In Vivo Cell Growth and Pharmacologic Determinants of Clinical Response in Acute Myelogenous Leukemia

By Judith E. Karp, Ross C. Donehower, John P. Enterline, Gregory B. Dole, Michael G. Fox, and Philip J. Burke

A predictable increase in the proliferative rate of malignant cells remaining after initial cytoreduction in vivo forms the rationale for timed sequential therapy (TST) with 1-B-D-arabinofuranosylcytosine (ara-C) for adult acute myelogenous leukemia (AML). The relationship between in vivo leukemic cell growth, intracellular ara-C metabolism, and clinical response to ara-C-containing TST was evaluated by comparing AML marrow cell growth kinetic and biochemical pharmacologic determinants obtained before therapy (day 0) and at the predicted peak of in vivo postdrug residual tumor proliferation (day 8). Serial measurements of DNA synthesis and net intracellular ara-C metabolism demonstrated marked increases in both determinants in day 8 residual tumor when compared with the pretreatment cells for newly diagnosed adults achieving complete remission but not for TST-refractory patients. The interrelationship of AML cell proliferation and biochemical pharmacology together quantitate cytotoxicity measured by both achievement and duration of remission and serve to predict eventual clinical outcome in response to TST with ara-C where both growth and favorable pharmacokinetics are intrinsic to the success of the drug schedule.

THE DEPENDENCY of the proliferative status of malignant cell populations and the antitumor effect of the cell cycle–dependent drug 1-B-D-arabinofuranosylcytosine (ara-C) has been demonstrated in rodent14–16 and human5,17 acute myelogenous leukemias (AML) both in vitro and in vivo. Effective ara-C cytotoxicity results from intracellular drug triphosphorylation (ara-cytosine triphosphate [CTP]), which then inhibits DNA polymerases,8,12 and is directly incorporated as a fraudulent nucleotide into DNA, with maximal DNA damage resulting during active DNA replication.15–17 The link between cell growth kinetics, net intracellular ara-C metabolism, and ara-C antitumor effect is the basis for ara-C-containing timed sequential therapy (TST) for adult AML at the Johns Hopkins Oncology Center.3,8,10,11 The design of TST rests on the hypothesis that cytoreduction with the initial drug in sequence stimulates the growth of residual tumor and promotes sensitivity of the primed residual tumor to cell cycle–active agents.8,12,13 The second drug in sequence is timed to coincide with this predictable tumor regrowth.2,3,8,10,18

Laboratory models of TST have demonstrated that both normal and malignant cells that proliferate in response to a growth-stimulated milieu are more inhibited by ara-C than are cells maintained in a steady state.7,9,16,20 Studies of intracellular ara-C metabolism in this system demonstrate a differential biochemical response in the growth-perturbed state that discriminates ara-C–sensitive from ara-C–refractory leukemia bone marrow cells obtained before therapy in patients with AML.9 While leukemic myeloblasts from both clinically sensitive and resistant patients are stimulated to proliferate by culturing them in serum containing drug-induced humoral stimulatory activity (HSA), only those marrow cells from patients with ara-C–sensitive AML demonstrate enhanced intracellular formation and retention of ara-CTP. Thus this in vitro model, designed to parallel the in vivo drug-perturbed milieu intrinsic to successful TST, supports the premise that such induced proliferation renders cells more sensitive to the effects of optimally timed ara-C. This enhanced sensitivity appears to be the result of both the S-phase specificity of ara-C and net intracellular drug metabolism.

To test in the patient the validity of this laboratory observation, we have studied the proliferative status and net ara-C activation of leukemic myeloblasts remaining in the bone marrow of adults with AML following initial cytoreductive drug priming, compared those measurements with values obtained before therapy, and determined the relevance of this increased cell growth and drug metabolism to the clinical outcome with TST. Our findings in residual AML in vivo parallel the in vitro model of TST,9 which demonstrates an association of growth and pharmacologic behavior that culminates clinically in enhanced ara-C cytotoxicity in sensitive AML, while refractory cells demonstrate uncoupling of these determinants in the in vivo drug-perturbed state.

MATERIALS AND METHODS

Patients and therapy. From July 1984 through October 1987, a total of 110 newly diagnosed, nonpreviously treated (NPT) adults (median age, 55 years; range, 19 to 79) with the diagnosis of AML (French-American-British classifications M1 to M7) were consecutively admitted to the Adult Leukemia Service of the Johns Hopkins Oncology Center for intensive timed sequential induction chemotherapy with 2 g/m2/72-hr continuous infusion of ara-C3,4 plus daunorubicin, 45 mg/m2/day for three days beginning day 1, and followed by amsacrine, 200 mg/m2/day for three days beginning day 8,21 and were evaluated with respect to clinical outcome after treatment. All patients received the same induction regimen. A second cycle of TST consisting of continuous-infusion ara-C plus daunorubicin beginning day 1 and followed by 2 g/m2/72-hr ara-C infusion beginning day 10 was administered after recovery from induction therapy.22 All patients received antileukemic therapy as...
part of the clinical investigation approved by the Joint Committee on Clinical Investigation of The Johns Hopkins University School of Medicine in accord with assurance approved by The US DHHS and following fully informed consent to participate in active investigative therapy. Of these 110 NPT patients, 13 (12%) did not survive induction therapy and were therefore not considered evaluable for response.\textsuperscript{9,12,21} Of the remaining 97, 76 (78%) were studied and were considered evaluable with respect to the interrelationship of growth, pharmacologic parameters, and clinical response. The remaining 21 (22%) patients did not have pretreatment (day 0) and/or day 8 postinitial cytoreductive drug studies performed; five patients had severe myelofibrosis before therapy, nine required emergency initiation of cytoreductive therapy for leukostasis, and seven had insufficient cells present for study on day 8. Thus, the exclusion of these patients from the study should not bias study results. Of the 110 consecutively admitted patients, 72 (65%) achieved complete remission (CR) while 25 (23%) had no response (NR) to induction therapy. When only the 97 evaluable patients were examined, a 74% CR rate (72/97) was achieved; of these 72 CR patients, 63 (87.5%) received the second cycle of TST in early first CR, while 13 (52%) of the 25 NR group also received the second TST cycle. Of the 76 patients studied and evaluated for in vivo induced changes in growth and ara-C pharmacology plus clinical outcome, all had at least 80% malignant marrow blasts before treatment. CR of at least 3 months' duration, defined by Acute Leukemia Group B criteria, was achieved following induction therapy in 56 (74%) of these NPT adults, while the remaining 20 patients had NR to TST (or CR less than 3 months); 67 (88%) of these 76 received the second TST cycle. This CR rate for the studied, evaluable patients was equivalent to the CR rate achieved for the entire clinically evaluable group of adults with AML who received induction TST. The median follow-up for all studied, evaluable patients is 16 months (range, 3 to 44).

Bone marrow cell cultures. Marrow cells were collected into RPMI 1640 from all patients before therapy (day 0) and at the time of predicted peak drug-induced residual tumor cell proliferation (day 8 postinitial drug) by routine needle aspiration. To ensure that marrow cells were free from contamination by peripheral blood blasts, marrow spicules were isolated, and cells isolated from the spicules were dispersed as single cells by drawing through a 25-gauge needle. Each cell suspension was prepared and studied individually. Whole marrow cell suspensions (1 x 10^6 cells/mL for labeling index studies, 2.5 x 10^6 cells/mL for ara-CTP formation and retention studies) were cultured in duplicate in sterile plastic Falcon 2063 and 2057 tubes (Falcon Labware, Oxnard, CA) with specific sera at 15% concentration. Cultures were maintained in a humidified atmosphere containing 5% CO2 at 37°C.

Specific sera collection. Marrow cells were incubated with 15% autologous sera obtained at the time of marrow aspiration. Before therapy (day 0) and on day 8, concomitant with bone marrow aspiration, 50 mL of whole blood was collected from each patient and allowed to clot; the serum was separated under sterile conditions and stored immediately at −70°C as necessary until use.

Labeled index determinations. \[^{1}H\] ara-C and \[^{3}H\] thymidine (dThd) leukemic blast labeling indices (LIs) were determined for AML marrow cells from 76 patients (56 CR, 20 NR) on day 0 before therapy and again on day 8 at the predicted peak of tumor proliferation following initial drug administration as previously described.\textsuperscript{18,19,20} In brief, aliquots of monodispersed AML marrow nuclei incubated with 15% autologous serum and either \[^{1}H\] ara-C, 1 \(\mu\)Ci/mL (5-6-H cytosine; Amersham Corp., Arlington Heights, IL; specific activity, 11.2 Ci/mmol), or \[^{3}H\] dThd, 0.1 \(\mu\)Ci/mL (methy-\[^{3}H\] New England Nuclear, Boston; specific activity, 2 Ci/mmol) for 75 minutes at 37°C in a humidified atmosphere containing 5% CO2. Following incubation, the cells were washed carefully in phosphate-buffered saline three times and lightly cytocentrifuged (1,000 g, five minutes) onto slides coated with gelatin. Autoradiographs were prepared with Kodak NTB-2 photographic emulsion, exposed for 21 days, developed, and stained with Giemsa. The \[^{1}H\] ara-C and \[^{3}H\] dThd LIs of leukemic marrow cells were determined by counting the number of cells per 1,000 that contain > 5 grains overlying the nucleus. Background labeling is estimated by the number of grains present in a cell-free area equivalent to the area of the leukemic blast nucleus. Background labeling is negligible for \[^{1}H\] dThd (≤ 1 grain) and is ≥ 2 to 3 grains for \[^{3}H\] ara-C. Cells labeled with \[^{1}H\] ara-C generally contain ≥ 10 grains overlying the nucleus, and cells labeled with \[^{3}H\] dThd generally contain ≥ 25 grains. Results are expressed as percentages of labeled blasts. The SE for this method is ± 1% in our laboratory.

Intracellular ara-CTP levels. \[^{1}H\] Ara-C (5,6-3H cytosine; Moravec Biochemicals, City of Industry, CA; specific activity, 18 Ci/mmol) was added to duplicate cultures of AML marrow cells from 45 patients (33 CR, 12 NR) with adequate aspiratable numbers of leukemic cells obtained on day 0 and again on day 8 to yield a final ara-C concentration of 1 \(\mu\)mol/L. Because ara-C entry intracellularly is linear over one hour and intracellular ara-CTP formation with subsequent retention continues with a plateau over the following one to two hours,\textsuperscript{23,25} cultures were incubated at 37°C in humidified 5% CO2 for three hours and then centrifuged at 5,000 g for five minutes, and the ara-C-containing medium was decanted. Half of the cultures were resuspended in fresh medium without drug and were returned to the incubator to be analyzed one hour later for ara-CTP retained following the end of drug exposure in the manner described later. The other half of the cultures were subjected to 0.8-mol/L perchloric acid precipitation. Following centrifugation, the supernatant liquid containing the acid-soluble cell constituents was transferred to another tube where it was neutralized with KOH to a pH of approximately 7. The insoluble salt was removed by centrifugation, and the second supernate was immediately ready for high-performance liquid chromatographic analysis of ara-C nucleotide levels as detailed previously.\textsuperscript{8,9} The coefficient of variation for replicate samples is ≤ 10% in our laboratory. This method is similar to those widely used for chromatographic separation of nucleotides.\textsuperscript{23-28}

Statistical methods. Comparison of cell growth kinetic and intracellular ara-C biochemical parameters obtained for day 8 cells relative to values obtained for day 0 cells (Δ day 8/day 0) were analyzed by using Student's \(t\) test and Fisher's exact \(t\) test. The relationships between in vivo drug-induced changes in growth and pharmacologic parameters and clinical outcome (CR, NR) were also analyzed by using Student's and Fisher's exact \(t\) tests.

The relationships between the in vivo growth and pharmacologic determinants and duration of CR achieved with two cycles of TST were examined by using the aforementioned analyses when CR duration was treated as a discrete variable (9-month breakpoint). These relationships were also examined by the Kaplan-Meier life table analysis\textsuperscript{29} when CR duration was viewed as a continuous variable from NR through longest ongoing CR duration (43 months). In these evaluations only those patients who were clearly NR (as defined earlier) or whose duration of CR in response to TST could be clearly defined were included. Nine of 56 (16%) CR patients who had \[^{1}H\] dThd and \[^{3}H\] ara-C LI's measured and five (15%) of those same CR patients who also had intracellular ara-CTP levels measured during induction TST died during intensive TST in early CR.\textsuperscript{11,22} Thus, a total of nine of 76 (12%) studied CR and NR patients were not evaluable for CR duration as a criterion of clinical response and were therefore excluded from such analyses.

**RESULTS**

**Measurements of DNA synthesis.** The in vivo response to initial cytoreductive therapy for the 56 CR and 20
NR patients with respect to drug-induced growth perturbation and drug-induced changes in ara-C incorporation into residual AML marrow cell DNA (Fig 1). The initial drug induced a meaningful increase in day 8 residual AML blast proliferation, as measured by the $[^{3}H]$ dThd LI, relative to that detected for the pretreatment (day 0) population for the CR patients, while no such increase was detected for the NR subgroup ($P < .009$). These data support those of our previous developmental trials with respect to the presence and magnitude of predicted peak residual tumor cell LI following initial drug administration and the relationship of that drug-induced proliferation to clinical response to TST. Similarly, initial cytoreduction resulted in enhanced ara-C incorporation into DNA by the residual tumor cohort, as measured by the $[^{3}H]$ ara-C leukemic blast LI, only in those patients achieving CR ($P = .002$). Further, patients whose blast cells demonstrated a significant in vivo drug-induced increase ($\Delta$ day 8/day 0 $\geq 1.2$) in $[^{3}H]$ dThd LI ($P < .006$) or $[^{3}H]$ ara-C LI ($P < .00004$) in response to initial drug were significantly more likely to achieve a CR, while significant deterioration of these parameters ($\Delta$ day 8/day 0 $\leq 0.8$) and thus divergence from the predicted proliferative response ($P < .005$) or drug incorporation ($P < .00005$) by the residual day 8 marrow cohort was associated with NR and clinical ara-C refractoriness (Table 1).

Fig 1. Change detected in vivo during TST in residual AML marrow cell $[^{3}H]$ dThd and $[^{3}H]$ ara-C labeling LIs from day 0 to day 8 that was induced by initial cytoreductive drug. The ratio of induced change ($\Delta$) is depicted for each of 56 NPT patients achieving CR and 20 NPT NR patients. Mean $\Delta[^{3}H]$ dThd and $[^{3}H]$ ara-C LIs for CR and NR subgroups are depicted by horizontal bars. The shaded area represents $\Delta$ of 0.80 to 1.20; nonshaded areas represent significant change for an individual $\Delta$. There was a significant in vivo drug-induced increase in $[^{3}H]$ dThd LI (mean $\Delta$, 1.51) and an even greater increase in $[^{3}H]$ ara-C LI (mean $\Delta$, 2.28) detected for CR patients, while no such increases were detected for NR patients.

Table 1. Comparison of Response Relative to In Vivo Drug-Induced Changes in Measurements of DNA Synthesis by Day 8 Residual Leukemic Marrow Cells

<table>
<thead>
<tr>
<th>Change</th>
<th>CR</th>
<th>NR</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta[^{3}H]$ dThd LI</td>
<td>1.51 (0.42-4.43)$^f$</td>
<td>1.00 (0.04-2.44)</td>
<td>&lt;.009</td>
</tr>
<tr>
<td>No. with $\Delta \geq 1.2$</td>
<td>37/56 (66%)</td>
<td>6/20 (30%)</td>
<td>&lt;.006</td>
</tr>
<tr>
<td>No. with $\Delta \leq 0.8$</td>
<td>7/56 (12.5%)</td>
<td>9/20 (45%)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>$\Delta[^{3}H]$ ara-C LI</td>
<td>2.25 (0.72-6.76)</td>
<td>1.16 (0.07-2.83)</td>
<td>.002</td>
</tr>
<tr>
<td>No. with $\Delta \geq 1.2$</td>
<td>50/56 (89%)</td>
<td>8/20 (40%)</td>
<td>&lt;.00004</td>
</tr>
<tr>
<td>No. with $\Delta \leq 0.8$</td>
<td>2/56 (4%)</td>
<td>9/20 (45%)</td>
<td>&lt;.00006</td>
</tr>
</tbody>
</table>

*CR, 56 patients; NR, 20 patients.
†Paired two-tailed t test (mean $\Delta$ values, CR v NR); Fisher’s exact t test (no. CR patients v no. NR patients).
‡Day 8/day 0; change induced by initial drug in vivo.
§LI, percentage of labeled leukemic blasts.
| Mean in vivo drug-induced change ($\Delta$) for all CR or NR marrow populations.
| Range for individual CR or NR marrows.
there was significant enhancement of quantitative three-hour intracellular ara-CTP formation (mean Δ, 2.7; range, 0.5 to 21.6; P < .002) and subsequent one-hour ara-CTP retention (mean Δ, 3.5; range, 0.6 to 27.2; P < .0001) in the day 8 residual AML population relative to steady-state day 0 marrow cells, with a meaningful trend toward increased relative percent retention (R/F) by the day 8 cohort as well (mean Δ, 1.7; range, 0.4 to 4.6; P < .055). In contrast, the 12 NR patients as a group had no such pharmacologic enhancement detected in the drug-primed day 8 cohort and, in fact, had significant deterioration in relative percent retention (mean Δ, 0.7; range, 0.08 to 1.1; P < .005) when compared with the day 0 population. While the magnitude of drug-induced change in intracellular ara-CTP formation was not meaningfully greater for CR than for NR patients (mean Δ, 2.7 v 1.8, respectively), the increase in active drug retention detected in the CR subgroup (mean Δ, 3.5) was significantly greater than that seen for NR patients (mean Δ, 1.1; range, 0.02 to 5.8; P < .02). Similarly, a markedly greater enhancement in relative ara-CTP retention was detected for CR patients in comparison with the NR counterparts (mean Δ, 1.7 v 0.7, respectively; P < .0001).

The biochemical pharmacologic behavior of day 8 cohorts relative to the steady-state day 0 populations in response to initial drug related directly to clinical outcome of TST. Significant in vivo drug-induced increases (Δ ≥ 1.2) in intracellular ara-CTP formation occurred in 23 of 33 (70%) CR patients but only four of 12 (33%) NR individuals (P < .035); similarly, 27 of 33 (82%) CR patients had significant enhancement in day 8 AML intracellular ara-CTP retention, whereas only two of 12 (17%) NR patients had such response to initial cytoreduction (P < .00003). Conversely, seven of 12 (58%) NR patients had significant deterioration (Δ ≤ 0.8) in the amount of ara-CTP retained intracellularly by the drug-primed day 8 residual tumor population, while only one of 33 (3%) CR patients evinced this decreased retention (P < .00015).

Relationship of in vivo induced change in growth and pharmacology to CR duration. While the achievement of CR in response to antileukemia induction therapy is a necessary first step toward an ideal clinical outcome, it is the duration of drug-unmaintained CR that reflects the magnitude of tumor cell kill and defines disease-free survival and cure. Examination of the relationship of in vivo drug-perturbed growth kinetics and net intracellular ara-C metabolism to unmaintained CR duration in response to two-cycle TST\(^{19,22}\) addresses whether these measurements are useful to accurately predict long-term remissions or cure for individual adults with AML by ara-C-containing TST.

When CR duration was treated as a discrete variable and a 9-month breakpoint was used to compare those patients who were clearly NR plus those with unequivocally short CR (≤9 months) as a group with those whose CR duration was >9 months, differences in both in vivo growth and pharmacologic behavior were again noted (Table 3). The group with substantial CR duration tended to have greater drug-induced increases in day 8 residual AML \({}^{[3]H}\) dThd LI (mean Δ, 1.7 v 1.2; P < .015), \({}^{[3]}H\) ara-C LI (mean Δ, 2.5 v 1.6; P < .0009), and intracellular ara-CTP retention (mean Δ, 5.2 v 1.6; P < .006) than did those less clinically responsive to

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**Table 2. Comparison of Day 0 and Day 8 Measurements of Intracellular ara-C Metabolism Relative to Clinical Outcome**

<table>
<thead>
<tr>
<th>ara-CTP</th>
<th>Day 0</th>
<th>Day 8</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular ara-CTP formation (pmol/10(^6) cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR†</td>
<td>5.6† (0.2-27)</td>
<td>10.1 (0.5-32)</td>
<td>&lt;.002</td>
</tr>
<tr>
<td>NR</td>
<td>9.7 (1.3-48)</td>
<td>8.7 (0.1-17)</td>
<td>NS</td>
</tr>
<tr>
<td>Intracellular ara-CTP retention (pmol/10(^6) cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>3.6 (0.2-19)</td>
<td>6.9 (0.6-25)</td>
<td>.0001</td>
</tr>
<tr>
<td>NR</td>
<td>6.8 (0.8-48)</td>
<td>4.2 (0.1-11)</td>
<td>NS</td>
</tr>
<tr>
<td>Relative retention (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>63 (10-100)</td>
<td>77 (38-164)</td>
<td>&lt;.055</td>
</tr>
<tr>
<td>NR</td>
<td>72 (43-100)</td>
<td>50 (6-84)</td>
<td>&lt;.005</td>
</tr>
</tbody>
</table>

*Paired two-tailed t test (day 8 v day 0 values).
†CR, 33 patients; NR, 12 patients.
‡Mean for all CR of NR marrow populations.
§Range for individual CR or NR marrows.
∥Intracellular ara-CTP retention/intracellular ara-CTP formation (R/F).

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![Fig 2. Change detected in vivo during TST in residual AML marrow net intracellular ara-C metabolism from day 0 to day 8 that was induced by initial cytoreductive drug (Δday 8/day 0). The ratios of induced change for quantitative intracellular ara-CTP retention (ΔR) and for relative ara-CTP retention (ΔR/F) are depicted for each of 33 CR and 12 NR patients, with mean ΔR and ΔR/F depicted for each subgroup by horizontal lines. The shaded areas represent significant change for an individual Δ. In vivo drug-induced enhancement of net biochemical pharmacology was detected for CR patients (mean ΔΔ, 3.5; mean ΔΔ/F, 1.7), similar to the Δ day 8/day 0 growth kinetic perturbation (Fig 1). In contrast, there was no such meaningful enhancement of ΔR (mean ΔΔ, 1.1), and in fact, significant deterioration in ΔΔ/F (mean ΔΔ, 0.7) was detected in day 8 relative to day 0 marrow populations from NR patients.](www.bloodjournal.org)
TST. Moreover, achievements of significant increases (Δ ≥1.2) in cell proliferation and net drug metabolism in response to initial drug were related to achievement of CR >9 months, with trends for the three levels of clinical outcome (NR, CR ≤9 months, CR >9 months) detected in relation to the identified in vivo behavior patterns.

When clinical outcome was evaluated as a continuous variable from NR through ongoing CR, differences in behavior patterns of growth and pharmacologic parameters were also demonstrated. With Kaplan-Meier analyses,²⁸ patients who achieved significant drug-induced increases (Δ day 8/day 0, ≥1.2) in residual tumor proliferation ([3H] dThd LI), nonexponential distribution, Wilcoxon P < .003), ara-C incorporation into DNA ([3H] ara-C LI, log rank P = .0001), quantitative intracellular ara-CTP retention (log rank P < .003), and relative retention (R/F, log rank P = .007) had a greater probability of having longer CR durations in response to ara-C–containing TST than did those patients for whom such meaningful enhancement by initial drug priming of cell growth kinetics and net intracellular drug metabolism could not be detected (Fig 3).

**DISCUSSION**

These studies of human AML marrow cells examine the interrelationships between cell growth kinetics, intracellular biochemical pharmacology of the S-phase–specific antimeabolite ara-C, and clinical response to ara-C-containing TST. In vivo measurements of growth and pharmacologic alterations that occur over time in response to initial cyto-

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**Table 3. Relationship of In Vivo Drug-Induced Change in Growth and ara-C Metabolism to the Spectrum of Clinical Response to TST**

<table>
<thead>
<tr>
<th>Clinical Response*</th>
<th>Determinations</th>
<th>Sensitive (CR &gt;9 mos)</th>
<th>Refractory (CR ≤9 mos)</th>
<th>NR</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurements of DNA synthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ[H] dThd LI ≥1.2</td>
<td>22/31</td>
<td>7/16</td>
<td>6/20</td>
<td>&lt;.008</td>
<td></td>
</tr>
<tr>
<td>Trend chi-square (P) = 8.44 (,&lt;.004)§</td>
<td></td>
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</tr>
<tr>
<td>[3H] ara-C-LI ≥1.2</td>
<td>29/31</td>
<td>13/16</td>
<td>8/20</td>
<td>&lt;.0009</td>
<td></td>
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<tr>
<td>Trend chi-square (P) = 17.6 (&lt;.0003)</td>
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</tr>
<tr>
<td>Intracellular ara-C metabolism</td>
<td></td>
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<tr>
<td>Δara-CTP formation</td>
<td>≥1.2</td>
<td>12/18</td>
<td>6/10</td>
<td>4/12</td>
<td>NS</td>
</tr>
<tr>
<td>Trend chi-square (P) = 2.99 (0.084)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δara-CTP retention</td>
<td>≥1.2</td>
<td>15/18</td>
<td>6/10</td>
<td>2/12</td>
<td>&lt;.004</td>
</tr>
<tr>
<td>Trend chi-square (P) = 12.5 (&lt;.0005)</td>
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</tr>
<tr>
<td>ΔRelative retention</td>
<td>≥1.2</td>
<td>12/18</td>
<td>5/10</td>
<td>0/12</td>
<td>&lt;.007</td>
</tr>
<tr>
<td>Trend chi-square (P) = 12.2 (&lt;.0005)</td>
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</table>

Values include all patients who achieved CR and survived both cycles of TST in CR (47 with LI determination; 28 with determinations of intracellular ara-C metabolism) and exclude all CR patients who did not survive the second cycle of two-cycle TST (died in early CR). All NR patients are included.

*bClinically ara-C sensitive, CR >9 months; clinically ara-C refractory, CR ≤9 months (short CR) and NR.
†Fisher’s exact test (CR >9 months v short CR plus NR).
‡Day 8/day 0; change induced by initial drug in vivo.
§Trend chi-square (P value) for CR >9 months. CR ≤9 months, and NR as levels of clinical response.

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**Fig 3. Relationship between in vivo drug-induced pharmacologic behavior (Δ day 8/day 0) as measured by differential change in quantitative intracellular ara-C retention (ΔR ≥1.2) and probability of remaining in unmaintained CR after ara-C–containing TST. Of 40 patients evaluable for CR duration (excluding five who died during TST in early first CR), 23 had ΔR ≥1.2, while the remaining 17 had no significant in vivo drug-induced increase in ΔR; of the 23 with ΔR ≥1.2, CR was achieved in 21 (91%), with nine still in first CR from 4+ to 39+ months after TST. Of 17 with ΔR <1.2, only seven (41%) achieved CR, and only one remains in CR at 43+ months. In this figure depicting the Kaplan-Meier analysis, for those achieving CR, CR duration was evaluated as a continuous variable from shortest (4+ months) through longest (43+ months) CR. The median follow-up for this study to date is only 16 months; at the time of this analysis, median CR duration after TST for those patients with meaningfully enhanced net ara-C metabolism in vivo (ΔR ≥1.2) is 14 months, which is significantly longer than the median of 8 months detected for those who achieved CR with ΔR <1.2 (chi-square, 12.51; P = .0006). In light of the median 16-month follow-up, there are too few patients in either arm thus far with ≥20 months’ CR duration to be confident of the proportions depicted after that time. In spite of this current limitation, these curves based on ΔR behavior induced in vivo during TST remain significantly different both in terms of median CR duration (log rank P < .003) and in terms of nonexponential distribution (Wilcoxon P < .0004).**

**Toxic therapy during TST are feasible and can be evaluated in a significant proportion of adults with acute leukemia.** With this approach, we have identified patterns of ara-C activation and net metabolism in the kinetically perturbed residual tumor remaining after initial cytoreduction that may discriminate clinical sensitivity from clinical resistance to ara-C–based TST. The coupling of increased proliferation and enhanced intracellular drug disposition in the day 8 marrow cell population in clinically sensitive patients contrasts with the divergence of these determinants after initial drug priming in the refractory subgroup. Thus, these data obtained in vivo during TST validate both the design and the clinical applicability of our in vitro model that examines net intracellular ara-C metabolism in pretreatment AML marrow populations. In that model, serum that contains the drug-induced HSA that occurs in vivo is used as the growth-promoting milieu in vitro.⁷,¹⁸,²⁰,³⁴ Both assay systems ultimately support the hypothesis that the increased growth that follows initial drug therapy in TST confers a salutary effect on eventual ara-C cytotoxicity that translates into an improved clinical outcome when the target cell population is capable of a favorable pharmacologic response. The variability in intracellular active drug formation and
retention by pretreatment AML populations regardless of ultimate clinical responsiveness is consistent with the premise that cells within an unperturbed pretreatment tumor mass encompass a wide range of capability from self-renewal to some degree of terminal differentiation that leads to multiple levels of expression of a number of determinants. Heterogeneity in the biochemical enzymatic machinery that determines net ara-C metabolism may be a function of variable cell growth kinetics, a well-described characteristic of pretreatment leukemic cell populations that may contribute to the failure of isolated measurements of either biologic determinant to consistently relate to clinical outcome. This reflects both patient-to-patient variability and, more importantly, cellular heterogeneity within an individual tumor population that likely obscures the detection of drug-resistant subpopulations.

The day 8 population, in contrast to the pretreatment mass, is enriched for cells with high self-renewal capacity and relative resistance to initial drug. In vivo studies in both rodent and human AML have defined the occurrence and timing of residual tumor proliferation following cytoreductive therapy and have supported the notion that this remaining cell cohort can be recruited to DNA synthesis and thereby to cytotoxicity. Previously, these studies have concentrated on the relationships between initial drug dose, initial tumor kill, presence and magnitude of drug-induced cell proliferation, and clinical outcome of TST. The current studies confirm our earlier observations and expand these findings to include the recruitment of the initial drug-selected residual AML cohort to enhanced conversion of ara-C to its active cytotoxic metabolites in patients with sensitive AML. The ability to measure these determinants when at least some of the confounding heterogeneity has been overcome may contribute to the more consistent relationship with clinical outcome of TST derived from both our in vitro model and our in vivo studies during TST.

In keeping with data from our previous in vivo AML trials and in contrast to our pretreatment in vitro model where a growth-promoting milieu is ensured by culture in a standardized reagent that maximizes target cell proliferation, CR and NR subgroups differed with respect to the occurrence and amount of increase in day 8 AML cell DNA synthesis over that detected for the steady-state day 0 tumor. As noted in the in vitro model, intracellular ara-CTP formation was increased in the day 8 tumor relative to pretreatment cells for both CR and NR patients, with a tendency for greater enhancement in cells from sensitive patients. This trend may relate to the consistently increased proliferation of the remaining day 8 tumor fraction in the CR subgroup but not in the NR subgroup. Nonetheless, the presence and magnitude of increased ara-CTP formation does not clearly differentiate clinically ara-C–sensitive from ara-C–refractory adult AML patients in terms of either achievement or duration of CR in response to TST.

Determinations of absolute and relative intracellular ara-CTP retention as the measurement of net drug metabolism in the day 8 marrow populations predict the overall depth of tumor kill and thus directly relate to eventual clinical outcome of ara-C–based TST. A significant increase in activated ara-C retention by the perturbed, proliferative day 8 AML cells relative to the heterogeneous tumor present before therapy was detected for 80% of those achieving CR, particularly those who achieved substantial (>9 months) unmaintained CR duration. The degree of this effect on net intracellular ara-C pharmacology relates to the spectrum of clinical response, with relatively less favorable behavior likely to be detected in the short CR subgroup and deterioration in net drug disposition in the NR patients. These in vivo findings closely parallel those documented in vitro where only those pretreatment AML marrow cells from CR patients that are growth stimulated by HSA evince enhanced net intracellular ara-CTP retention. While the increase in ara-CTP retention measured in CR patients may reflect a heightened intracellular stability of triphosphorylated drug in the growth-perturbed state, the paradoxical decrease in ara-CTP retention detected in the majority of day 8 cohorts relative to the day 0 populations from NR patients is less readily explained but may relate to increased levels of degradative enzymes or to an efflux of active drug from the cell. Such potential defects cannot be determined from our data but will be the subject of further investigation.

From our present studies, we conclude that sensitive AML populations demonstrate a direct linkage of growth and pharmacologic behavior that culminates in enhanced ara-C cytotoxicity in the drug-perturbed state in vivo during TST while ara-C–refractory cells demonstrate no such consistent enhancement of these determinants. While our in vitro models measure the proliferative and pharmacologic behavior of cell populations cultured in a growth-optimized milieu where drug-induced HSA is exogenously provided and stimulation thus ensured, the in vivo determinations reflect the actual behavior of individual patients' AML populations in response to timed sequential drug administration. These in vivo measurements of interrelated growth and pharmacology reflect the overall depth of tumor kill as measured by both achievement and duration of CR and, in combination with our in vitro measurements, may further serve to accurately predict the eventual clinical outcome in response to TST with ara-C where both determinants of response are intrinsic to the designed drug schedule.

ACKNOWLEDGMENT

We wish to thank Theresa Ferland and Nicola Edwards for their expert technical assistance and Elisa Fullenkamp and Lisa Butzner for their expert secretarial assistance.

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