Timed Sequential Therapy of Human Leukemia Based upon the Response of Leukemic Cells to Humoral Growth Factors

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SUMMARY

Consecutive studies of timed sequential therapy were conducted in 44 adults with acute myelocytic leukemia. The rationale was based on evidence that bone marrow proliferation in vivo relates temporally to induced humoral stimulatory activities, and on models that demonstrate increased cytotoxicity of cytosine arabinoside to myeloblasts cultured in stimulatory serum. In the initial study (26 patients), humoral stimulation induced by cyclophosphamide reached peak concentrations on Days 8 through 10. When the cycle-active drug cytosine arabinoside was infused at that predicted time, complete remissions were induced in 46% of patients, tumor cells cleared in 81%, and unmaintained remissions of 8.5 months were achieved. In an ongoing study (18 patients) with daunorubicin and cytosine arabinoside as the initial drugs in the sequence, the time of measured serum stimulation and the second infusion of cytosine arabinoside beginning on Day 8 correlated directly with an increased in vivo tumor-labeling index. In this study, in vivo tumor-labeling indices correlated with serum proliferative activities in 13 of 15 patients studied. Complete remissions were obtained in 50%, tumors cleared in 78%, and remissions with projected durations of >18 months were achieved without therapy following the initial single induction course. These results suggest that the predictable kinetics of induced humoral stimulation that correlates in time with increased leukemic cell growth permits coupling the administration of an S-phase drug with increased growth of residual tumor. The proper role of further drug therapy in patients with acute myelocytic leukemia in remission requires further examination.

INTRODUCTION

Studies in rodents and humans have demonstrated the value of the sequential administration of antitumor agents (17, 26–28, 31, 38, 40–43, 48, 56). Data from laboratory models and clinical experiences have been gathered to provide additional rationale to this approach. During drug-induced aplasia in humans and rodents, activities in sera that affect DNA synthesis in hematopoietic cells have been identified (4–6). Serum stimulatory activity is first noted immediately after the induction of aplasia when only “stromal” elements are present in the bone marrow biopsy. There is evidence that these supporting cells are the source of these or similar factors (7, 54), although production at sites distant from the bone marrow have not been excluded (2, 8, 15). Maximum levels of stimulator are attained by Day 9 after drug in man, the magnitude (150 to 300% of normal) being related to the degree of resultant aplasia (4). Peak activity is temporally associated in vivo with the proliferative phase of marrow recovery and an increased LI3 of normal marrow granulocytic elements (4). It affects “recruitment” of granulocytic forms into cell cycle in vitro, but neither causes nor interferes with cellular maturation in that series (24). Decline in serum stimulatory activity after aplasia induced by CY is first noted by Day 12, coinciding with the end of the marrow’s proliferative phase. The in vitro effects of stimulatory serum and its consistent temporal coincidence with in vivo granulocyte proliferation (4) seemed clinically applicable to the therapy of hematopoietic cancer. In our experience, malignant cells respond consistently in vitro to these induced serum factors which influence tumor proliferation (23, 24). That leukemic cells can be affected by stimulatory factors has also been noted by others (33). Since most antileukemic drugs act on proliferating cells, their efficacy should be enhanced by exploiting the relationship between induced humoral stimulation and an enlarged tumor-growth fraction.

The positive relationship between the antitumor effects of cytotoxic chemotherapy and a large growth fraction has been well demonstrated in animals (17, 18, 28, 38, 41–43). In recent clinical studies in humans, a high LI predicted a favorable response to antileukemic therapy (21, 22, 48, 49), while an indolent tumor mass at presentation portended a poor outcome (22). The initial requirement to increase this growth fraction may be to counter the effect of a circulating inhibitor, an activity detected in the body fluids of leukemic patients by various assays (12, 16, 23, 24, 32, 34).

In our in vitro model, the cytotoxic effect on human leukemic myeloblasts was enhanced in those cells initially cultured in stimulatory serum relative to cells initially cul-

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tured in pretreatment inhibitory serum (23). These studies were consistent with the hypothesis that induced humoral stimulation of leukemic cells in vitro recruits a greater tumor growth fraction and thereby increases the cytotoxicity of cycle-dependent drugs. The in vivo temporal predictability of this in vitro proliferative humoral effect should help direct the administration of sequential drugs for tumor cell kill, providing the cells are biologically sensitive to the effects of the agent (6).

To explore the antitumor potential of sequential therapy timed to coincide with peak circulating stimulatory activities, we conducted studies that have culminated in therapeutic models. Individual drugs identified by their antitumor potency, their presumed mechanism of action, and their clinically limiting toxicity are used in a sequence based on knowledge of the growth of the neoplastic cell. Prolonged unmaintained remissions in AML have been achieved.

**MATERIALS AND METHODS**

**Patient Selection and Therapy**

Adults with AML (myelocytic, myelomonocytic, monocytic) who had received no previous therapy with the drugs used in these studies were enrolled without regard to clinical condition or age. The 44 patients had a mean age of 54.1 year (range, 11 to 76), with 41% being >60 yr of age. The diagnosis of acute leukemia was based on an evaluation of the clinical findings and the morphological abnormalities seen in the blood and the bone marrow examination. All patients received either CY (Mead Johnson Co., Evansville, Ind.) or DRN (Cancer Therapy Branch, National Cancer Institute) or ara-C. In the initial study (1973 to 1974), 26 patients were treated with CY, 75 mg/kg, followed by ara-C, 90 mg/kg, by a 72-hr infusion begun on Day 9. On Days 14 and 15, VCR, 0.025 mg/kg, was injected in a running i.v. infusion (CY-AC5-O). In the present ongoing study (begun in 1975) in which 18 patients have been treated, ara-C, 45 mg/kg, by a 72-hr infusion, and DRN, 1.0 mg/kg, every day for 3 days, are given simultaneously. On Day 8, ara-C, 45 mg/kg, is again infused over a 3-day period (AC-D-AC5). All patients were supported with blood products and appropriate antibiotics when indicated.

**Criteria of Response**

A CR was defined by the lack of clinical signs of leukemia, a peripheral blood smear with a normal cellular differential, and a bone marrow aspirate which showed recovery of all normal elements and a blast percentage of less than 5. If evidence of leukemia persisted, the effect was termed NR. If patients died prior to the recovery of normal elements but showed no evidence of leukemia on physical, peripheral blood smear, and bone marrow aspirate examinations, they were classified as TETE.

A specimen of bone marrow was aspirated before the administration of each drug to ascertain the effectiveness of the preceding agent. The cellularity and contents were evaluated on the Wright’s-stained smear and the hematoxylin and eosin-stained clot section.

Duration of remission was the time from fulfillment of the requirements of CR to relapse.

**Analysis of Sequential Therapy**

**Sera.** Prior to therapy and 3 times a week until the peripheral WBC stabilized, 10 ml of whole blood were collected from each patient and allowed to clot. The sera were separated and stored immediately at -70°. At the end of the collection period, all sera were assayed simultaneously to determine the effect of the sequential sera on [3H]TdR incorporation by varied marrow cells in the assay system. In CY-AC5-O, sequential sera were obtained from 21 of the 26 patients. In AC-D-AC5, sera were obtained from all patients undergoing therapy. Normal sera were obtained from 30 volunteers and pooled. When these 30 normal sera were assayed individually for their effects on [3H]TdR incorporation by bone marrow cells, each fell within the range of 1 S.E. of the activity of the pool.

**Scintillation Assay.** The effect of sera on [3H]TdR and, in some experiments, on [3H]ara-C incorporation by normal and leukemic bone marrow cells was measured by scintillation-counting technique (4). Control studies in this laboratory have demonstrated that the activities of distinct, induced, humoral factors that influence cell proliferation are measurable by this method (39).

In these assays, either 1.0 μCi of [3H]TdR (specific activity, 1.9 Ci/m mole) or 1.0 μCi [3H]ara-C (specific activity, 16 Ci/m mole) was added to replicate tubes of Roswell Park Memorial Institute Medium 1640 and incubated with the cultured cells (1.0 × 10⁷/ml) at 37° in a 7% CO₂-humidified atmosphere. Sera to be evaluated were added to attain a concentration of 30%. After 18 hr of incubation, the cultures were terminated by immersion in ice, and the cellular contents of each tube were collected under vacuum on a glass fiber filter. Each tube was washed with cold 0.9% NaCl solution, and the wash was added to the filter. Following 3 washings with 0.9% NaCl solution, the acid-insoluble materials on the filter were precipitated by 3 washings with cold 5% trichloroacetic acid and were then washed 3 times with absolute ethanol. The dry filter was transferred to a screw-top vial to which 6 ml of scintillation fluid (Instagel) were added, cooled, and counted in a liquid scintillation spectrometer. Results are expressed as the percentage of cpm of [3H]TdR incorporated into the acid-insoluble precipitate after 18 hr of culture relative to the cpm incorporated by cells in control normal or pretreatment serum.

**Preparation of [3H]TdR Microautoradiographs**

Aliquots of aspirated bone marrow tumor cells were added to 5 ml of heparinized tissue culture media (Roswell Park Memorial Institute Medium 1640, 30% autologous serum) containing [3H]TdR at a concentration of 0.1 μCi/ml. After 1 hr and 15 min of incubation at 37° in a 7% CO₂-humidified atmosphere, the cells were washed, resuspended in phosphate-buffered saline (containing per liter: CaCl₂, 100 mg; KH₂PO₄, 200 mg; KCl, 200 mg; MgCl₂, 6H₂O, 100 mg; NaCl, 8 g; and Na₂HPO₄, 2H₂O, 1.15 g, filter sterilized), and spun in a cytocentrifuge at 1500 rpm for 5 min onto slides coated with gelatin. Autoradiographs were pre-
pared with Kodak NTB-2 photographic emulsion and allowed to incubate for 21 days; then they were developed and stained with Giemsa. The [\(^{3}\text{H}\)]TdR LI was determined by counting the number of cells per 1000 that contained 5 or more grains overlying the nucleus. Background labeling was estimated by the number of grains present in a cell-free area equivalent to the area of the myeloblast nucleus. Greater than 90\% of all cells scored contained approximately 50 grains. Results are reported as percentage of labeled myeloblasts. The standard error of this method in our laboratory is ±1\%.

Determination of the LI was attempted in all patients who entered the AC-D-ACK study. Fourteen of these patients had residual tumor cells in the bone marrow aspirate obtained just prior to the administration of the 2nd drug in the sequence (Day 8, prior to the infusion of ara-C) which allowed an accurate evaluation of any alteration in the LI of the tumor.

RESULTS

Timed Sequential Therapy

With data that normal and leukemic bone marrow cells respond similarly in vitro to induced serum stimulation (24), and that myeloblasts stimulated in vitro are more sensitive to S-phase active drug (23), a timed sequential regimen based on the temporally predictable occurrence of humoral stimulation was devised (CY-AC\(_9\)-O).

CY-AC\(_9\)-O. CY was used to induce humoral stimulatory activity. ara-C was infused at the time of predicted peak serum stimulation on Days 9 through 11 to kill potentially sensitive proliferating myeloblasts. VCR was then given at the time of the 2nd peak of serum stimulation following ara-C. No maintenance therapy was given after induction of remission (Chart 1).

The effects of sequential sera on the incorporation of [\(^{3}\text{H}\)]TdR bone marrow cells demonstrated the synchrony between peak humoral stimulation and the time of administration of ara-C and VCR to the 21 evaluable patients treated with CY-AC\(_9\)-O (Chart 2). As predicted, the ara-C had been infused at the time of peak serum stimulatory activity, when small numbers of tumor cells were present in most aspirated marrows.

A constant pattern was produced by ara-C. When assayed, sera obtained during the infusion of ara-C, which contained in vivo pharmacological amounts of that drug [0.12 to 0.25 \(\mu\text{g/ml}\) by microbiological assay (20)], caused marked suppression of marrow cell [\(^{3}\text{H}\)]TdR incorporation. With decay of this activity, further stimulation synchronous with the VCR injection persisted through early bone marrow recovery. Subsequent sera inhibition of DNA synthesis coincided with a maturing bone marrow and peripheral WBC recovery.

These sequential sera affected DNA synthesis of both normal and leukemic bone marrow cells similarly. The incorporation of [\(^{3}\text{H}\)]TdR by sera obtained from 21 patients before treatment with CY-AC\(_9\)-O was compared with that induced by sera obtained from those patients just prior to the ara-C infusion on Day 9 (Chart 3). Pretreatment sera from 7 of 21 patients stimulated uptake, while 14 inhibited DNA synthesis. In those patients in whom an antitumor effect had been achieved, sera obtained prior to the infusion of ara-C either remained stimulatory, had reached stimulatory levels, or was more stimulatory relative to pretreatment values. Those patients either achieved a CR, or, with only stromal elements present in a marrow space free of tumor, died before the character of the recovery cells...
Timed Sequential Therapy

Chart 2. The effect of serial sera of 21 patients with AML treated with CY-AC-O on the DNA synthesis of normal hematopoietic cells. Prior to the infusion of ara-C, there was a 35% increase in the incorporation of [3H]TdR into myeloblasts, compared with pretreatment values. This stimulation became maximal during the period of VCR injection and persisted until early proliferative forms were present in the bone marrow. (ER, early recovery). Inhibition of DNA synthesis was synchronous with the nonproliferative marrow phase (MR, middle recovery; LR, late recovery) and subsequent peripheral blood granulocyte reconstitution. In most instances, CY produced reduction but not total clearing of leukemic forms from the bone marrow prior to ara-C injection. Bars, estimated cellularity of the bone marrow aspirate. The bar labeled Elastic, difficulty in distinguishing malignant from normal myeloblasts at this time of cohort marrow cell reconstitution. O, mean WBC; •, mean cpm [3H]TdR incorporation by assay cells; bar, S.E.

could be identified. There was close correlation between the incorporation of both [3H]TdR and [3H]ara-C by leukemic myeloblasts (Chart 4). Sera from those who entered CR increased the uptake of both isotopes. In contrast, the sera of 3 of the 4 patients in whom tumor was not cleared from the marrow aspirate during therapy inhibited the uptake of [3H]TdR and [3H]ara-C by both normal and leukemic cells prior to the infusion of the S-phase-dependent drug.

AC-D-AC<sub>4</sub> CY-AC<sub>4</sub>-O therapy failed to clear tumor cells from 19% of the patients treated. With the availability of a drug with high activity as a single agent, CY was replaced with DRN. ara-C and DRN were combined as the initial drugs to reduce the numbers of both dividing and indolent cells, and to induce humoral stimulation. The 2nd infusion of ara-C was begun on Day 8, the day observed in the previous studies in patients with leukemia to be the time of peak serum stimulation, and continued for 72 hr (AC-D-AC<sub>4</sub>).

Results of Therapy

The antitumor effect of the drug sequences was evaluated in all the patients entered into these studies. A group was designated TETE in order to include in the evaluation those patients whose bone marrow aspirate contained no tumor cells following the administration of the 2nd drug in the sequence (ara-C).

CY-AC<sub>4</sub>-O and AC-D-AC<sub>4</sub> induced CR in 46 and 50% of patients, respectively (Table 1). A marked reduction in tumor mass had been achieved, as leukemic cells could not be detected in 81 and 78% of patients treated with CY-AC<sub>4</sub>-O and AC-D-AC<sub>4</sub>, respectively.

The median duration of CR was 8.5 (0.9 to 29.2) months in those patients treated with CY-AC<sub>4</sub>-O, with a median survival of >1 year (13 months).

In the ongoing AC-D-AC<sub>4</sub> study, 5 of 9 patients who achieved CR remain free of evidence of disease at 11.0, 11.8, 13.8, 21.0, and 22.3 months. Four have relapsed at 1.0, 4.0, 6.2, and 18.2 months.

Relation of Therapeutic Result and Serum Factors

The effects of pretreatment sera and sera containing induced humoral factor activities were analyzed with regard to the therapeutic outcome achieved (Table 2). Eight initial sera stimulated DNA synthesis, 20 were within normal range, and 16 inhibited proliferation of leukemic myeloblasts in culture. After the initial treatment, the vector of
serum activity was upward (stimulatory) in 26, unchanged in 10, and downward and inhibitory of DNA synthesis in 8.

The pretreatment sera of 12 of the 21 patients who achieved CR induced [3H]Tdr incorporation by leukemic myeloblasts which were within the range of the effect of normal sera. Four inhibited proliferation, while 5 produced incorporation above normal.

When compared with these pretreatment values, 16 sera obtained after initial therapy, just prior to the 2nd drug, produced a significant increase ($p < 0.005$) in [3H]Tdr incorporation, with 13 stimulatory, 8 within the normal range, and none remaining that had inhibitory activity on Day 9. Tumor cells were present at that time in most aspirated samples of marrow. Following the 2nd ara-C infusion, when only stromal cells were present in the bone marrow aspirates, 18 sera were stimulatory and 3 were normal.

The ara-C infusion failed to clear tumor cells from 9 patients (NR). The pretreatment sera of 4 of these were within the normal range, while 5 were inhibitory. The sera of 6 of these patients prior to the 2nd treatment produced even greater inhibition than pretreatment, 1 remained inhibitory, while 2 remained unchanged and within the normal range. When compared with normal, the inhibitory sera of 3 patients obtained subsequent to the 2nd therapy were then within the normal range, while 1 previously normal became inhibitory of [3H]Tdr incorporation. Sera were not obtained from 3 patients at that time because of clinical exigencies.

Correlation of Change of Tumor LI with Serum Activity and Clinical Response

Measurements of changes in the tumor LI during chemotherapy were made in those patients treated with AC-D-AC₆.
Leukemic cells were aspirated from bone marrows to determine the LI both prior to therapy and on Day 8 just prior to the infusion of ara-C. Tumor cells were present in 15 of the 18 patients at that time. Analysis of the autoradiographs revealed no correlation between the initial LI and the eventual response to therapy (Table 3). The LI in those patients who entered CR ranged between 5.0 and 21.0, and between 1.0 and 13.7 in those who evidenced NR. Of the 9 patients who achieved CR, 7 had tumor cells present in the Day 8 aspirate. Each demonstrated an increase in the in vivo LI prior to the infusion of the S-active drug (range, 117 to 337% > pretreatment LI, p < 0.025). In contrast, a decrease in the growth fraction of the tumor was observed in 2 of the 4 patients whose tumors were unresponsive to treatment.

The change in proliferative activity in sera correlated with the direction of change of the tumor LI in 13 of the 15 patients monitored. As with the LI, the ultimate result of treatment was not predicted by the proliferative activity of the initial sera. Nonetheless, both the LI and in vitro serum activity had increased relative to the initial value in all who responded. Twelve of 15 patients, including all who entered CR, had significant increases in the percentage of cells undergoing DNA synthesis in vivo, with a corresponding change in serum activity. Although the direction of change of serum proliferative activity and cell growth was similar, no direct quantitative association could be established.

**DISCUSSION**

These clinical trials and laboratory correlates are consistent with the notion that tumor cells are more sensitive to the effects of cytotoxic drugs when they are in a proliferative phase. The results suggest that our in vitro data (23) parallel the situation achieved in the patient with AML, that stimulation assayed in serum at specific times correlates with cell growth, and that this timing can be used to improve the therapeutic effects of drugs in sequence.

**CY-AC9-O.** It was assumed that the growth fraction of leukemic cells at the time of initial clinical presentation would be relatively low, and the total mass of tumor would be high (10, 11, 13, 40, 46). CY, a cell-cycle phase nonspecific agent that affects both dividing and nondividing cells (41), was given to provide the humoral stimulus to tumor cell proliferation and to overcome the effects of pretreatment inhibitory factors, either directly or indirectly, by reducing the initial tumor load (Chart 1).

The drugs were given in a sequence suggested by animal models (17, 18, 26, 28, 38, 41-43) and studies in children with acute leukemia (27, 37). Following CY, ara-C was infused on Days 9 through 11, the time of peak humoral effect and tumor DNA synthesis. The 72-hr infusion was chosen to span the generation time of leukemic myeloblasts (25). Those cells surviving ara-C exposure then proceeded through the cell cycle to encounter VCR 48 hr after ara-C at the time of maximal serum-stimulating activity induced by that drug. VCR exerts its effect 12 to 24 hr after a single dose by irreversibly arresting cells in mitotic metaphase, killing susceptible cells in cycle, as well as preventing their further recruitment from the G1 state (27).

In 12 of 26 patients, CY-AC9-O therapy induced a CR that persisted without drug maintenance for a median duration of 8.5 months (Table 1). In only 5 of 26 patients were there leukemic cells present in the bone marrow aspirate 72 hr after the completion of the ara-C infusion.

Although maximal serum stimulation and, therefore, presumed peak in vivo leukemic cell proliferation occurred at the time of the greatest sensitivity of the emerging normal marrow cells, extended aplasia did not ensue. In one sense,
Table 3
Correlation of tumor growth fraction in vivo with DNA synthesis induced in myeloblasts by sera obtained during sequential treatment of leukemia with AC-D-AC*

<table>
<thead>
<tr>
<th>LI (in vivo)</th>
<th>Effect of sera on [3H]TdR incorporation in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case no.</td>
<td>Pre-Rx*</td>
</tr>
<tr>
<td>CR 1</td>
<td>21.0</td>
</tr>
<tr>
<td>2</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>20.0</td>
</tr>
<tr>
<td>4</td>
<td>11.1</td>
</tr>
<tr>
<td>5</td>
<td>29.6</td>
</tr>
<tr>
<td>6</td>
<td>9.5</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>13.2</td>
</tr>
<tr>
<td>9</td>
<td>15.0</td>
</tr>
<tr>
<td>NR 10</td>
<td>12.5</td>
</tr>
<tr>
<td>11</td>
<td>12.0</td>
</tr>
<tr>
<td>12</td>
<td>13.7</td>
</tr>
<tr>
<td>13</td>
<td>10.0</td>
</tr>
<tr>
<td>Died TETE</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>17.5</td>
</tr>
<tr>
<td>16</td>
<td>11.5</td>
</tr>
<tr>
<td>17</td>
<td>8.1</td>
</tr>
<tr>
<td>18</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* Pre-Rx*, pretreatment.
* Vector, the direction of activity over time of [3H]TdR incorporation by bone marrow tumor cells in vivo (LI), or effected in vitro by various sequential sera.
* NC, no cells were obtained by bone marrow aspiration on Day 8.
* ↑, increased; →, unchanged; ↓, decreased.

Table 4
Combination drug therapies in AML

<table>
<thead>
<tr>
<th>Investigator (present study)</th>
<th>No. of patients</th>
<th>No. of CR</th>
<th>CR %</th>
<th>Induction drugs</th>
<th>Consolidation drugs</th>
<th>Maintenance drugs</th>
<th>Median remission (mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burke et al. (present study)</td>
<td>26</td>
<td>12</td>
<td>46</td>
<td>CY, ara-C, VCR</td>
<td>CY, ara-C, VCR</td>
<td>CY, ara-C, VCR</td>
<td>8.5</td>
</tr>
<tr>
<td>Whitecar et al. (50)</td>
<td>39</td>
<td>17</td>
<td>53</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>9.1</td>
</tr>
<tr>
<td>Gluckman et al. (14)</td>
<td>30</td>
<td>16</td>
<td>53</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>12.0</td>
</tr>
<tr>
<td>Powles et al. (36)</td>
<td>107</td>
<td>42</td>
<td>38</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>ara-C, DRN</td>
<td>6.3</td>
</tr>
<tr>
<td>Burke and Owens (6)</td>
<td>15</td>
<td>8</td>
<td>53</td>
<td>CY, ara-C, VCR</td>
<td>CY, ara-C, VCR</td>
<td>CY, ara-C, VCR</td>
<td>8.7</td>
</tr>
<tr>
<td>Wiernick et al. (52)</td>
<td>33</td>
<td>16</td>
<td>49</td>
<td>DRN*</td>
<td>DRN</td>
<td>DRN</td>
<td>6.8</td>
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<tr>
<td>Wiernick and Serpick (53)</td>
<td>33</td>
<td>15</td>
<td>46</td>
<td>DRN, ara-C, TG</td>
<td>DRN, ara-C, TG</td>
<td>CY, guanazole</td>
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<td>Whittaker and Slater (51)</td>
<td>22</td>
<td>11</td>
<td>50</td>
<td>DRN</td>
<td></td>
<td></td>
<td>2.6*</td>
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<tr>
<td>Manaster et al. (30)</td>
<td>29</td>
<td>13</td>
<td>45</td>
<td>CY, ara-C, VCR</td>
<td>CY, ara-C, VCR</td>
<td>BCNU, CY</td>
<td>20.0</td>
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<tr>
<td>Vogler et al. (49)</td>
<td>206</td>
<td>75</td>
<td>36</td>
<td>ara-C, TG, DRN</td>
<td>ara-C, TG, DRN</td>
<td>MTX</td>
<td>6.0</td>
</tr>
<tr>
<td>Bodey et al. (3)</td>
<td>66</td>
<td>32</td>
<td>48</td>
<td>CY, ara-C, VCR, P</td>
<td>CY, ara-C, VCR</td>
<td>6MP, CY, ara-C, VCR</td>
<td>14.5</td>
</tr>
<tr>
<td>Levi et al. (29)</td>
<td>22</td>
<td>9</td>
<td>41</td>
<td>ara-C, TG, HU</td>
<td>ara-C, TG</td>
<td>ara-C, TG</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Randomized study.
* These data are not included in the determination of the median duration of remission.
* P, prednisone; TG, thioguanine; MTX, methotrexate; 6-MP, 6-mercaptopurine; BCNU, 1,3-bis(2-chlorethyl)-1-nitrosourea; HU, hydroxyurea.

...this resiliency of the hematopoietic precursor cell provides a therapeutic index. Because the disease produces total cellular replacement of the bone marrow, successful tumor ablation coincides with absolute bone marrow aplasia. Further increments of drug are limited only by toxicities other then myelosuppression, already complete. Concentrations of both DRN and ara-C in excess of those producing granulocytopenia can be given to encounter the finite (9) tumor cell during the period of its greatest vulnerability without affecting the stem cell in an irreversible manner (55).

AC-D-AC*. With the knowledge that irreversible bone marrow aplasia would not result with the CY-AC9-O schedule, it seemed warranted to replace CY with a more active antileukemic agent. Since with these studies both CY and ara-C had been shown to induce humoral activities, it ap-
peared that production of stimulating factor related to bone marrow aplasia, and not to the specific myelosuppressive agent was used. Therefore, to increase the initial serum stimulation as well as direct antitumor effect, DRN was selected and combined with ara-C as the initial drug. DRN was given by a dose and schedule shown most effective in single-agent trials (52, 53). In this regard, drug sequences with similar timing using DRN or Adriamycin followed by an infusion of ara-C have proved extremely effective (31, 56) in the therapy of AML.

In this study, the initial LI did not predict response to therapy and eventual outcome. However, as Vogler et al. (49) suggested, adequate cytoreductive chemotherapy may have offset this prognostic sign. Our data suggest that the initial chemotheraphy reduced the tumor mass and its associated inhibitor (12, 16). When assayed, this tumor reductation was associated with increased serum stimulatory activity and an increase in the tumor LI.

In the 16 patients whose tumor growth change was measured, those who entered complete remission demonstrated an increase in leukemic cell proliferation prior to the 2nd drug, while 2 of the 4 who remained unresponsive became less proliferative. Serum of 14 of the 16 patients had activity which affected DNA synthesis that paralleled the changes in the tumor LI. In those patients who demonstrated response to the initial drug, subsequent drug administration was coupled with the predicted peak in vivo stimulatory activity.

In contrast, in those patients with persistent tumor, sera were more inhibitory at the time of the administration of the cycle-active drug than at presentation. This persistence of inhibitory activity, possible elaborated by the resistant tumor (12), apparently dampened action of any induced stimulatory substance.

Clinical Results. From these studies, we cannot directly attribute our clinical results to the induction of serum stimulation. Any causal relationship to induced cell growth in vivo remains to be proved. Although in vitro models in our laboratory (23) and the in vivo autoradiographs data in these studies support the concept that drug cytotoxicity is increased at the time of predictable tumor stimulation, other factors must also be considered. Variability of initial biological sensitivity (6, 45), sequential drug effect on cell cycle kinetics (27), development of resistance (44), and drug pharmacokinetics related to the high dose of ara-C (35) must also influence the final result.

When compared with reported studies conducted during the same period, the initial results of CY-AC9-O are similar (3, 8, 14, 29, 30, 36, 49–53). Most therapies, many including similar drugs, produced complete remissions in approximately 50% of patients (median 46%, Table 4). However, all except that of Wiernick and Serpick (53) used drug therapy during remission. The median unmaintained remission duration of 8.5 months obtained with CY-AC9-O compares favorably with those studies that included a consolidation therapy and/or a period of maintenance therapy until relapse (median, 8.6 months; range, 3.5 to 20.0 months). This implies not only that the cell kill achieved with 1 course of CY-AC9-O was large, but that the efficacy of additional treatment during remission requires examination. This consideration is germane, since the unmaintained remissions achieved with 1 course of chemotherapy in the ongoing AC-D-AC9 study are of long duration. By assuming immediate relapse of all patients currently in remission, the minimum median unmaintained remission duration would be 13 months, and by actuarial analysis can be projected to exceed 18 months. Recent communications from other centers investigating similar drugs in sequences that encompass the time-span of AC-D-AC9 (31, 56) report comparable results. Since the value of early continued therapy has not been established, additional factors must be considered in study design. These include the clinical well-being of the patient during remission, retention of tumor sensitivity to the active drugs (ara-C) (44), and prevention of accrued toxicity of the agents (DRN) (19). Reserving these drugs until early relapse, or for use at a future date for cytoreductive therapy in remission, may have a great impact on survival. In addition, in our patients, both humoral and cellular immunological competence has been preserved after remission induction (1). A favorable milieu for function of the patients’ intrinsic immunological surveillance mechanism has been maintained. This unperturbed responsiveness may improve the results obtained with adjuvant therapy (47). The merit of these concepts is presently being examined.

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REFERENCES


Timed Sequential Therapy of Human Leukemia Based upon the Response of Leukemic Cells to Humoral Growth Factors


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