Detection of Cytarabine Resistance in Patients With Acute Myelogenous Leukemia Using Flow Cytometry

By Francis Lacombe, Francis Belloc, Patrice Dumain, Maryse Puntous, Pascale Cony Makhoul, Marie-Claude Saux, Philippe Bernard, Michel R. Boisseau, and Josy Reiffers

Cytarabine (Ara-C) is currently used in the treatment of adult acute myeloid leukemia (AML). To predict the results of induction chemotherapy, it could be useful to detect leukemic cells that are resistant to Ara-C in patients with AML. Using a bromodeoxyuridine/DNA (BrdUrd/DNA) staining method in flow cytometry (FCM), we have developed a cell resistance index to Ara-C (RI). The technique has been applied to 121 bone marrow (BM) samples from patients with de novo AML treated by a regimen containing Ara-C and daunorubicin (DNR). Ninety-seven patients achieved a complete remission (CR), and 24 patients did not and were considered drug-resistant (DR). The BM cells collected at diagnosis were cultured for 48 hours and underwent BrdUrd/DNA analysis.

ACUTE MYELOGENOUS leukemia (AML) is considered a heterogeneous disease. The response to therapy differs greatly among patients. This could be due to the differing sensitivity of leukemic cells to drugs and/or the differential proliferative rate of the leukemic blast progenitors versus normal cells.

Cytarabine (Ara-C) is one of the most effective agents for treating adult AML.1 Its active metabolite, Ara-CTP, inhibits DNA synthesis by competition with deoxyadenosine triphosphate (dCTP) to DNA polymerase2 and/or by incorporation in DNA strands.3,3 Ara-C incorporation in DNA correlates strongly with cytotoxicity.3,4 Pharmacokinetic studies have shown that there is a differential intracellular retention of Ara-CTP in normal versus tumor cells.7,8 These studies have also defined the optimal doses, schedule, and mode of administration of Ara-C.9 Whatever the molecular effects of Ara-C and the schedule for therapy, they mainly result in DNA synthesis inhibition.

Various biochemical mechanisms of Ara-C resistance have been described in experimental animal tumor models or in vitro systems, including enhanced degradation of Ara-C or its metabolites into the inactive Ara-U by high levels of cytidine deaminase,10,11 deficiency in deoxycytidine kinase,11-13 and high levels of intracellular dCTP pool.14,15 However, there are few data to accurately define the mechanisms of Ara-C resistance in patients with AML.16,17 The remission duration of patients achieving complete remission (CR) has been correlated with the ability of their blast cells to retain intracellular Ara-CTP.18,19 Response to therapy with Ara-C has been correlated with Ara-C deamination20 and with the activities of deoxycytidine kinase and deaminase.21 Despite the fact that Ara-C resistance is closely related to the lack of DNA synthesis inhibition,17 few correlations between inhibition of DNA synthesis and response to treatment with Ara-C have been described.22,23 The response to chemotherapy including Ara-C depends on the proliferative rate24 and on the sensitivity of leukemic blast progenitors to Ara-C.25 In vitro labeling with H-thymidine or DNA content histogram analyses have failed to correlate leukemic cell proliferative rate and treatment outcome.24,26 On the contrary, it has been proved that in vivo infusion of bromodeoxyuridine (BrdUrd) and immunolabeling of bone marrow (BM) biopsies using a monoclonal anti-BrdUrd antibody leads to better estimation of cell-cycle parameters of the blast cells,26,27 which could be correlated with clinical outcome. Similarly, the in vitro effect of Ara-C on blast-cell clonogenicity has been shown to be correlated with clinical outcome.28,29 In flow cytometry (FCM), the use of BrdUrd/DNA staining with a monoclonal anti-BrdUrd antibody and propidium iodide (PI)30-33 allows efficient simple determination of the cell-cycle phases and the evaluation of the DNA synthesis rate. Alterations in the DNA synthesis rate through the S-phase can be evaluated quickly and accurately by FCM from bivariate BrdUrd/DNA distribution, since the amount of BrdUrd incorporated over a short period is proportional to the amount of DNA synthesized during that period.34 Cell resistance to Ara-C has been evaluated from BrdUrd/DNA distributions in cell lines that are sensitive or resistant to Ara-C.35,36 To reliably evaluate the degree of leukemic cell resistance to Ara-C in clinical practice, it is necessary to determine the proportion of S-phase cells that are resistant to Ara-C and the level of BrdUrd incorporation in cells that are resistant to Ara-C. We recently described a computerized method for the analysis of BrdUrd/DNA bivariate distributions of HL60 cell lines sensitive or resistant to low or high doses of Ara-C.37 The degree of resistance
is determined automatically from an index of resistance to Ara-C (RI). We have shown that it is possible to accurately analyze the BrdUrd/DNA distributions, and hence determine from the RI small numbers of cells slightly resistant to Ara-C.

Here, we report a study of leukemic cells from 121 newly diagnosed patients with AML. After short-term culture, cells were incubated with various Ara-C concentrations, then processed for BrdUrd/DNA distribution analysis by FCM. Having calculated the RI, we were able to determine which patients were sensitive and resistant to conventional doses of Ara-C and, to a great extent, those who did not achieve CR. Preliminary results on a smaller series of patients have been reported elsewhere.35

**MATERIALS AND METHODS**

*Patients.* The study included 121 patients with previously untreated de novo AML who were treated in our institution between January 1989 and March 1993 and considered eligible for the response to induction chemotherapy. Patients who failed to achieve a CR were classified according to the proposals of Preiser.36 Excluded from the study were 51 other patients who were not assessable for response to chemotherapy: 34 who did not receive intensive induction chemotherapy for medical reasons, and 17 who died during the first week of treatment (inadequate trial category) or died in aplasia (hypoplastic death category). In the same period, 82 other patients with de novo AML, treated and considered eligible for response to induction chemotherapy in our institution, could not be studied for in vitro BrdUrd incorporation into their blast cells either for logistic problems (52 cases), for insufficient density of BM samples (18 cases), or for poor quality of their data (12 cases). There was no difference in the rate of CR and drug resistance (DR) between the 121 studied and 82 unstudied patients.

All of the patients gave informed consent. Their disease was classified according to the recommendations of the French-American-British (FAB) Committee.37 The clinical and hematological characteristics are summarized in Table 1. For patients aged =55 years, induction chemotherapy consisted of daunorubicin (DNR; 60 mg/m²/d, bolus, 3 days) and Ara-C (100 mg/m²/d, continuous infusion, 10 days).38 Patients older than 55 years of age were given Ara-C (100 mg/m²/d, continuous infusion, 7 days) combined with either DNR (50 mg/m²/d, bolus, 3 days) or idarubicin (8 mg/m²/d, bolus, 5 days).39 Twelve patients achieved a CR only after a second course of chemotherapy according to the same schedule. CR was defined as the presence of less than 5% blast cells in a cellular BM smear, and the absence of circulating blasts and extramedullary leukemic cell infiltration.

**BM collection and preparation.** Before induction chemotherapy, BM aspirates were sampled on sodium heparinate tubes. Bone marrow mononuclear cells (BM-MNC) were isolated by centrifuging row mononuclear cells (BM-MNC) were isolated by centrifuging of 5637 bladder cell line.40 Cell concentration was adjusted to 1 x 10⁶ cells/mL and cells were cultured for 48 hours in a humidified 5% CO₂, 37°C warmed incubator before in vitro treatment.

**Cell incubation with Ara-C and BrdUrd.** Cell viability after a 48-hour culture was assessed by trypan blue exclusion. Approximately 1 mL of the culture (adjusted to 1 x 10⁶ viable cells/mL) was dropped in four tubes. Two samples were incubated without Ara-C, one sample was incubated with 0.1 µg/mL Ara-C for 3 hours, and one was incubated with 1 µg/mL Ara-C for 3 hours. To verify the validity of the doses of Ara-C chosen for the in vitro incubations with leukemic blast cells, sequential measurements of Ara-C plasma concentrations were performed by high-pressure liquid chromatography after a 24-hour continuous infusion of Ara-C (100 mg/m²/d) for 15 patients. The mean value of plasma Ara-C concentrations was 0.085 ± 0.070 µg/mL (range, 0.015 to 0.36). The samples were then washed in RPMI 1640 to remove the drug. Finally, the blast cells were pulse-labeled with 10 µmoL BrdUrd for 30 minutes at 37°C, except one negative control that was incubated without BrdUrd or Ara-C. After washing and centrifugation, cells were fixed in 50% (vol/vol) ethanol in phosphate-buffered saline and stored at -20°C.

**BrdUrd/DNA staining.** The cells were stained as previously described.41 Briefly, the cell DNA was partially denatured using HCl, labeled by indirect immunolabeling using anti-BrdUrd and anti-mouse IgG-fluorescein isothiocyanate (FITC) F(ab')₂, and counterstained by PI. Cells were analyzed by FCM the same day.

**FCM assay.** Cells were analyzed with an ATC 3000 cell sorter (Odum-Brucker, Wissensbourg, France). An argon ion laser (2025 Spectra Physics, Les Ulis, France) was adjusted to emit 500 mW at 488 nm. Green fluorescence was measured through a band-pass filter (530 nm) to measure the amount of bound anti-BrdUrd antibody. Red fluorescence was measured through a long-pass 600-nm filter to determine the amount of bound PI. The bivariate BrdUrd/DNA (green/red) fluorescence distributions were displayed as dot plots (256 x 256 channel arrays). Each analysis was performed on a minimum of 50,000 cells. Doubles were eliminated by gating all data on the DNA fluorescence peak-area cytogram. The green signal was corrected for red contamination by electronic compensation until the bivariate FITC/PI histogram assumed a horizontal shape (mean green fluorescence of G1 and G2M approximately equal).

**Analysis of BrdUrd/DNA bivariate distribution: calculation of RI.** We used a personal software program detailed elsewhere,42 which

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**Table 1. Clinical and Hematologic Characteristics of Patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DR</th>
<th>CR</th>
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<tr>
<td>Total no. of patients</td>
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<td>97</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>43</td>
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</tr>
<tr>
<td>M6</td>
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<td>6</td>
</tr>
<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>57.4 ± 20.0</td>
</tr>
<tr>
<td>Median</td>
<td>67</td>
<td>57</td>
</tr>
<tr>
<td>Range</td>
<td>17–80</td>
<td>20–80</td>
</tr>
<tr>
<td>Blast cells (%)</td>
<td>Mean ± SD</td>
<td>81.4 ± 13.2</td>
</tr>
<tr>
<td>Median</td>
<td>86.5</td>
<td>71</td>
</tr>
<tr>
<td>Range</td>
<td>56–95</td>
<td>30–100</td>
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<tr>
<td>WBC (10⁹/L)</td>
<td>Mean ± SD</td>
<td>77.3 ± 106.9</td>
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<tr>
<td>Median</td>
<td>36.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Range</td>
<td>1.0–470.0</td>
<td>0.5–351.0</td>
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Fig 1. BrdUrd/DNA bivariate distributions of HL60 cells incubated 3 hours with or without Ara-C. (A) Negative control (no BrdUrd incubation). (B) HL60 cells not incubated with Ara-C. (C and D) HL60 cells incubated with 0.1 and 1 ng/mL of Ara-C, respectively. All calculations were performed from data corresponding to a box enclosing the cell population in S-phase (S-Box).

Table 2. BrdUrd/DNA Analysis of Patients (global results)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Total (N = 96)</td>
<td>DR (n = 15)</td>
</tr>
<tr>
<td>S-phase (%)</td>
<td>19.0 ± 10.4</td>
</tr>
<tr>
<td>R[0.1]</td>
<td>8.0 ± 7.6</td>
</tr>
<tr>
<td>R[1]</td>
<td>3.4 ± 4.2</td>
</tr>
</tbody>
</table>

Assessed by analysis of BrdUrd/DNA distributions using FCM.

**Evaluation of proliferative rate of leukemic progenitors.** Using the BrdUrd/DNA labeling technique, the detection of significant differences in the cell proliferative rate is difficult when the percentage of cells in the S-phase is low. We considered that a minimum amount of cells in the S-phase (3%) was needed to perform accurate calculation of the RI. Thus, the patients were divided into two groups: group A consisted of 25 patients (20.7%) with no detectable proliferative activity (12 with a loss of cell viability and 13 with <3% of S-phase cells) after a 48-hour culture in 5637-CM; group B consisted of 96 patients (79.3%) with ≥3% cells in the S-phase. In group A, there were significantly more DR patients than in group B: nine of 25 (36%) versus 15 of 96 (15.6%) (P < .025). Within the group B, for CR patients the mean ± SD of the percentage of S-phase cells was 18.0% ± 9.8%. For DR patients, the mean ± SD of the percentage of S-phase cells was 24.2% ± 11.9%. The difference is significant (P < .03) (Table 2). The percentages of S-phase cells for the CR and DR patients are shown in Fig 2. For the 85 patients who achieved a CR after one course of chemotherapy, 13 (15.3%) were in group A and 62 (74.7%) in group B; for the 12 patients who achieved a CR after two courses of chemotherapy, three (25%) were in group A and nine (75%) in group B. The difference was not significant.

**RESULTS**

The aim of this work in a series of 121 patients with de novo AML was to study the relationships between the response to induction chemotherapy including conventional doses of Ara-C and the data (ie, proliferative activity and degree of sensitivity to Ara-C) provided by a new FCM method for analyzing leukemic cells.
DETECTION OF Ara-C-RESISTANT CELLS

Evaluation of RI. For the 96 patients with ≥3% S-phase cells, the RI could be calculated. Typical results are illustrated in Fig 3 and 4, which show BrdUrd/DNA bivariate histograms obtained from two patients: one achieved CR (Fig 3) and the other was resistant to chemotherapy (Fig 4). On these histograms, the S-phase boxes used for RI calculation are indicated. When possible, two RIs were calculated for each patient and termed RI[0.1] and RI[1] according to the two doses of Ara-C used for incubation with leukemic cells (ie, 0.1 μg/mL and 1 μg/mL). In our series, 81 patients (84.4%) achieved CR and 15 (15.6%) were considered DR. The mean ± SD values of RI[0.1] were 6.4 ± 6.3 for CR patients and 18 ± 8.8 for DR patients. The mean ± SD values of RI[1] were 2.3 ± 2.0 for CR patients and 9.7 ± 7.4 for DR patients. The differences were highly significant (P < .0001) for the two values of RI between CR and DR patients (Table 3).

In Fig 5, the two corresponding values of RI[0.1] and RI[1] for each patient are plotted. The best cut-off RI value to discriminate CR and DR patients was calculated to be 8 for RI[0.1] and RI[1] using the likelihood ratio method. The patients were thus divided into three groups: group 1—RI[0.1] and RI[1] less than 8; group 2—RI[0.1] ≥8 and RI[1] less than 8; and group 3—RI[0.1] and RI[1] ≥8. In group 1 (63 patients), we found one DR and 62 CR patients. In group 3, all patients were DR. In group 2 (24 patients), 19 patients achieved CR and five were DR. The distribution of DR and CR patients between groups 1 and 3 was significantly different (P < .0001). In group 1, 56 patients (65.9%) achieved CR after one course of chemotherapy and six (50%) after two courses; in group 2, 16 patients (18.8%) achieved CR after one course and three (25%) after two courses.

Fig 3. BrdUrd/DNA bivariate distributions of a CR patient. (A) Negative control. (B) Leukemic blast cells not incubated with Ara-C. The S-Box indicated is the same for all distributions. (C and D) Leukemic blast cells incubated with 0.1 and 1 μg/mL of Ara-C, respectively. The corresponding values of RI are indicated.

Fig 4. BrdUrd/DNA bivariate distributions of a DR patient. (A) Negative control. (B) Leukemic blast cells not incubated with Ara-C. The S-Box indicated is the same for all distributions. (C and D) Leukemic blast cells incubated with 0.1 and 1 μg/mL of Ara-C, respectively. The corresponding values of RI are indicated.

In Fig 5, the two corresponding values of RI[0.1] and RI[1] for each patient are plotted. The best cut-off RI value to discriminate CR and DR patients was calculated to be 8 for RI[0.1] and RI[1] using the likelihood ratio method. The patients were thus divided into three groups: group 1—RI[0.1] and RI[1] less than 8; group 2—RI[0.1] ≥8 and RI[1] less than 8; and group 3—RI[0.1] and RI[1] ≥8. In group 1 (63 patients), we found one DR and 62 CR patients. In group 3, all patients were DR. In group 2 (24 patients), 19 patients achieved CR and five were DR. The distribution of DR and CR patients between groups 1 and 3 was significantly different (P < .0001). In group 1, 56 patients (65.9%) achieved CR after one course of chemotherapy and six (50%) after two courses; in group 2, 16 patients (18.8%) achieved CR after one course and three (25%) after two courses.

DISCUSSION

Predicting remission outcome in AML requires rigorous evaluation of the predictive tests used. Many prognostic factors have been described, but the proliferative activity and chemosensitivity of leukemic cells are usually considered as the most decisive parameters. Using only a 48-hour culture of the sample and a single FCM test based on BrdUrd/DNA staining, we have been able to analyze simultaneously these two decisive parameters in leukemic cells.

In this work, we have studied the relationships between the clinical response to induction chemotherapy of 121 patients with de novo AML treated with a standard Ara-C/anthracycline induction chemotherapy, the proliferative activity, and the sensitivity to Ara-C of leukemic cells. All samples were processed in the same culture conditions using modifications of the Nara technique: the BM-MNC were not depleted in T lymphocytes, the 5637 supernatant of bladder cell line was used to stimulate leukemic blast progenitor growth, and the duration of blast-cell culture was only 48 hours. In our hands, these culture conditions increased the number of cells in S-phase in the majority of cases, and thus allowed us to improve the sensitivity and reproducibility of the analysis of BrdUrd/DNA histograms. Moreover, we were able to confirm that the cells studied after a 48-hour culture were almost all leukemic cells as assessed by microscopic inspection and, in some cases, immunophenotype analysis (data not shown). In some cases, no proliferation of the leukemic cells was found after a 48-hour culture of the BM...
Table 3. BrdUrd/DNA Analysis of Patients Classified According to Sensitivity to Ara-C

<table>
<thead>
<tr>
<th>Group 1*</th>
<th></th>
<th>Group 2*</th>
<th></th>
<th>Group 3*</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>CR (n = 62)</td>
<td>(Mean ± SD)</td>
<td>DR (n = 5)</td>
<td>(Mean ± SD)</td>
<td>CR (n = 19)</td>
</tr>
<tr>
<td>S-phase (%)</td>
<td>8.6 ± 17.1 ± 10.3</td>
<td>28.3 ± 5.9 ± 7.3</td>
<td>21.0 ± 7.3</td>
<td>23.9 ± 14</td>
<td></td>
</tr>
<tr>
<td>R(0.1)</td>
<td>4.5 ± 3.5 ± 2.3</td>
<td>12.4 ± 2.8 ± 5.4</td>
<td>16.0 ± 5.4</td>
<td>23.6 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>R(1)</td>
<td>0.6 ± 1.6 ± 1.6</td>
<td>1.6 ± 1.5 ± 1.9</td>
<td>4.3 ± 1.9</td>
<td>13.9 ± 4.0</td>
<td></td>
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</tbody>
</table>

Assessed by analysis of the inhibition of BrdUrd incorporation into leukemic cells by Ara-C using FCM.

*Group 1: R(0.1) and R(1) < 8; group 2: R(0.1) > 8 and R(1) < 8; group 3: R(0.1) and R(1) > 8.

samples, and a higher number of DR patients was found in this group of 25 patients. This result can be explained by the low rate of proliferative leukemic cells, which leads to a low sensitivity to the action of Ara-C on S-phase cells.

To accurately measure the proliferation of blast cells in culture, the BrdUrd/DNA technique in FCM is a more efficient method than single labeling using PI as a DNA probe. In a group of 96 patients with measurable cell proliferation, we found a higher percentage of S-phase cells in DR than in CR patients. This parameter, which has been described as determining the duration of CR, seems to also play a role in the attainment of CR in our series. Moreover, from preliminary studies of our series, we confirm that the percentage of S-phase cells after a 48-hour culture of the BM samples is the main factor in determining the duration of CR (data not shown). Recently, by using ³H-thymidine to measure the spontaneous proliferative activity of blast cells after 3-days culture, Löwenberg et al also reported that a high proliferative rate is an unfavorable prognostic factor for CR. However, in this latter study and recently in that of Hunter et al, low rates of autonomous proliferation were associated with CR. Our culture conditions are not comparable to those in these studies, but the BrdUrd/DNA technique could help to evaluate rapidly and accurately the proliferative status of leukemic cells cultured with or without growth factors. Similarly, the self-renewal capacity of leukemic blast progenitors has been reported to be associated with prognosis in AML. The higher the self-renewal capacity, the higher the probability of failure. Our data corroborate these findings.

In vivo studies of blast-cell proliferative activity probably play a much greater role than in vitro studies in determining response to therapy. Raza et al, using in vivo infusion of BrdUrd, measured S-phase duration and total cell-cycle time of leukemic cells. Their data showed that patients with a low proliferating leukemia had longer remissions than patients with a high proliferating one. Using a visual method of analyzing BrdUrd incorporation into proliferative cells, they accurately identified leukemic blast cells in heterogeneous samples, but the procedure was time-consuming and did not provide quantitative information on the incorporation of BrdUrd. Riccardi et al used in vivo infusion of BrdUrd and studied cell-cycle characteristics by FCM. Their results were in agreement with those obtained using ³H-thymidine.

Fig 5. Diagram of all RI values. For each patient situated on the x-axis according to increasing values of R(0.1) and R(1), R(0.1) and R(1) values are plotted. Three groups could be composed: group 1—R(0.1) and R(1) < 8; group 2—R(0.1) > 8 and R(1) < 8; group 3—R(0.1) and R(1) > 8. The only DR patient in group 1 is borderline. For some patients, only one RI value was available, but their classification in a specific group was possible by considering that R(1) is always lower than R(0.1).
incorporation, but no quantitation of the incorporation of BrdUrd after treatment by Ara-C was done. We also used BrdUrd and FCM to analyze DNA synthesis of leukemic cells, but found that in vitro experiments only provided good quality data and that it was necessary to perform a test of cell sensitivity to Ara-C.

Ara-C chemosensitivity of leukemic cells is the other important component for obtaining CR in AML. As cells resistant to Ara-C continue to synthesize DNA despite the presence of Ara-C, the efficacy of Ara-C on leukemic cells can be evaluated from the inhibition of the DNA synthesis rate in cells incubated with Ara-C. Inhibition of 3H-thymidine incorporation into DNA is a standard method for evaluating the DNA synthesis rate, and has been used with cell lines and leukemic blast cells. It has been shown that Ara-C-resistant cells can be detected using a BrdUrd/DNA assay. The effect of Ara-C on BrdUrd incorporation into DNA has been reported, and this method has been applied clinically. Recently, we have proved that it is possible to quantify the resistance of HL60 leukemic cells to Ara-C using the BrdUrd/DNA assay and a dedicated software program. We showed that it was essential to determine the following simultaneously: (1) the percentages of cells remaining in the S-Box after treatment by Ara-C, which represent the cells potentially resistant to a given dose of Ara-C; and (2) the extent of BrdUrd incorporation in these cells alone, which is the best way to assess their degree of resistance to a given dose of Ara-C. From these findings, we developed a RI of leukemic cells incubated with various doses of Ara-C. The RI was independent of the shape of the BrdUrd labeling distribution, and could detect partially resistant homogeneous populations of blast cells, as well as resistant cells among sensitive ones. Applying this new method of BrdUrd/DNA bivariate histogram analysis to our series of patients, we found a good correlation between the remission outcome of 96 patients and the RI values. In a series of 20 children with acute lymphoblastic leukemia (ALL), Katano et al determined the sensitivity of S-phase cells to Ara-C by measuring the percentages of the residual S-phase cells capable of incorporating BrdUrd after incubation with various concentrations of Ara-C. Unlike us, they did not find a significant correlation between the percentages of S-phase cells and the in vitro sensitivity to Ara-C. Three reasons may explain these opposing results: (1) no BM culture was performed in the series of Katano et al; (2) the proliferative activity and the sensitivity to Ara-C are perhaps not the same for the leukemic cells of AML and ALL; (3) the detection of Ara-C resistance by only the determination of the percentages of the residual S-phase cells capable of incorporating BrdUrd after incubation with Ara-C is not sufficient, and also requires the calculation of the intensity of BrdUrd incorporation by leukemic cells after Ara-C incubation.

The resistance to anthracyclines has also been described to be correlated with the outcome of AML treatment using the detection of the GP 170 protein expression. In a smaller series of de novo AML patients treated with the same regimen including Ara-C and anthracyclines, we simultaneously studied Ara-C resistance using our test and GP 170 activity reverted by verapamil to measure the accumulation of anthracyclines by FCM. In our hands, the Ara-C resistance test was the best predictive test of remission outcome and was not correlated with GP 170 activity (data not shown).

The method of analyzing BrdUrd/DNA distributions described here to quantitate the inhibition of DNA synthesis in cells incubated with Ara-C represents a convenient in vitro method to predict in vivo resistance to Ara-C. It could be used as a test for resistance to treatment with Ara-C or other drugs acting on DNA synthesis in patients with AML. Many other ways to study Ara-C resistance exist, but they do not involve cell-by-cell analysis of this resistance (dosage of Ara-C metabolites, incorporation of 3H-thymidine) or are very time-consuming (clonogenic assays). Our test rapidly assesses (48 hours) both of the essential characteristics of AML: kinetic properties and sensitivity to Ara-C of individual blast cells. Its association with the determination of other known prognostic factors could lead to a strategy of treatment adapted to each individual case, and help to improve the prognosis of AML.

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Detection of cytarabine resistance in patients with acute myelogenous leukemia using flow cytometry

F Lacombe, F Belloc, P Dumain, M Puntous, PC Makhoul, MC Saux, P Bernard, MR Boisseau and J Reiffers

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