Prognostic Value of Chromosomal Findings in Ph¹-positive Chronic Myelocytic Leukemia¹

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SUMMARY

Chromosome examinations were performed on bone marrows from 88 patients with Ph¹-positive chronic myelocytic leukemia (CML). As a group, Ph¹-positive CML patients with some cytogenetically normal cells in the marrow survived much longer than those without such cells in their marrow. The survival for patients whose first bone marrow exhibited only metaphases with a Ph¹ and other karyotypic abnormalities was significantly shorter than that for patients whose marrow exhibited only metaphases with a Ph¹ and an otherwise normal karyotype or patients whose marrow contained both categories of cells. The shorter the interval between the diagnosis of CML and the first chromosome examination, the greater the frequency of karyotypically normal cells in the bone marrow. Karyotypic progression in CML was a common phenomenon, whereas a reversion was very rare. On the basis of the findings obtained, the early diagnosis and treatment of CML are indicated, both possibly being helped by the chromosomal findings in the marrow. Furthermore, a combination of the chromosomal data and the marrow cell differential may serve as an important prognostic index in CML.

INTRODUCTION

Chromosomal studies in CML² have led to a reclassification of the disease, so that it is now generally accepted that Ph¹-positive and Ph¹-negative CML constitute apparently 2 different clinical entities (8, 25, 26). Furthermore, Ph¹-positive CML consists of 2 distinct stages, i.e., a chronic stage and a blastic phase. The chronic stage is considered by many investigators (5, 17, 29) to be preleukemic rather than a truly leukemic one, and the blastic phase as very similar to AML (2, 14, 17, 25, 31), but more resistant to chemotherapy (2, 25, 28). In addition, the chronic form of CML is chiefly associated with a Ph¹-positive but an otherwise normal karyotype, whereas the blastic phase, even though it can also be associated with such a karyotype (12, 25, 26), is often accompanied by additional cytogenetic abnormalities other than the Ph¹ (11, 12, 18, 25, 26, 30). These findings, together with the occasional presence of additional chromosomal abnormalities in the chronic stage, have made the significance of karyotypic abnormalities in the blastic phase obscure. Despite these complicated karyotypic situations, additional chromosomal abnormalities appearing in the later stages of CML have been shown to be a poor prognostic sign (30).

Recently, we have reported that the presence of normal metaphases among the abnormal ones in the marrow was crucially important in terms of the patients' survival and response to chemotherapy in AML (19). If Ph¹-positive but otherwise cytogenetically normal cells play a role similar to that of normal metaphases in AML, how does this affect the clinical aspects of CML? Are the former cells, in fact, leukemic or not? Moreover, if normal cells, Ph¹-positive and otherwise normal cells, and cells with a Ph¹ and additional abnormalities have a different significance, in terms of the patients' response to chemotherapy, then the chromosomal findings could possibly be utilized in the staging of this disease. Although others have addressed themselves to some of these questions (4—6, 9, 30, 31), complete answers to these are still lacking; this paper not only presents additional chromosomal data in Ph¹-positive CML but also provides an analysis of the findings, based on the experience of our clinic and laboratory, which may be of help in the understanding of the course and therapy of CML.

MATERIALS AND METHODS

Chromosomes of bone marrow from 88 patients with Ph¹-positive CML (59 males and 29 females) were analyzed during the period between September 1970 and November 1974. Except for 1 patient, all were seen at Roswell Park Memorial Institute. The patients' ages ranged from 7 to 72 years (median, 43) for the males, and from 16 to 70 years (median, 44) for the females. A high percentage of the patients studied by us was first seen at our Institute before they had received any specific therapy for the leukemia. Thus, only 8 of our patients had been treated prior to cytogenetic examination and the inclusion or exclusion of the data on these patients did not affect materially the conclusions reached. Almost all of the patients received some form of chemotherapy during the course of the CML, the exact therapy being tailored to the requirements and indications of each case, so that the course of treatment varied from patient to patient.

The methods for chromosome analysis have been described in previous papers (19—23). A direct method of

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² The abbreviations used are: CML, chronic myelocytic leukemia; AML, acute myelocytic leukemia.

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preparation for chromosome analysis was used in most instances, but a short-term culture technique (12 to 18 hr) was also utilized occasionally. Chromosomes of 25 cells were usually counted in each sample. Examinations with less than that number of metaphases were also included, but those with less than 4 scorable cells were excluded from the study. The chromosome analyses were repeatedly re-evaluated through the course of the disease.

Metaphases were classified according to their chromosomal constitution: (a) those with a normal chromosomal constitution; (b) those with a Ph'-chromosome (Ph'-positive) and an otherwise normal karyotype; (c) those Ph'-positive and with additional karyotypic abnormalities; and (d) Ph'-positive metaphases with a missing Y-chromosome. It is possible for any given patient to have in the marrow all or, what was observed most frequently, only 1 of the cell types just described. Equivocal metaphases were excluded from consideration. Ph'-positive marrows, including those with a missing Y, were considered to be hypodiploid when at least 3 such cells with the same karyotype were found in a marrow.

Some statement may be in order regarding the chromosomally normal cells in the bone marrow of the CML patients. Although banding analysis was not performed in the initial phase of the study (1970—1971), and the results were based on "standard" Giemsa staining of the chromosomes, several observations led us to believe that the normal cells encountered were, indeed, normal karyotypically. Reexamination of destained slides of some of these patients with Q- and/or G-banding has confirmed the diploid nature of these metaphases. Furthermore, since 1972, all of the slides have been examined with various banding methods; additionally, we have not encountered a single instance in which apparently diploid cells in the bone marrow of CML, observed with "standard" Giemsa staining, did not turn out to be so when examined with banding.

Survivals after the 1st chromosomal examination were compared among the patient groups discussed above. Survival curves were made according to life table methods (1). A modified Wilcoxon test (3) was utilized to compare the differences between the survival curves, as well as to evaluate the differences in the length of the period from the date of diagnosis to the date of examination among the various patient groups.

RESULTS

Chromosomal Findings in Ph'-positive CML. A total of 254 examinations were performed; 17 were from 4 patients with Ph'-positive CML but with a missing Y-chromosome. The latter examinations were evaluated separately and will constitute a future report. Thus, of the 237 examinations which were performed on the patients without missing sex chromosomes, 161 were from the 55 males and 76 were from the 29 females (Table 1). The average number of examinations was 2.9/male (range, 1 to 10) and 2.6/female (range, 1 to 5). The results of the chromosome examinations of the marrows were as follows: (a) 60% were characterized by the presence of only Ph'-positive cells without any other chromosomal abnormalities; (b) 34% by Ph'-positive cells, partially or totally involved by other karyotypic abnormalities; and (c) only 6% by cells containing a normal chromosome constitution in addition to the Ph'-positive cells. Relatively more marrows of the 1st-mentioned type, i.e., only Ph'-positive cells in bone marrows, and less of those with a Ph' but with other karyotypic abnormalities were observed in male than in female patients with CML. Thus, 8 male patients exhibited at least 1 normal metaphase in the marrow during their clinical course, whereas only 1 female patient did so. Two of these male patients and 16 female patients had at least 1 Ph'-positive cell with other chromosomal abnormalities in the marrow. Two of the male patients had every type of cell in the marrow. The remaining 31 male and 12 female patients had only Ph'-positive cells without any other cytogenetic abnormalities in the marrow.

The differences between the sexes in the distribution of patients among the various groups were not significant but only suggestive (0.05 < p < 0.10), according to a χ² test with a correction for continuity. The median of the duration medians (the time duration from the date of diagnosis to the date of examination, or in case the patient had more than 1 examination, the median of such time durations) was 20 months for males and 18 months for females. There was no

| Table 1 |

Distribution of bone marrow examinations classified by the presence or absence of normal metaphases, Ph'-positive and otherwise normal metaphases, and Ph'-positive metaphases with other additional abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Normal only</th>
<th>Normal and Ph'-positive</th>
<th>Ph'-positive without other abnormalities</th>
<th>Ph'-positive but all abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, 55 patients</td>
<td>4 (2.5)*</td>
<td>8 (5.0)</td>
<td>1 (0.6)</td>
<td>101 (62.7)</td>
</tr>
<tr>
<td>Female, 29 patients</td>
<td>0</td>
<td>2 (2.6)</td>
<td>0</td>
<td>40 (23)</td>
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<tr>
<td>Total examinations</td>
<td>4 (1.7)</td>
<td>10 (4.2)</td>
<td>1 (0.4)</td>
<td>141 (59.5)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, indicate the percentages of each group.
significant difference between the sexes (0.50 < p < 0.60).

Survival in Ph<sup>+</sup>-positive CML with and without Other Karyotypic Abnormalities. The life table survival curves for the various patient groups are given in Chart 1. The remarkable survival of the patients with some normal cells in the marrow is apparent. The median survival following the 1st chromosome examination was 31.5 months for patients with only Ph<sup>+</sup>-positive cells and without other chromosomal abnormalities, 25.5 months for those with Ph<sup>+</sup>-positive cells with or without other cytogenetic changes, and 7.5 months for those patients with Ph<sup>+</sup>-positive cells but all with other chromosomal abnormalities. The median survival time was not obtained for the 4 patients with Ph<sup>+</sup>-positive cells and a missing Y and the 7 patients whose marrow contained some cells with a normal chromosome constitution, since only 1 patient (with a missing Y) was dead at the time of this report. The median survival for the patients with Ph<sup>+</sup>-positive cells, all with other karyotypic anomalies, was significantly shorter (p < 0.01) than that of the patients with Ph<sup>+</sup>-positive cells but otherwise chromosomally normal, and that of the CML patients with Ph<sup>+</sup>-positive cells with or without additional karyotypic changes (p < 0.05). However, the median survival for the Ph<sup>+</sup>-positive patients who had some normal cells in the marrow was significantly (p < 0.01) longer than that for all other Ph<sup>+</sup>-positive patients with or without chromosomally abnormal cells (Chart 1).

The intervals between the date of diagnosis of the Ph<sup>+</sup>-positive CML and the date of the 1st chromosome marrow examination are given for each group of patients in Table 2. The median interval was: (a) 1.6 months for the patients with a missing Y; (b) 4.7 months for those with some normal metaphases in the marrow; (c) 15.0 months for those whose marrow had only Ph<sup>+</sup>-positive cells without any other karyotypic changes; (d) 17.0 months for those with Ph<sup>+</sup>-positive cells containing some cells with other chromosomal changes; and (e) 40.0 months for those in whom all of the cells were characterized by other karyotypic anomalies in addition to the Ph<sup>+</sup>.

The intervals were significantly shorter for the patients with some normal metaphases in the marrow than those for the other 3 groups (p < 0.01), according to a Wilcoxon test. However, the difference between the intervals for the patients in Group a and for those in Group c and the difference between the intervals for the patients in Group e and those in Group c were not significant, but only suggestive (0.05 < p < 0.10) in both instances; and that between the patients in Group e and those in Group d was not significant (0.20 < p < 0.30) either.

Chromosomal Transition in CML. For those patients on whom more than 1 examination was performed during the course of the disease, the transition of the chromosomal expression of bone marrow was examined. Most com-

### Table 2

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</tr>
</thead>
<tbody>
<tr>
<td>Normal metaphases present (6 males, 1 female)</td>
<td>45</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Missing Y (4 males)</td>
<td>48</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1.6</td>
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<tr>
<td>Only Ph&lt;sup&gt;+&lt;/sup&gt;-positive cells (36 males, 21 females)</td>
<td>40</td>
<td>4</td>
<td>9</td>
<td>12</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>59</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Ph&lt;sup&gt;+&lt;/sup&gt;-positive with and without other abnormalities (4 males, 5 females)</td>
<td>44</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>17.0</td>
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<td></td>
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<tr>
<td>All cells Ph&lt;sup&gt;+&lt;/sup&gt;-positive and abnormal (7 males, 2 females)</td>
<td>54</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>40.0</td>
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commonly, the same chromosomal type was observed in succeeding examinations. When a transition did occur, that from a marrow containing exclusively Ph'-positive cells without any other chromosomal changes to one with some additional karyotypic abnormalities and then to a marrow consisting exclusively of abnormal Ph'-positive cells seemed to be the most common pathway. Reversion of the chromosomal picture in the bone marrow from one of exclusively abnormal Ph'-positive cells to one containing some Ph'-positive cells without other abnormalities was relatively rare. Even rarer was reversion to a marrow consisting exclusively of Ph'-positive cells without other chromosomal changes. The rarest was the reversion of a Ph'-positive marrow of any type to one in which any number of normal metaphases was present.

Correlation of Chromosome Picture with Cellular Differential of Bone Marrow. A correlation was attempted between the chromosomal findings and the percentage of myeloblasts plus promyelocytes, mature granulocytes, and proerythroblasts plus erythroblasts in the marrow, respectively. The median value of myeloblasts plus promyelocytes was 12% in marrows with Ph'-positive cells but without any other chromosomal anomalies, 19.5% in those with cells containing some additional karyotypic changes, and 23% in marrows consisting exclusively of karyotypically abnormal Ph'-positive cells (Chart 2). That for mature granulocytes was 43, 31.5, and 25%, respectively; and that for proerythroblasts plus erythroblasts was 13, 14, and 8%, respectively. No correlation was detected between the percentages of the kind of cells and those of abnormal (or normal) metaphases in marrows with a mixed population of karyotypically normal and abnormal Ph'-cells. The proportions of patients with more than 30% myeloblasts plus promyelocytes at the 1st examination were compared among the patients with only Ph'-positive cells without any other chromosomal changes and those patients with only abnormal Ph'-positive cells in the marrow. Thus, 4 out of 9 of the latter patients had more than 30% such cells, whereas only 1 out of 59 of the former patients did so. In other words when the cell differential showed less than 30% myeloblasts plus promyelocytes, a marrow with only Ph'-positive but otherwise karyotypically normal cells is most likely; whereas, when the differential contained more than 30% such cells, the chances that the marrow will contain only Ph'-positive cells without other chromosomal changes, or Ph'-positive cells with and without such changes, or exclusively abnormal Ph'-cells, are almost equal. The difference was highly significant at the 0.001 level, according to a direct calculation of probability (15).

Some Aspects of Ph'-positive CML with Chromosomally Normal Cells in the Bone Marrow. All of the patients who had cytogenetically normal cells in their bone marrow upon initial examination are still alive at the time of this writing. In 3 of these patients (2 males, 1 female), with substantial numbers of normal cells in the marrow, the percentage of these cells decreased and that of Ph'-positive cells increased with clinical and hematological progression of the disease. One of the male patients had 95% normal and 5% Ph'-positive cells in the bone marrow when first diagnosed. When the disease progressed, i.e.,. enlargement of the spleen, progression of the anemia, and an increase in the WBC count, the percentage of Ph'-positive cells arose to 50%, and with further progression, to nearly 85%. However, the response to therapy was excellent in this patient, and his marrow became nearly normal cytogenetically. The other male patient originally had 2% Ph'-positive cells in his bone marrow and, within 1 year, they increased to 70%, paralleling progression of his disease, both clinically and hematologically. In all the other patients with some normal cells in the marrow, progression of the CML was usually accompanied by a decreased proportion of normal cells, whether the Ph'-positive cells did or did not contain or further develop chromosomal changes. Of importance is the observation that the cytogenetically normal cells have persisted in all of the patients who had such cells on initial examination, even after prolonged courses of therapy.

In this series, an apparently karyotypic normal marrow was achieved in 4 male patients, after successful therapy. All of these patients had a mixture of normal and Ph'-positive cells in the marrow prior to such therapy. One of the patients later exhibited 100% Ph' positivity and died 57 months after he had been first diagnosed mistakenly as having AML; another patient is still living with 100% chromosomally normal cells in his marrow 18 months after the diagnosis; and the other 2 patients have had karyotypically normal marrows for only a few months.

DISCUSSION

In this study, an analysis of the chromosomal data obtained in a series of patients with Ph'-positive CML has not only revealed the probable importance of normal cells in the marrow to the course of this disease, but has also strongly indicated that the clinical progression of CML is usually accompanied by concomitant progression of the karyotypic
Among the 88 patients with Ph'-positive CML studied in this series, 4 male patients had a missing Y in addition to a Ph' in their marrow cells. Since a missing Y may affect the prognosis of CML (7, 10, 13, 16, 23, 24), we have separated these patients from the other male patients with CML. No female patients had a missing sex chromosome in the marrow.

Karyotypically normal cells were encountered early in the course of CML, as indicated by the short interval between the diagnosis and the chromosomal examination. Furthermore, the presence of these normal cells appeared to endow this group of patients with a much longer survival than that seen for patients without normal cells in their marrow. It is possible that the Ph'-positive cells may be more sensitive to therapy than the karyotypically normal cells and, hence, repopulation of the marrow with normal cells, when the leukemic cells decrease in number or totally disappear from the marrow, takes place. That this may, indeed, occur is pointed to by the emergence of cytogenetically normal marrow cells in 4 CML patients with some normal cells in their marrows prior to initiation of the chemotherapy for the disease.

As an extension of the above, and in confirmation of the findings of others (4—6, 17, 28, 30, 31), additional chromosomal changes besides the Ph' were usually seen in advanced stages of CML, commonly in the blastic phase. The latter was particularly frequent in those patients whose marrow contained not only Ph'-positive cells but also additional cytogenetic abnormalities. Thus, the present study points to the need for an earlier diagnosis of CML than is presently available in the preponderant number of cases with this disease, since, apparently, at a very early stage, chromosomally normal cells will usually be encountered; and the survival of these patients, when appropriately treated, appears to be significantly longer than when the disease is diagnosed at a relatively late stage. At present, normal metaphases are encountered in the marrow of only a small percentage of CML patients, whether the disease is in the chronic or blastic phase (11, 12, 18, 27); or, when present, they are rather scanty (21, 22, 30). In the later stages of Ph'-positive CML, normal cells are only rarely encountered in the marrow, and the presence of additional cytogenetic abnormalities is indicative of progression of the CML, usually into the blastic phase.

The intervals from the date of diagnosis to the date of the first examination were significantly shorter for the patients with some normal cells in their marrow than for those with Ph'-positive and otherwise chromosomally normal cells (Table 2). This fact indicates that normal metaphases are usually present in the marrow much more frequently at an early stage of the disease than at a later one and that they will disappear from the marrow unless the CML is successfully treated (6, 14, 27); a long survival, in fact, has been reported in patients with normal metaphases among the Ph'-positive cells following appropriate therapy (9, 20). However, reports indicating that the normal cells may persist for long periods without treatment of the CML have also been published (5, 9).

The median survival after the 1st examination was significantly shorter for the patients with only abnormal Ph'-positive cells as compared to that of the patients with only Ph'-positive cells with or without some other karyotypic abnormalities (Chart 1). This fact is reminiscent of our findings in AML (19), in which condition any normal cells in the marrow were vitally important to the success of chemotherapy and, more importantly, to the survival of the patients. The Ph'-positive but otherwise karyotypically normal cells in CML appear to have a role similar to that of the normal cells in AML (19), even though the former cells must themselves become the target for therapy when their number is too high and especially when the patient has normal metaphases besides the Ph'-positive cells in the marrow.

The distribution of the percentages of myeloblasts plus promyelocytes among Ph'-positive but otherwise chromosomally normal marrows seemed to follow a normal distribution up to the 30% point (Chart 2); this observation and the influence of the percentage of such cells on the patients' prognosis indicated that from a practical aspect, a bone marrow with more than 30% of such cells should be considered as in the blastic phase. A Ph'-positive but otherwise chromosomally normal marrow was more likely to be seen when there were fewer than 30% myeloblasts plus promyelocytes; when there were more than 30% such cells, the chances for the bone marrow to be only Ph'-positive or containing some or all cells with other karyotypic changes were almost equal. About 10% of the marrows with only Ph'-positive cells were accompanied by more than 30% myeloblasts plus promyelocytes, and more than one-half of the marrows with only chromosomally abnormal Ph'-positive cells were accompanied by less than 30% of such cells. These findings indicate that the former cells can undergo a blastic transformation without further chromosomal changes (5, 11, 26). Thus, these Ph'-positive cells can be compared to normal cells in the bone marrow of normal individuals before developing acute leukemia. Both types of cells behave like normal cells, and without acquiring (further) chromosomal changes, such subjects can develop AML or the blastic phase of CML.

In addition to the chromosomal constitution, the cell differential of the marrow seemed to correlate with the patient's prognosis. Out of the 9 patients with only karyotypically abnormal Ph'-positive cells in the marrow, 5 died within 6.3 months, 4 of whom had more than 30% myeloblasts plus promyelocytes at their 1st examination. The other 4 survived more than 6.4 months, all of whom had less than 30% such cells. There were also 9 patients whose 1st bone marrow examinations exhibited more than 30% myeloblasts plus promyelocytes. Among them, 5 died within 6.3 months, 4 of them being patients with only chromosomally abnormal cells in the marrow (the same patients as the above 4). None of the other 4 patients who had survived 1.6, 3.0, 7.4, and 15.8 months, respectively, and were living at the time of this study or, when last seen, had only cytogenetically abnormal cells in the marrow. Thus, we believe that, for a more complete appraisal of the prognosis Ph'-positive CML, an evaluation of both the chromosomal findings and the marrow cell differential is indicated.

The results of the present study were based solely on Ph'-
positive CML. Ph"-negative CML probably constitutes a separate entity, differing in many respects from the Ph"-positive disease, including a very poor prognosis (20). Even though in the preponderant number of patients with Ph"-negative CML the initial and subsequent cytogenetic studies reveal a diploid picture, including those performed with banding techniques, the significance of the diploidy in the marrow is of a different nature than in Ph"-positive CML. Hence, in this paper, the proposed hypothesis regarding the significance of cytogenetically normal cells in the bone marrow of CML applies only to the Ph"-positive disease.

Based on the findings of the present study, we believe that every effort should be directed toward the early detection and diagnosis of Ph"-positive CML due to the following reasons: (a) marrows with normal cells are mostly found in the very early stages of the disease; (b) improvement in the cellular karyotypic picture is very difficult to achieve; and (c) the hope for the permanent cure of the disease must reside in the eradication of the Ph"-positive cells and the restoration of a normal marrow.

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