1971, the chromosomes were identified with different banding techniques: GAG (G bands by acetic saline using Giemsa), QFA (Q bands by fluorescence using quinacrine), and RFA (R bands by fluorescence using acridine orange) according to the nomenclature of the Paris Conference (1971) Supplement (1975).[14] Silver staining of the nucleolus organizer regions (NOR banding) was attempted according to the method of Tantravahi et al.[15]

Colony assays for myeloid precursor cells in BM and blood were performed according to the technique of Pike and Robinson.[16] Colonies of more than 100 cells were scored. Values for normal marrow were 8–15 colonies/$10^5$ nucleated cells. Cells giving rise to colonies in vitro were designated CFU-C (colony-forming units culture).

## RESULTS

Karyotype analyses of BM and blood cells were performed at different times during the $6\frac{3}{4}$ yr following diagnosis (Table 2).

In 1971, at diagnosis, 50 BM cells were analyzed. All of them showed a deletion of one of the G-group chromosomes, which was assumed to be the $Ph^1$ chromosome (Fig. 1). No banding technique was operational at that time, and therefore definite identification of the Gq- was not achieved. The Y chromosome appeared to be rather large, as confirmed by Q banding (Fig. 2), and the

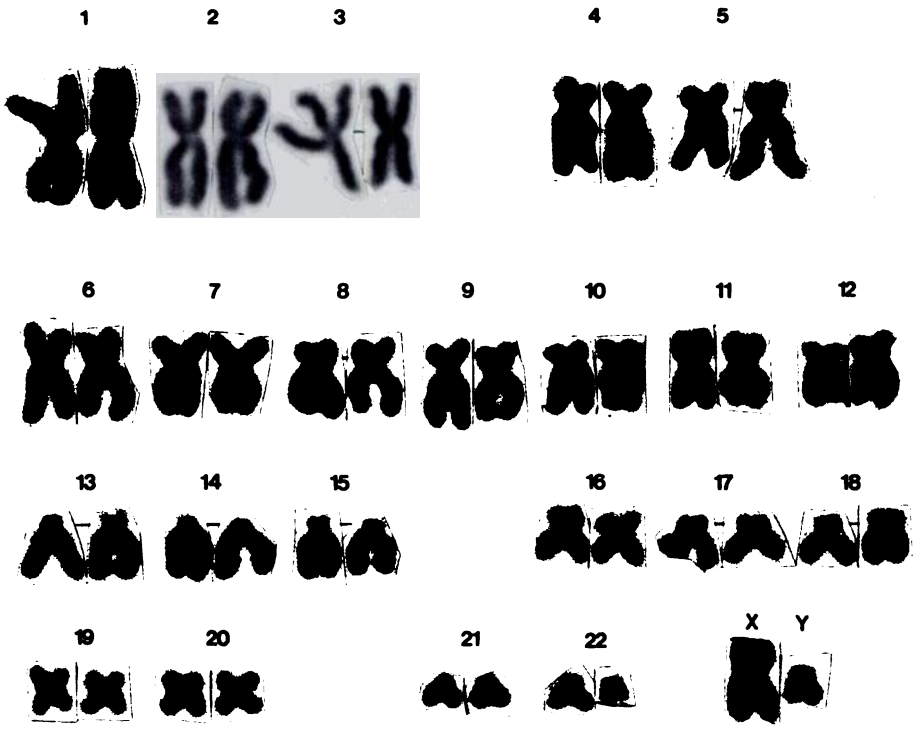


**Fig. 1. Karyotype of first BM culture, 1971. Deleted G-group chromosome (Gq− or $Ph^1$ chromosome) was found in all metaphases. Standard Giemsa staining.**

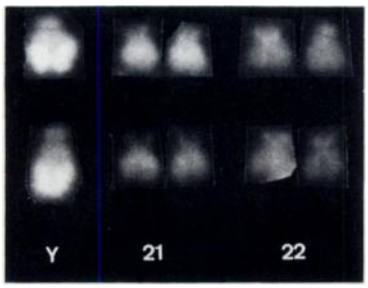


**Fig. 2. G-group chromosomes of two metaphases from BM culture, 1975. Note absence of deleted chromosome. Q banding with quinacrine.**

other chromosomes were scored as normal. For obvious reasons a possible 9q+ could not be detected. Later attempts to band the old preparations did not succeed.

In 1975, none of the 64 metaphases analyzed in BM, blood, and spleen showed the Ph$^1$ chromosome; only a normal karyotype was observed (Fig. 2). Clinically, the patient presented with a picture of CML during its chronic phase (Table 1).

In December 1976, during blastic transformation, 22 of the 23 cells analyzed in the BM contained 49 chromosomes with trisomy of chromosomes 9, 10, and

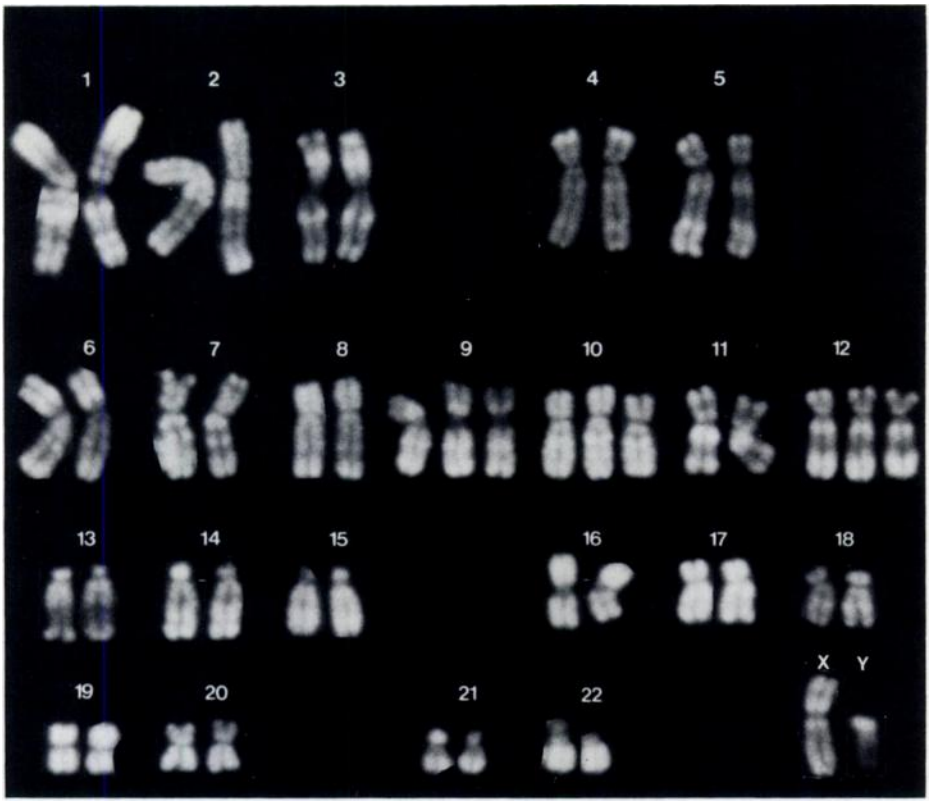


**Fig. 3. BM karyotype during blast transformation, 1976: 49,XY,+9,+10,+12. R banding with acridine orange.**

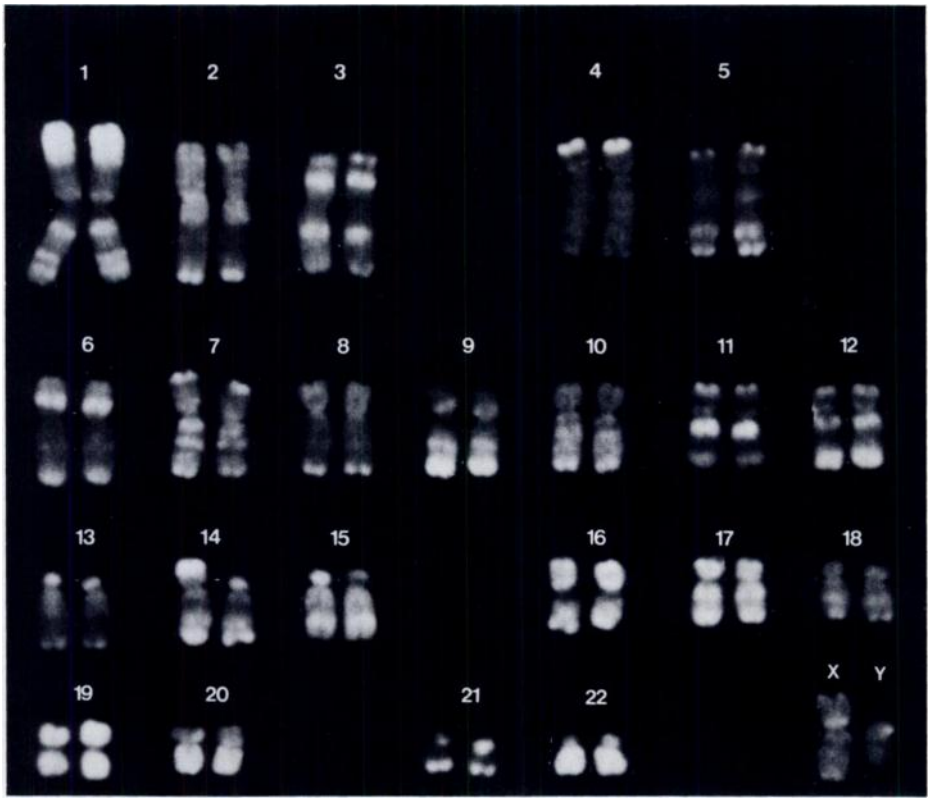


**Fig. 4. Karyotype of abnormal clonal population discovered in August 1977: 46,XY,14p+. R banding with acridine orange.**

12 but no $Ph^1$ (Fig. 3). Chemotherapy resulted in severe BM hypoplasia and subsequent clinical remission accompanied by the disappearance of the abnormal clonal population (May 1977). In August 1977 a new clonal aberration (14p+) appeared in 50% of the cells (Fig. 4). Definite identification of this anomaly was not achieved. Combination of the banding techniques suggested a translocation onto 14p11 of either the terminal part of chromosome 9q (band 9q33 → qter) or the terminal part of chromosome 4p (band 4p53 → pter), but a more complex rearrangement cannot be excluded. NOR banding showed absence of silver staining on the 14p+. Concomitant with the development of this new clone, an increase in myeloid blast cells and normoblasts in peripheral blood was observed (Table 1). A relapse of the blast crisis was foreshadowed in November 1977, when 15% of the metaphases showed the reappearance of the ancient clone with the 49,XY,+9,+10,+12 karyotype characteristic of the first blast crisis. This clone became prominent in December (second blastic phase) and disappeared after chemotherapy (February 1978).

In December 1977, 7.5% of the mitoses showed a new chromosomal aberration: 45,XY,−5,−17,+mar. The new marker chromosome appeared as the result of a translocation of the entire short arm of chromosome 5 (5p) onto the entire long arm of chromosome 17 (17q), abbreviated t(5p;17q). The break-

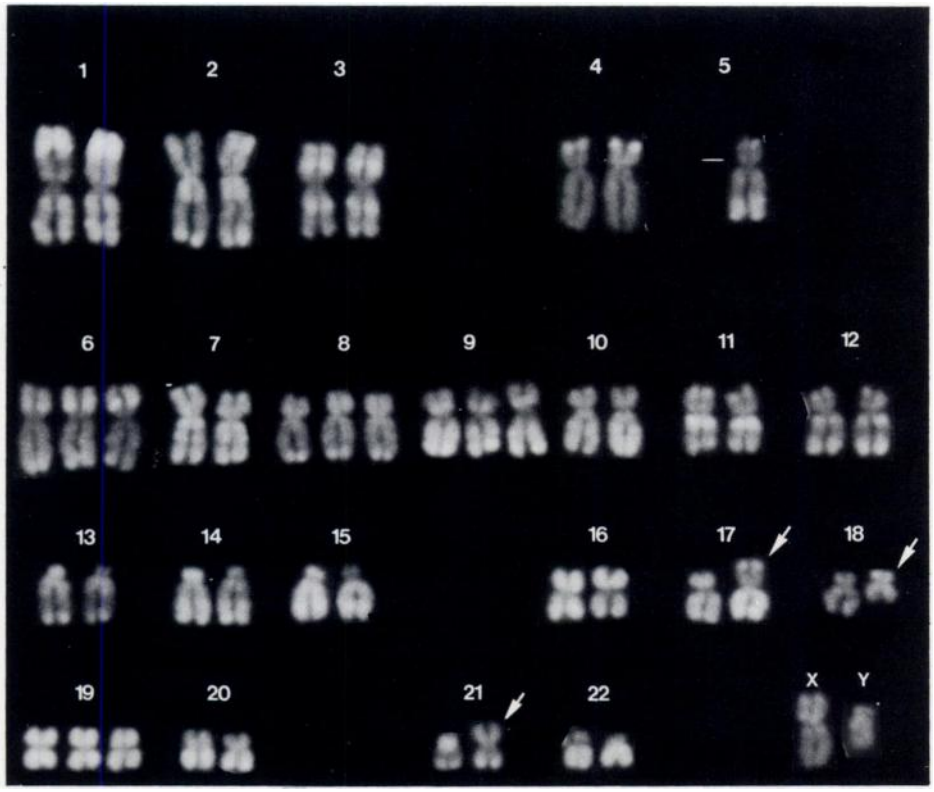


**Fig. 5. Karyotype of subclone with 49 chromosomes, derived from the stem line with t(5p;17q), that became prominent in February and March 1978: 49,XY,−5,+6,+8,+9,−17,+t(5p;17q), t(18;21)(q21;p11),+19. Chromosome t(5p;17q) and der (18) and der (21) arrows. R banding with acridine orange.**

points were at or very near the centromeres. Both chromosome arms (5p and 17q) were complete; whether the centromeres belong to chromosome 5 or 17 or to both is unclear (Fig. 5).

During the period of BM hypoplasia following chemotherapy further clonal evolution of this cell line was observed (Table 3). Analysis of the sequential

**Table 3. Clonal Evolution of the Stem Line 45,XY,t(5;17)**

| No. of Chromosome | Karyotypic Changes | No. of Cells Analyzed on: | | | |
|---|---|---|---|---|---|
| | | 2/8/78* | 2/27/78† | 3/13/78* | 3/23/78* |
| 45 | t(5;17)‡ | 3 | 12 | 0 | 3 |
| 46 | t(5;17) +8 | 0 | 1 | 0 | 0 |
| 49 | t(5;17) +6 +8 +9 +19 | 4 | 10 | 15 | 1 |
| 49 | t(5;17) +6 +8 +9 +19 t(18;21)§ | 2 | 13 | 21 | 29 |
| 49 | t(5;17) +6 +8 +9 12p$^+$ +19 | 0 | 4 | 5 | 0 |
| 50 | t(5;17) +6 +8 +9 +12 +19 | 1 | 4 | 0 | 0 |
| 51 | t(5;17) +6 +8 +9 + 9 + 12 +19 | 3 | 1 | 0 | 0 |

*Unstimulated blood culture.
†Uncultured BM cells.
‡Stands for −5,−17,+t(5p;17q).
§Stands for t(18;21)(q21;p11).

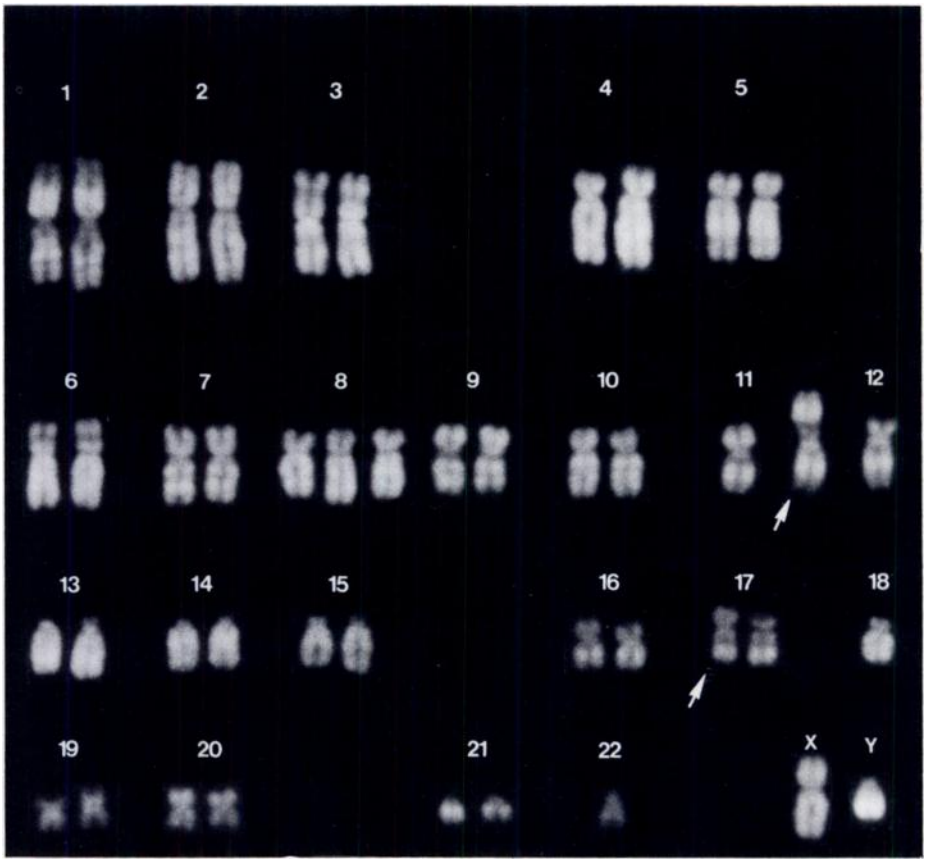


**Fig. 6. Karyotype of clonal population discovered in March 1978: 44,XY,+8,−11,−12,+t(11q;12q),17p+,−18,−22. Q banding with quinacrine.**

changes showed that the 49-chromosome subclone had evolved in three directions: +12, 12p+, and t(18;21). The subclone with t(18;21) became the dominant population in later analyses (Fig. 5). The translocation between chromosomes 18 and 21 was apparently balanced: t(18;21)(q21;p11). As shown in Figs. 2 and 3 the chromosome 21 involved had only small satellites with dull fluorescence. Careful analysis of the karyotypes seemed to indicate that the proximal part of 21p remained on the der(21) on which the terminal part of 18q was translocated. Satellites were never clearly seen on the long arm of der(18).

In March 1978 still another karyotypic change was observed in four cells: 44,XY,+8,−11,−12,+t(11q;12q), 17p+,−18,−22 (Fig. 6). The translocation chromosome t(11q;12q) contained complete long arms of both chromosomes involved (11 and 12), placing the breakpoints at or very near the centromeres. C banding showed only one centromere. Studies of the 17p+ chromosome by Q and R banding strongly suggested a translocation of a part of the long arm of chromosome 22 onto 17p; exact breakpoints could not be determined. This last clone, as well as the stem line with the t(5p;17q), had two normal chromosomes 14 and no $Ph^1$ chromosome, which indicates that these clones were also

independently derived from a cell with a normal karyotype. Random breakage of chromosomes was also found rather frequently in the four final analyses.

Lymphocyte cultures performed during and after chemotherapy showed many more instances of random breakage and aneuploidy than the BM metaphases. In BM the high rate of cell division probably eliminated damaged cells quite rapidly.

Colony assays for myeloid precursors showed the classical increase in number of colonies during CML and a decrease during blastic transformation. During remission the number of colonies returned to normal. Surprisingly, the number of colonies remained normal in the second blastic phase. Cytogenetic analysis of these colony cells showed that 14 of the 21 mitoses analyzed carried the 45,XY,t(5p;17q) karyotype, an indication that the majority of the myeloid precursors belonged to a particular subpopulation. A similar observation was made 3 mo later when the colony cells again belonged to the last developed clonal population with the t(11q;12q).

## DISCUSSION

Three different aspects make this clinical and cytogenetic observation remarkable: first, the fact that complete conversion from Ph$^1$+ CML into Ph$^1$− CML occurred in the interval 1971–1975 without an episode of BM aplasia and without a clinical or hematologic remission of any significant length of time; second, the favorable therapeutic response of the blast transformation leading to hematologic, clinical, and cytogenetic remission; and third, the development in the patient's final 6 mo of different cytologic and clinical changes accompanied by the proliferation in BM of new clonal subpopulations identified by chromosomal markers.

*Ph$^1$ conversion and CML diagnosis.* In almost 90% of CML, transformation of one stem cell[17] results in a Ph$^1$+ clone that gradually overgrows preexistent hemopoiesis, leading to myeloid hyperplasia. Maturation of myeloid cells occurs, and for 2–4 yr the disease can relatively easily be maintained therapeutically in a chronic stage. When blastic transformation ultimately follows, it is in most cases heralded and accompanied by additional cytogenetic abnormalities. Our patient characteristically fits the clinical patterns of Ph$^1$+ CML if no account is taken of the loss of the Ph$^1$ chromosome.

Two additional categories of CML can be distinguished: (1) Ph$^1$−CML has a poor prognosis and a rapid evolution into an acute phase. Median survival is only 8 mo, compared to 40 mo for Ph$^1$+ CML.[4] Our patient, who converted to a Ph$^1$− condition, did not fulfill the clinical criteria of this subgroup. (2) In rare cases the transformed clone, marked by Ph$^1$, does not dominate the normal stem cell population and remains a minority. Five such cases of mosaicism have been reported,[6 12] characterized by long periods (6–10 yr) of therapeutically induced complete remission and by long survival without the occurrence of a blast crisis in the observation period. An episode of iatrogenic BM hypoplasia was reported in three of the five cases. The findings in our patient contrast with these observations in that no mosaicism was found in 1971, when all mitotic BM cells were Ph$^1$+, nor after 1975, when all cells were Ph$^1$−; also, no episode of bone marrow hypoplasia (nor a prolonged remission) was noted.

During the blastic phase there was no return of a $Ph^1+$ cell population and the newly acquired cytogenetic abnormalities appeared in a $Ph^1-$ stem cell. In May and June 1971 there was no other patient with CML for whom chromosome analysis was carried out. Therefore we feel confident that laboratory mistakes are excluded and that at that time our patient had the $Ph^1$ chromosome in most if not all of his BM cells.

All attempts to explain cytogenetic and clinical findings of this case must be more or less hypothetical because of the lack of cytogenetic observations between 1971 and 1975. One explanation could be that a $Ph^1+$ CML was cured with eradication of the $Ph^1+$ clone and that this patient later developed an acute myeloid leukemia. Another explanation could be that the $Ph^1+$ clone regressed with concomitant reappearance of normal stem cells ($Ph^1-$) in the hematopoietic tissues; subsequently a new transformation of one or more stem cells may have resulted in a $Ph^1-$ CML cell line more virulent than the $Ph^1+$ one and overgrowing it. The fact that the new cytogenetically abnormal subpopulations that later emerged stemmed from a $Ph^1-$ cell also supports the notion of two successive transformations on different stem cells. In favor of the AML hypothesis are the absence of a $Ph^1$ chromosome and the complete remission obtained by chemotherapy accompanied by the return of a normal karyotype. Nevertheless, we did not retain this interpretation on clinical grounds: from diagnosis (1971) until the first blastic phase there was no remission in the patient's disease; hyperactive myelopoiesis was always present as evidenced by the total and differential blood counts. In 1975 the clinical picture was that of a florid CML, splenomegaly had become resistant to drug therapy and a splenectomy was performed, the LPA was low, and cytogenetic analysis showed only cells with a normal karyotype, suggesting a $Ph^1-$ CML. Furthermore, both times when remission of blast crisis was achieved the hematologic picture returned to a CML pattern in chronic phase, and the postmortem pathologic analysis of the lymph nodes did not show a monotonous blast infiltration but a variegated myeloid picture with different stages of maturation of the cells.

*Blastic transformation.* The cytogenetic abnormalities that developed during the blastic phase did not help in discriminating between AML and $Ph^1-$ CML blast crisis. In both diagnoses, trisomy of C-group chromosomes, regression of the abnormal clone during remission, appearance of new clones, and clonal evolution have been described.[18 21] Trisomy 8 is the most common trisomy C reported in AML as well as in CML blast crisis.[18 21] Here the hyperdiploid karyotype showed trisomies of chromosomes 9, 10, and 12, which have also been described individually in both diagnoses[18,22-24] but not in this particular association. In this observation trisomy 8 was found in clones that appeared later in the evolution of the disease (Figs. 5 and 6).

Twice, 1 yr apart, the patient developed a blast crisis accompanied by the rapid increase in BM of the same abnormal clone with the 49,XY,+9,+10,+12 karyotype. Both times this subpopulation responded to chemotherapy and disappeared from the proliferating pool at the benefit of cells with a normal karyotype and, the second time only, cells derived from another clone with the t(5p;17q). This is one of the few cases of documented cytogenetic remission of CML in blast crisis.

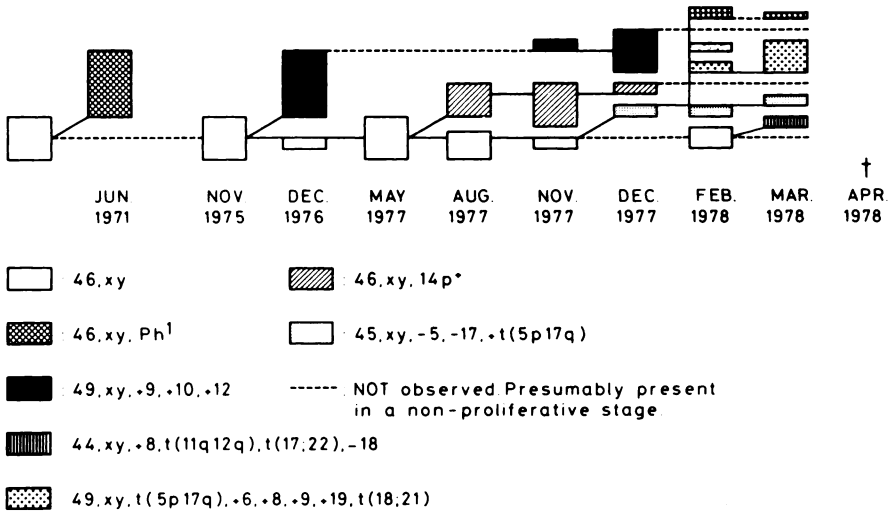


**Fig. 7. Schematic representation of different clonal populations found in BM mitotic cells of our patient from June 1971 to March 1978.**

*Development of new clonal populations.* Figure 7 represents a scheme of the appearance and development of karyotypically different subpopulations as they were observed in our patient between June 1971 and March 1978. At least five times a new clone appeared, apparently stemming from a cell with a normal karyotype. This is at variance with the usual pattern in CML, where stepwise changes are observed in a $Ph^1$+ stem cell, similar to what we found in our last cytogenetic analysis of this patient (Table 3).

Cytogenetic analysis gives information on the population of cells undergoing mitosis in vivo or in short culture, so that only a limited number of cells can be analyzed. Therefore a subpopulation of cells that is poorly represented or is in a nonproliferative stage may escape detection. This explains why, although at one particular time we failed to find cells with a normal karyotype, these cells may later, after chemotherapy, represent the majority of the proliferative pool. Similarly, this explains why the "blastic" karyotype with 49 chromosomes, apparently eliminated from BM in May 1977, could reappear in November 1977 together with a relapse of the blast crisis.

It is not likely that the hyperdiploid clones with trisomy of chromosomes of the C group or trisomy 17, 19, etc. are therapeutically induced, since they have been seen in AL and in blast crisis of CML in untreated patients and in patients treated with widely different approaches. In cases of random structural aberrations, like our 14p+, the role played by therapy in the initiation of the abnormal clone is difficult to evaluate. In any case, the abnormality must have started in a stem cell still capable of proliferation and furthermore gaining proliferative advantage over the other stem cells.

The 14p+ clone arose in August 1977 and became prominent in November, but in December relapse of the blast crisis rapidly invaded the BM with cells belonging to the 49,XY,+9,+10,+12 karyotype, and the 14p+ clone appears to have been transient.

The hypodiploid clone with the characteristic t(5p;17q) presents a few interesting features. It was observed for the first time during the second blast crisis, when most of the mitotic cells belonged to the "blastic" clone with 49 chromosomes. At the same time, we did not observe the decrease in CFU-C seen during the previous blast crisis. Karyotyping of the colony cells showed mainly mitoses belonging to this new minor clone. Unfortunately, because morphology of the colonies was not studied, the maturation capabilities of these cells in vitro, and presumably in vivo, were not ascertained. Events during induction of remission cannot be satisfactorily monitored because many cells are damaged. Nevertheless, during the hypoplastic phase we found in blood culture of our patient two groups of metaphases—cells with a normal karyotype and cells with t(5p;17q). A few weeks later, most of the BM and blood metaphases showed a hyperdiploid karyotype derived from this particular stem line (Table 3). This suggests that this clone was not only relatively more resistant to chemotherapy than the other, which disappeared, but also that it had the property of rapidly acquiring new chromosomes by nondisjunction.

The fifth cell line, discovered 1 mo before the patient's death, appeared less malignant than the hyperdiploid cells proliferating at that time. The deterioration of the patient's health was not caused by blastic transformation but by generalization of the disease.

Close cytogenetic follow-up of this CML case turned out to be particularly instructive. Apart from the $Ph^1$ conversion and the favorable response to chemotherapy of two blast crises, this case represents also, to the best of our knowledge, the first observation of a transient clone (the 14p+) that developed independently of blast transformation and receded not under chemotherapy but because of the rapid takeover by a more malignant clonal population. Later, the extensive clonal evolution stemming from the t(5p;17q) population indicated a loss of mitotic control in this particular stem line and acceleration of the leukemic process.

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