The Comparison of 6-Mercaptopurine with the Combination of 6-Mercaptopurine and Azaserine in the Treatment of Acute Leukemia in Children: Results of a Cooperative Study

Prepared for the Leukemia Chemotherapy Cooperative Study Group A by


The development of new chemotherapeutic agents for use in the treatment of acute leukemia has created a need both for extensive clinical trials of the antileukemic drugs and for the establishment of methods of evaluation of subsequent clinical responses. In order that the newer agents could be tested on a large series of patients with childhood leukemia within a short period of time, the Leukemia Chemotherapy Cooperative Study Group A was organized under the guidance of the Clinical Studies Panel of the Cancer Chemotherapy National Service Center. Study Group A included investigators from 11 institutions throughout the United States (table 1). The protocol of the study was designed by the original members of the study group and approved by the Clinical Studies Panel. Subsequent revisions in the protocol were discussed and agreed upon by all of the investigators participating in the study.

The first study undertaken by Study Group A was an attempt to determine whether or not the addition of azaserine to 6-mercaptopurine is of value in the treatment of acute leukemia in children. The effectiveness of each treatment program was judged by the initial remission rate and the duration of remissions, according to the criteria for evaluation adopted by the group. The purpose of this report is to present the results of this first study.

6-Mercaptopurine has previously proved itself an active agent against acute leukemia.1,2 Azaserine, or O-diazoacetyl-L-serine, is an antibiotic isolated from a culture broth of Streptomvces, and its mode of action appears to be that of a glutamine antagonist by inhibiting the donation by glutamine of an amino group in the conversion of formyl-glycinamide ribotide to formyl-glycine amidine ribotide.3,4 Azaserine was shown to be relatively ineffective as an antileukemic agent when used alone in the treatment of acute leukemia in

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children, since transient partial remissions were seen only occasionally. However, a synergistic effect against transplanted mouse leukemia L 1210 and mouse sarcoma 180 was demonstrated when azaserine was combined with 6-mercaptopurine. An additive action resulting from the concurrent administration of these drugs was further supported by laboratory evidence that the two compounds affected the synthesis of nucleic acids at different levels.

METHODS

The case material was accumulated during the period from December, 1955, to March, 1957. The patients with acute leukemia chosen for study were previously untreated and 14 years of age or under. All cell types of acute leukemia were included. Patients who had evidence of severe hemorrhagic manifestations or fulminating disease were excluded from the study if the investigator felt that treatment with adrenocortical steroids was preferable. Those patients who were considered suitable for antimetabolite therapy were selected randomly for treatment with 6-mercaptopurine or with the combination of 6-mercaptopurine and azaserine. Each patient was followed by the responsible investigator in accordance with the accepted protocol, and specific data were recorded on standardized forms which summarized the preliminary observations on each patient, the initial history and physical examination, a complete blood count including a platelet count, a bone marrow examination and a urinalysis. Optional but desirable additional tests were a blood urea nitrogen or non-protein nitrogen level, a serum uric acid level, and roentgenograms of the skeleton and chest. Out-patient visits were requested at intervals of 2 to 4 weeks, and the follow-up data included an evaluation of the clinical and hematologic findings and the performance rating of the patient.

The drug dosage for both 6-mercaptopurine and azaserine was 2.5 mg. per kilogram of body weight daily by mouth. When 6-mercaptopurine was used alone it was given continuously for at least four weeks unless the patient's condition deteriorated during that time and other therapy was deemed advisable. When the combination of drugs was given, the patients were managed in a similar way. If mouth lesions occurred, the azaserine dosage was halved or stopped temporarily for two or three days. A patient was considered treated if he had been on therapy for at least four weeks. A patient was considered inadequately treated if his therapy was discontinued before four weeks, and such cases were not used in the present evaluation.

The onset of remission or relapse was determined by criteria as published by the Clinical Studies Panel of the Cancer Chemotherapy National Service Center. These criteria are summarized in table 2. The response to therapy of each patient was determined by the attending investigator in conference with other members of the group.

The remissions were evaluated according to four categories: (a) bone marrow, (b) peripheral blood, (c) physical findings and (d) clinical status of the patient. The remissions were divided into complete, partial and clinical remissions, as designated in table 2. The duration of a complete remission was measured from the time that a "one" rating was present in all four categories simultaneously until the first evidence of relapse. The children were seen every two to four weeks and evaluated with respect to the four categories mentioned above.

The status of the disease when the patient was first seen was considered mild, moderate or advanced, according to its rating in the four categories used for remission. Mild disease had a "two" rating in one or more of the categories. Moderate disease had "three" rating in one or two categories. Advanced disease had "three" ratings in three of the four categories.

RESULTS

During the period from December, 1955, to March, 1957, 250 cases of acute leukemia were seen by the investigating groups. The number of patients contributed by each group is shown in table 1. Of these, 82 were
Table 1.—Investigators of the Leukemia Chemotherapy Cooperative Study Group A and the Patients Contributed by Each

<table>
<thead>
<tr>
<th>Hospital and city</th>
<th>Investigators</th>
<th>Total number of patients seen (and % of total)</th>
<th>Total number of patients evaluated (and % of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children's Hospital Society, Los Angeles, Calif.</td>
<td>P. Sturgeon C. B. Hyman C. A. Brubaker</td>
<td>78 (31.2)</td>
<td>53 (42.4)</td>
</tr>
<tr>
<td>University Hospital Ann Arbor, Mich.</td>
<td>F. H. Bethell R. M. Heyn D. E. Pearson</td>
<td>37 (14.8)</td>
<td>15 (12.0)</td>
</tr>
<tr>
<td>Bobs Roberts Hospital Chicago, Ill.</td>
<td>M. I. Pierce R. E. Carter</td>
<td>28 (11.2)</td>
<td>12 (9.6)</td>
</tr>
<tr>
<td>Babies Hospital New York, N. Y.</td>
<td>J. A. Wolff A. Sitarz</td>
<td>13 (5.2)</td>
<td>9 (7.2)</td>
</tr>
<tr>
<td>Memorial Center New York, N. Y.</td>
<td>J. H. Burchenal M. L. Murphy C. T. C. Tan</td>
<td>39 (15.6)</td>
<td>8 (6.4)</td>
</tr>
<tr>
<td>Stanford University Hospital San Francisco, Calif.</td>
<td>B. E. Hall D. R. Hales</td>
<td>15 (6.0)</td>
<td>9 (7.2)</td>
</tr>
<tr>
<td>New York Hospital New York, N. Y.</td>
<td>C. H. Smith M. Erlandson</td>
<td>8 (3.2)</td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>M. D. Anderson Hospital Houston, Tex.</td>
<td>G. Taylor M. P. Sullivan W. W. Sutow</td>
<td>15 (6.0)</td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>University Hospitals Iowa City, Ia.</td>
<td>C. D. May H. G. Cramblett M. Lyman</td>
<td>10 (4.0)</td>
<td>4 (3.2)</td>
</tr>
<tr>
<td>Children's Hospital Washington, D. C.</td>
<td>E. C. Rice S. Leikin G. H. Guin</td>
<td>4 (1.6)</td>
<td>4 (3.2)</td>
</tr>
<tr>
<td>Children's Hospital Denver, Colo.</td>
<td>E. C. Beatty, Jr.</td>
<td>3 (1.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>****</td>
<td><strong>250 100.0%</strong></td>
<td><strong>125 100.0%</strong></td>
</tr>
</tbody>
</table>

not used for the study because of (a) previous antileukemic treatment, (b) a need for initial steroid therapy, (c) death before any therapy could be started, or (d) the inability of the patient to return for continued care. One hundred and sixty-eight patients were begun on a study regimen. Of these, 30 were on the study less than 4 weeks either because their deteriorating condition required other therapy or because the patient expired during that time. Thirteen patients were lost from the study because they failed to return for care or because errors were made in drug administration. The remaining 125 patients received four weeks or more of therapy and form the basis of this report. A summary of the case material is given in table 3. The disease status at the time the patients were first seen is shown in table 4.

Of the 168 patients given a study drug initially, 84 received 6-mercaptopurine alone and 84 the combination of 6-mercaptopurine and azaserine. Of the 125 patients evaluated in the study, 67 had 6-mercaptopurine alone, and 58, 6-mercaptopurine and azaserine. Of those achieving complete remissions,
Table 2.—Criteria for Evaluation of Response to Therapy

A. Bone marrow (differential on 200 cells)
1. Reduction in the number of blasts to less than 10 per cent with lymphocytes to less than 20 per cent; essentially normal-appearing granulopoiesis, erythropoiesis and thrombopoiesis.
2. Improvement as evidenced by an increase in normal myelopoiesis to more than 30 per cent of total nucleated cells and a reduction in the number of blasts and lymphocytes to less than 70 per cent.
3. No improvement, or less than that sufficient to qualify for A 2.

B. Peripheral blood
1. Return to and maintenance for more than one month of:
   a. Hemoglobin greater than 11 Gm. per cent for children under 15 years, or 10 Gm. per cent for infants under 2 years.
   b. Granulocyte levels in excess of 1500 per cu.mm.
   c. Platelet counts greater than 100,000 per cu.mm.
   d. Absence of leukemic cells.
2. Improvement as evidenced by an increase in normal granulocytes to levels as in B 1 b, and maintenance of hemoglobin at levels of 9 Gm. per cent or better for more than one month.
3. No change, or less than B 2.

C. Physical findings
1. Subsidence of all evidence of leukemic infiltration.
2. 50 per cent or more reduction in physical measurement of organ with the greatest leukemic infiltration.
3. No change.

D. Clinical symptoms
1. No symptoms ascribable to leukemia.
2. Definite improvement though still symptomatic.
3. No change.

Complete Remission: A 1, B 1, C 1, D 1
Partial Remission: A 1 or 2, B 1 or 2, C 1 or 2, D 1 or 2
Clinical Remission: D 1 or 2

Relapse: Complete remission shall be terminated when:
1. Leukemic cells in marrow increase to 20 per cent or more, or the total number of leukemic cells and lymphocytes exceeds 50 per cent.
2. In the peripheral blood the leukemic cells appear in excess of 10 per cent of the differential count, or the total number of leukemic cells and lymphocytes exceeds 70 per cent.
3. Definite evidence of leukemic infiltration (other than CNS) occurs.

30 were on 6-mercaptopurine alone and 28 on the combination of drugs. Of the patients included in the evaluation who received 6-mercaptopurine, 44.8 per cent had complete remissions, 23.9 per cent had partial remissions, 16.4 per cent had clinical remissions, and 14.9 per cent were failures. Comparable
Table 3.—Analysis of Patients Seen by the Leukemia Chemotherapy Cooperative Study Group A from December, 1955, to March, 1957

1. Put on a study drug, received four or more weeks of therapy and used for evaluation ........................................ 125

2. Put on a study drug and treated for four weeks but either had errors made in drug administration or failed to return for care ......................... 13

3. Put on a study drug but treated for less than four weeks:
   a. Expired while on therapy ........................................ 14
   b. Changed to steroids because of deteriorating condition .......... 16

4. Not given a study drug because of previous therapy, need for steroids initially, death before any therapy could be initiated or inability of the patient to return for continued care .......................... 82

Total number of patients seen .......................................... 250

Table 4.—Disease Status of Patients When First Seen

A. Status of total number of patients seen:
   1. Advanced ......................................................... 206
   2. Moderate ....................................................... 33
   3. Mild .............................................................. 2
   4. Insufficient data to rate ......................................... 9

Total ................................................................. 250

B. Status of 168 patients put on a study drug and their drug category. The figures in parentheses refer to the 125 patients evaluated in the study.

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Advanced</th>
<th>Moderate</th>
<th>Mild</th>
<th>Insufficient Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-MP</td>
<td>68 (54)</td>
<td>16 (13)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6-MP and Azaserine</td>
<td>79 (55)</td>
<td>5 (3)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total ........................................ 84 (67) .............. 84 (58)

figures for those patients receiving the combination of drugs are 48.3 per cent complete remissions, 25.9 per cent partial remissions, 15.5 per cent clinical remissions, and 10.3 per cent failures. These findings are summarized in figure 1.

Of the patients given 6-mercaptopurine, 61.2 per cent attained “one” marrow ratings, as compared with 67.2 per cent of those receiving the combination of drugs.

The median duration of the complete remissions for the combination therapy is 4.12 months compared to 2.75 months for 6-mercaptopurine alone. The range covered by the duration of the remissions in the 6-mercaptopurine group is from 0.75 month to 25 months in one patient who is still in remission. When 6-mercaptopurine and azaserine were combined, remissions ranged from 0.75 month to 27.5 months. The mean for the duration of remissions is 4.12 months for 6-mercaptopurine alone and 5.89 months for the combination of drugs.
With the use of standard statistical tests,* there is no significant difference either in the number of remissions obtained or in the duration of the complete remissions for the two drug groups.

The initial disease status of the patient at the time therapy was begun did not influence the remission rate or the duration of remissions between the two drug groups. Although there was a larger number of patients with a moderate disease status receiving 6-mercaptopurine alone, an analysis of the outcome of those patients in the moderate category in both drug programs demonstrated no advantage of moderate over the advanced disease status.

The survival time from onset of disease until death for the 58 patients who had complete remissions on the study program has been reported for 48 thus far. Ten of the 58 are still alive at this writing, and one patient is still in his initial remission at 25 months. Only two patients who did not achieve complete remissions in the groups studied are still alive. The median survival time for all patients who received 6-mercaptopurine initially, including those treated for less than 4 weeks, is 12.75 months, whereas that for the group receiving the combination of drugs is 9 months. Because the subsequent treatment programs varied considerably once a patient was removed from the cooperative study, no conclusions can be drawn from the survival times referable

*Standard t-tests, rank t-tests, and ridit confidence intervals were applied. For the standard t-test with \( p = 0.05 \), the critical \( t \)-value is 2.008, and the computed \( t \)-value, 1.4043. On applying the rank t-test and using the same \( p \) value, the critical value was found to be 3.841, and the computed value, 2.6128.
Fig. 2.—Survival time of patients treated with 6-mercaptopurine initially compared to those receiving 6-mercaptopurine and azaserine initially. Both curves include patients treated for less than four weeks. Solid line, 77 patients receiving 6-mercaptopurine; broken line, 77 patients receiving the combination of drugs (no death date in one case).

to the initial therapy given. Of the complete remission group, all patients survived 7 months or longer. Figure 2 represents the comparison of the survival times of the two drug groups comprising the 155 patients studied.

Figure 3 illustrates the difference seen when patients not treated for 4 weeks are included in the survival curve. Both drug programs are grouped together here. The median survival in the group of patients excluding those not treated for 4 weeks is 12 months, compared with 10.5 months when they are included. This difference appears to be explained largely by the fact that the majority of the patients not treated for 4 weeks expired within the first two or three months of their disease onset. Beyond this period the survival curves appear parallel.

Discussion

The value of a cooperative study group in accumulating a large number of patients in a short period of time has been demonstrated in both this study and that recently reported by Frei et al. Within a year's time it was possible to acquire a significant number of cases for the comparative study of treatment programs. Although a loss of sample does not affect the comparison of two treatment programs, it does extend the time needed for the completion of a given study. Because of the large number of cases lost to final evaluation
in this study (50 per cent of the total number seen), it becomes apparent that greater care should be exercised in the use of these patients as study material. The option to treat severely ill patients with steroids initially presents a problem of selection. Although this selection allows for a final evaluation of less severe cases, the primary purpose of the study is to compare two drug programs. Because of randomization, the omission of these cases will only influence survival time and not the relative effects of the two therapeutic programs. Experience with the antimetabolite compounds supports the fact that a longer period of therapy may be necessary to achieve clinical and laboratory improvement compared with some of the immediate beneficial effects seen with steroid therapy. However, it is interesting that only 19 patients were given steroids initially instead of antimetabolites. In addition, of the 16 patients who were started on antimetabolites but changed to steroid therapy in less than a month because of a deteriorating status, only 5 improved and acquired a better status. The remaining 11 continued a downhill course to death. This exclusion again affects the ultimate survival time rather than the comparison of two drug programs (fig. 3). Another problem is the loss of patients because of previous therapy. This loss appears to be necessary if the study drug has been used previously and the patient is already in a remission or if the patient has already become resistant to that particular therapy.
The number of adequately treated patients achieving a complete remission in this study is comparable to or better than that reported in other series using 6-mercaptopurine.\textsuperscript{1,2} It should be noted that the duration of a single remission as determined in this study represents only one phase of the disease, and many children went on to additional remissions with subsequent therapy. The survival time as shown in figure 2 supports this fact. Because of the rigid criteria for a complete remission, many of the patients who achieved a “one” marrow and an excellent clinical status for extended periods of time were kept in the partial remission group. This was often due to the peripheral blood and/or physical findings.

Since the percentage of remissions in the two treatment groups was approximately the same, a greater response of the leukemic process to either of the treatment regimens was not shown.

**Summary**

The results of the first study of the Leukemia Chemotherapy Cooperative Study Group A for the evaluation of two treatment programs in acute leukemia are reported. The cases were accumulated in a period of 15 months. One hundred and twenty-five of the 168 patients started on the study were considered adequately treated and suitable for analysis. The 43 cases excluded from evaluation consisted of patients whose therapy was changed to steroids, those expiring while on treatment, and those lost for follow-up or drug errors. In 125 cases of previously untreated acute leukemia in children, no significant difference was seen in the percentage of complete remissions obtained when 6-mercaptopurine was used alone or when 6-mercaptopurine and azaserine were used in combination.

The duration of the remissions obtained with the combined therapy was not significantly longer than when 6-mercaptopurine was used alone.

**Summario in Interlingua**

Es reportate le resultatos del prime studio del Cooperativa de Chemotherapia Anti-Leucemic, Gruppo de Studio A pro le evalutation de duo programmas therapeutic in leucemia acute. Le casuistica eseva accumulate in le curso de un periodo de 15 menses. Initialmente, 168 patientes eseva includite in le studio. Cento vinti-cinque de istes eseva considerate como adecuatemente tractate e appropriate pro le analyse. Le gruppo de 43 patientes non includite in le evalutation final consisteva de casos in que le therapia eseva cambiate in favor del uso de steroides, del casos del patientes qui moriva durante le tractamento, e del casos non disponibile al observation sequential o in que errores de medication habeva occurrirte. In 125 casos de previemente non-tractate leucemia acute in patientes pediatric, nulle significative differentia eseva trovate inter le procentage de remissions obtenite quando 6-mercaptopurina eseva usate sol e le procentage de remissiones obtenite quando 6-mercaptopurina eseva usate in combination con azaserina.

Le duration del remissiones obtenite per medio del therapia combinate non eseva significativemente plus longe que illo obtenite per medio de 6-mercaptopurina sol.
ACKNOWLEDGMENT

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REFERENCES

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