Resistance of leukemia cells to cytosine arabinoside (ARA-C) may be due to any one or combination of biochemical processes, which in certain instances may be substantially reversed by an appropriate increase in ARA-C dosage. Based on these and other laboratory observations indicating pharmacologic synergy between sequential high-dose ARA-C and asparaginase (HiDAC—ASNase), a therapeutic program was developed for the treatment of patients with acute nonlymphocytic leukemia (ANLL) refractory to conventional doses of ARA-C, as well as patients with high risk ANLL and advanced acute lymphocytic leukemia (ALL). Treatment consisted of 3-hr intravenous infusions of 3 g/sq m of ARA-C given at 12-hr intervals for 4 doses, followed by 6,000 IU/sq m ASNase given i.m. at hour 42. The same schedule was repeated on day 8. In 32 induction attempts, only 4 patients proved to be truly refractory, i.e., failed to achieve substantial leukemia cell cytoreduction.

Complete remissions were achieved with HiDAC—ASNase in 9 of 13 patients with refractory ANLL, 6 of 9 patients with antecedent hematologic disorders, and 3 of 10 patients with advanced ALL. These include 9 of 14 patients who had either failed induction or who had relapsed on active ARA-C therapy and 6 of 8 patients who had had no prior exposure to ARA-C. The median duration of unmaintained remission in ANLL was 5 mo. In a patient with central nervous system (CNS) leukemia, there was clearance of cerebrospinal fluid (CSF) blasts without intrathecal therapy, suggesting a role for HiDAC in CNS prophylaxis. In general, toxicity was tolerable and reversible. These data suggest that HiDAC—ASNase is an exceptionally effective and well tolerated regimen in leukemic patients with antecedent hematologic disorders and in those refractory to conventional doses of ARA-C.

The most effective commercially available antimitabolite for the treatment of acute nonlymphocytic leukemia (ANLL) is 1-[3'-deoxy-3'-arabinofuranosyl]cytosine (ARA-C). Used as a single agent, a daily treatment for 5-7 days results in complete remission (CR) in 25% of previously untreated patients. Retreatment following failure of an initial induction attempt or relapse following a period of CR during which there is active ARA-C therapy is usually associated with a much lower frequency of response due to acquired drug resistance.

Studies with malignant cells in vitro have shown that resistance to ARA-C may be overcome by a 10-100-fold increase in ARA-C concentration above that effective against “ARA-C-sensitive” neoplasms. The clinical application of such an approach must consider toxicity to normal organs. Studies of ARA-C toxicity in mice and in man indicate that a greater than 30-fold increase in unit dose is tolerable provided that the duration of exposure and number of doses are appropriately limited.

Our previous studies have shown that the cytotoxicity of high-dose ARA-C (HiDAC) to murine leukemia cells both in vitro and in vivo can be significantly potentiated by the sequential administration of the protein synthesis inhibitor, asparaginase (HiDAC—ASNase). This effect, which is distinctly schedule-dependent, results in a marked enhancement of ARA-C’s antitumor effect without a concomitant increase in toxicity to normal organs. This article expands the initial pilot study of the combination in patients refractory to conventional doses of ARA-C and those with a history of antecedent hematologic disorders. A preliminary summary of these data has been presented.

MATERIALS AND METHODS

A total of 32 patients (22 ANLL and 10 ALL) were treated. There were 13 patients with ANLL who had not had an antecedent hematologic disorder. Prior to HiDAC—ASNase, the median number of antileukemic drugs they had received was 5 (range 3-8 drugs). All but 2 had prior chemotherapy-induced CR; the median duration for all prior remissions with maintenance therapy was 210 days (range 0-1,055 days). There were 9 patients with ANLL following an antecedent hematologic disorder. They had previously been treated with a median of 2 drugs (range 0-7). Despite these attempts, none had achieved a prior remission. The median and...
average age for the 22 patients with ANLL was 32 and 36 yr, respectively, with a range of 6–67 yr. There were 10 males and 12 females. Prior to the induction attempts with HiDAC→ASNase, there were serious infections in 3 patients, central nervous system (CNS) leukemia in 3, and extensive organ infiltration in 2, one of which was associated with jaundice (total bilirubin 8.3 mg/dl). Severe coagulopathy was present in 3 patients: 2 with disseminated intravascular coagulation (DIC) treated with heparin and 1 patient with Trousseau’s syndrome, manifested as multiple deep vein thromboses and pulmonary embolus, which progressed despite active anticoagulation with heparin, streptokinase, and urokinase. The coagulopathies subsided once marrow aplasia was achieved from the chemotherapy. All but 1 patient was anemic and 11 were severely thrombocytopenic. Five patients had hyperleukocytosis (≥50,000/μm³).

There were 4 adult male and 2 female patients with relapsed ALL; their median age was 26 (range 16–62 yr). All but one had prior therapy with ASNase both for remission induction as well as in the tandem methotrexate/asparaginase (MTX/ASNase) maintenance program⁶; they were all clinically resistant to ASNase. Two patients were refractory to standard dose ARA-C (SDAC). Three patients had a past history of treated CNS leukemia. Two patients were in their first relapse, 3 were in their second relapse, and 1 had failed 2 prior induction attempts. The median duration of all prior remissions was 360 days (range 0–390). One patient relapsed with a leukemic infiltrate in the optic nerve and bilateral testicular leukemia. Four children with advanced ALL were similarly treated. In contrast to the adults, all had 3–5 prior induction attempts with other drugs and two had relapsed while on maintenance regimens that included SDAC. They all had extensive prior treatment with ASNase, both for induction as well as in the tandem MTX/ASNase maintenance program⁶,⁷ and were clinically resistant to this drug. Remission durations prior to HiDAC→ASNase therapy were brief, with a median of 180 days (range 50–840 days). All 4 children had prior CNS prophylaxis, consisting of whole brain radiation and intrathecal MTX. In spite of this, 2 children sustained 2 CNS relapses each prior to treatment with HiDAC→ASNase.

Eligibility for treatment with HiDAC→ASNase included an unequivocal diagnosis of acute leukemia with marrow blasts greater than 50% or unequivocal organ infiltration, e.g., chloroma or CNS leukemia along with 25%–50% marrow blasts. The CALGB definition of marrow relapse and remission was used.² The duration of remission was dated from the time of notation of the CR. Thereafter, surveillance marrows were usually repeated at monthly intervals. Having achieved a complete or partial remission in the patients with ANLL, there was usually no attempt to “maintain” the remission with further chemotherapy, as most patients had exhausted available conventional therapy.

The treatment regimen is outlined in Table 1. The first infusion of HiDAC was begun in the evening, usually 7 p.m., so that the i.m. asparaginase could be administered during the early afternoon. If the patient had had prior treatment and/or an allergic reaction to the E. coli enzyme, asparaginase from Erwinia carotovora was substituted. One course of therapy was administered over a 42-hr period and was repeated on day 8.

### RESULTS

Response to therapy is summarized in Table 2. The diagnosis of an antecedent hematologic disorder (and prior chemotherapy) did not appear to influence response to therapy. Nine of 13 patients (70%) with ANLL and 6 of 9 patients (66%) with an antecedent hematologic disorder achieved a CR (Table 2). Sorting all patients according to prior ARA-C exposure (Table 2) showed a slight advantage for those who had had no prior treatment with ARA-C (6 CR in 8 patients, 75%) versus those who had (9 CR in 14 patients, 64%). The latter patients had either failed a recent induction or reinduction attempt or had relapsed while on active therapy with SDAC, i.e., within 30 days of having received a 5-day course of 100 mg/sq m ARA-C along with a thiopurine for remission maintenance.

The most immediate response was a rapid cytoreduction of peripheral blasts, chloromas, gingival hypertrophy, and organomegaly, as well as the pain associated with leukemic infiltration of joints. Impres-

### Table 1. Sequential High-Dose ARA-C and ASNase (HiDAC → ASNase)

<table>
<thead>
<tr>
<th>Time</th>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2 hr</td>
<td>ARA-C</td>
<td>3 g/sq m</td>
</tr>
<tr>
<td>12–15 hr</td>
<td>ARA-C</td>
<td>3 g/sq m</td>
</tr>
<tr>
<td>24–27 hr</td>
<td>ARA-C</td>
<td>3 g/sq m</td>
</tr>
<tr>
<td>36–39 hr</td>
<td>ARA-C</td>
<td>3 g/sq m</td>
</tr>
<tr>
<td>42 hr</td>
<td>ASNase</td>
<td>6,000 IU/sq m</td>
</tr>
</tbody>
</table>

The ARA-C was reconstituted in preservative-free saline and infused intravenously. ASNase is given i.m. The course of treatment is repeated on day 8.

### Table 2. Overall Responses in Patients With ANLL Treated With HiDAC → ASNase

<table>
<thead>
<tr>
<th>No.</th>
<th>CR</th>
<th>PR</th>
<th>AP</th>
<th>ID</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
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<td>1</td>
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</tr>
<tr>
<td>22</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>64.3%</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>75%</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Treated with 100–200 mg/sq m daily for 5–7 days.
† Failed prior induction attempt with 1 (4-dose) course of HiDAC → ASNase.
‡ ARA-C 100–300 mg/sq m daily for 5–7 days in combination with other drug(s); one patient failed one course of HiDAC → ASNase.
sive lysis of blasts was frequently evident after the first infusion.

Those patients who achieved a CR or PR and who had exhausted conventional chemotherapy were not given any maintenance therapy. The median duration of unmaintained complete remission was 150 days, with a range of 40–240 days. One patient with chronic myeloid leukemia (CML) blast crises was treated with 5 monthly courses of chemotherapy following CR; he remained in CR for 9 mo.

In spite of extensive prior therapy, 9 of 10 patients with ALL sustained brisk cytoreduction of blasts and achieved marrow hypoplasia. Three patients regener-

ative lysis of blasts was frequently evident after the first infusion. Three patients regener-

ated with blasts, 3 entered complete remission, and 3 entered partial remission. The durations of the 3 CRs were 180, 240, and 330 days (on maintenance ther-

apy). Despite prior CNS prophylaxis, 6 of the 9 patients had 1 or more CNS relapses prior to HiDAC→ASNase. Although no direct (i.t.) CNS therapy was administered during treatment with HiDAC→ASNase, there were no instances of recurrent CNS leukemia during this time.

Toxicity associated with induction therapy is shown in Table 3. Nausea and vomiting was universal during the first infusion and tended to be considerably less or not problematic with the subsequent three infusions. Total alopecia was usually evident within the first 30 days. Drug-associated fever, as high as 103°F, was coincident with drug administration. Conjunctivitis was initially manifested as pain and photophobia within 48–72 hr of drug administration and was followed by marked injection. Untreated, the signs and symptoms usually lasted 4–5 days; however, treatment with glucocorticoid eye drops produced significant relief within 24 hr. Mild diarrhea lasted several days and appeared to be aggravated by lactose-containing foods. One patient had signs and symptoms of an acute abdomen, which were preceded by diarrhea. There was only one instance of severe CNS toxicity, consisting of a generalized seizure followed by coma lasting 24 hr. There were other possible contributing features to this CNS toxic effect, including prior whole brain radiation, concurrent intrathecal MTX during HiDAC infusion, fever to 104.4°F, the syndrome of inappropriate antidiuretic hormone (ADH) (serum sodium 118 meq/liter) with serum and urine osmolality of 253 and 594, respectively, and hypokalemia (7.7 mg/dl) related to rapid tumor lysis.

When modest elevations (25%–50% increase) in hepatic enzymes, SGOT, SGPT, and/or alkaline phosphatase occurred, they usually returned to normal within 14 days and were not of any clinical consequence. Coincident with the rapid lysis of blasts, the serum calcium dropped 1–2 mg/dl during the first 4–5 days of therapy. In most instances, there was a concomitant increase in serum phosphate consistent with the tumor lysis syndrome. Lactate dehydrogenase (LDH) was elevated in 15/16 patients prior to therapy (range 293–19, 578 U), the highest values occurring in the patients with ALL. These abnormal values rapidly normalized over the first 2–5 days of therapy. One white patient developed gradual, but marked, hyperpigmentation within the 2-mo period following therapy, and another developed dry desquamation of the skin. One patient developed severe pain and tenderness in neck and leg muscles, which resolved slowly over a 2.5-wk period. Doppler examination of leg veins failed to reveal any thrombotic problems and serum creatine-phosphokinase (CPK) enzyme analysis remained normal throughout. This was associated with a drop in serum calcium from 9.4 mg/dl before therapy to 7.6 mg/dl. There were no allergic reactions to the i.m. ASNase; however, the only patient who received i.v. ASNase developed an immediate hypersensitivity reaction. There were no coagulopathies associated with chemotherapy. Serial monitoring disclosed a slight decrease in antithrombin III activity and antigen and a slight prolongation of the thrombin clotting time.

There were prompt and profound drug-induced cytopenias; the nadir occurring within the first 1–2 wk. All patients required red blood cell and platelet trans-
fusions. In patients achieving a CR, the median time to granulocyte and platelet recovery (≥1,000/cu mm and ≥75,000/cu mm, respectively) was 32 (range 17–64) and 31 (range 15–64) days, respectively; this being measured from day 1 of therapy. During the period of granulocytopenia, 31 of the 32 induction attempts were associated with fever. In 7 instances, neither an organism nor focus of infection could be detected. There were 2 deaths from disseminated sepsis contracted during the period of marrow hypoplasia. Other infectious disease complications were those commonly seen in the immune compromised host. There was only one instance of severe thrombocytopenic bleeding; a cerebrovascular hemorrhage occurred during marrow hypoplasia resulting in an induction death.

**DISCUSSION**

The development of drug resistance is one of the major obstacles to the control and cure of acute leukemia. An understanding of the mechanisms of drug action and its corollary, drug resistance, is useful in the design of optimal therapeutic regimens. As such, the nucleoside ARA-C is nontoxic and is subject to both anabolism and catabolism. Consequentially, the end result of ARA-C action on the cell is determined by the net balance of these enzyme actions as well as the presence/absence of competing metabolites affecting ARA-C incorporation into DNA. A number of laboratory experiments indicate that resistance to the concentrations of ARA-C, as achieved during conventional antileukemic protocols, can be overcome by a substantial increase in the extracellular concentration (dose) of the drug. These high extracellular concentrations used in experiments in vitro are achievable in vivo and are therapeutically useful in leukemic mice without significant toxic effects to normal organs, provided that the number of doses is suitably limited.

Peak plasma levels following the administration of SDAC in patients range between 0.2 and 1 μM. This is of importance, as in some patients, there may be competition between ARA-C and elevated levels of the normal nucleoside, dCyd. In a survey of plasma deoxycytidine (dCyd) concentrations in leukemic patients, levels ranging from 0.5 to 4 μM have been found (the lower level of detection being 0.5 μM). As the Km of ARA-C for dCyd kinase is higher than that of dCyd, the concentration of drug vis-à-vis the normal metabolite will be a critical parameter in determining ultimate outcome. In studies with L5178Y in vitro, Chu and Fischer found that dCyd could reverse the toxic effects of low levels of ARA-C, providing further importance in consideration of dosage. Peak plasma levels achieved during the 3-hr infusion of 3 g/sq m average 115 μM.

Likewise, in a resistant subline of L5178Y that had an increased intracellular pool size of deoxycytidine triphosphate (dCTP), a fourfold increase in the dose of ARA-C resulted in cytotoxicity equivalent to that from lower doses in the parental strain. As similar conditions have been noted in other murine and human leukemias, various pharmacologic means for reducing the pool size of dCTP have been used in order to enhance the cytotoxic effect of ARA-C. Such studies have used thymidine, hydroxyurea, and deazauridine. However, escalation of the dose of ARA-C alone would result in levels of intracellular ARA-CTP analogous to that achieved with so-called "biochemical modulation." The resultant higher intracellular ARA-CTP/dCTP ratios would lead to enhanced competition for the catalytic site of DNA polymerase, as well as increased incorporation of ARA-C into DNA with consequent increased inhibition of DNA synthesis and loss of the clonogenic potential of leukemia cells.

Studies of the combination of HiDAC and ASNase in leukemic mice and cell culture have indicated a distinct schedule-dependent synergy: the maximal therapeutic interaction being achieved when the ASNase is administered after the ARA-C but before the cells have had the opportunity to recover from the ARA-C effect. As the observed therapeutic synergy against the tumor is noted without any increase in toxicity to normal organs, a drug–drug interaction, resulting in a substantial improvement in the therapeutic index for the combination, is suggested.

Given the above-noted laboratory and clinical observations, the HiDAC—ASNase protocol was developed (Table 1). Our clinical data indicate a substantial therapeutic effect in patients who have had recent failure of standard doses of ARA-C. This high frequency of response would suggest “relative,” dose-dependent drug resistance rather than absolute drug refractoriness. The 64%–75% CR rate is higher than that which one would expect in patients with refractory or recurrent leukemia as well as those with an antecedent hematologic disorder. This response rate is equivalent to that obtainable in previously untreated good risk patients with ANLL treated with standard induction regimens that include conventional dose ARA-C and an anthracycline antibiotic.

Despite the substantial dose escalation, the duration of granulocytopenia and thrombocytopenia was similar to that observed with other regimens using SDAC, with most patients recovering with adequate granulocytes and platelets within 1 mo of starting therapy.
Levels of ARA-C in the CSF were 160% of the plasma concentration 2–3 hr after drug infusion, a level considerably higher than that achieved with SDAC. The clearance of cerebral spinal fluid (CSF) blasts in one patient with systemic therapy alone, as well as the rapid resolution of testicular leukemia in a patient with T cell ALL, suggests the potential utility of HiDAC for sanctuary prophylaxis. The coupling of this effect with the observed effect of systemic ASNase against CNS leukemia may serve to enhance the overall utility of this combination for this purpose.

The pattern of bone marrow recovery was unusual in several of these patients following HiDAC→ASNase therapy. Typically, in patients entering complete remission, the posttreatment bone marrow examination shows marked hypoplasia, followed by regeneration of all three cell lines with less than 5 blasts/100 nucleated bone marrow cells; this was the case for most patients entering remission in this study. However, four patients showed a pattern of cytodestruction to hypoplasia but with persistent blasts from 20% to 50%.

This cohort of blasts persisted from 2 to 8 wk in the regenerating marrow, but eventually disappeared, leaving a typical M1 marrow. The combined picture of a regenerating marrow with a significant fraction of blasts in these several patients presented a dilemma with regard to a clinical decision for further cycles of therapy, yet clearance of the persistent blast population without additional therapy occurred. In two previously untreated patients with ANLL, there was minimal cytodestruction, yet the patients entered CR, suggesting drug-induced leukemic cell maturation.

The question of ASNase potentiation of HiDAC in ANLL is currently being investigated in a randomized clinical trial. Although the overall CR rate for ASNase as a single agent in ANLL is in the range of 10%, approximately 50% of the patients will sustain an initial cytodestruction.

This effect of ASNase may be correlated with hypoasparaginemia noted in untreated patients with ANLL, indicating an initial dependence of the leukemia cell on exogenous asparagine. Thus, ASNase might be useful during initial induction therapy, especially if the observed schedule-dependent synergy between HiDAC and ASNase noted in the laboratory is applicable in the clinical setting. A comparison of the reported CRs after 4–8 doses of HiDAC alone versus HiDAC→ASNase would suggest a therapeutic advantage for the HiDAC→ASNase regimen. Preliminary data from our phase I trial and subsequent confirmatory data from Karanes et al. noted only 4 CRs in 19 patients after 4–8 consecutive doses of HiDAC. This is in contrast to 15 CR in 22 patients with ANLL in the current series. Extension of the number of doses of HiDAC alone to 12 has been associated with a response rate comparable to HiDAC→ASNase; however, this has been associated with an increased incidence of toxicity to normal organs, most notably the CNS, tending to diminish the therapeutic index.

The results obtained in the present study indicate that resistance to “conventional” doses of ARA-C is a “relative phenomenon” overcome by a suitable increase in dose. The tolerable toxicity reported here indicates that a 30-fold increase in unit dose is clinically tolerable provided that the number of doses is limited. These high doses result in a 200-fold increase in plasma drug levels, which may not only overcome systemic drug resistance but may also be capable of penetrating pharmacologic sanctuaries in therapeutically useful concentrations.

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RL Capizzi, M Poole, MR Cooper, F 2d Richards, JJ Stuart, DV Jr Jackson, DR White, CL Spurr, JO Hopkins and HB Muss

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