Cytokinetically Based Induction Chemotherapy and Splenectomy for Childhood Acute Nonlymphocytic Leukemia

By Gary V. Dahl, David K. Kalwinsky, Sharon Murphy, A. Thomas Look, Sergio Amadori, Mahesh Kumar, Robert Novak, Stephen L. George, Clara Mason, Alvin M. Mauer, and Joseph V. Simone

A four-drug regimen, based on cell kinetic principles, induced complete remissions in 68 of 95 children (72%) with acute nonlymphocytic leukemia (ANLL). Patients entered remission after 2–5 weekly cycles of vincristine-daunorubicin (day 1) followed by sequential cytosine arabinoside and 6-azauridine (days 4–7). With continuation therapy of monthly vincristine-doxorubicin-cyclophosphamide, weekly cytosine arabinoside, and daily 6-mercaptopurine, the median duration of complete remission was 10 mo and the median survival time 21 mo. Portal triaditis, evident in 11 of 23 patients with liver biopsies, was associated with long remissions. A larger spleen size (>5 cm) and a higher myeloblast labeling index (>10%) at diagnosis were clearly related to shorter durations of remission. Splenectomy within 1 mo of remission had no statistically significant effect on the frequency of relapse or length of remission. Patients without central nervous system (CNS) leukemia at diagnosis, all treated prophylactically with intrathecal methotrexate, had a low frequency of initial CNS relapse (3/56, 5%). The 2-yr disease-free survival rate is 29% (20 of 68 patients attaining complete remission). Fifteen patients have completed 2.5 yr of therapy, and each remains in continuous complete remission, off treatment, for 1–36+ mo. This induction chemotherapy was as effective as more intensive regimens, with the advantage of less toxicity and shorter periods of hospitalization.

In 1976 we asked three questions in a new treatment plan for children with acute nonlymphocytic leukemia (ANLL). Can chemotherapy based on cell kinetic principles improve the frequency of remission induction and extend lengths of complete remission? Does splenectomy early in remission prolong remission duration? Will periodic intrathecal methotrexate, administered alone, prevent central nervous system (CNS) leukemia? The remission induction regimen was suggested by observations that recruitment and synchronization of leukemia cells by chemotherapy may increase the low number of cells in S-phase and improve cell kill by S-phase-specific agents.1,2 Splenectomy was undertaken because of the possibility that ANLL depends on the spleen for sustained growth and because of suggestive evidence that the procedure might prolong remission duration.3

Central nervous system leukemia occurs as often in ANLL, per unit of time at risk, as in acute lymphocytic leukemia (ALL).4,5 Since preventive CNS irradiation limits tolerance for chemotherapy and has had little impact on the quality or duration of survival among patients with ANLL,4 we sought an effective, less toxic alternative. Periodic intrathecal chemotherapy was the logical choice to control the disease without undue effects on marrow reserves.

In ANLL, clinical and biologic prognostic features have been relatively unimportant compared to experience with childhood ALL.7-9 Some investigators have reported associations between response to therapy and age, sex, morphological classification, leukemia or platelet count, splenomegaly, cytokinetic findings, and the presence or absence of bleeding or infection.10,11 More recent studies have linked the probability of response to in vitro growth characteristics of leukemic bone marrow and to specific chromosome abnormalities.12 These findings have been controversial and no reliable set of prognostic factors has emerged. Consequently, an additional objective of this study was to relate an array of presenting features to initial treatment response and duration of remission.

MATERIALS AND METHODS

Ninety-five consecutive untreated patients, all less than 20 yr of age, were admitted to the study from January 1976 to February 1980. ANLL was diagnosed when greater than 25% of the cells infiltrating the bone marrow were leukemic and showed myeloid, monocytic, promyelocytic, or erythroid differentiation, as determined by morphological and cytochemical examinations. Cell types were classified by the French-American-British (FAB) committee system13 after study of Wright-staining characteristics and reactivity with peroxidase and α-naphthol acetate (non-specific) esterase.

Central nervous system leukemia was diagnosed when leukemic blasts were found in Wright-stained slides of spinal fluid prepared from 2–5-ml cytocentrifuged samples. If the cellular morphology was questionable, a second sample was obtained a few days later. Lumbar punctures were performed at diagnosis of ANLL, each month during the first 6 mo of treatment, and then every 3 mo until all therapy was stopped. Intrathecal methotrexate (MTX) was instilled with all lumbar punctures except the first. With relatively
administered to all patients on months 31 and 32. Chemotherapy was given therapy (Pred. 100 mg/sq m. i.v.) monthly for 6 mo and then every 3 mo throughout the remainder of the treatment course. If patients had CNS involvement at diagnosis, they received MTX (12 mg/sq m, i.t.) until the spinal fluid was cleared at blasts, and then monthly for 30 mo. Late intensive therapy (Pred. 100 mg/sq m, p.o., daily x 5; VCR, 2 mg/sq m on day 1; MTX, 7.5 mg/sq m daily x 5; 6-MP, 500 mg/sq m daily x 5) was administered to all patients on months 31 and 32. Chemotherapy was given i.v. unless indicated otherwise. VCR, vincristine; Dauno, daunorubicin; ara-C, cytosine arabinoside; 6-Az, 6-azauridine; Adria, Adriamycin (doxorubicin); Cyclo, cyclophosphamide; 6-MP, 6-mercaptopurine; Pred, prednisone; MTX, methotrexate; CNS, central nervous system.

frequent examination of spinal fluid, CNS leukemia was usually diagnosed before clinical signs or symptoms appeared.

All investigations were approved by the Clinical Trials Committee of St. Jude Children’s Research Hospital; informed consent was obtained from both patients and their parents, or the patients only if they were over 17 yr old.

**Treatment**

An outline of the protocol is presented in Fig. 1. Remission induction therapy was usually administered for 2–5 wk. If leukemic cells were still present in the bone marrow 24 hr after the fourth dose of cytosine arabinoside (ara-C), the entire weekly course was repeated. Patients failing to enter complete remission after 6 wk of therapy, or showing progressive disease during induction, were given methotrexate (MTX) followed by l-asparaginase for up to 6 wk. If remission was not achieved with this combination, the patients were dropped from the study.

As soon as patients attained complete remission, they were randomized for splenectomy; there was no further stratification because of the lack of unambiguous prognostic factors. Splenectomy, together with a liver biopsy, was performed during the first month of continuation therapy, received 2400 rad of cranial irradiation with intrathecal MTX prior to cessation of chemotherapy.

**Criteria for Response**

Complete remission was defined as a normal blood count, normal cerebrospinal fluid, no physical signs of leukemia, and a marrow with active hematopoiesis and no identifiable leukemic cells. Relapse was signified by the presence of greater than 5% leukemic blasts in the marrow or any extramedullary, histologically proven leukemia. Bone marrow aspirates were obtained every 3 mo during continuation therapy, every 3 mo for the first year after cessation of treatment, and every 6 mo thereafter. Lumbar punctures were done simultaneously with marrow aspiration throughout the treatment course.

**Supportive Care**

During induction therapy, supportive care consisted of standard usage of antibiotics and blood products, with two exceptions. Chemoprophylaxis with trimethoprim-sulfamethoxazole (Bactrim) was begun by day 14 and continued throughout therapy, and platelet transfusions were not used prophylactically.

**Determination of Labeling Indices**

Labeling indices (LI) were determined at diagnosis by a standard autoradiographic technique. Background grain counts typically averaged less than 2/cell. The percentage of labeled (>5 grains) leukemic blasts was determined from 1000 cells by a single trained observer. The more differentiated, nondividing elements (metamyelocytes, bands, and segmented neutrophils), as well as monocytes, lymphocytes, and erythroid cells, were excluded from the denominator of cell counts. The studies were repeated at 72 hr after administration of VCR-daunorubicin (Dauno) and at 24 hr after the fourth dose of ara-C (168 hr after VCR-Dauno).
Statistical Analysis

All patients, including 4 who did not receive chemotherapy, were assessed for initial treatment response. The duration of remission was measured from the date a remission bone marrow examination was obtained. Patients withdrawing from the study were considered to have relapsed at that time. The log-rank test\(^{15}\) was used to compare complete remission distributions among categories of any presenting feature and to compare remission durations among the treatment groups. Comparisons among the categories of variables with respect to response rate were done with ordinary Chi-square tests appropriate for two-dimensional contingency tables. Estimates of the probability of surviving beyond any specified time were obtained by using the Kaplan-Meier estimate of survival function.\(^{16}\) Changes in labeling indices were evaluated with the paired-sample Wilcoxon signed rank test.\(^{17}\)

RESULTS

Initial Clinical Features

At diagnosis, the 95 patients ranged in age from 3 mo to 19 yr 11 mo (median 8.8 yr). Fourteen children were black, and 42 were boys (M:F = 1:1.3). Eighteen had white blood counts over 100,000/cu mm. No child had Down’s syndrome and only one (with erythroleu-
kemia) had the Philadelphia chromosome. Of 14 patients presenting with CNS disease, 9 had an M-2 FAB type, 4 an M-4, and 1 an M-5. Other features are listed in Table 1.

Remission Induction

Sixty-eight patients (72%) attained complete remission, 66 after 2–5 weekly cycles of chemotherapy and 2 after added treatment with MTX and L-asparaginase. Of the remaining 27 children, 8 died within 14 days after admission and 12 failed to achieve marrow hypoplasia despite 3–5 wk of therapy. Induction failures are classified in Table 2. Promyelocytic leukemia, the most difficult type to treat, accounted for 5 of 15 cases of drug-refractory disease.

Table 1 shows the influence of age, sex, leukocyte count, platelet count, FAB type, and other possible prognostic factors on remission induction. Response rates were significantly better for patients whose platelet counts were \(\geq 100,000/cu\) mm \((p = 0.03)\) and for those who lack coagulation abnormalities \((p < 0.01)\). Leukocyte count at diagnosis, a prominent factor in a
recent study, had no demonstrable effect on initial responses ($p = 0.38$).

**Remission Duration**

With follow-up periods of 20–67 mo (to October 15, 1981), the median duration of complete remission for all 68 responders was 10 mo and median survival 21 mo. There were 48 relapses, no deaths, and 2 withdrawals during remission. The Kaplan-Meier estimate of the probability that any patient who achieved remission will be in continuous complete remission for 2 yr or more is $0.29 \pm 0.11$ (Fig. 2). Fifteen patients have completed therapy, and after subsequent disease-free intervals of 1–36 mo, all remain in remission.

Two patients who were successfully treated for CNS relapse have been in continuous hematologic remission for 31 and 39 mo. Both are off therapy after receiving late intensive chemotherapy and CNS irradiation, and both remain in CNS remission.

Spleen size ($<5$ or $\geq 5$ cm below the left costal margin) and initial leukemic blast labeling index (LI $<10\%$, $\geq 10\%$) were the only presenting features that correlated significantly with treatment outcome (see Table 1). Patients with smaller spleens had proportionally fewer relapses and a longer median length of complete remission ($p = 0.01$); an LI of $<10\%$ likewise was associated with a lower relapse rate and longer remission durations ($p = 0.026$). There was no correlation between spleen size and LI or leukocyte count at diagnosis.

**Effects of Splenectomy**

Of the 68 patients who achieved remission, 51 were eligible for splenectomy and 26 were randomized to receive the procedure (2 later refused). Of the 17

Table 2. Reasons for Remission Induction Failure

<table>
<thead>
<tr>
<th>Reason</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate trial (none or &lt;2 wk)</td>
<td>10</td>
</tr>
<tr>
<td>Two refused therapy</td>
<td></td>
</tr>
<tr>
<td>Two died before receiving therapy</td>
<td></td>
</tr>
<tr>
<td>Six died before 2 wk of therapy (1 with Fanconi’s anemia)</td>
<td></td>
</tr>
<tr>
<td>Failed to achieve marrow hypoplasia</td>
<td>12</td>
</tr>
<tr>
<td>Achieved hypoplasia but recovered with blasts</td>
<td>3</td>
</tr>
<tr>
<td>Died secondary to marrow hypoplasia</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
</tr>
</tbody>
</table>

completed therapy, and after subsequent disease-free intervals of 1–36 mo, all remain in remission.

Of the 68 patients who achieved remission, 51 were eligible for splenectomy and 26 were randomized to receive the procedure (2 later refused). Of the 17
ineligible patients, 15 refused randomization and 2 had chronic fungal infections. The group randomized for splenectomy contained none of the children whose spleens were >5 cm below the left costal margin and none who were 18 yr of age or older. Otherwise, the distribution of presenting features was comparable to that among patients not having splenectomy. The median time of hospitalization was 5 days; there were no surgical complications.

Three of the 24 spleens contained discrete leukemic foci; only one of these was enlarged. All 3 patients with positive spleens had early relapses (at 5, 6, and 7 mo). Although lacking definite leukemic foci, spleens of 3 other patients contained sinusoidal cells that were morphologically atypical and possibly malignant; these children relapsed in 8, 10, and 13 mo. None of the remaining 18 spleens showed evidence of leukemic involvement, and the median duration of complete remission for this small group of patients was 20+ mo. Fourteen patients had spleens that were more than twice the normal weight for age; however, there was no correlation between spleen weight or clinical size and the presence of leukemic foci.

The effect of splenectomy on the duration of complete remission is shown in Fig. 3. For purposes of comparison, the 68 patients who achieved remission were divided into three groups; randomized patients receiving splenectomy \( (n = 24) \), randomized patients not receiving the procedure \( (n = 25) \), and patients who were not randomized \( (n = 17) \) or refused splenectomy after randomization \( (n = 2) \). The relapse rates for these categories were not significantly different \( (p = 0.29) \).

Leukemic cells were not identified in any liver biopsy samples. Mononuclear inflammatory infiltrates were found in the triads of 11 of 23 patients and were significantly related to longer periods of complete remission \( (p < 0.01) \).

**Effect of CNS Prophylaxis**

Remission induction rates and sites of initial relapse for patients with or without meningeal involvement at diagnosis are compared in Table 3. With intrathecal MTX as the only prophylactic measure, the frequency of initial CNS relapse for patients without CNS involvement at diagnosis was low \( (3/56, 5\%) \). The relapses occurred at 8, 10, and 19 mo and were treated

<table>
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<th>CNS Leukemia at Diagnosis</th>
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<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>81</td>
<td>14</td>
<td>95</td>
</tr>
<tr>
<td>Achieved complete remission</td>
<td>56</td>
<td>12</td>
<td>68</td>
</tr>
<tr>
<td>Initial relapses</td>
<td>39</td>
<td>9</td>
<td>48</td>
</tr>
<tr>
<td>CNS</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
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<td>0</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>34</td>
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with 4 weekly doses of intrathecal MTX followed by monthly intrathecal MTX. One child had a marrow relapse soon after CNS relapse, and the remaining 2 are in complete remissions of 26 + 4 weekly doses with CYTOKINETIC THERAPY. Twelve of the 14 patients with CNS leukemia at diagnosis or prior to marrow remission attained complete remissions. Of the 2 who failed to respond, 1 refused treatment after 2 courses and the other died of sepsis before completing the second course of chemotherapy. The median duration of complete remission for patients with early CNS leukemia, 10 mo, is identical to that for patients without the complication. Subsequent relapses in the CNS accounted for 3 of the 9 relapses in this group; because of periodic surveillance lumbar punctures for MTX instillation, no CNS relapse was symptomatic.

The 12 patients requiring therapeutic intrathecal MTX at diagnosis or during induction did not fare well. Three had subsequent CNS relapses, with leukemia cells again found in their spinal fluid after 2, 2, and 11 mo of remission. All 3 subsequently relapsed in the bone marrow at 9, 12, and 1 mo after CNS relapse.

Toxicity

The weekly courses of induction chemotherapy, usually given in the outpatient clinic, were well tolerated. Although 83 of 95 patients required hospitalization during induction to receive intravenous antibiotics for fever and neutropenia, the median number of hospital days was low (14, range 1–70). Complications during continuation therapy were rare, with an occasional patient admitted for treatment of infection associated with neutropenia.

Cytokinetic Findings

There was substantial variability among pretreatment labeling indices in the 84 patients in whom it was measured. The median pretreatment LI was 7.7 with a minimum of 0.4 and a maximum of 36.1. For the 61 patients who also had a LI measured at 72 hr, the median change was negligible (−0.4) and not significantly different from zero (Wilcoxon signed ranks test, p = 0.76). Labeling indices were increased in 29 patients and decreased in 32 at 72 hr.

After 1 wk of therapy (168 hr), the LI had increased in 44 of the 52 patients studied at both times. The median increase was 5.85, which was significantly different from zero (Wilcoxon signed rank test, p < 0.001). The median LI at 168 hr was 14.1 with a minimum of 1.3 and a maximum of 35.4.

Although the LI did not predict CR, patients with an initial LI <10 had significantly longer remissions than patients with initial indices of ≥10 (p = 0.02 by log-rank test). Similarly, patients whose LI at 168 hr had increased by more than 50% had significantly longer remissions than those whose indices did not increase by this much (p = 0.03 by log-rank test). Additional details will be given in a subsequent publication on cell kinetics.

DISCUSSION

Recent efforts to improve remission induction rates for patients with ANLL have featured intensified schedules of chemotherapy, combining ara-C with an anthracycline with or without 6-thioguanine. Conceivably, the same goal could be achieved by manipulating the cell cycle of leukemic blasts. In the present study, VCR and Dauno were given simultaneously to destroy a portion of the leukemic cell population so that the growth fraction would be higher 72 hr later when ara-C was administered to kill cells in S-phase. Alternating doses of 6-Az and ara-C were then given over 4 days to achieve a pyrimidine blockade, theoretically leading to repeated synchronization and increased cell kill. The resulting rate of remission induction, 72%, compares favorably with rates being obtained with more intensive empirically derived regimens. Despite the lack of any indication that the VCR-Dauno combination had recruited a greater portion of leukemia cells into S-phase by 72 hr, in the majority of patients there was a significant increase in the median LI from a 7.7 to 14.1 after one induction cycle. This may be interpreted as evidence that sequential therapy with ara-C and 6-Az was partially synchronizing leukemia cells in the DNA synthesis phase of the mitotic cycle.

Although used to treat a variety of hematologic or neoplastic disorders, splenectomy has never been tested in a randomized controlled fashion as therapy for ANLL. Spleen cells have been reported to produce “microenvironments” capable of stimulating hematopoiesis and thus might be expected to provide a favorable milieu for malignant cells that survive chemotherapy. Our findings indicate no therapeutic advantage from splenectomy following attainment of complete remission. The apparent benefit from splenectomy observed in an earlier clinical trial at this center may have been offset by the adoption of more intensive chemotherapy with anthracyclines and ara-C. In the previous study, the spleen was found to be a major site of leukemic infiltration during remission (8 of 14 spleens and 5 of 14 livers had leukemic foci). By contrast, we found leukemia in only 3 of 24 spleens and in none of the liver specimens. It may be important that nearly half of the liver biopsies did contain inflamma-
tory mononuclear cell infiltrates in the portal triads, and all but one of the patients with this feature remain in CR. Interestingly, none of the patients with leukemic foci had evidence of triaditis. Liver dysfunction during induction therapy has been correlated with longer remissions so the inflammatory reactions could indicate that these patients were exposed to higher, and possibly more effective, levels of drugs that are normally detoxified by the liver.

Although irradiation is effective in preventing CNS leukemia in patients with ANLL, this modality has had little impact on the quality or duration of survival, owing to the rapid occurrence of marrow relapse. Moreover, craniospinal irradiation is poorly tolerated by children with ANLL, most likely because of decreased marrow reserve following irradiation of an appreciable volume of skull and vertebral marrow. This study provides strong evidence that intermittent intrathecal MTX will prevent CNS relapse in patients without overt meningeal involvement at diagnosis. Of the 56 patients in this group, only 3 (5%) relapsed initially in the CNS. Since each case occurred after 6 mo of complete remission, when the interval between intrathecal therapy was increased, the failures were most likely due to inadequate prophylaxis. Periodic intrathecal MTX was not as effective for patients with CNS leukemia at diagnosis. Of 9 relapses among these 12 patients, 3 occurred in the CNS at 2, 2, and 11 mo after remission induction. A combination of intrathecal MTX and ara-C, or perhaps early CNS irradiation, might prolong the duration of complete remission for this potentially high-risk group.

Despite complex intensive programs of therapy, early marrow relapse continues to mark the clinical course of ANLL. Median durations of remission in recent studies have rarely exceeded 1 yr, although Weinstein et al. project an exceptional 24 mo. Except for the significant proportion of long-term survivors (29%), our data show little improvement over studies in the literature.

Applying the schema proposed by Preisler, we analyzed the categories of induction failures (Table 2). Nearly half of these patients (10% of those entered in the study) received an inadequate trial of chemotherapy because of poor clinical condition or refusal of treatment. Fifteen patients (16% of those entered) showed absolute or relative resistance to induction therapy. No single factor adequately explains this finding, although 5 of the 15 patients who were resistant to therapy had promyelocytic leukemia, consistent with previous findings. No other FAB type was prominent in this group.

A platelet count of greater than 100,000/cu mm at diagnosis was correlated with a favorable response to induction chemotherapy: 21 of 23 achieved CR. The most likely explanation is that a high platelet count reflects a smaller tumor mass infiltrating the marrow. Of the 20 patients with coagulation abnormalities at diagnosis, 5 had promyelocytic leukemia and 3 others died of hemorrhagic complications without an adequate trial of therapy. Of the 12 remaining children, 8 achieved CR. Aside from their common occurrence in patients with promyelocytic leukemia, abnormal coagulation studies at diagnosis did not appear to be related to increased drug resistance, but they did serve to identify a group of patients who might benefit from more vigorous support to lessen hemorrhagic complications that prevent administration of effective chemotherapy.

A shorter duration of CR was significantly related to an initial LI of 10% or more, an association that has been noted before, but one that is not widely recognized. Murphy et al. and Amadori et al. found no relationship between initial LI and remission duration in earlier studies; however, their study groups were small and treatment differed considerably from our regimen.

A high rate of cell proliferation provides a plausible mechanism for the pathogenesis of early relapse. It would favor selection and overgrowth of drug-resistant mutants among cells surviving chemotherapy; alternatively, resistant cells present at diagnosis in very small numbers would have rapid growth potential once the drug-sensitive population was destroyed. An enlarged spleen (>5 cm) at diagnosis was also significantly related to a shorter remission duration. The lack of correlation between spleen size and LI or leukocyte count suggests that this feature does not merely reflect tumor volume. In contrast to findings reported by Keating et al., the number of cycles of induction chemotherapy in this study had no discernible effect on length of remission.

We conclude that the cytokinetically based induction therapy was as effective as more intensive regimens of ara-C and anthracyclines, with the advantage of less toxicity and shorter periods of hospitalization. Less residual leukemia, as determined from splenic and hepatic involvement, was noted for this treatment as compared to our earlier, preanthracycline splenectomy study. Moreover, splenectomy early in remission did not significantly influence the duration of complete remission, so we do not recommend it as a therapeutic modality. We have demonstrated that CNS leukemia can be effectively prevented and treated with intrathecal MTX alone. As the durations of complete remission become longer, it may be necessary to reconsider CNS irradiation for patients with CNS leukemia at diagnosis.
To address the problem of early relapse, we suggest more intensive treatment for patients with high labeling indices or large spleens at diagnosis. Early intensification of induction chemotherapy, using an investigational drug such as VP16-213 or AMSA, followed by a more intensive continuation regimen, might forestall drug-resistant cells to arise.

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