PAPERS AND ORIGINALS

Treatment of Acute Lymphoblastic Leukaemia

Comparison of Immunotherapy (B.C.G.), Intermittent Methotrexate, and No Therapy after a Five-month Intensive Cytotoxic Regimen (Concord Trial)

Preliminary Report to the Medical Research Council by the Leukaemia Committee and the Working Party on Leukaemia in Childhood*

British Medical Journal, 1971, 4, 189–194

Summary

One hundred and ninety-one cases of acute lymphoblastic leukaemia were entered in a trial in which, for five months, all received cytotoxic therapy with prednisolone, vincristine, mercaptopurine, L-asparaginase, and methotrexate (the latter in high dosage followed by folinic acid). Patients were then randomized to receive immunotherapy (B.C.G.), twice-weekly methotrexate, or no further treatment.

One hundred and seventy-seven patients (93%) achieved full remission and at the time of analysis, 26 months from the beginning of the trial, 143 were still alive, including 70 in their first remission. Median "post-intensive" remission lengths were 17 weeks (no treatment), 27 weeks (B.C.G.), and 52 weeks (methotrexate). The prolongation of remission by methotrexate was most evident in those patients with low initial white cell counts. B.C.G. seemed to cause lymphocytosis but was without other conspicuous effect. The incidence of toxic reactions is reported, including an unusually low rate of anaphylaxis with L-asparaginase.

These preliminary results are discussed and compared with those of similar trials.

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Professor L. J. Witts, chairman of the Leukaemia Committee, and Professor J. V. Dacie, chairman of the Working Party on Leukaemia in Adults, took part in the planning and supervision of the trial.

The data were collected together by Dr. N. B. Ikoku and Miss M. Gilham at the Leukaemia Trials Office, Royal Marsden Hospital, under the supervision of Dr. H. E. M. Kay. This report was drafted by Dr. Kay. Statistical analysis was carried out by Mr. Peto and Dr. Pike.

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Introduction

Improvements in the treatment of acute lymphoblastic leukaemia have been reported from many centres using a variety of different regimens (Henderson and Samaha, 1969; Holland, 1971; Pinkel *et al.*, 1971). The broad conclusion from these reports is that intensive treatment is called for, while the work of Mathé *et al.* (1968) suggested that immunotherapy may be an effective form of treatment if it follows a course of cytotoxic drugs. On the basis of these observations a multicentre randomized controlled clinical trial was planned to evaluate the use of B.C.G. as a form of immunotherapy after a particular regimen of intensive cytotoxic treatment. Patients were admitted to the trial from January 1969 to August 1970. Of the 191 patients entered 143 (75%) were still alive (1 March 1971) and 70 (37%) were still in their first remission. It is clearly premature to reach final conclusions but some preliminary observations can be reported.

Intake to Trial

The trial was limited to patients aged 12 months and over with untreated and newly diagnosed acute lymphoblastic leukaemia. When the cytological diagnosis was uncertain the patients were regarded as having lymphoblastic leukaemia if they were under 20 years of age but myeloblastic if they were 20 or over. Blood and bone marrow smears stained by May-Grünwald-Giemsa and periodic-acid Schiff were all examined

centrally by one haematologist (H.E.M.K.) to confirm the provisional diagnosis; in fact only three cases had to be excluded because of diagnostic disagreement.

Visceral involvement, including mediastinal enlargement, did not disqualify patients from admission to the trial, and it is possible that a few cases that might elsewhere be considered cases of lymphosarcoma with bone marrow involvement were included.

Twenty-four centres in England, Wales, Scotland, and Northern Ireland participated. With the exception of three West Indian children and one child with a West African father, all were of Caucasoid stock.

Treatment

The scheme of treatment is set out in Fig. 1. All patients started on a period of cytotoxic drug treatment lasting 21 weeks or, in the event of delay, slightly longer, and if then still in first remission they were allocated either to a group receiving immunotherapy (Glaxo freeze-dried B.C.G.) or to a control

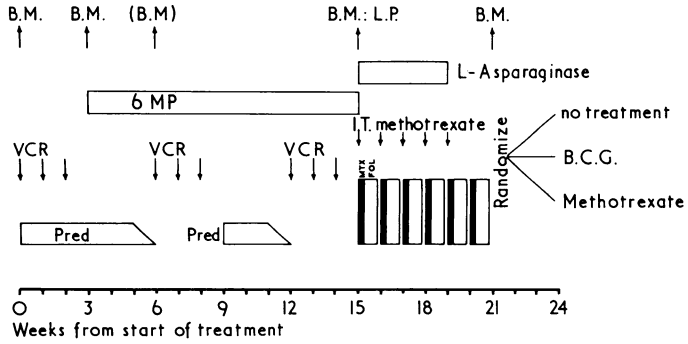

FIG. 1—Treatment protocol. See text for full details and dosages. B.M. = Bone marrow. L.P. = Lumbar puncture. MP = Mercaptopurine. MTX = Methotrexate. FOL = Folinic acid. Pred. = Prednisolone. VCR = Vincristine. I.T. = Intrathecal

group. The control group received either no further therapy or twice-weekly methotrexate. The choice of control regimen was selected initially by each centre; some considered a "no treatment" group both ethical and informative; others thought it unjustified to discontinue treatment and the methotrexate maintenance regimen was preferred. The allocation to these groups was done at our central office as soon as the presentation data on the patients were available. This allowed us to balance the patients between the groups for sex and age, and to ensure that individual centres had about equal numbers of B.C.G. and control patients. Subject to this balancing allocation was at random.

In the early stages this produced a division of cases of approximately 2:1:1 between the B.C.G., methotrexate, and "no treatment" groups. Later more cases were entered at centres choosing the methotrexate control; moreover, in September 1970 an option was given to centres which had chosen the no treatment control to switch to maintenance methotrexate for new patients and a number did so. At this stage the randomization was adjusted to aim at final equal numbers of B.C.G. and methotrexate patients. This should not invalidate any comparisons between the groups, though it must be recognized that the different groups were not entirely comparable with regard to the periods at which the patients were admitted to the trial. The final numbers in the main group of analysed cases were: 52 B.C.G., 18 no treatment, and 52 methotrexate.

CYTOTOXIC DRUG REGIMEN

Induction of remission was by the orthodox combination of prednisolone (40 mg/m² oral daily) and vincristine (1·5 mg/m² intravenously weekly) for three weeks; the regimen then switched to mercaptopurine (70 mg/m² oral daily) and prednisolone for the next three weeks, steroid being tailed off during the last week. A bone marrow aspirate was taken after three weeks, and again after six weeks in those not already in full remission at three weeks, and provided the six-week marrow showed improvement the patient continued on the protocol treatment. Those failing to show any sign of remission at six weeks were given other treatment at the discretion of the treating physician.

After induction of remission, a period of cytoreductive treatment not needing hospital admission was continued to the end of the 15th week from diagnosis. Two drugs at a time were always given, to obtain the benefit of possible synergism and to avoid loss of control in cases resistant to any one drug (Henderson and Samaha, 1969). Mercaptopurine, vincristine, and prednisolone were given at the times shown in Fig. 1—the mercaptopurine and prednisolone at the same dosage as above, but the vincristine dose reduced to 1·0 mg/m² in weeks 7-9 and 13-15.

Patients were then readmitted to hospital for investigation and for intensive therapy. In some instances a delay after the completion of the mercaptopurine regimen (see Fig. 1) was needed before the patient could begin the next stage of treatment. This consisted of six courses of methotrexate and folinic acid each lasting a week, with the addition of L-asparaginase (Bayer) (30,000 or 6,000 units/m² daily—see below) during the first four weeks. Each course of methotrexate consisted of six doses of 40 mg/m² intravenously or intramuscularly every eight hours, followed by folinic acid 24 mg/m² intravenously or intramuscularly three times a day for four days. After one day with no treatment a further course was started; but if marrow depression was prolonged the next course was delayed, and if necessary the methotrexate schedule was reduced by one dose. Not all patients were able to tolerate this regimen and those receiving less than five of the six courses were excluded from the randomized part of the trial.

The reason for stopping L-asparaginase after the first four weeks of intensive therapy was the need to give a least immunosuppressive treatment immediately before immunotherapy.

Five weekly doses of intrathecal methotrexate (8 mg/m²) were included in this phase of treatment as prophylaxis against meningeal disease. Supportive treatment, including blood transfusions and antibiotics, was given as needed.

AMENDMENT DURING COURSE OF TRIAL

In addition to the opportunity to change the type of treatment given to the control group, one further amendment was made during the course of the trial. The dose of L-asparaginase was reduced after the first 50 cases from 30,000 to 6,000 units/m² daily. The reasons for this change were toxicity at the higher dose and cost (see Symposium International sur la L-Asparaginase, 1970).

RANDOMIZED PHASE

The choice of Glaxo freeze-dried B.C.G. as a form of non-specific immunotherapy and its preparation, dose, and mode of inoculation were dictated by the following arguments and circumstances. (1) From the original series published by Mathé *et al.* (1968, 1970) it seemed that B.C.G. alone was as effective as B.C.G. combined with blast cells or blast cells alone. (2) Grave practical difficulties prevented the use in a multicentre trial of pooled blast cells. (3) At the start of the trial the Pasteur Institute vaccine used by the French could not be distributed for our general use and we therefore decided to use the freeze-dried preparation made by Glaxo. This differs from the Pasteur vaccine in a number of ways (see Table I). (4) Hesitation to scarify large areas of skin, as was done by Mathé's group, led us to adopt percutaneous inoculation by Heaf gun.

TABLE I—*Comparison of B.C.G. Preparations*

	Pasteur	Glaxo
Culture	Superficial	Deep
Glycerol	Present	Absent
Solid weight	75 mg/ml	10 mg/ml
Viable bacilli	10^8/ml	$1{\cdot}5 \times 10^8$/ml
Form	Liquid	Freeze-dried
Inoculation	Scarification	Percutaneous gun

This method is easier to perform and is less traumatic, and reasonable uniformity of practice can be assured. (5) The contents of one ampoule were administered by the application of 40 points of a Heaf gun set at 2 mm (1 mm in children under 2 years). On the basis of information then available it was calculated that the absorbed number of *living* bacilli would be of the same order as that delivered by the French workers.

Maintenance methotrexate was given twice a week either orally or intramuscularly in the maximum tolerated dose up to 30 mg/m^2.

Patients assigned to the no treatment group were followed up and observed in the same way as were patients on the other regimens.

TREATMENT OF RELAPSED CASES

From the end of the first remission, whether due to haematological or meningeal relapse, treatment was at the discretion of the physician at each centre. However, a suggested continuation, which has been used for most cases, was induction of remission by prednisolone and vincristine followed by cyclical six-week courses of mercaptopurine, cyclophosphamide, and methotrexate (except for patients relapsing from methotrexate maintenance) with repeated two-week courses of prednisolone and vincristine every 14 weeks.

Follow-up records are kept on all patients until death so that duration of survival as well as of remission may be analysed in future reports.

Investigations

Initial investigations included full blood count and a bone marrow aspirate, chest *x*-ray examination, and a number of biochemical tests. Thereafter blood counts were recommended at weekly intervals during weeks 1-15, at least thrice weekly during the intensive therapy phase, and after randomization twice weekly for the first four weeks, once weekly for the second four weeks, and thereafter every four weeks. Participants were encouraged to plot all blood counts on semilogarithmic paper (Galton, 1960), which is valuable in anticipating severe myelodepression. The use of Xerox copies of these charts also helps the central secretariat to assimilate the progress of the patients.

Bone marrow aspirates were taken initially for diagnosis, after three weeks' treatment and again after six weeks' treatment if the three-week marrow did not show complete remission, at the start and end of the intensive phase of treatment, and at eight-week intervals thereafter.

Lumbar puncture was avoided, unless positively indicated by symptoms, until the beginning of the intensive therapy.

Skin tests were performed by the multiple puncture (Heaf gun) technique. The antigens used were: tuberculin (P.P.D. mammalian 2 mg/ml—that is, 100,000 units/ml), depot P.P.D. 5 mg/ml, depot *Candida albicans* somatic protein 5 mg/ml, and *Candida albicans* culture filtrate 5 mg/ml. Tests were carried out immediately before the beginning of the first intensive methotrexate course and if negative were repeated eight weeks after the beginning of the randomized treatment. Results were read after 72 hours, at the end of one week, and weekly thereafter.

Immunological Effects of B.C.G.

Evidence that the B.C.G. regimen did or did not cause some stimulation of the immune system is of three kinds.

(1) Weekly or twice-weekly blood counts during the eight weeks after the end of intensive cytotoxic therapy were carried out on most patients. The mean lymphocyte levels (calculated on a logarithmic scale) at all counts after the first week showed an increase among the B.C.G.-treated cases over both the methotrexate and no treatment groups (Fig. 2). The difference between the B.C.G. group and the combined methotrexate and no treatment group averages about 700/mm^3, and though there is a wide variation from case to case and in individual patients from week to week the difference is statistically significant ($P<0{\cdot}01$) after the fourth week.

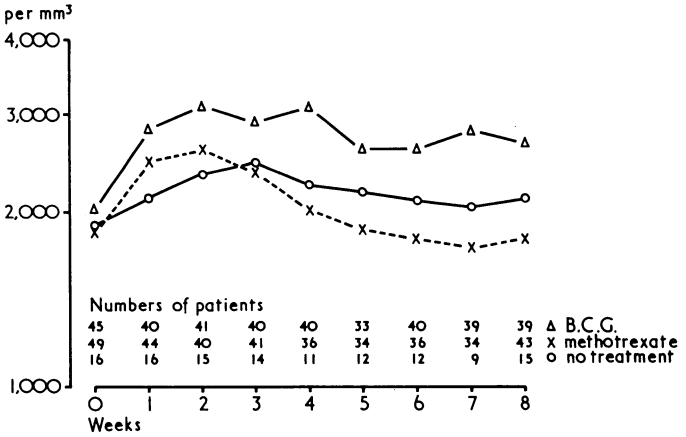

FIG. 2—Effect of "post-intensive" treatment on absolute blood lymphocyte counts. Plotted points are the mean values for all relevant patients whose lymphocyte counts were done at the stated time. (All calculations were done on a logarithmic scale.)

(2) Delayed hypersensitivity to the two candida antigens and two tuberculin preparations was determined by skin testing at week 15 and again in the negative reactors at week 30 (more precisely, eight weeks after the end of intensive therapy). The use of a depot preparation enabled a change of immune status from negative to positive to be observed between these times—this could have happened as a result of discontinuing immunosuppressive drugs or because of the stimulation caused by B.C.G.; in practice this change was only rarely recorded. Most changes from negative to positive represent the difference of the two distinct tests at weeks 15 and 30.

The results of the tuberculin testing are given in Table II. For a variety of reasons not all those negative at week 15 were, in fact, tested again at week 30; but, as expected, most negative reactors treated with B.C.G. converted to positive by week 30, though 6 out of 19 did not, which accords with the slight reactions seen at the B.C.G. sites in some patients. Six out of 12 patients having methotrexate also converted from negative to positive, presumably owing to the reduction of immunosuppression. In those having no further treatment only one out of seven converted.

Fewer conversions in the response to candida antigens were recorded (see Table II) and no significant differences are seen. Unfortunately, the numbers in each group are too small for definite

TABLE II—*Delayed Hypersensitivity Responses*

	Treatment after Randomization		
	Methotrexate	B.C.G.	None*
Tuberculin:			
Week 15, positive	8/52	11/52	2/17
Week 15, negative; week 30, negative	6	6	6
Week 15, negative; week 30, positive	6	13	1
Candida:			
Week 15, positive	13†/52	14†/52	5‡/17
Week 15, negative; week 30, negative	8	11	5
Week 15, negative; week 30, positive	2	5	2

*One patient was not tested at week 15.
†Two cases negative at week 30.
‡One case negative at week 30.
For a variety of reasons many cases were not retested at week 30.

conclusions, but it would seem that twice-weekly methotrexate is at least not detectably immunosuppressive.

(3) The response of lymphocytes to phytohaemagglutinin has been tested in a number of cases by Jones *et al.* (1971). No significant differences were recorded between the different groups.

Results

Criteria for Assessment of Results.—In this report the criteria for assessing the benefits of therapy are: (1) the rate of remission induction, remission being defined as (*a*) bone marrow aspirate with less than 5% blast cells, (*b*) peripheral blood showing no lymphoblasts, over 500/mm³ neutrophils, and over 75,000/mm³ platelets, and (*c*) no symptoms or physical signs definitely attributable to leukaemia; (2) the incidence of serious toxic reactions to the drugs and other complications; and (3) duration of remission from the end of the intensive cytotoxic therapy until relapse (due to bone marrow or meningeal disease)—post-intensive remission. (N.B. In some reported series the duration of remission ignores previous relapse due to meningeal disease and thus cannot be compared with the duration in this trial.)

The response to treatment of the 191 patients before the "randomized" phase is analysed in Table III by age and sex. Sixty-nine of these were excluded for various reasons (Table III) from the main analysis.

TABLE III—*Progress of Trial Cases before Randomization*

	Both Sexes	M.	F.	Age in Years			
				1	2-10	11-20	21+
Total No.	191	116	75	13	129	37	12
Withdrawals before randomized phase:							
"No veins"	3	1	2	0	3	0	0
Parents' or patient's request	3	1	2	0	2	0	1
Physician's request	1	1	0	0	0	1	0
Failed to remit	14	9	5	1	5	4	4
Remitted and relapsed*	24	16	8	1	17	4	2
Regimen not tolerated	20	13	7	0	15	4	1
Age	4	2	2	—	—	—	4
Randomized	122	73	49	11	87	24	—

*Including asymptomatic meningeal disease; the first lumbar puncture was scheduled at week 15.

Four were withdrawn before the randomized phase of the trial for social reasons—three because the patient or parents would not agree to admission to hospital for the intensive methotrexate treatment and one because the referring doctor had second thoughts—and a further three because of lack of suitable veins for intravenous therapy. Twenty-four patients relapsed or were found to have asymptomatic meningeal disease at first lumbar puncture and 20 further patients did not tolerate one or other phase of the cytotoxic treatment. These have all been excluded from the main analysis, though many continue in satisfactory remission.

All but 4 of the 12 patients over the age of 20 had been withdrawn or died before the randomization phase and the first three of these chanced to be assigned to the methotrexate group. We therefore decided to exclude also these older patients from the main analysis of results so as to retain homogeneity and comparability between the groups.

REMISSION INDUCTION

Of the 191 patients, 177 (93%) achieved full remission within 15 weeks—120 in three weeks, a further 42 by six weeks, and seven between six and 15 weeks (71%, 25% and 4% respectively of "timed" remitters); in eight cases the onset of remission could not be timed exactly because of failure to procure all the necessary bone marrow samples or because of an equivocal marrow aspirate. Three patients died before remission could be achieved in the first six weeks and a further seven died before week 16.

COMPLICATIONS DURING INTENSIVE CYTOTOXIC THERAPY

When infection occurred it was usually in conjunction with leucopenia, and varied in intensity from trivial—for example, conjunctivitis and skin sepsis—to fatal—for example, septicaemia and pneumonia. These are listed in Table IV according to the phase of treatment. The severity of and risk from any of these infections is difficult to assess but some indication of the frequency of severe infections is gained from the figures for proved septicaemias (positive blood culture) and fatalities.

Neurotoxicity from vincristine was noted, especially in older patients (Table V). Four of the episodes were listed as severe.

TABLE IV—*Infective Complications Occurring after the Beginning of Treatment*

Drug	Approx. Period at risk (patient-weeks)	Total No. of Infections	Septicaemia	Fatal
Prednisolone, Vincristine, Mercaptopurine	2,500	85	3	3 (pneumonia 2, septicaemia 1)
Methotrexate, L-Asparaginase	900	49	7	3 (septicaemia 2, measles 1)
Methotrexate	1,422	27	1	1 "Pneumonia"
B.C.G.	1,366 (3,261)	27 (61)	0	0
No treatment	473	7	0	0

TABLE V—*Neurotoxicity*

	No. of Cases	Ages
Vincristine:		
Mild	4	2, 3, 4, 20
Moderate	2	2, 40
Severe	4	4, 16, 18, 37
Intrathecal methotrexate:		
Meningism	8	5, 6, 11, 15, 15, 17, 19, 20
L-asparaginase:		
Tremor in both hands	1	2
Coarse tremor and titubation of head	1	3
Coarse tremor of hands	1	6
Transient loss of consciousness	1	11
Diplopia. Muscle weakness	1	14
Weakness in legs. 8th nerve toxicity	1	18
Cerebellar signs and slurring of speech	1	20

Meningism during intrathecal therapy was especially noted among adolescents and probably indicates that the intrathecal dose of methotrexate should be limited to a maximum of 15 mg rather than being related to surface area.

A number of unusual reactions occurred during L-asparaginase and methotrexate therapy. These included tremors, weakness, and some localizing signs. The origin of these disturbances is not at once apparent, but since they were commoner on the larger dose of L-asparaginase and ceased when the drug was discontinued, and since also lesser symptoms such as mood changes are common during L-asparaginase therapy but are not particularly associated with methotrexate, it would seem that L-asparaginase is the prime cause. A subsidiary role may be assigned to methotrexate, which may have toxic effects on the nervous system, and just possibly to the presence of local leukaemic lesions undergoing necrosis.

Other complications resulted almost entirely from depression of the bone marrow during both the mercaptopurine and the methotrexate regimens. Delay during the methotrexate phase owing to myelotoxicity is indicated in Table VI. Patients having fewer than five methotrexate courses or taking more than 10 weeks to get through the courses represented deviations from the trial protocol. The effect of this will be considered in a later paper.

B.C.G. was well tolerated, and apart from one recent case of iritis after 26 weeks of B.C.G. administration there were no complications.

TABLE VI—*Intensive Methotrexate Treatment Deviations*

Delay (in Days)	No. of Cases
6 courses:	
0	79
7	13
8-14	19
15-21	8
22-28	3
>28	4
5 courses only	3
4 courses or less	10

RANDOMIZATION

Randomization of the 122 cases included in the analysis resulted in the grouping shown in Table VII.

TABLE VII—*Comparability of Randomized Groups*

	Methotrexate	B.C.G.	No Treatment
Total No.	52	52	18
Age in Years 1	3	7	1
Age in Years 2-10	39	37	11
Age in Years 11-20	10	8	6
Sex Male	31	30	12
Sex Female	21	22	6
Presentation:			
Leucocyte count:			
0−	24	21	6
6,000−	13	16	6
21,000+	15	15	6
Mediastinal enlargement	4	3	0
Platelets <50,000/mm³	31	32	11
Transfusion	6	5	3
Splenic or hepatic enlargement	39	40	7
Infection	22	12	7
Haemorrhage	27	26	9
During cytotoxic therapy:			
Transfusion	28	28	7
Infection	29	22	5

DURATION OF REMISSION AFTER RANDOMIZATION

The time between the end of intensive cytotoxic therapy and relapse is shown in Fig. 3 for the randomized patients. It can be seen that the patients continuing on twice-weekly methotrexate remained in remission for considerably longer (median 52 weeks) than those in the other two groups ($P<0.01$). A few cases in the no treatment group relapsed relatively rapidly but thereafter this group and the B.C.G. group had a similar rate of relapse, and overall there was no statistically significant difference; the median post-intensive remission durations were 17 and 27 weeks respectively, each with a standard error of the order of six weeks.

The rate of relapse was independent of age but correlates, as expected, with the initial level of the leucocyte count (Table VIII). A high white cell count at presentation tends to be associated with shorter remissions in all treatment groups, and the benefits of methotrexate maintenance are most apparent in those having a low initial leucocyte count. The relapse rates of the methotrexate group in relation to the combined B.C.G. plus no treatment group are as follows:

Initial Leucocyte Count	Ratio of Relapse Rates. B.C.G. + "No Treatment": Methotrexate (±S.E.)
0−	15·5:1 (4·4:1-95:1)
6,000−	3·3:1 (1·5:1-7·9:1)
12,000+	1·1:1 (0·7:1-1·8:1)

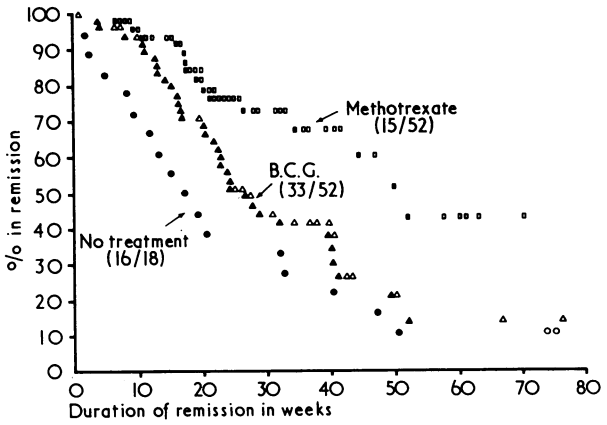

FIG. 3—Remission duration after intensive cytotoxic therapy. The percentages in remission at the different times have been calculated by standard actuarial methods. Each symbol represents one patient—open symbols are for patients still in their first remission, closed symbols for relapsed patients. Figures in parentheses are number relapsed at 1 March 1971 and total number in group respectively.

Relapse was due to recurrence of bone marrow disease (with or without meningeal leukaemia) in 45 out of 64 patients and to meningeal disease alone in 18; one patient died in remission. A higher proportion relapsed with meningeal leukaemia in the methotrexate group, an effect which can be attributed to the more prolonged suppression of bone marrow disease in this group, which provided a longer opportunity for the manifestation of meningeal leukaemia.

Discussion

The principal objective nowadays in treating acute lymphoblastic leukaemia is to achieve long-term remissions in the maximum number of patients, some of whom may be permanently cured. Consequently it must be emphasized that this is only a preliminary report on a trial that is still continuing, and that certain judgements must remain in suspense; in particular it would be hazardous to assume from the data in Fig. 3 that maintenance methotrexate will in the long run prove superior to the other regimens; indeed, the very small numbers of deaths after randomization indicate no better long-term prognosis.

The effectiveness of the regimen of cytotoxic therapy can be judged by the incidence of induced remissions (177/191 or 93%), which is in accordance with general experience, and by the length of post-intensive remission whether the patients are on B.C.G. or receiving no maintenance therapy. The median lengths of remission in these two groups (27 and 17 weeks) are similar to those following the VAMP (vincristine, Amethopterin (methotrexate), mercaptopurine, and prednisone) and BIKE (two cycles of therapy given over a total period of five months using methotrexate, mercaptopurine, and cyclophosphamide) regimens (20 weeks), in which the period of cytotoxic drug therapy was also about five months (Freireich *et al.*, 1968). They are somewhat shorter than those of other regimens,

TABLE VIII—*Remission after Randomization*

Initial Leucocyte Count	Methotrexate				B.C.G.				No Treatment				All Treatments			
	n	r	e	r/e	n	r	e	r/e	n	r	e	r/e	n	r	e*	r/e
0−	24	1	13·75	0·07	21	12	12·83	0·94	6	6	3·04	1·98	51	19	28·42	0·67
6,000−	13	3	7·69	0·39	16	10	7·65	1·31	6	5	4·01	1·25	35	18	20·47	0·88
21,000+	15	11	6·46	1·70	15	11	6·33	1·74	6	5	2·23	2·24	36	27	15·11	1·79
All†	52	15	27·59	0·54	52	33	26·73	1·23	18	16	9·68	1·65	122	64	64·00	1·00

n = No. of patients.
r = No. of patients relapsing.
e = Expected No. of patients relapsing, calculated on the assumption of exponential distribution of time to relapse—this has previously been shown to be a good approximation. Similar results are obtained if the non-parametric approach of Peto and Peto (1971) is adopted.
r/e = Relative relapse rate.
*Standardized for treatment.
†Expected values standardized for initial leucocyte count.

such as POMP (prednisone, Oncovine (vincristine), methotrexate, and Puri-nethol (mercaptopurine)) (34 weeks) (Henderson, 1969), and the best regimen of group B (45 weeks) (Holland and Glidewell, 1968), in which, however, a longer period of treatment was given with consequently fewer patients surviving to enter the period with no maintenance therapy. These patients have, moreover, been "selected" for good prognosis by having survived a long time before being taken off therapy (independent of its effectiveness), and valid comparisons are not possible on the basis of the published results.

It may be asked whether the treatment reduced the number of leukaemic cells to a residue small enough to succumb to immune attack. Calculation must be based on many questionable assumptions, but given a cell number of 10^{11} at relapse (most relapses were diagnosed early by bone marrow aspiration) on day 120 (17 weeks) and a doubling time of four to six days, then the cell residue would be $10^{11} \div 2^{120/4}$ or $10^{11} \div 2^{120/6}$—namely, 100-100,000 cells. These are the sort of cell numbers which might, from a comparison with animal experiments, be dealt with by the immune system.

In retrospect the scheme of treatment can be criticized on a number of counts, and a report on a parallel trial by Mathé *et al.* (1971) has mentioned some of these. For example, it is possible that the combination of methotrexate with L-asparaginase was deleterious since, at one dose level in an experimental mouse leukaemia, drug antagonism has been found (Capizzi *et al.*, 1970). However, a beneficial result of the placing of L-asparaginase in this schedule of treatment has been to diminish the incidence of anaphylaxis. In this trial only one probable and one possible anaphylactic reaction was noted; this is in contrast to the usual experience, where an incidence of 10-20% has been found. The reduction is probably attributable either to the length of the preceding chemotherapy or to the simultaneous use of methotrexate.

The regimen of high-dose methotrexate with folinic acid was an attempt to compress into a short period of time an antileukaemic attack and to kill those cells which are not susceptible to the usual doses (Hryniuk and Bertino, 1969). The scheme of treatment is certainly expensive and has been considered too traumatic. However, in the long run the total pain and discomfort is perhaps no more than that of more prolonged methotrexate regimens and it remains to be seen whether this type of schedule has a part to play in an antileukaemic regimen. Certainly the anti-central nervous system prophylactic treatment is much less than is required to prevent the major part of the incidence of meningeal disease. Whether it might be effective in those patients having minimal latent central nervous system disease and curable blood disease may become evident in the long term. Meanwhile the data of Pinkel (1971) encourage a more energetic attack on the central nervous system to avert meningeal leukaemia.

The best group in the randomized series was that on maintenance methotrexate. It is most important to note that within this group the height of the initial leucocyte count was critical. Those with a W.B.C. count in excess of 21,000 do little better than those on the other regimens (estimated improvement only 10%). The best remissions are almost all among those with a low initial count. At this point it is worth noting that, though the prognostic importance of the initial blast count has been known for many years (Bethell, 1953), this factor has not always been seen to be taken into account in trial reports.

In the B.C.G. and no treatment groups the effect of the initial W.B.C. count is less apparent. B.C.G. as used in this trial seems to be virtually without effect and it is possible that this form of immunotherapy was insufficiently stimulatory. In the initial French trial the results suggested that B.C.G. alone was as effective as more elaborate forms of immunotherapy (3/8 long-term remissions compared with 4/12) but data were not available on the appropriate dosage. The apparent difference between the results in the different trials may be attributable to the properties or the mode of inoculation of the Pasteur B.C.G. vaccine, which may be a more powerful non-specific stimulant (Bluming *et al.*, 1971). It should be noted, however, that in the study of Bluming and his co-workers the dose of the two forms of B.C.G. is very different, and that the method of administration (and hence the dose) of Glaxo B.C.G. differs from that used in this trial.

The results to date of this trial therefore emphasize the uncertainties of using such a variable agent as B.C.G., especially when the desired effect is only a by-product of the specific immunization, and when there are such extreme differences in response to treatment between subgroups of patients.

Our immediate aims are to follow this trial to its conclusion and, in particular, to examine the effectiveness of the treatment in relation to the immune reactions and other observed features of the disease. The lessons learned from the preliminary results of this trial are being applied to current and future trials, in which also the necessity for adequate antimeningeal treatment is being examined.

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