Differences in Cell Cycle Characteristics Among Patients With Acute Nonlymphocytic Leukemia

By Azra Raza, Yogesh Maheshwari, and Harvey D. Preiser

The proliferative characteristics of myeloid leukemias were defined in vivo after intravenous infusions of bromodeoxyuridine (BrdU) in 40 patients. The percentage of S-phase cells obtained from the biopsies (mean, 20%) were significantly higher \( (P = .00003) \) than those determined from the bone marrow (BM) aspirates (mean, 9%). The post-BrdU infusion BM aspirates from 40 patients were incubated with tritiated thymidine in vitro. These double-labeled slides were utilized to determine the duration of S-phase \( (T_s) \) in myeloblasts and their total cell cycle time \( (T_c) \). The \( T_s \) varied from four to 49 hours (mean, 19 hours; median, 17 hours). Similarly, there were wide variations in \( T_c \) of individual patients ranging from 16 to 292 hours (mean, 93 hours; median, 76 hours). There was no relationship between \( T_s \) and the percentage of S-phase cells, but there was a good correlation between \( T_s \) and \( T_c \) \( (r = .8) \). Patients with relapsed acute nonlymphocytic leukemia (ANLL) appeared to have a longer \( T_s \) and \( T_c \) than those studied at initial diagnosis. A subgroup of patients at either extreme of \( T_s \) were identified who demonstrated clinically documented resistance in response to multiple courses of chemotherapy. We conclude that \( T_s \) and \( T_c \) provide additional biologic information that may be valuable in understanding the variations observed in the natural history of ANLL.

From the Section of Cell Biology, Department of Hematologic Oncology, Roswell Park Memorial Institute, Buffalo.

Submitted April 24, 1986; accepted January 15, 1987.

Address reprint requests to Dr Azra Raza, Head, Section of Cell Biology, Department of Hematologic Oncology, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/6906-0016$3.00/0

Blood, Vol 69, No 6 (June), 1987: pp 1647-1653

1647
were counted from each biopsy section from at least five different fields by a single observer. A cell was counted as positively labeled if it showed any brown staining overlying the nucleus. Control samples were treated with only the primary or only the secondary antibody or just 3-amino-9-ethylcarbazole (AEC). There was no positive labeling detected in any of the control sample sections.

**Determination of** $T_{c}$: $T_{c}$ was determined in 40 leukemic patients as follows. The post-BrdU infusion BM aspirate was processed in Ficoll-Hypaque as described before. Cells at a concentration of 1 x 10$^5$/mL were resuspended in RPMI 1640 and 10% FCS. One hundred microliters of [methyl-3H]-thymidine (stock solution, 20 μCi/mL; specific activity, 60 Ci/mmol; Amersham Corp, Arlington Heights, IL) was added to 1 mL of the cell suspension along with 10 μL of 10-8 mol/L fluorodeoxyuridine (FUdR) and incubated for one hour at 37°C in 5% CO$_2$. After the incubations were completed, cells were washed thrice in cold saline and once in phosphate-buffered saline. Three drops of this suspension were placed on Alcian blue–coated coverslips and fixed in 70% ethanol for ten minutes. These coverslips were first processed by the RPMB technique described before and then coated with Nuclear Track Emulsion (NTB2; Eastman Kodak, Rochester, NY) and exposed in the dark for 48 hours at ~70°C. Kodak D19 developer was used to develop the samples, and after the samples were fixed, the coverslips were mounted onto glass slides with Elvolan 51-50 (Dupont) and examined under a fluorescence microscope. At least 2,000 positively labeled cells were counted per slide by a single observer. A cell was considered positively labeled for BrdU incorporation if there was any fluorescence detected over its nucleus and positive for 3H-thymidine ($^3$H-tr) incorporation if at least five or more silver grains were detected on its nuclear surface. The cells considered positive for double labeling demonstrated both fluorescence and silver grains over their nuclei. $T_{c}$ was then calculated by the following formula as described by Wimber and Quastler: $T_{c} = (DL + HTdtr) × t/BrdU$, where DL is the number of cells demonstrating double labeling, $HTdtr$ is the number of cells only showing $^3$H-tr incorporation, BrdU is the number of cells revealing only BrdU incorporation, and t is the time interval between the two labels. The interval t in patients who received a two-hour BrdU infusion was taken as two hours, and for individuals who received a one-hour infusion of BrdU, it was one hour. The reason we were able to keep this interval constant was because of the extreme care we exercised in obtaining the samples immediately at the end of the BrdU infusion and subsequently handling all samples on ice. Even cell washes were conducted in refrigerated centrifuges with cold media. Since minor delays ranging from a few minutes to an hour could in theory occur, we performed the double labeling of samples kept on ice at various times. Immediate post-BrdU infusion BM samples were incubated with $^3$H-tr, and the cell kinetic data thus obtained were compared with data obtained from the same specimen that was placed on ice for 15, 30, 60, and 120 minutes and then incubated with $^3$H-tr. Results indicate that no differences were noted for delays of up to two hours as long as the samples were kept on ice. Long delays, however, never occurred in our experience because the protocol was very strictly followed, all specimens being incubated with $^3$H-tr within 30 minutes.

**Control samples.** Samples of PB, BM aspirates, and biopsies were obtained from all 40 patients immediately at the end of the BrdU infusion. All samples were placed on ice and transported to the laboratory in the same building. Subsequent cell separation procedures as well as cell washes were conducted in refrigerated centrifuges with cold media. Since minor delays ranging from a few minutes to an hour could in theory occur, we performed the double labeling of samples kept on ice at various times. Immediate post-BrdU infusion BM samples were incubated with $^3$H-tr, and the cell kinetic data thus obtained were compared with data obtained from the same specimen that was placed on ice for 15, 30, 60, and 120 minutes and then incubated with $^3$H-tr. Results indicate that no differences were noted for delays of up to two hours as long as the samples were kept on ice. Long delays, however, never occurred in our experience because the protocol was very strictly followed, all specimens being incubated with $^3$H-tr within 30 minutes.

**Statistical methods.** Analysis was performed by using the terminal analysis package, a set of interactive computer programs. A two-tailed $t$ test was performed to compare the means of the percentage of cells in S-phase from the bone marrow aspirates and the BM biopsy samples. Similarly, a two-tailed $t$ test as well as Mann-Whitney and Spearman correlations were performed to compare the $T_{c}$, $T_{r}$, LI from BM aspirates, LI from BM biopsy samples versus other clinical or biologic parameters such as the FAB type, height of the WBC count at presentation, cytogentics, and so on.

**RESULTS**

**Estimation of S-phase cells in vivo.** Figure 1 shows an RPMB-processed BM aspirate sample after BrdU infusion in a patient. The use of immunoperoxidase-conjugated secondary antibody and counterstaining permits evaluation of the morphology of the S-phase cells. Using these slides, we were able to assess the percentage of S-phase cells from therapy and only received supportive care and/or 1 g hydroxyurea orally everyday until the time of death. Twelve patients received remission induction therapy with cytosine arabinoside (araC) at 100 mg/m²/d by continuous intravenous infusion and adriamycin at 30 mg/m²/d intradurally for seven (7 + 3) or ten (10 + 3) days plus 3.0 g/m²/d by intravenous infusion for a period of 75 minutes every 12 hours for six days (HDaraC), and one patient also received three days of daunorubicin (DNR) at 45 mg/m²/d intravenously for the last three days after HDaraC therapy (HDaraC + DNR). Of the 24 newly diagnosed ANLL patients who received the remission induction therapy described earlier, eight entered complete remission (CR), 11 had resistant disease (RD), and five patients died during induction (“other” failures). The treatment failures were classified according to our previously described classification system, which provides for recognizing those individuals who failed therapy because of persistent leukemia (RD) versus patients who died during induction therapy or during the hypoplastic phase induced by chemotherapy (“others”). CR was defined according to the Cancer and Leukemia Group B (CALGB) criteria described before.

All of the 11 ANLL patients studied at the time of relapse received remission induction therapy. Seven were treated with HDaraC, and four received mitoxantrone, 12 mg/m²/d, intravenously for five days. Two of these latter patients also received 5-azacytidine at 100 mg/m²/24 hr by continuous intravenous infusion for three days after five days of mitoxantrone therapy. Four patients achieved CR (3/7 CRs in the HDaraC-treated group and 1/4 CR in the mitoxantrone-treated group), five had RD (2/7 in the HDaraC-treated group and 3/4 in the mitoxantrone-treated group), and two patients treated with HDaraC died during induction or were “other” failures.

**Clinical data.** Prior to administering the BrdU infusion, a diagnosis of acute leukemia was established in every case. Of the 40 cases being reported in this paper, 37 had ANLL with a French-American-British (FAB) staging of M1 through M7, and three patients had blastic crisis of CML. Twenty-six patients had newly diagnosed ANLL, and 11 patients were studied at the time of relapse of ANLL. Of the 26 newly diagnosed ANLL patients, 24 received remission induction therapy, whereas two patients refused chemo...
matched BM aspirates and plastic-embedded biopsy samples in individual patients. These data are presented for the 40 leukemic patients in the first two columns of Table 1. Clearly, higher LI values were obtained from the biopsy samples (mean, 23%) than from the respective BM aspirates (mean, 9%) in individual patients. The difference in the means of the two groups as determined by the t test was statistically significant with a P value of .00003. In addition to this striking difference, it is important to note in Table 1 that there is no consistent relationship between the two values in individual patients. For example, patient 1 (Table 1) had 4% S-phase cells in the aspirate and 16% in the biopsy sample, whereas patient 2 had a LI of 15% in the aspirate and 17% in the biopsy sample.

**Measurement of cell cycle times.** Tc, as well as Tc, were determined on 40 leukemic patients. Table 1 provides this information in detail. Thirty-seven patients had a diagnosis of ANLL, 26 having been studied at the time of initial diagnosis and 11 at the time of relapse. Three patients were studied in blastic crisis of CML. If all the leukemic patients studied are considered together, one can see from Table 1 that there are wide variations in both Tc as well as Tc between individuals, although the mean and median values for each correspond well with what has been reported in the literature (mean Tc, 19 hours; median, 17 hours; and mean Tc, 93 hours; median 76 hours). The Tc ranges from four to 49 hours, and the Tc ranges from 16 to 292 hours. Table 1 also compares the cell cycle characteristics of newly diagnosed and relapsed ANLL patients as well as three individuals studied during blastic crisis of CML. These last three patients demonstrate shorter Tc and Tc as compared with the ANLL group. Although not statistically significant because of the few patients studied, it appears that both Tc and Tc of the leukemic cells in relapsed individuals are longer than that for newly diagnosed ANLL (mean of 22 hours v 18 hours for Tc and mean of 117 hours v 87 hours for Tc, respectively).

Since Tc is a derivative of both Tc and LI, we determined its relationship to these two parameters separately. Although there was no correlation between Tc and LI (r = .4), there appeared to be a statistically significant correlation (r = .8)
between $T_s$ and $T_c$. Figures 2A and 2B show these comparisons. We also assessed the relationship between $T_s$ and $T_c$ and the FAB type, the cytogenetic karyotype, and the height of the WBC count at presentation. There was no unique relationship between any of these values. For example, in nine newly diagnosed ANLL patients with FAB-M2, the median value for $T_s$ was 12 hours, and that for $T_c$ was 72 hours. Similarly, in ten newly diagnosed ANLL patients with FAB-M4, the median values for $T_s$ and $T_c$ were 15 and 70 hours, respectively.

Relationship of cell cycle characteristics to clinical course of the disease in ANLL. Although the means of $T_s$ and $T_c$ for all leukemic patients studied approached closely with what has so far been reported in the literature, it is clear that there are distinct differences between individual patients. For example, the leukemic cells of seven patients had $T_s$ values that differed from the population mean value by more than 1 SD (ie, either less than 23 hours or greater than 157 hours). All seven patients (Table 1, patient numbers 4, 7, 8, 20, 29, 32, and 34) failed to enter CR after chemotherapy, six because of persistent leukemia after therapy and one because of early death. Five of the six treatment failures had a very long $T_s$ (173, 196, 203, 213, 282 and 292 hours, Table 1), whereas one had a very short $T_c$ (16 hours). Although chemotherapy produced marrow aplasia in the latter patient, leukemic cells rapidly repopulated the marrow.

The values of $T_s$ and $T_c$ for patients studied at the time of initial diagnosis of ANLL and the outcome of remission induction therapy are presented in Table 2. It appears at a glance that both $T_s$ and $T_c$ are shorter (mean $T_s$, 16 hours; mean $T_c$, 66 hours) in patients who achieved CR as compared with those who had RD (mean $T_s$, 21 hours; mean $T_c$, 104 hours). Of note in the RD group is that two patients had a very long $T_s$ (196 and 283 hours), whereas two patients had a very short $T_c$ (16 and 38 hours), which suggests a possible role of cell cycle-related “kinetic resistance” to therapy in these individuals.

**DISCUSSION**

The proliferative characteristics of malignant cells were studied in 40 leukemic patients. The data presented in this paper led to three important observations. First, the percentage of S-phase cells was much higher in BM biopsy specimens than in simultaneously obtained BM aspirates. Second, there was great variability in cell cycle times of individual patients ranging from very short to very long durations of both the S-phase and the cycling times of cells. Finally, there was no direct relationship between the length of the cell cycle and the percentage of cells actively synthesizing DNA.

In previous studies, we have demonstrated that the in vitro BrdU LI were similar to the in vitro ³HTdr LI. In the present study, the in vivo BrdU labeling of BM aspirates provides data comparable with studies that have used ³HTdr in vivo. For example, the mean LI in BM aspirates after a BrdU infusion in this study is 9% compared with the mean LI after a ³HTdr infusion reported in the literature ranging between 5.9% to 7%. Similarly, the observation of a higher LI from matched BM biopsy samples of these patients confirms the findings of previous investigators who have addressed the question of hemodilution of the aspirates.

Many attempts have been made to relate the proliferative characteristics of leukemic cells as measured by the LI of BM aspirates with treatment outcome. The results have been conflicting. Obviously, one reason for this lack of relationship may be that the value for the percentage of S-phase cells was obtained from BM aspirates, which as noted earlier may provide inaccurate information. Even if

### Table 2. Cell Cycle Studies on Newly Diagnosed Patients With Acute Nonlymphocytic Leukemia Who Received Remission Induction Therapy v Outcome of Treatment

<table>
<thead>
<tr>
<th>S. No.</th>
<th>$T_s$ (h)</th>
<th>$T_c$ (h)</th>
<th>$T_s$ (h)</th>
<th>$T_c$ (h)</th>
<th>$T_s$ (h)</th>
<th>$T_c$ (h)</th>
<th>$T_s$ (h)</th>
<th>$T_c$ (h)</th>
<th>$T_s$ (h)</th>
<th>$T_c$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>29</td>
<td>132</td>
<td>2</td>
<td>25</td>
<td>100</td>
<td>1</td>
<td>13</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>75</td>
<td>4</td>
<td>34</td>
<td>283</td>
<td>6</td>
<td>11</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td>48</td>
<td>5</td>
<td>21</td>
<td>91</td>
<td>17</td>
<td>9</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>16</td>
<td>80</td>
<td>8</td>
<td>4</td>
<td>16</td>
<td>24</td>
<td>11</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>42</td>
<td>13</td>
<td>12</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>9</td>
<td>32</td>
<td>14</td>
<td>18</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>9</td>
<td>41</td>
<td>15</td>
<td>24</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>28</td>
<td>112</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>13</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16</td>
<td>66</td>
<td>21</td>
<td>104</td>
<td>13</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>16</td>
<td>61</td>
<td>21</td>
<td>92</td>
<td>11</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** CR, complete remission; RD, resistant disease; Others, death during induction; S. No., patient no.; $T_s$, duration of S-phase in hours; $T_c$, duration of total cell cycle time in hours.
the LI were accurate, it is not the only determinant of the biologic behavior of leukemic cells since it provides no information about \( T_s \) or \( T_c \), which are far more important measurements than the LI alone. The most striking illustration of this can be observed in Table 1, where both patients 7 and 8 have 7% S-phase cells in the BM aspirate and 25% S-phase cells in their biopsy specimens, yet their \( T_s \) (49 \( v \) 4 hours) and \( T_c \) (196 \( v \) 16 hours) are entirely unrelated. One patient (no. 7) takes approximately eight days to double his leukemic burden, whereas the other (no. 8) is doubling his leukemic cell mass every 16 hours. Hence, in order to acquire a comprehensive view of the cell cycle characteristics in ANLL patients, all three characteristics, i.e. LI as well as \( T_s \) and \( T_c \), must be taken into account.

Furthermore, conclusions regarding cell cycle characteristics in newly diagnosed ANLL patients have in the past been based on approximately 20 patients from nine different studies. Because the dose level and schedule of \( ^3 \text{H} T \text{d}r \) infusions as well as the methodology of sample processing was quite different in each study, comparisons of such data are difficult. Since the methodology we used is considerably more efficient than the double-labeled autoradiography or the estimation of labeled mitosis used in previous studies, we were able to study large numbers of patients uniformly.

After administration of BrdU and in vitro incubation of BM aspirates with \( ^3 \text{H} T \text{d}r \), we used our previously described double-label technique to measure the \( T_s \) and \( T_c \) in 40 leukemic patients. The internal \( t \) between the two labels was maintained at a constant by obtaining the BM samples immediately at the end of the BrdU infusion and subsequently handling samples on ice. All cell washing procedures were conducted with cold media in refrigerated centrifuges so that metabolic activity in the cells remained arrested until the start of incubation with the second label (\( ^3 \text{H} T \text{d}r \)) in vitro at 37\(^\circ\)C.

Cell cycle characteristics were thus measured on 40 leukemic patients, 26 of whom were studied at the time of initial diagnosis of ANLL, 11 at the diagnosis of relapsed leukemia, and three individuals in blastic crisis of CML. Of the 26 newly diagnosed ANLL cases studied, 24 subsequently received remission induction therapy. It appears that 11 patients who survived therapy (CR + RD only) had a \( T_c \) of less than 80 hours, and seven of these 11 patients achieved CR (Table 2). On the other hand, among eight patients who had a \( T_c \) longer than 80 hours and who survived therapy (CR + RD), only one entered remission, and seven demonstrated clinical resistance. This seems to indicate that patients with a \( T_c \) longer than 80 hours tend to respond unfavorably to chemotherapy. In fact, of six ANLL patients who had an extraordinarily long \( T_c \) (173, 196, 203, 213, 282, and 292 hours, Table 2), five demonstrated resistance to chemotherapy, and one patient died during treatment.

On the other hand, patients at the other extreme of \( T_c \) do not respond well either. For example, the patient with \( T_c \) of 16 hours (no. 8, Table 1) achieved aplasia after chemotherapy, but his BM was repopulated by rapidly dividing leukemic cells. This patient is particularly interesting because he achieved aplasia twice after chemotherapy, and each time his BM was repopulated by leukemic cells. The regrowth rate of his leukemic cells as measured by the daily increase in myeloblasts from his PB both times was consistent with a \( T_c \) ranging between 19 to 21 hours (manuscript in press). This value correlated strongly with a cycling time of 16 hours for the myeloblasts we had determined prior to administration of any chemotherapy. It must be remembered that for the calculation of \( T_c \) we utilized the LI value from biopsy samples rather than BM aspirates. Had we used the LI value from the BM aspirate in this patient, the calculated \( T_c \) would have been 57 hours rather than 16 hours. Since the clinical and biologic behavior of his leukemic cells was more consistent with a cycling time of 16 hours, this case validates the use of biopsy specimens for a more accurate assessment of the percentage of cells actively engaged in DNA synthesis.

Table 1 also compares the cell cycle characteristics of newly diagnosed and relapsed ANLL patients as well as three individuals studied during blastic crisis of CML. The three CML patients demonstrate a shorter \( T_s \) and \( T_c \) compared with the ANLL group, an observation consistent with the fact that, although chemotherapy often produces marrow aplasia, rapid regrowth of blastic crisis clones often occurs. Similar to previously reported results, both the \( T_s \) and \( T_c \) in relapsed ANLL patients were longer than that for newly diagnosed ANLL (22 \( v \) 18 hours for \( T_s \) and 177 \( v \) 87 hours for \( T_c \), respectively). Therefore, selection of leukemic clones that are slowly cycling may be an additional explanation for the frequent resistance to chemotherapy observed in these patients.

It must be remembered, however, that the biologic properties of leukemic cells represent only one of several factors that help to determine response. It is only possible to say that some cell cycle characteristics may be identified that if present will result in an unfavorable response to therapy, but if absent does not guarantee CR. Therefore we may see patients who have cell cycle characteristics compatible with a favorable response to chemotherapy yet who fail to enter remission because of other reasons (such as metabolic drug resistance of leukemic cells or a very high leukemic cell mass at the time of treatment). The clinical implications of identifying a subset of patients who will manifest resistance to therapy either because of very slowly cycling cells or unusually rapid proliferation regardless of other favorable prognostic factors are then obvious. For example, if this information can be obtained prior to therapy, both remission induction as well as consolidation/maintenance regimens could be altered to suit the individual needs of patients. Thus patients with a long \( T_c \) may benefit from prolonged treatment utilizing low doses of drugs. On the other hand, individuals with very rapidly dividing leukemias may be candidates for intermittent therapy with intensive courses of S-phase--specific agents. Finally, the duration of remission in patients who achieve CR may at least in part depend upon the regrowth rate of the residual leukemic cells; however, the present study is too young for comment on this aspect.

One last concern that needs to be addressed in the assessment of cell cycle characteristics is the question of the actual growth fraction in each patient. We assumed it to be 100% in the calculations of \( T_c \). This assumption was based on
the observations reported in three elegant studies \cite{1,2,3} that have shown that the noncycling or G0 compartment of cells in ANLL is relatively small. For example, Clarkson et al \cite{4} administered ^3HdR by continuous infusion for eight to ten days and demonstrated that 90% to 98% BM cells were labeled at the end of this period. This left a definite but insignificant proportion of 2% to 8% noncycling cells in the G0 compartment. Although it would be ideal to measure even this small subcompartment in every patient, at the present time the risk of administering BrdU by continuous infusion for long periods of time is not justified. Second, it must be remembered that the methods we utilized in our studies as well as those in the past can only yield information about the average behavior of a cell population. No information can be obtained about the rate of DNA synthesis or duration of cell cycle time in individual cells. It is possible that the 2% to 8% unlabelled cells in the study of Clarkson et al were very slowly cycling rather than being truly quiescent. Therefore, similar to past experience and assuming all cells to be in cycle, we have estimated the average cell cycle times of leukemic cells in a population.

In summary, therefore, cell cycle characteristics were determined for 40 leukemic patients. There were distinct differences in both the T_{c} as well as the T_{p} of individual patients. A subgroup of individuals was identified with either a very long or very short T_{p} who demonstrated clinical drug resistance in response to multiple courses of chemotherapy. Because these measurements can now be obtained within a reasonable time frame, they will contribute greatly in understanding the biologic behavior and the natural history of acute leukemias as well as assist in tailoring therapy to suit the needs of individual patients.

ACKNOWLEDGMENT

The authors would like to thank Drs Mayers and Bankert for producing the monoclonal anti-BrdU antibody. Thanks are also due to C. Grande for expert technical help and Grace Kuwik for excellent secretarial assistance.

REFERENCES

27. Saunders EF, Lampkin BC, Mauer AM: Variation of prolif-
Differences in cell cycle characteristics among patients with acute nonlymphocytic leukemia

A Raza, Y Maheshwari and HD Preisler