To the Editor:

In a recent article, Kufe et al demonstrated that the incorporation of cytosine arabinoside (Ara-C) into DNA is the single most powerful predictor of cell lethality and correlates with the amount of Ara-CTP formed. Although the studies were well designed and executed, we would like to point out that one must exercise great caution in order to avoid over-interpretation. The studies were not performed on freshly obtained human leukemic cells as suggested by the title of the manuscript, but rather on HL-60 cells, which, although originally derived from a patient with promyelocytic leukemia, are now a tissue culture line. If one were to apply the studies reported by Kufe et al as a model for human leukemic cells, then one would expect the amount of Ara-CTP formation to be directly proportional to nuclear incorporation of Ara-C into DNA, which would be directly proportional to the inhibition of DNA synthesis, which would in turn determine the treatment outcome with Ara-C. Unfortunately, this is not the case. We have performed similar studies on P388 murine leukemia cells, as well as on freshly obtained human leukemic cells obtained with acute myelocytic leukemia (AML) and the results are contrary to those Kufe et al found in their studies on HL-60 cells.

P388 cells sensitive (P388-S) and resistant (P388-R) to Ara-C were maintained in RPMI 1640 and passed every seventh day. The P388-R line was established by the addition of increasing doses of Ara-C to the liquid culture medium every week. After 20 passages, it was repeatedly confirmed that the cells were able to grow despite the continuous presence of 5 μg/mL of Ara-C. Measurements of 1H-thymidine labeling index (LI), 1H-Ara-C index, Ara-CTP formation, and inhibition of DNA synthesis were performed on both lines as previously described. Table 1 provides the results.

It is clear that, whereas by increasing the concentration of 1H-Ara-C (1.0, 3.0, 5.0, and 7.5 μg) the number of labeled cells increased in the P388-R line (8%, 21%, 26%, and 65%), the number of cells labeled in the P388-S line progressively decreased (52%, 42%, 19%, and 16%). The inhibition of DNA synthesis was maximal at the lowest concentration in the sensitive line, and increasing the Ara-C concentration had no additional effect, whereas no significant inhibition was noted at any concentration in the P388-R line. The amount of Ara-CTP formed in the cells of both cell lines, on the other hand, increased progressively with exposure to higher concentrations of Ara-C. Hence, studies of the P388-S cell line cannot necessarily provide data that can be used to anticipate the outcome of similar studies in the P388-R cell line and vice versa.

Additionally, we studied bone marrow samples from 49 AML patients prior to therapy with high-dose Ara-C (3 g/m² twice a day for six days) for LI, 3H-Ara-C index, and inhibition of DNA synthesis in vitro. There was no consistent relationship between the number of 1H-Ara-C grains per cell or the percentage of labeled cells and either the outcome of therapy or the inhibition of DNA synthesis in individual patients. We measured Ara-C incorporation into DNA by means of autoradiography and grain counting over individual cells, in contrast to Kufe et al, who extracted the DNA and isolated DNA containing Ara-C. It must be remembered that only macromolecules incorporated into DNA will be detected by autoradiography. The fact that the 1H-Ara-C index correlated well with 1H-Tdr LI (r = .87) is further proof that we were only measuring Ara-C incorporated into the DNA.

Ara-C formation measured for some of these patients was not related to nuclear incorporation of Ara-C as represented by the number of 1H-Ara-C grains per cell or the percentage of cells labeled by 1H-Ara-C. For example, in five AML patients with median 1H-Ara-C grains per cell of 44, 65, 50, 22, and 44, the Ara-C-CTP formation was 130, 82, 114, 155, and 349 pmol/10⁷ cells, respectively. Lastly, Ara-C formation was not related to induction outcome, as already reported by our group, at least for combination chemotherapy containing Ara-C.

In conclusion, our data demonstrate that even studies performed on sensitive and resistant cells of the same tissue culture line yield significantly different results and certainly have little relationship to freshly obtained human leukemic cells. Therefore, studies performed on tissue culture lines can only be interpreted as representing the properties of the cell line under study and cannot be used to provide data that can be immediately extended to the design of therapeutic regimens for patients.

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Table 1. Relationship Between Ara-C Concentration In Vitro and Ara-CTP Formation, Nuclear Incorporation of Ara-C, and Inhibition of DNA Synthesis in P388 Leukemia Lines

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>1H-Thd LI (%)</th>
<th>Dose of Ara-C (μg/mL)</th>
<th>1H-Ara-C Index (%)</th>
<th>Inhibition of DNA Synthesis (%)</th>
<th>Ara-CTP (pmol/10⁷ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388-S</td>
<td>54</td>
<td>1.0</td>
<td>52</td>
<td>97.0</td>
<td>982.2</td>
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<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>42</td>
<td>97.6</td>
<td>2,821.3</td>
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<td></td>
<td></td>
<td>5.0</td>
<td>19</td>
<td>99.1</td>
<td>4,138.6</td>
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<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>16</td>
<td>99.9</td>
<td>5,537.2</td>
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<tr>
<td>P388-R</td>
<td>61</td>
<td>1.0</td>
<td>8</td>
<td>0.0</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>21</td>
<td>17.0</td>
<td>29.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>26</td>
<td>0.0</td>
<td>41.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
<td>65</td>
<td>2.6</td>
<td>68.8</td>
</tr>
</tbody>
</table>

Ara-CTP measurements were kindly provided by Dr. Y. Rustum.
To the Editor:

The correspondence by Drs Raza, Spiridonidis, Zhao, and Preisler raises several issues regarding the intracellular metabolism of cytosine arabinoside (ara-C). Ara-C is an effective inhibitor of eukaryotic DNA replication.1,2 We have demonstrated that the extent of ara-C incorporation that ara-CTP is only a weak competitive inhibitor of DNA polymerase.2 We have confirmed that the extent of ara-C incorporation that ara-CTP is only a weak competitive inhibitor of DNA polymerase.2 We have also shown that the extent of ara-C incorporation into DNA correlates with inhibition of DNA synthesis and loss of clonogenic survival.3,4 The incorporated ara-C residue alters DNA template function.6 As reported in our recent publication in Blood, we have monitored the relationships among ara-CTP pools, formation of (ara-C)DNA and cytotoxicity.7 We have performed these studies with a population of cycling cells. The interpretation of a similar study using cycling and noncycling cells would be more complex. Ara-CTP synthesis would occur in the absence of (ara-C)DNA formation for the noncycling cell population. Thus, the use of ara-CTP pool measurements alone might be misleading when monitoring a mixed population of cycling and noncycling leukemic cells from clinical samples. In contrast, measurement of the end product, (ara-C)DNA, monitors drug effect only on the cycling, and therefore sensitive, cell population.

It is obvious that incorporation of ara-C into DNA is dependent upon the formation of ara-CTP. Other factors affecting (ara-C)DNA formation would include rate of cell proliferation, time of drug exposure and intracellular dCTP levels. The data by Raza et al using sensitive and resistant P388 cells demonstrate that the resistant cell synthesizes less ara-CTP than the sensitive cell.3,4 It is not clear, however, whether the percent [3H]ara-C index represents an absolute measure of ara-C incorporation into DNA. The authors make this assumption without performing direct measurements of (ara-C)DNA formation. For example, the percent [3H]ara-C index decreases with increasing ara-C concentration. Our studies monitoring direct incorporation of ara-C into DNA using cesium sulfate density gradient centrifugation have demonstrated a direct relationship with drug concentration and time of exposure.5,6 Further interpretation of the percent [3H]ara-C index as compared to our method of analysis will be required and we have initiated a collaboration with Dr. Preisler’s laboratory to study their P388 cells.

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REFERENCES


SURFACE PHENOTYPE AND CYTOCHEMISTRY OF T CELLS

To the Editor:

Landay and coworkers recently reported that human T lymphocyte subpopulations exhibited different cytotoxic patterns of staining for lysosomal enzymes.1 They studied cells bearing epitopes recognized by two monoclonal antibodies, D12 and 2D2. This subpopulation, previously shown to belong to a subset of cells capable of suppressing T cell proliferation,2 showed an increased proportion of cells positive for the scattered granular pattern of reactivity of the acid hydrolases acid phosphatase (AP) and alpha naphthyl acetate acid esterase (ANAE) and was almost completely negative for the dotlike pattern of reactivity of these enzymes. Thus, the authors suggested that there was a significant correlation among surface antigen phenotype, expression of lysosomal enzymes, and functional properties of this T cell subpopulation.

We have recently evaluated ANAE activity in a human helper cell population also defined by two monoclonal antibodies, Leu3 and Leu8. Previous studies have demonstrated that the effective helper
Ara-C incorporation into DNA [letter]
A Raza, C Spiridonidis, SC Zhao and HD Preisler

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