

The Effectiveness of Combinations of Antileukemic Agents in Inducing and Maintaining Remission in Children with Acute Leukemia

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THE BROAD objective of the present study was to determine whether combinations of chemotherapeutic agents could improve the frequency and duration of complete remission in children with acute lymphocytic leukemia. The complete remission induction rate for the more effective remission inducing agents, prednisone and vincristine, approaches 60 per cent (table 8). Patients who do not enter remission have a significantly shorter survival.^{1,2} In the present study an effort was made to increase the initial complete remission rate by using prednisone and 6-mercaptopurine (6-MP) in combination.

While drug administration during complete remission will prolong the duration of remission,¹ relapse always occurs. A patient who relapses on drug treatment is generally refractory to subsequent administration of the same drug. In the present study, three remission maintenance programs were employed in an attempt to delay the development of this drug refractoriness or resistance (fig. 1). For details of the rationale for these programs, see Discussion. Briefly, the first program consisted of combined concurrent 6-MP and methotrexate (MTX) in full doses. This has been shown to be at least as good as the best single agent for remission maintenance, 6-MP.³ The second remission maintenance program included 6-MP alternated at 28-day intervals with MTX. Drug resistance to antimetabolites probably results from the selec-

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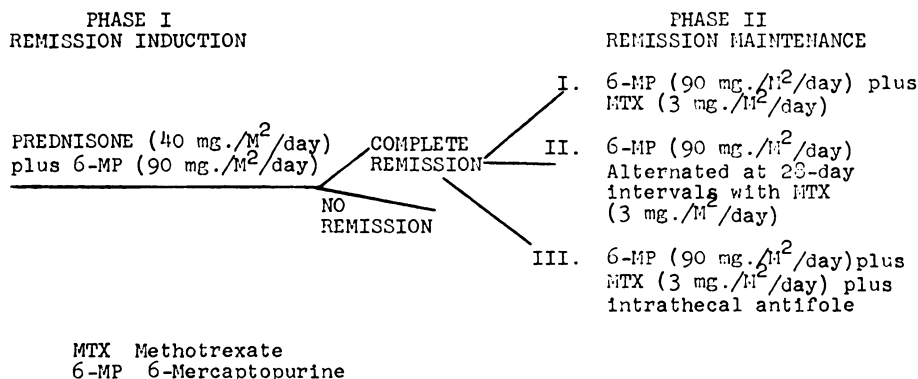


Fig. 1.—Experimental design.

tion of those few cells in the initial leukemic cell population which are drug resistant. Since it takes a period of time before such cells reach relapse levels (a minimum of 6 weeks in a previous study¹), it is reasonable to hypothesize that alternating therapy would successively prevent the ascendance of resistant cells and prolong remission. Several studies of alternating chemotherapy at longer intervals suggest that survival is improved.^{4,5}

The third remission maintenance program consists of intrathecal folic acid antagonist administration in addition to combined oral antimetabolite administration. MTX and 6-MP are largely excluded from the spinal fluid. Clinical meningeal leukemia is being observed with increasing frequency,⁶ and it is probable that subclinical leukemic infiltration of the meninges is much more common.⁷ These leukemic cells in the subarachnoid space are exposed to low levels of antimetabolites, an ideal circumstance for the development of resistance. When this occurs, these cells may reenter the systemic circulation and produce relapse. Intrathecal antifolate was administered to this group of patients to markedly reduce and hopefully eradicate the leukemic cells in the subarachnoid space and thus delay resistance or relapse.

Experimental Design

The protocol prepared for the study is outlined in figure 1.* All patients less than 20 years of age with acute lymphocytic leukemia who came under the care of physicians participating in Acute Leukemia Cooperative Group B were considered for the study. To be included, patients must have active acute leukemia—i.e., must not be in remission and must not have had prior antimetabolite therapy.

All drugs were given on a surface area basis, since the evidence suggests that this unit is most appropriate for cancer chemotherapeutic agents.^{8,9} Conventional doses of 6-MP and prednisone were used for remission induction. Selected hematologic and chemical laboratory procedures were performed at regular intervals. At 28 days, and if indicated before, a bone marrow examination was performed. If there were less than 10 per cent leukemic cells and less than 20 per cent lymphocytes in the marrow (complete remission or Al marrow¹⁰), the patient entered Phase II. If a complete remission marrow was not achieved, treatment was continued until day 42 when the same procedure was

*The protocol which includes the details of the experimental design can be obtained thru the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Table 1.—Disposition of Patients by Institution

Institution	Total No. Entered on Study	No. Accepted for Analysis in Phase I	No. Entered in Phase II*	No. Accepted for Analysis in Phase II
Bowman Gray Sch. of Med.	10	9	9	6
Children's Hosp. of Philadelphia	28	26	15	11
Dartmouth Medical College	5	5	4	4
Jefferson Medical College	4	3	3	3
Mayo Clinic	17	15	11	10
Medical College of Va.	17	13	9	9
Mount Sinai Hosp. (N. Y.)	8	8	6	6
National Cancer Institute	34	33	28	28
Roswell Park Mem. Inst.	11	11	9	9
State Univ. N. Y. (Brooklyn)	32	31	22	20
<i>Total</i>	166	154	116	106

*This number differs from the number accepted for analysis in Phase I by the number who did not achieve remission and, therefore, did not qualify for Phase II, or for 10 patients who achieved remission but were not started on Phase II for various reasons.

followed. The study was terminated for patients who did not achieve complete remission by the 42nd day.

Patients who qualified for Phase II—i.e., those who achieved a complete remission marrow in Phase I—were randomly allocated to the three remission maintenance programs depicted in fig. 1. Treatment programs I and II were “blinded.” Full conventional doses of 6-MP and MTX were given concurrently in programs I and III since it had previously been demonstrated that this combination was well tolerated during remission induction.³ Thus, at full dose, patients on treatment programs I and III received twice as much antimetabolite per unit time as did patients on treatment program II. All drugs (save for the intrathecal antifole) were given orally in a single dose per day.

Intrathecal folic acid antagonist was administered to all patients on treatment program III at the onset of Phase II, one week later, and at 28-day intervals thereafter. During the first third of the study, aminopterin at a dose of 2.5 mg./M² was used, and during the remainder of the study MTX at a dose of 12 mg./M² was employed. Oral MTX was not given on the day of and for the four days following the intrathecal antifole dose. The doses, both oral and intrathecal, were modified or interrupted for toxic manifestation (for details, see protocol). Therapy was continued in Phase II until relapse occurred, as evidence by greater than 70 per cent leukemic cells and lymphocytes in the marrow (A3 marrow by criteria for response¹⁰). When relapse occurred, the study was terminated for that patient.

It was decided that a 12-week difference in remission duration would be of biomedical significance. In order to determine a 12-week difference with 95 per cent confidence and with a less than 20 per cent chance of missing such a difference if it exists, at least 25 patients must be included in each of the three Phase II treatment programs.¹¹ The study was so designed.

RESULTS

A total of 166 patients were entered on study by the ten participating institutions (table 1). Eighty-nine per cent of these were acceptable for analysis in Phase I, and of the 116 entering Phase II, 106 (90 per cent) were acceptable for analysis.

Eighty-two per cent of the patients achieved a complete remission of bone marrow in Phase I, (table 2). While the peripheral blood did not always

Table 2.—*Response in Phase I: Remission Induction with Combination 6-MP and Prednisone*

Total number of patients	154
No. achieving complete remission*	127 (82%)
No. achieving partial remission*	13 (9%)
No response	14 (9%)
Death in phase I	8 (5%)

*Bone marrow criteria only.¹⁰

reach complete remission levels at the time Phase II therapy was started, such did occur shortly after the onset of Phase II in all but eight patients. Only 14 patients did not achieve a bone marrow remission, eight of whom (5 per cent of the total) died in Phase I.

The proportion of patients and their response in the various categories known or suspected to influence prognosis are presented in table 3. The distribution of our patients in the various categories is similar to that of other large reported patient populations. In general, increasing age adversely affects response in acute lymphocytic leukemia,¹² and Chi square analysis confirms this relationship for the present study ($p = 0.01$).

The height of the white blood cell count at the time of diagnosis or at the onset of treatment is inversely related to duration of survival¹³ and remission (fig. 6). The complete remission rate, however, was not affected by the height of the initial white cell count in this study (table 3).

The duration of therapy to complete remission marrow is presented in table 4. Marrow examinations were performed routinely on the 28th and 42nd day of therapy and at other times when indicated. Forty-three per cent of the patients achieved complete remission marrows by the 30th day.

The distribution of patients by Phase II treatment program into the various prognostic categories is given in table 5. It is apparent that the three patient samples are comparable for these factors.

The duration of remission for the three treatment programs is presented in figure 2. There are no significant differences, and the median duration of remission for all three treatment programs is approximately 30 weeks. When these three groups are combined and compared to 6-MP maintenance following remission induction with prednisone¹ (fig. 3), the two curves are similar. Thus the combinations of agents were not superior to 6-MP given alone. The effect of the three treatment programs on survival from onset of Phase I to death is presented in fig. 4. Again, there is no difference and the median duration for all three treatment programs was 56 weeks.

The effect of initial response—that is, response to Phase I—on survival is presented in fig. 5. The median survival for patients achieving complete remission in Phase I is 56 weeks as compared to 28 weeks for those not achieving complete remission ($p = 0.01$). This difference largely develops during the first several weeks after the start of treatment. However, the difference between the two survival curves in fig. 5 continues to widen after 8 weeks—that is, after the remission induction period has been completed.

Table 3.—Response to 6-MP and Prednisone for Patients Classified by Initial Status

Initial Status	No. of Patients	Complete Remission	
		No.	Per Cent
<i>Age (Years)</i>			
0-4	67	64	96
5-9	59	44	75
10-14	21	15	71
15-19	7	4	57
<i>Time from Onset of Symptoms to Onset of Treatment (Weeks)</i>			
Less than 2	20	17	85
2 to 4	59	45	76
4 to 6	23	22	96
6 to 10	21	19	91
Greater than 10	31	24	76
<i>White Cell Count at Onset of Treatment*</i>			
Below 5,000	36	30	83
5,000-9,999	26	19	73
10,000-19,999	24	20	83
20,000-49,999	35	30	86
50,000-99,999	18	16	89
100,000-299,999	7	6	86
> 300,000	5	3	60
<i>Platelet Count at Onset of Treatment</i>			
Less than 25,000	58	50	86
25,000-49,000	42	35	83
50,000-99,000	29	22	76
Greater than 100,000	25	20	80

*The number of patients here totals 151 rather than 154 since a pretreatment WBC count was not obtained in 3 patients.

Table 4.—Duration of Therapy to Complete Remission Marrow in Phase I

	Days from Onset of Treatment					
	0-9	10-19	20-29	30-39	40-49	50-59
No. of patients	2	11	41	36	30	7

This is probably explained by the fact that (1) most of the patients who responded in Phase I are still in remission whereas those who did not respond in Phase I probably still have active disease by the eighth week, and (2) those who do not respond in Phase I are demonstrably not responsive to two of the five effective antileukemic agents, whereas the responding group may be expected to further benefit from 6-MP and corticosteroids.

The relative frequency of meningeal leukemia while on maintenance therapy (Phase II) is given in table 6. Definite or probable meningeal leukemia occurred in 17 per cent of patients in treatment programs I and II but in only 1 of 35 (3 per cent) patients in treatment program III ($p = 0.05$).

Table 5.—Comparability of Patients for the Three Treatment Programs in Phase II

Prognostic Factors	I. Combined	II. Alternating	III. Combined and Intrathecal Antifole
<i>No. of Patients</i>	37	34	35
<i>Age (Years)</i>			
0-4	21	15	21
5-9	10	13	9
10-14	5	5	3
15-19	1	1	2
<i>Sex</i>			
Male	22	20	22
Female	15	14	13
<i>Time from Onset of Symptoms to Onset Treatment (Weeks)</i>			
<2	6	3	7
2-3.9	16	12	10
4-5.9	6	6	7
6-8.9	3	5	4
9	6	8	7
<i>Initial (Prior to Phase I) White Cell Count</i>			
Less than 5,000	9	11	9
5,000-9,999	6	8	5
10,000-19,999	7	2	7
20,000-49,999	7	6	9
50,000 and over	8	7	5

The toxicity which occurred during remission for the three treatment programs is presented in table 7. Leukopenia, thrombopenia and mucosal ulceration occurred more frequently in programs I and III than in II ($p = 0.01$). Downward dose adjustment in these programs was almost invariable, and the average maintenance dose for these programs was about 60 per cent as opposed to 91 per cent of initial dose in program II. This is consistent with the fact that the initial antimetabolite dose per unit time for programs I and III is twice that of program II, and suggests that the drugs are additive with respect to effect on the host (see Discussion). Neurologic toxicity presumably related to the intrathecal medication occurred in four of the patients in treatment program III. The neurologic manifestations included seizures, and one patient died in status epilepticus. One patient has a persistent neurologic deficit; the remaining two patients, both of whom had seizures, have recovered. Five patients died of toxicity, four from infection following toxic myelosuppression in addition to the one from seizures. An additional four patients died in remission: one of disseminated varicella, one of interstitial pneumonia in the absence of myelosuppression, and two died suddenly at home for unknown reasons.

The duration of remission was not significantly effected by the age of the patient or by the duration from symptomatic onset to diagnosis. However,

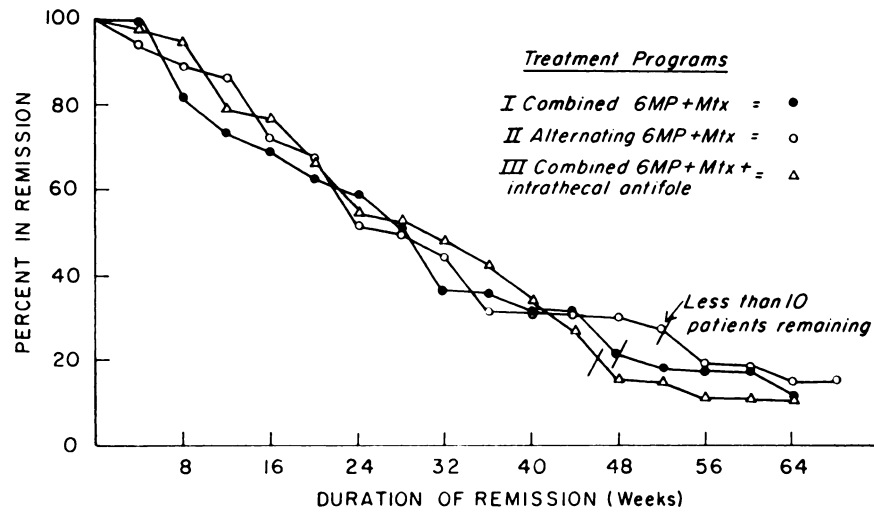
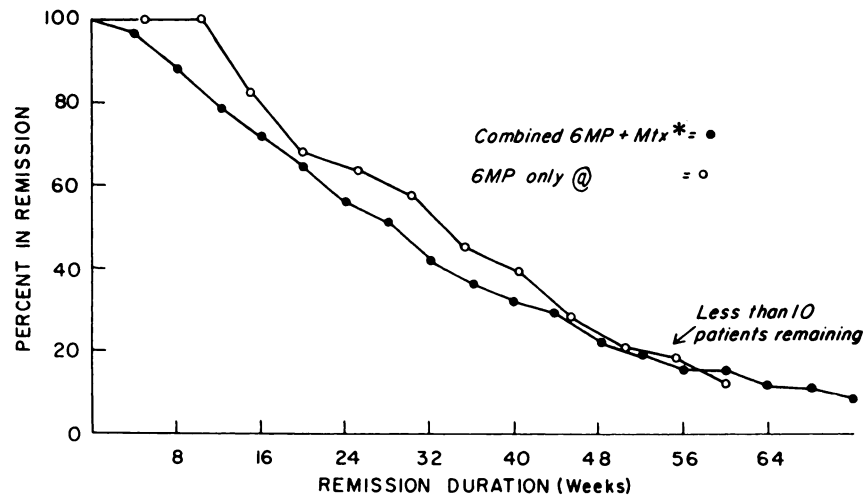


Fig. 2.—The effect of the three remission maintenance programs on remission duration.



* Includes all three remission maintenance programs in present study

@ Taken from previous study(1)

Fig. 3.—Comparison of the effects of combination (Mtx 6-MP) and single (6-MP) drug treatment in remission on remission duration.

the magnitude of the pretreatment white cell count had an adverse effect on the duration of complete remission ($p = 0.01$) (fig. 6).

DISCUSSION

The complete remission rate for various agents used singly and in combination as treatment for children with acute lymphocytic leukemia is presented in table 8. All of these studies were performed by the same group of investigators (Leukemia Chemotherapy Group B) on the same patient cate-

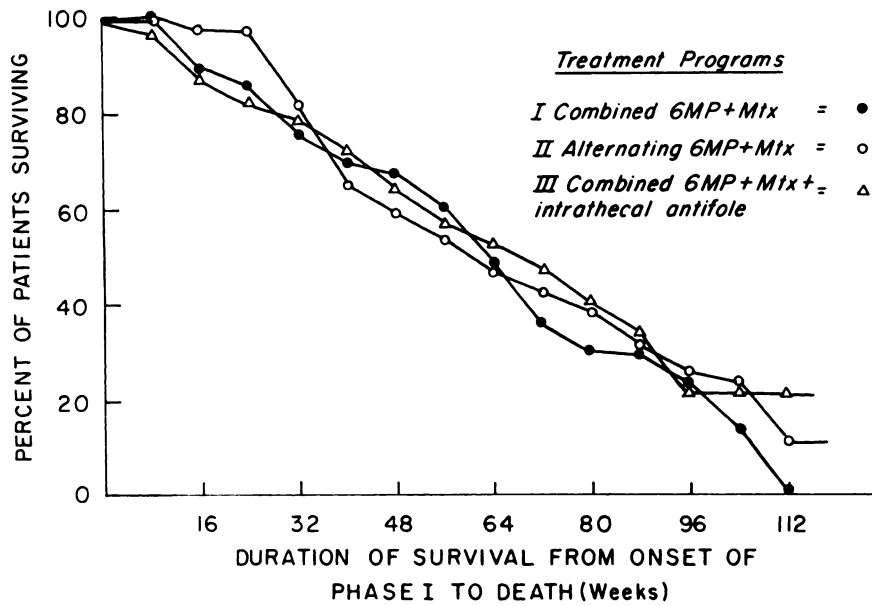


Fig. 4.—The effects of the three remission maintenance programs on survival.

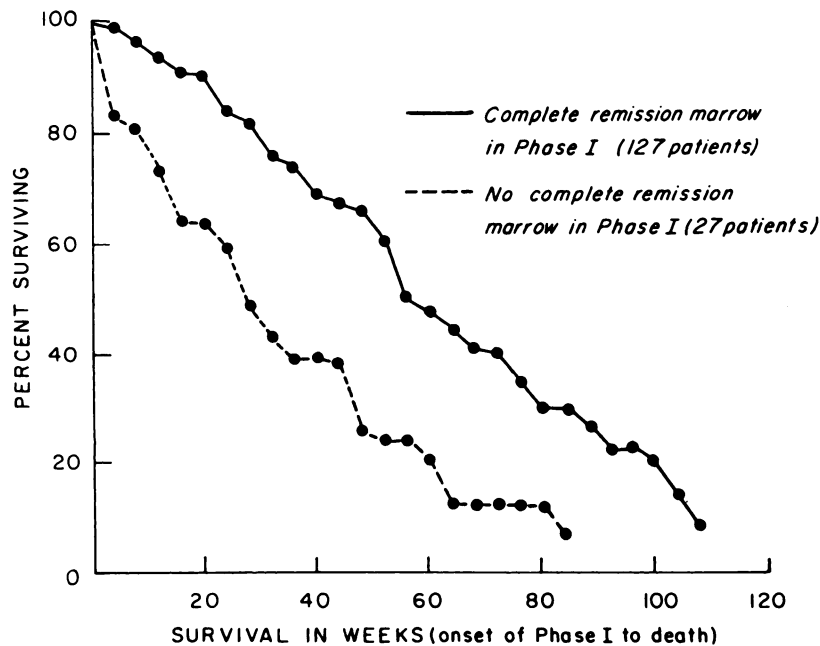


Fig. 5.—Effect of response in Phase I on survival.

gory (children with acute lymphocytic leukemia without prior treatment) during the past six years. For each combination the remission rate is equal to or greater than that calculated assuming independent drug action. The complete remission rate for prednisone and 6-MP in the present study was 82 per cent and in a current study of prednisone and vincristine, 84 per cent.¹⁵

Table 6.—*Meningeal Leukemia*

Maintenance Treatment Program	No. of Patients	No. with Meningeal Leukemia Overall	Probable
I. Combined	37	3	2
II. Alternating	34	6	1
III. Alternating plus Intrathecal antifole	35	1	0

*Clinical syndrome of increased intracranial pressure plus an increase in mononuclear cells in the spinal fluid.

Table 7.—*Toxicity in Phase II*

Treatment Category	Total No. of Patients	Episodes of WBC Depression to		Platelet Depression to less than 75,000	Mucosal Ulceration
		1000-2000	0-1000		
I. Combined	37	22	9	9	9
II. Alternating	34	6	2	2	2
III. Combined plus Intrathecal antifole	35	18	4	12	15

Treatment Category	Gastrointestinal Symptoms	Neurological Toxicity	Average Maintenance dose (% of full dose)	Deaths in Phase II	
				Toxic	Other
I. Combined	4	0	56	2	3
II. Alternating	4	0	91	0	0
III. Combined plus Intrathecal antifole	9	4	62	3	1

Thus, with present therapy, over 80 per cent of patients can achieve a complete remission with initial treatment. In addition to the obvious value of this to the patient, it should be emphasized that failure to respond to initial treatment may be associated with a significantly reduced survival (fig. 5).^{1,2} The fact that there are five agents capable of producing complete remission in acute childhood leukemia, that combinations of these agents tend to be "synergistic," and that a complete remission rate approaching 85 per cent can be reached with two agents has suggested that multiple combinations might be capable of eradicating the entire leukemic cell population. Skipper and his associates have extensively and quantitatively studied this problem in a model system (L1210 mouse leukemia).¹⁴ As a result of the above clinical and experimental data, intensive multiple combination chemotherapy studies have been initiated in patients with acute leukemia.¹⁶⁻¹⁸

The objective of the remission maintenance (Phase II) part of the study was to prolong remission—that is, to delay the development of relapse and leukemic cell drug resistance. Combination chemotherapy was employed (1) because in certain bacterial diseases (e.g., tuberculosis) combinations of effective agents may markedly delay the development of resistance,¹⁹ and (2) because there is suggestive evidence that long-lasting remissions are more frequent in patients receiving combination MTX or 6-MP or combination 6-MP and asaserine than with 6-MP alone.^{3,20}

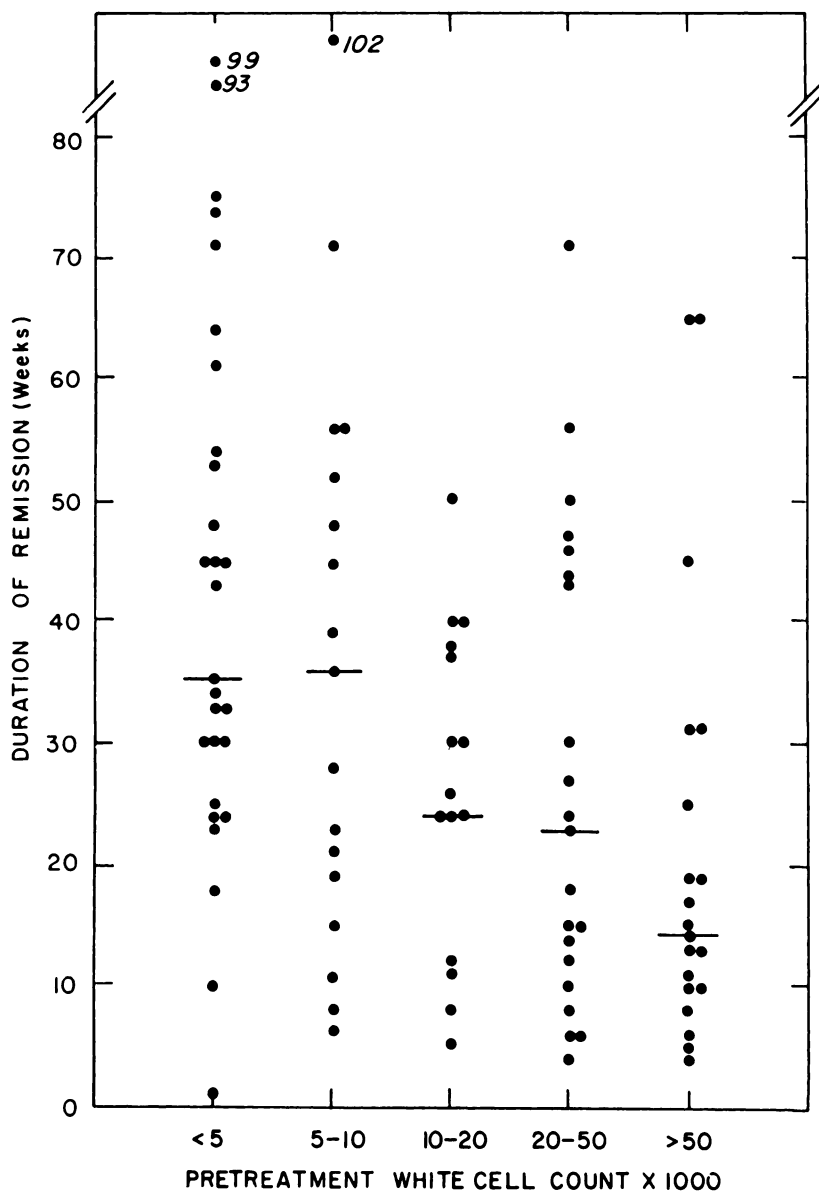


Fig. 6.—Effect of pretreatment white count on duration of remission.

Alternating treatment between MTX and 6-MP at four-week intervals (Treatment Program II), was employed in an effort to prolong remission. The major rationale for this alternating treatment is that leukemic cell resistance to a given agent may be delayed if treatment with the agent is interrupted or alternated. The four-week interval was chosen because in a previous clinical study it took a minimum of six weeks exposure to 6-MP before relapse developed.¹ In the present study the surviving fraction of leukemic

Table 8.—*Combination Chemotherapy in Acute Lymphatic Leukemia of Childhood*

Agent (s)	Number of Patients	Complete Remission		Reference
		No.	%	
MTX	48	10	21%	(3)
6-MP	43	12	27%	(3)
VCR	81	38	47%	(2a)
Pred	72	41	57%	(1)
MTX + 6-MP	39	17	45% (42%)*	(3)
Pred + 6 MP	154	127	82% (69%)	(This report)
Pred + VCR	63	53	84% (77%)	(15)

MTX = Methotrexate; 6-MP = 6-Mercaptopurine; VCR = vincristine.
Pred = Prednisone.

*Per cent in () is the complete remission rate (CRR) calculated assuming independent drug action.

$$CRR_{A+B} = CRR_A + CRR_B \frac{(100 - CRR_A)}{100}$$

Data from Leukemia Chemotherapy Group B.

cells following remission induction with 6-MP and prednisone have presumably developed some degree of "resistance" to 6-MP. Were 6-MP only continued these surviving, partially 6-MP resistant leukemic cells would proliferate, perhaps with the continuing selection of cells with greater degrees of resistance to 6-MP, until relapse occurred. If, on the other hand, MTX is substituted, as in the alternating program (Program Number II), the surviving fraction may be controlled and perhaps further reduced. Since there is no clinical or experimental evidence for cross-resistance between 6-MP and MTX,^{3,21} there is no reason to believe that the 6-MP resistant cells would be less sensitive to MTX. In fact, in some rodent leukemia systems exposure to 6-MP enhances the antileukemic effect of MTX.²¹ The critical question is whether, after 6-MP treatment is interrupted, the surviving, partially 6-MP resistant leukemic cells change, either in the direction of increasing or decreasing 6-MP resistance. If the degree of 6-MP resistance does not change or continues to increase during MTX administration, the alternating program will fail. This will also obtain if the proportion of cells resistant to 6-MP of the surviving fraction increases during MTX administration. In bacterial systems, drug resistant variants are generally less "hardy" than the original sensitive cell lines, and in mixed populations in the absence of the drug the sensitive bacteria will be ascendent. In some experimental rodent leukemia studies the reverse is true; that is, the drug resistant cell lines will rapidly predominate in an originally mixed population.²²

If, on the other hand, drug resistance in man is temporary, alternating therapy may be effective. Thus, a temporary, reversible increase in dihydrofolic reductase occurs in human leukemic cells exposed to MTX and is perhaps related to drug resistance.²³ In these circumstances, interrupted or alternated MTX may prevent accumulation of MTX resistance and prolong remission. Similarly, if human drug resistant leukemic cells decrease proportionally in favor of sensitive cells in mixed populations, as is true in some

bacterial systems, interrupted or alternated drug treatment should delay resistance. The above are some of the considerations which prompted the present study of the effects of alternating antimetabolite therapy on remission duration. In this clinical study, alternating chemotherapy did not prove superior to concomitant MTX and 6-MP (fig. 1). However, Zuelzer^{4,24} and Brubaker⁷ have reported promising results with the alternating use of three or more agents.

The third remission maintenance program consisted of combined MTX and 6-MP plus intrathecal folic acid antagonist administration. Clinical evidence of leukemic involvement of the arachnoid membranes occurs in 17 per cent of children with acute lymphocytic leukemia, and such invasion is observed at autopsy in at least 45 per cent of patients.^{6,7} Thus, major involvement of this site occurs in half of the patients, and it is probable that small, subclinical degrees of meningeal involvement occur frequently in the remaining half.²⁵ Since both MTX and 6-MP are largely excluded from the central nervous system, these meningeal leukemic cells are exposed to low concentrations of the drugs, an ideal circumstance for the development of resistance. It is hypothesized that relapse from remission results from the successful reentry and multiplication of these leukemic cells in the systemic circulation and main body compartments. Treatment Program III was designed to prevent or delay relapse by the intrathecal administration of a folic acid antagonist at 28-day intervals to patients in remission. The fact that remissions were not prolonged suggests that the above is not a common mechanism of relapse or that intrathecal administration of folic acid antagonists are not effective in markedly reducing or eliminating leukemic cells in the central nervous system. It has been demonstrated that with meningeal leukemia the infiltration involves the surface of the subarachnoid membrane and the Virchow-Robin spaces and thus is exposed to cerebrospinal fluid.⁷ This is consistent with the observation that folic acid antagonists introduced into the lumbar subarachnoid space produce complete but temporary regression of the clinical and laboratory manifestations of meningeal leukemia.^{26,27} Rieselbach et al.²⁸ demonstrated that the volume of injected solution was the most important factor in achieving rapid and extensive cerebrospinal fluid drug distribution. However, even with the relatively large volumes used in this study, it is doubtful that the folic acid antagonists injected into the lumbar subarachnoid space produce effective drug concentrations in the ventricles and cortical subarachnoid space.²⁸ This, plus the fact that meningeal leukemia tends to recur rapidly after treatment with intrathecal folic acid antagonists, suggests that most but not all of the meningeal infiltrate is eliminated by such treatment. While meningeal leukemia occurred less frequently in those patients receiving intrathecal folic acid antagonists (Treatment Program III), the prophylactic use of intrathecal therapy is not recommended because of the development of neurotoxicity in a minority of patients (table 7).

Since there was no difference between the remission maintenance programs, all of which involved the use of both MTX and 6-MP, these were combined and compared to a previous study wherein 6-MP only was used for remission maintenance (fig. 2). It is apparent that the combination of MTX

and 6-MP is not superior to 6-MP in prolonging remissions. This comparison must be qualified by the fact that different agents were used for remission induction. Thus prednisone was used for remission induction for 6-MP maintenance and 57 per cent of patients entered complete remission.¹ In the present study prednisone plus 6-MP was used and 82 per cent of patients entered complete remission. It is probable that the better remission-inducing programs result in an increased increment of patients in remission whose overall responsiveness to chemotherapy is less. However, when only 6-MP was used to induce remission and the complete remission rate was 27 per cent, the median duration of remission was only 18 weeks.³

CONCLUSIONS

1. Combinations of effective agents produce at least an additive increase in complete remission rates over that which can be achieved when the agents are used individually.
2. Patients who do not achieve complete remission with initial treatment have a significantly shorter survival.
3. Alternating MTX and 6-MP at 28-day intervals during remission does not prolong the duration of remission over that of combined concurrent 6-MP and MTX.
4. The administration of folic acid antagonists intrathecally at 28-day intervals during antimetabolite maintained remission did not prolong the duration of remission. Meningeal leukemia, however, occurred significantly less frequently in these patients.
5. The duration of combined 6-MP and MTX maintained remission is not greater than that of 6-MP maintained remission.
6. The toxicity of 6-MP and MTX in combination in patients in remission is additive.

The above conclusions were drawn from this comparative study of combinations of chemotherapeutic agents in children with acute lymphocytic leukemia.

SUMMARIO IN INTERLINGUA

1. Combinationes de agentes efficace resulta in al minus un augmento additive del proportion de remissiones complete in comparison con illos effectuable quando le agentes es usate individualmente.
2. Patientes in qui le tractamento initial non resulta in un remission complete ha un significativamente reduce expectation de superviventia.
3. Le duration del remissiones non es prolongate si durante illos cursos de 28 dies de methotrexato es alternate con equalmente longe cursos de 6-mercaptopurina in comparison con le duration del remissiones durante le quales 6-mercaptopurina e methotrexato es administrate concurrentemente.
4. Le administration intrathecal de antagonistas de acido folic a intervallos de 28 dies durante remissiones mantenite con antimetabolitos non prolongava le duration. Tamen, in iste patientes leucemia meningeae occurreva significative-mente minus sovente.
5. Le duration de un remission mantenite per un combination de 6-mer-

captipurina e methotrexato non excede le duration de un remission mantenite per 6-mercaptopurina sol.

6. Le toxicitate de 6-mercaptopurina e methotrexato in uso combinate in patientes in remission es additive.

Le supra-listate conclusiones esseva derivate ab iste studio comparative de combinationes de agentes chimotherapeutic in juveniles con aucte leucemia lymphocytic.

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