Significance of Ph⁻⁻Negative Marrow Cells in Ph⁺⁻Positive Chronic Granulocytic Leukemia

By Joseph E. Sokal

Fifty-six of 195 Ph⁺⁻positive patients with chronic granulocytic leukemia were found to have Ph⁻⁻-negative metaphases in marrow aspirates on one or more occasions. In 22 cases, Ph⁻⁻-negative cells were found prior to initiation of antileukemic therapy. Five patients were in the blastic stage of the disease when Ph⁻⁻-negative mitoses were seen. The finding of Ph⁻⁻-negative cells appeared to be related principally to short duration of CGL and to administration of antileukemic therapy (conventional agents and doses, in most cases). Ph⁻⁻-negative cells were usually not found more than 2 yr after the diagnosis of leukemia, but in a few cases, they were seen as long as 5–10 yr after diagnosis. Only a minority of metaphases analyzed were Ph⁻⁻⁻negative, except in the case of 6 patients who transiently had 50% or more Ph⁻⁻-negative cells after antileukemic therapy. The presence of Ph⁻⁻⁻negative cells in marrow was not associated with any survival advantage in this series.

CHRONIC granulocytic leukemia (CGL) is a disease of clonal origin. In the majority of cases, karyotypic studies of marrow at the time of diagnosis show only leukemic metaphases, characterized by the Ph¹ chromosome; remaining normal cell populations, if present, appear to have been suppressed. The translocation responsible for the appearance of the Ph¹ chromosome is usually the only demonstrable karyotypic abnormality in “early” CGL. As the disease progresses, however, additional, more abnormal subclones often appear. These may include aneuploid or pseudodiploid populations, lines characterized by double Ph¹, etc. It is generally agreed that the development of such additional chromosomal abnormalities constitutes an unfavorable sign in CGL.¹⁻³

In contrast to the many studies of the import of additional chromosomal abnormalities in CGL, relatively little information is available on the significance of persisting normal cells in the marrow of patients with Ph⁺⁻-positive CGL. There have been suggestions from anecdotal reports of long survival⁴⁻⁶ and from one short-term study of a small group of patients⁹ that demonstration of Ph⁻⁻-negative metaphases in marrow implies a significantly better prognosis than the finding of only Ph⁺⁻-positive mitoses. On the basis of such data, some authors have assumed that treatment which results in the appearance of significant numbers of Ph⁻⁻⁻negative cells will, thereby, improve survival.¹⁰ However, there have been no reports of relatively large series of patients with some Ph⁻⁻⁻negative marrow cells, followed long enough to define their survival with confidence. The present study was undertaken to obtain such data. Chromosomal studies of marrow aspirates from more than 200 patients with CGL were reviewed, and 56 Ph⁺⁻-positive patients were identified who had normal metaphases on 1 or more occasions. With one exception, the fate of these patients is known, and they have been followed until death or for at least 3 yr.

MATERIALS AND METHODS

Chromosomal study of bone marrow aspirates has been performed routinely since 1961 as part of the workup of patients referred to the Roswell Park Memorial Institute with known or suspected CGL. Most patients with CGL followed regularly at the Institute have had several such studies during the course of their disease, in conjunction with routine follow-up marrow aspirations or aspirations performed for various specific indications. During the period covered by this report (1961–1977), 195 Ph⁺⁻-positive patients were registered. Roughly half of these patients were referred to Roswell Park Memorial Institute prior to receiving any treatment for their disease. The others had had one or more courses of therapy, usually with busulfan, prior to study.

Chromosomal examination was not considered valid unless there were at least four metaphases suitable for analysis. The methods for chromosome study have been described previously.¹¹⁻¹² They varied somewhat during the period covered by this report. A direct marrow preparation was used in the majority of cases, and a short-term culture technique (12–18 hr) in the remainder. During the first decade of these studies, banding analysis was not performed; since 1972, banding studies were performed routinely. Destaining and re-examination of a few of the older slides with banding techniques confirmed the original reports of normal metaphases.¹³ An average of 26 metaphases were analyzed per examination (range 4–54).

Survival curves were calculated by the actuarial method. Since the purpose of this study was to determine the influence of chromosomal patterns on the course of leukemia, patients who died of other causes (e.g., cardiovascular disease) were considered as alive and lost to follow-up on the date of death. Survival was calculated from the date of the first chromosomal analysis showing a Ph⁻⁻⁻negative cell. In the case of two-fifths of the study patients, this was essentially the same as the date of diagnosis; thus, their survival could be compared directly with that of other Ph⁺⁻⁺positive patients. In the case of patients whose Ph⁻⁻⁻negative cells were found one or more years after treatment of CGL, however, this introduced some negative bias, since the risk of death is lower during the first year of "typical" CGL than it is later in the course of the disease.¹⁴ This can be corrected for (see "Survival," below) and appeared preferable to calculating survival of such patients from their dates of diagnosis, which could have introduced major bias toward longer survival.

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RESULTS

There were 56 patients who had at least 1 apparently normal metaphase (i.e., diploid and Ph'-negative) on 1 or more occasions during the study period. This represented 29% of the Ph'-positive patients studied during this period, but a substantially smaller percentage of the total number of studies, since most of these patients had other chromosomal analyses in which no Ph'-negative mitoses were detected (see below). The group consisted of 37 males and 19 females, a sex ratio not significantly different from that of the other patients with Ph'-positive CGL. They ranged from 7 to 78 yr of age, with a median of 44 yr; this was also similar to the remainder of the patient population.

Twenty-two of these patients had Ph'-negative marrow cells detected prior to receiving antileukemic therapy, while 34 had been treated for leukemia (Table 1). In one-third of the former group, normal cells were found after the initial course of therapy, while in one patient (studied on several occasions), it was not until almost 9 yr later; the median time from diagnosis to the finding of Ph'-negative metaphases was 14 mo. In both groups, one-fourth of the patients had only a single Ph'-negative metaphase identified. None of the untreated patients had as many as 50% Ph'-negative cells, while 6 of the treated patients had a majority of normal metaphases ($p < 0.05$). Seventeen patients—a little less than half of those who underwent subsequent chromosomal studies—were found to have Ph'-negative metaphases on at least one later occasion. An increase in the number of Ph'-negative cells was recorded after treatment in 4 patients originally identified at the time of diagnosis (including 3 of the patients with only 1 normal metaphase on initial study), but in none of the later studies of patients whose Ph'-negative cells had been detected only after antileukemic therapy.

Although Ph'-negative metaphases were usually found during the first year or two after the diagnosis of CGL (84% of all our patients), there were a few exceptions. In 4 patients, such cells were first found more than 5 yr after diagnosis. The latest demonstration of a Ph'-negative metaphase in this study was almost 10 yr after diagnosis (1/25 mitoses, in a patient who had exhibited 20% Ph'-negative cells on a previous occasion). In this series of patients, who did not receive especially “aggressive” therapy during chronic stage disease, Ph' mosaicism was not a particularly persistent phenomenon. In only 6 patients were Ph'-negative metaphases detected over time spans in excess of 2 yr. The longest of these was in the case of a 33-yr-old man who had 8/50 normal mitoses on initial study at the time of diagnosis and 1/25, 5 yr later. He lived eight more years and had annual marrow studies, but Ph'-negative cells were not again detected.

The 6 patients who were found to have 50% or more Ph'-negative metaphases all exhibited this finding on only a single occasion. Two patients had received intensive therapy for accelerated/blastic disease. In 1 case of chronic stage disease, 50% of metaphases were normal after the initial course of busulfan (3 mo after diagnosis), and in 3 others, such findings were recorded 10–18 mo after diagnosis. Two of these 4 patients had 8% Ph'-negative mitoses on re-examination half a year later, but none after that.

Five patients were blastic (blasts, 20% in blood or marrow, or blasts + promyelocytes, 30% in blood) at the time that Ph'-negative metaphases were identified. Two of these had newly diagnosed disease, while the others were known to have had leukemia for 39–64 mo.

Survival

Forty-three of the study patients (77%) have died of leukemia. Seven died of other causes, 13–156 mo after detection of Ph'-negative metaphases (median, 24 mo). One was lost to follow-up at 6 mo, and 5 patients were alive at 37–121 mo (median, 50 mo). A total of 11 patients lived more than 5 yr and 3 of them, more than 10 yr. Figure 1 presents the survival curves for the untreated and the treated groups of patients from the time of the first finding of Ph'-negative marrow cells. Also shown in the figure is the survival from diagnosis of the entire population of 195 Ph'-positive patients registered at our Institute during the time period of

### Table 1. Features Associated With the Finding of Ph' Negative Metaphases Among 56 Patients With Ph' Positive CGL

<table>
<thead>
<tr>
<th></th>
<th>Previously Untreated Patients</th>
<th>Previously Treated Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (M:F)</td>
<td>22 (12:10)</td>
<td>34 (25:9)</td>
</tr>
<tr>
<td>Prior duration of CGL, months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from diagnosis (median)</td>
<td>0–3 (0)</td>
<td>2–107 (14)</td>
</tr>
<tr>
<td>Average no. of metaphases</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Only 1 Ph'-negative metaphase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>found</td>
<td>6 (27%)</td>
<td>10 (29%)</td>
</tr>
<tr>
<td>Ph'-negative cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>found in subsequent studies of</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>above patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% or more Ph'-negative cells</td>
<td>0</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Ph'-negative cells in two or more studies</td>
<td>8 (36%)</td>
<td>9 (26%)</td>
</tr>
<tr>
<td>Increase in Ph'-negative cells from first positive study</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No change or decrease</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Time span over which Ph'-negative cells recorded, months (median)</td>
<td>2–62 (19 mo)</td>
<td>7–43 (23 mo)</td>
</tr>
</tbody>
</table>
Fig. 1. Survival of 56 patients with Ph' negative metaphases in marrow at diagnosis or after treatment, from the date of chromosomal study. Two reference curves are shown: dashed line: survival from diagnosis of the entire population of Ph'-positive patients from which the study patients were drawn, for comparison with the curve for patients with Ph'-negative cells at diagnosis; dotted line: this curve shifted by 8 mo, for comparison with curve for patients with Ph'-negative cells after treatment (see text for explanation).

In this study (dashed line). It is obvious that there is no significant difference between any of these curves.

In the case of the previously treated patients, comparison of survival from chromosome study with that from diagnosis of the reference group introduces a negative bias. To eliminate this, a second reference curve was constructed. The principal effect of plotting survival from 1 yr after diagnosis instead of from the date of diagnosis, among typical Ph'-positive patients in the chronic stage of CGL, is to shift the survival curve approximately 7 mo to the left (Sokal, unpublished data). Therefore, to prepare a more suitable control curve for the group of 34 previously treated patients, whose median prior duration of disease was 14 mo, the reference curve was shifted by 8 mo (dotted line). This results in a minor, statistically insignificant advantage for the patients with Ph'-negative metaphases.

In a further effort to detect a prognostic influence of Ph' mosaicism, the 5 blastic patients were excluded, and survival of the remaining patients was correlated with the frequency of Ph'-negative metaphases. Among the previously untreated patients, 11 had more than 10% Ph'-negative metaphases. Seven of them have died of leukemia, 17–158 mo after diagnosis, and the median survival of this group will be between 45 and 54 mo. Six untreated patients had 20% or more Ph'-negative metaphases. Two of them died of other causes at 13 and 17 mo, 3 died of leukemia at 26, 38, and 54 mo, and 1 is alive at 45 mo, in the accelerated stage of the disease. The latter patient had the highest percentage of normal metaphases in the untreated group—43%. The longest survivors in this group (8, 10+, and 13 yr) had 7%, 7%, and 16% Ph'-negative metaphases on initial study. The numbers of patients in these subgroups are too small for formal analysis, but it is apparent that there is not a consistent correlation between frequency of Ph' negative metaphases and survival.

By combining untreated and previously treated patients, large enough groups could be defined to permit comparison of survival curves (Fig. 2). Twenty-eight patients, had more than 10% (and, at least 2) Ph'-negative metaphases, while 15 patients had 25% or more presumably normal dividing cells. Survival of these groups was not significantly different from that of the entire population of 51 patients, and there was no consistent trend in favor of patients with larger proportions of Ph'-negative cells. Survival of none of these groups differed significantly from that of all nonblastic Ph'-positive patients. Finally, the 4 patients in the chronic stage of CGL who (transiently) had...
50% or more Ph'-negative cells after conventional chemotherapy did not do particularly well. All died of leukemia within 37 mo of this finding, a trend toward poorer survival than that of any group in Fig. 2.

Thus, it appears that the presence of modest to moderate numbers of presumably normal marrow cells was not associated with any survival advantage in this series of patients with Ph'-positive CGL.

**DISCUSSION**

It is evident that Ph'-negative metaphases can be found in a substantial minority of patients with Ph'-positive CGL. The frequency in this series, 29%, is significantly higher than the 6% reported in an early study by Whang-Peng et al. On the other hand, Cunningham et al. reported a still higher figure (51%) in a group of 37 patients with recently diagnosed CGL. Ph'-negative cells may be seen in the blastic state as well as during the chronic stage of CGL. Five of our patients were blastic at the time such cells were first identified. Zaccaria et al. found Ph'-negative metaphases in 3 of 12 patients presenting in the blastic stage.

The finding of Ph'-negative mitoses appears to be related principally to two factors, the duration of CGL and antileukemic therapy. In 84% of our cases, less than 2 yr had elapsed since diagnosis. There was a distinct trend toward decrease in the percentage of Ph'-negative cells with time, and ultimately, such cells were no longer seen in the overwhelming majority of our patients. Antileukemic therapy was the factor that appeared to be responsible for the exceptions to the rule that normal metaphases decrease with time. Increases in the percentage of Ph'-negative cells in serial studies occurred only in association with treatment, and all our patients with 50% or more of such cells were in the treated group.

A variety of therapeutic agents appear capable of increasing the proportion of Ph'-negative cells in marrow aspirates. We have not studied the effects of different therapeutic schedules systematically, but work of others suggests that aggressive treatment and especially, use of combinations active in acute myeloblastic leukemia, is more likely to result in the appearance of normal metaphases. It should be noted, however, that this can also be achieved with conventional courses of single-agent chemotherapy and, even, with splenic irradiation or splenectomy.

Our data indicate that the finding of modest to moderate numbers of Ph'-negative metaphases in marrow of patients with Ph'-positive CGL is not associated with better prognosis. A similar conclusion was reached by Whang-Peng et al. over a decade ago. Additional evidence on this point is provided by the study of Cunningham et al. These investigators attempted to eliminate Ph'-positive cells from marrow of a selected group of patients with recently diagnosed disease. Among their “nonresponders” (patients who did not achieve major reduction in the percentage of Ph'-positive cells), half had some Ph'-negative metaphases at entry into the protocol, and almost all were found to have some normal cells after intensive therapy. However, the median survival of this group was less than 3 yr and the indicated 5-yr survival is only 10%. Thus it is clear that the presence of a minority of nonleukemic cells in the marrow does not delay the appearance of lethal transformations within the leukemic cell population.

Whether patients whose Ph'-positive population is reduced to a minority of the replicating cells have a more favorable outlook is not yet clear. None of our four patients with chronic stage disease who achieved 50% or more normal metaphases (transiently) did particularly well. The only reported series of such patients with sufficiently long follow-up for evaluation of survival is that of Cunningham et al. The 12 “responders” in that study, whose Ph'-positive metaphases were reduced to 0%-27% (median, 0), lived significantly longer than the nonresponders described above, and their median survival has not yet been reached (follow-up, 17-107 mo). However, these patients appear to have constituted a more favorable group prior to entry into the protocol, with smaller spleens at diagnosis and more slowly progressive disease. Thus, their survival might have been better even if such reductions in Ph'-positive cells had not been achieved. The fact that these reductions were only maintained for a few months in most of them suggests that other factors were responsible for their better survival.

The results of this study and data reported by others justify the following conclusions. It is now clear that Ph'-mosaicism is not rare in CGL. In fact, it is quite possible that the great majority of patients with “early” disease have residual normal stem cells in their marrow. These may not be seen on initial study because their proliferation is inhibited by homeostatic feedback from the greatly increased population of leukemic cells, but their presence often becomes evident after appropriate reduction of the total granulocyte mass releases such inhibition. In some patients, considerable numbers of normal cells may persist in the marrow for many years, but usually, their number decreases progressively with time. In most patients, only Ph'-positive cells can be found after the first 2 yr of disease. The presence of some Ph'-negative metaphases in marrow, either before or after treatment, has no prognostic significance at present. There is no
evidence that normal cells inhibit proliferation of the leukemic population or delay the emergence of the lethal clones of end-stage disease.

Although the presence of normal cells in marrow does not now confer a survival advantage to patients with CGL, it has considerable theoretical importance and may prove to be a critical determinant of prognosis in the future. The agents now used to treat CGL are marrow depressants with little or no specificity for leukemic versus normal precursor cells. When agents with true antileukemic activity (i.e., preferential toxicity for leukemic clones) become available, however, the presence of normal stem cells will make it possible to attempt to cure the disease by eliminating the Ph' positive clones. However, patients who have no residual normal cells and thus, are dependent on their leukemic cell population for granulocyte function, would not be eligible for curative treatment.

REFERENCES

Significance of Ph1-negative marrow cells in Ph1-positive chronic granulocytic leukemia

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