Cyclic Chemotherapy in Childhood Acute Leukaemia

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During the phase of complete remission children with acute leukaemia are in apparently full health and can take part in all normal childhood activities. Until a form of therapy is devised which can achieve cure in a significant proportion of these children, the most desirable clinical objective appears to be the prolongation of these remissions with the minimum of hospitalization and the minimum of toxic side-effects. We have been evaluating the cyclic use of oral mercaptopurine, methotrexate, and cyclophosphamide for this purpose since late 1964.

Methods and Criteria

All marrow aspirations were performed on the posterior iliac crest using the approach described by Bierman and Kelly (1956). Approximately 0.2 ml. was aspirated and immediate smears were made. In addition to Romanowsky methods the smears were stained by the PAS and Sudan Black techniques as described by Hayhoe, Quaglino, and Doll (1964). Classification of the cellular type of the leukaemia was attempted using the general criteria suggested by these authors. Venepunctures for diagnostic purposes were avoided, all peripheral blood tests being performed on finger-prick blood samples using standard haematological methods (Dacie and Lewis, 1963). Platelet counts were performed as described for capillary blood by Macfarlane and Biggs (1955). The platelet count, total white cell count, absolute neutrophil count, absolute blast cell count, and Hb were plotted on 4-cycle logarithmic graph paper, which also showed the drug treatment and patient's weight for ease of interpretation. During remission induction these tests were performed thrice weekly or daily; otherwise they were performed at fortnightly intervals. The criteria for remission were the absence of clinical symptoms attributable to leukaemia, together with an absence of blast cells on a carefully examined blood film, a platelet count consistently over 150,000/c.mm., a total neutrophil count consistently over 1500/c.mm., and a Hb over 10 g./100 ml. Latterly an additional criterion has been the presence of less than 3% of blast cells in a confirmatory marrow aspiration performed at the completion of remission induction. If a progressive fall in platelet

count or total neutrophil count occurred while on maintenance therapy the marrow was re-examined to distinguish between drug depression or early leukaemic relapse.

The criteria for systemic leukaemic relapse were the presence of even a single unequivocal blast cell on the peripheral blood film or of over 3% of blast cells in a marrow smear. These findings were invariably accompanied by thrombocytopenia or neutropenia but not necessarily clinical symptoms of leukaemia.

The criteria for meningeal leukaemic relapse were pleocytosis of the CSF. The majority of the cells on these occasions had the morphological criteria of blast cells when examined by the cytological method of Spriggs and Boddington (1959).

The length of first remissions and of total survival was measured by the number of completed weeks from the institution of initial treatment. A table was made showing the number of patients still in first remission at the end of each completed month and also the number of possible patients in their first remission at the end of each month. The latter figure was derived by subtracting from the starting number (31) those who had not yet completed the month in question but who were still in remission. From such a 'life-table' it can be seen what proportion of the possible patients are still in their first remission at the end of each monthly interval (Cutler and Ederer, 1958; Zuelzer, 1964). The time at which 50%, 25%, and 10% of the possible patients were still in remission was then calculated; the 50% figure corresponding to the *median length of the first remission*. This method of calculation permits an estimate to be made before waiting for all the patients to relapse. It has similarly been applied in the calculation of the median, 25%, and 10% periods of total survival in the present series. In addition, the median first remission and total survival durations have been calculated for the 'cohort' of patients who began treatment on this schedule over 12 months ago. It is of interest to follow a 'cohort' of such patients when so few relapse or die during the first year of observation (Zuelzer, 1964).

Treatment

Initial treatment in all patients was a standard course of oral prednisolone consisting of 60 mg. per day in divided doses for 10 days, followed by stepwise reduction at 10-day intervals, the course being completed after 40 days. In 6 of the earlier patients this course was given alone. In the majority it was given together with

Received September 1, 1967.

* Research Registrar in Childhood Leukaemia, in receipt of a grant from the Leukaemia Research Fund Limited.

INITIAL and CYCLIC TREATMENT SCHEDULES

FIG. 1.

mercaptopurine at a dose of 2.5 mg./kg. per day. In the 6 most recent patients, weekly intravenous vincristine (0.05 to 0.075 mg./kg. per week) was also given (see Table II). In 2 early patients not rapidly responding to prednisolone alone, vincristine was also given.

All patients with clinical and haematological remission were submitted to the scheme of cyclic maintenance therapy shown in Fig. 1. The period of 10 weeks on mercaptopurine, 2.5 mg./kg. per day, was chosen, since, when used in this way, relapse is unusual before this time (Freireich *et al.*, 1963). From similar considerations a period of 6 weeks was chosen for both methotrexate, 0.125 mg./kg. per day, and cyclophosphamide, 5 mg./kg. per day. When the first drug of the cycle, mercaptopurine, had been used as part of the initial remission-induction treatment, the start of the first cycle was timed from the first day of this treatment. This was the case in 24 out of the 32 patients in whom remissions were obtained.

The maintenance drugs were administered daily but not necessarily in equal daily doses. By spreading the correct dose for the weight over a period of 2, 3, or 4 days and by using half-tablets for mercaptopurine or methotrexate (25 and 1.25 mg., respectively) it was possible to adjust the dose to the nearest 2.5 kg. body weight, erring in general to the lower dose.

During the periods of remission the patients attended as out-patients at fortnightly intervals. If thrombocytopenia (<100,000/c.mm.) or neutropenia (<1000/c.mm.) developed, the maintenance drug was discontinued for 1 week. If the count fell further, a marrow aspiration was performed (see methods and criteria), otherwise treatment was still discontinued until the platelet count rose above 150,000/c.mm. and the absolute neutrophil count above 1500/c.mm. The drug in question was then restarted at half or two-thirds the original dose, or alternatively the next drug in the cycle was employed in full dosage if this change was due according to the cyclic schedule.

Meningeal relapse was treated with intrathecal methotrexate 0.25 mg./kg., given on alternate days until the cell count in the CSF was normal. Oral drugs were discontinued during this time.

Systemic leukaemic relapse was treated by weekly intravenous vincristine (usually 4 doses of 0.05, 0.05, 0.075, and 0.075 mg./kg.) and oral prednisolone (15 mg. q.d.s. × 10 days, 10 mg. q.d.s. × 10 days, 5 mg. q.d.s. × 10 days, 5 mg. b.d. × 10 days). After a second remission

was established the cyclic maintenance therapy was continued, omitting the drug on which relapse had occurred. Subsequent relapses were treated similarly with vincristine and prednisolone until the case was demonstrably refractory to each of the 5 drugs. When this point was reached no excessive efforts were made to prolong life.

TABLE I

Summary of Cases of Acute Leukaemia Treated since October 1964

Type	No.	No. Having Remission	No. in First Remission	No. Alive
Lymphoblastic	31	31	15	20
Myeloblastic	3	1	1	1
Myelomonocytic	1	0	0	0
	35	32 (91%)	16	21

Results

Table I shows the incidence of lymphoblastic, myeloblastic, and myelomonocytic types of acute childhood leukaemia when classified at the time of initial marrow examination by morphological and cytochemical criteria. One of the patients in the lymphoblastic group had originally presented as a case of localized lymphosarcoma. During the leukaemic phase of her disease, however, she has behaved in a manner clinically indistinguishable from the other patients with lymphoblastic leukaemia. This similarity in pharmacological response has also been reported recently by Jones *et al.* (1967). All those cases diagnosed as being lymphoblastic had a clinical and haematological remission lasting more than 2 months. Only one of the myeloblastic cases had a similar remission. Half of those achieving a remission are still in their first remission and two-thirds are still alive. The drugs used for inducing these first remissions are shown in Table II and the subsequent schedule of cyclic maintenance therapy in Fig. 1.

Table III and Fig. 2 relate to the duration of the first remission while being maintained on the cyclic

TABLE II
Drugs Used for Induction of First Remission

Drugs	No.
Prednisolone plus mercaptopurine	18
Prednisolone plus mercaptopurine plus vincristine ..	6
Prednisolone alone	6
Prednisolone plus vincristine	2
Total number of remissions obtained	32

schedule. The median length of the first maintained remission is 65 weeks (15 months) if considered for the cohort of 23 patients who began treatment more than 1 year ago, or 60 weeks (14 months) for the whole series of patients calculated by the life-table method (so as to exclude those patients who have not been on the schedule long enough to reach the point of median remission duration (see 'Methods and criteria')). The length of remission and total survival curves shown in Fig. 2 have also been calculated by this method. In addition to median

TABLE III
Summary of Cases with First Remission Maintenance on Cyclic Schedule

	No.	Median First Remission	No. Relapsing
Cohort starting less than 12 months ago	8	Not yet reached	1
Cohort starting more than 12 months ago	23	65* weeks	14
	31	60* weeks	15

No. starting cyclic schedule 32; No. withdrawn from schedule 1†; No. remaining on schedule 31.

* Calculated by the life-table method.

† Clinical toxicity from cyclophosphamide, withdrawn while in remission.

(50%) remission duration, the 25% and 10% levels of 108 weeks (25 months) and 126 weeks (29 months) are also shown though the latter relate, of necessity, to numbers of patients, which are too small for accuracy. The clinical quality of these remissions

FIG. 2.—First remission and total survival curves calculated by the life-table method. Superimposed on the table is a diagrammatic representation of the cyclic sequency of drug treatment, the drugs in question being indicated on the first cycle. The points on the 'survival' and 'remission' curves corresponding to 50%, 25%, and 10% levels are also indicated by arrows.

TABLE IV
Summary of Cases Treated for First Relapse

No. Treated	Drugs	No. Achieving Second Remission	Duration of Second Maintained Remission (wk.)		
			Median	Mean	Range
9	Vincristine + steroids	8*	9	9.4	4-14
4	Vincristine + steroids + mercaptopurine	4†	23	24	21-28

* Remission assessed from peripheral blood findings, see methods and criteria.

† Remission assessed by A1 marrow status in addition to above (Bisel, 1956), i.e. absence of identifiable leukaemic blast cells.

was extremely good. After a few weeks the signs of hypercortism disappeared and the children returned to their normal lives at home and at school.

Meningeal relapse subsequently occurred in 6 patients, 5 of whom were in haematological remission at the time. Of these 5, 4 have subsequently developed a haematological relapse 3, 4, 7, and 11 weeks after the meningeal episode had been successfully treated with intrathecal methotrexate. Table IV shows the results of treatment of all systemic leukaemic relapses with either vincristine plus prednisolone or with a combination of these two drugs plus oral mercaptopurine. Preliminary results suggest an improvement in the length of subsequent remission after the latter three-drug therapy. (More careful haematological surveillance is necessary with the three-drug combination, but no excessive haematological depression has so far been encountered.)

The median duration of the total survival is set out in Table V as well as being shown in Fig. 2. When considered for the cohort of patients starting treatment more than 1 year ago the median survival point has not yet been reached but must be in excess

of 82 weeks (19 months). The life-table calculation for the whole series gives the same figure, with 25% and 10% survival times of 126 weeks (29 months) and 143 weeks (33 months), respectively (Fig. 2).

The incidence of clinical and haematological toxicity encountered during the use of the three 'maintenance' drugs, mercaptopurine, methotrexate, and cyclophosphamide and with the 'induction' drug vincristine are shown in Table VI where they are related to the total number of patient-weeks on each drug and to the number of leukaemic relapses which occurred while on each drug.

The 31 patients constituting the present series maintained on cyclic therapy have been classified so as to show the percentage having possible initial 'favourable' or 'unfavourable' characteristics as regards response to treatment (Zuelzer, 1964; Frei *et al.*, 1965). These are set out in Table VII where they are compared to two other representative series. It appears that the present patients did not possess any much more favourable initial status as a whole. The oldest case in the present report, however, was 9 years compared to several of over 10 years in the other series.

TABLE V

Survival among Cases Having Cyclic Maintenance Therapy and Vincristine plus Prednisolone Relapse Therapy

	No.	Median Survival	No. Expired
Cohort starting less than 12 months ago	8	Not yet reached	None
Cohort starting more than 12 months ago	22	> 82* weeks	9
Total maintained on both schedules	30	> 82* weeks	9

No. on cyclic maintenance schedule 31; No. withdrawn from relapse schedule 1†; No. adhering to both schedules 30.

* Calculated by the life-table method.

† Withdrawn after first relapse for ethical reasons (autism).

Discussion

These results illustrate that the major clinical problem in childhood leukaemia is no longer how to induce a remission but how best to maintain it. Between 80 and 90% of children with lymphoblastic leukaemia achieve a complete remission when treated by combinations of prednisone + mercaptopurine (Zuelzer, 1964), prednisone + vincristine (Selawry, 1965), prednisone + methotrexate (Hartmann, 1966) or prednisolone, vincristine + mercaptopurine (Freireich, 1966). It is of interest that combinations of these drugs appear to be 'synergistic' with respect to the percentage of remissions achieved, thus exceeding the calculated remission rate, assuming independent drug action from each agent (Frei *et al.*, 1965).

TABLE VI
Incidence of Relapses and Clinical or Haematological Toxicity

	Mercaptopurine	Methotrexate	Cyclophosphamide	Vincristine*	Total
Average actual dose, mg./kg. per day	2.2	0.12	3.5	—	—
No. of patient-weeks on drug	817	404	355	144	1720
No. of systemic leukaemic relapses	4	6	5	—	15
No. of CNS leukaemic relapses	3	2	1	—	6
<i>Clinical Toxicity</i>					
Buccal ulceration (severe in 1 case)	0	12	0	0	12
Alopecia (severe in 1 case)	0	0	5	5	10
Painful micturition (haemorrhagic cystitis in one case)	0	0	3	0	3
Ataxia—transient	0	0	0	1	1
Muscular 'stiffness'—transient	0	0	0	2	2
Jaundice—not recurrent	1	2	0	0	3
Diarrhoea	2	1	1	0	4
Rash	1	1	0	0	2
Total clinical toxicity	4	16	9	8	37
<i>Haematological Toxicity</i>					
Neutropenia, polymorphs 500–1000/c.mm. ..	15	7	15	—	37
Neutropenia, polymorphs less than 500/c.mm. ..	5	2	3	—	10
Thrombocytopenia, platelets 50,000–100,000/c.mm. ..	1	13	6	—	20
Thrombocytopenia, platelets less than 50,000/c.mm. ..	2	4	1	—	7
Total haematological toxicity	23	26	25	—	74
Total clinical + haematological toxicity	27	42	34	8	111
Expressed per 100 patient-weeks on drugs	3.3	10.4	9.6	5.5	6.5

* Used only at times of active leukaemia by weekly injections together with daily oral prednisolone.

Continuous therapy beyond the period of remission induction with drugs such as prednisone, vincristine, or the newly described drug rubidomycin, results in poorly sustained remissions, with a median duration of 79 days or less (Table VIIA). It is well recognized that mercaptopurine, methotrexate, or cyclophosphamide are more effective for the maintenance of such remissions. The conventional application of these principles is to maintain a child with leukaemia on daily oral mercaptopurine until a relapse occurs. The median duration of this first remission is no greater than 33 weeks (7½ months) and would not be any longer with cyclophosphamide, methotrexate, or methotrexate plus mercaptopurine, if these are given in the usual daily doses by the oral route (Table VIIIB). It has recently been shown, however, that somewhat higher doses of methotrexate given by twice-weekly intramuscular injection increase the length of remission to five times that obtained by daily oral doses (Selawry, 1965). In spite of this obvious advance, such a régime is not without practical difficulties, and clinical toxicity is frequent (Zoger *et al.*, 1966). Intermittent, rather than continuous, dosage does not appear to result in a similar advantage in the case of mercaptopurine (Chevalier and Glidewell, 1967) or cyclophosphamide (Pierce *et al.*, 1966).

Cyclic maintenance therapy was pioneered by Zuelzer and Flatz (1960), Zuelzer (1964), and Brubaker *et al.* (1963). Their results, as regards

TABLE VII
Case Material of Present and Other Series of Leukaemic Patients

	Present Series	Frei <i>et al.</i> (1965)	Zuelzer (1964)
Total number	31	151	205
Initial WBC	%	%	%
<20,000/c.mm.	68	57	74
>20,000/c.mm.	32	43	26
>30,000/c.mm.	9.5	3.3	—
Initial platelet count			
>50,000/c.mm.	20	34	—
<50,000/c.mm.	80	66	—
Age at diagnosis			
Under 4 years	64	52	52
4 years or over	36	48	48
Interval from first symptom to treatment			
4 weeks and over	39	48	—
Under 4 weeks	61	52	—

The first mentioned category in each classification is thought to carry a more favourable prognosis as regards remission.

TABLE VIII

Duration of Median First Established Remission with Continuous Maintenance Therapy

Drug	Route	No. of Patients	Drug Used for Initial Induction	Median Remission (wk.)	Authors
A. Drugs used for induction of remissions					
Prednisone	Oral	16	Steroids*	11	Hyman <i>et al.</i> (1959)
Vincristine	Intravenous	37	Vincristine†	9	Karon (1963)
Daunomycin	Intravenous	28	Daunomycin‡	7	Tan (1966)
B. Drugs used for maintenance of established remissions					
Mercaptopurine	Oral	28	Steroids	33	Freireich <i>et al.</i> (1963)
Mercaptopurine	Oral	43	Steroids + mercaptopurine	24**	Fernbach <i>et al.</i> (1966)
Mercaptopurine	Oral	71	Steroids + mercaptopurine	26	Saunders <i>et al.</i> (1967)
Cyclophosphamide	Oral	34	Steroids + cyclophosphamide	24**	Fernbach <i>et al.</i> (1966)
Methotrexate	Oral	27	Steroids + vincristine	14**	Selawry (1965)
Methotrexate bi-weekly	intramuscular	22	Steroids + vincristine	74**	Selawry (1965)
Mercaptopurine plus methotrexate	Oral	37	Steroids + vincristine	30	Frei <i>et al.</i> (1965)

* Usually prednisone, administered daily.

† Administered weekly.

‡ Daunomycin (or Rubidomycin), administered weekly for maintenance.

** 'Complete' plus 'partial' remissions by these authors' criteria.

remission maintenance and total survival, were impressive at that time (Table IXA). The theoretical rationale has been discussed by Frei *et al.* (1965) in the context of an alternating régime of mercaptopurine and methotrexate at monthly intervals. A true understanding of the cell-kinetic considerations involved is probably beyond present methods of assessment, but a simple concept might be that resistance to a particular antileukaemic drug is only likely to develop while the patient is exposed to that drug. This, for instance, would mean that after 3 cycles (15 months) of the present schedule the total exposure to mercaptopurine would be 30 weeks (by which time half the patients would be expected to be resistant to it), while the total exposure to methotrexate and cyclophosphamide would have been 18 weeks (by which time many would also be resistant to each drug). This concept would be consistent with the median first remission of 14–15 months found in the present series and the similar durations in other comparable cyclic schedules (Table IX(A)).

Whether this is the correct theoretical explanation or not, the practical achievement in terms of duration of the first remission is to exceed greatly that which could be obtained by the oral use of a single maintenance drug alone (Table IX and Table VIII(B)). It may well be that overt relapse does not occur on cyclic therapy until some degree of resistance to all three drugs has developed. This may explain the similarity in total survival, as opposed to length of first remission, found in some

trials when sequential use of the same drugs has been compared to their cyclic use (Colebatch, 1966; Krivit *et al.*, 1966).

The median survival figure of over 19 months in the present series may likewise be compared to the rather similar median survival of 17 months recently reported by Saunders, Kauder, and Mauer (1967) who used the same 5 drugs as in the present series; but sequentially rather than cyclically. Their first remission, however, was maintained with mercaptopurine alone, resulting in a median duration of only 6 months.

Clinical and haematological toxicity have not proved excessive with the drugs used for either induction or maintenance, as is shown in Table VI. Recovery from these complications was invariable. With regard to mercaptopurine and cyclophosphamide our experience has been similar to that reported by Fernbach *et al.* (1966). Only one patient at an early stage of our experience with cyclophosphamide had to be withdrawn because of the development of haemorrhagic cystitis, alopecia, and pancytopenia while on this drug. With regard to methotrexate, one early patient suffered severe ulceration of her lip but subsequent occurrences of buccal involvement in other patients have been trivial, often responding rapidly to local application of folic acid solution (3 mg./ml. 'leukovorin', Lederle).

The future developments in treatment of acute leukaemia probably lie in intensive régimes designed with the hope of complete eradication of the cellular

TABLE IX
Duration of Median First Remission with Intermittent and Multiple Drug Therapy

Maintenance Drugs	Duration on Each Drug	No. of Patients	Median Remission (wk.)	Author
A. Cyclic therapy following induction with steroids or steroids + mercaptopurine				
Steroids, methotrexate, mercaptopurine	6 wk.	111	60	Brubaker <i>et al.</i> (1963)
Mercaptopurine, methotrexate	3 mth.	175	48	Zuelzer (1964)
Mercaptopurine, methotrexate	4 wk.	34	30	Frei <i>et al.</i> (1965)
Mercaptopurine, methotrexate, vincristine*, steroids, cyclophosphamide	6 wk.	75	65 +	Krivit <i>et al.</i> (1966)
Vincristine*, mercaptopurine, cyclophosphamide, methotrexate	6-12 wk.	27	78 (S)	Colebatch (1966)
Mercaptopurine, methotrexate, cyclophosphamide ..	6-10 wk.	31	60-65	Present series
B. Intensive therapy following induction with steroids or steroids + vincristine				
1000 rad.I.M. + I.T. methotrexate				
Mercaptopurine, methylGAG*, cyclophosphamide vincristine*, steroids (in succession)		13	96	Mathé <i>et al.</i> (1966)
Mercaptopurine + I.M. methotrexate (continuously)		39	78 +	Jacquillat <i>et al.</i> (1967)
Steroids + Vincristine* + I.T. methotrexate (reinduction)		15	52 +	Djerassi <i>et al.</i> (1967)
Methotrexate*, cyclophosphamide* (intermittent monthly)		21	23 +	Hananian, Holland, and Sheeche (1965)
Methotrexate*, mercaptopurine*, cyclophosphamide*, BCNU* (in succession, then discontinued)		21	23 +	
Vincristine* + methotrexate* + mercaptopurine + steroids (combination (VAMP) then discontinued)		11	21	Henderson (1966)
Methotrexate*, mercaptopurine*, cyclophosphamide* (sequential x 2 (BIKE) then discontinued)		12	24	" "
Vincristine* + methotrexate* + mercaptopurine + steroids (combination (POMP) discontinued after 12 months)		17	60-70	" "

S = median survival rather than median remission; * = I.V. therapy; I.M. = intramuscular; I.T. = intrathecal; MethylGAG = methyl glyoxalbisdiguanylhydrazone; BCNU = 1,3-Bis (2-chloroethyl) -1-Nitrosourea (NSC-409962). Remaining abbreviations as in Table II.

phase of leukaemia by means of multiple anti-leukaemic agents used in optimum modes of administration. If such a form of treatment is developed the present object of mere remission maintenance would become superseded. This objective does not yet appear to have been attained except in the case of perhaps two or three patients who have remained in unmaintained remission for over 800 or 1000 days following intensive combination therapy (Freireich, 1966). The majority of patients submitted to régimes of intensive therapy do not yet show improved duration of their first (unmaintained) remission when compared to the present and other series of patients maintained on continuous therapy (Table IX). Nor does cyclic therapy necessarily prejudice a patient against the possibility of their becoming a 'long-term survivor' (over 5 years). Among 42 such long-term survivors collected by Burchenal and Murphy (1965) who were still living and without active leukaemia, 8 (19%) had been treated by cyclic therapy.

Summary

The cyclic use of oral mercaptopurine, metho-

trexate, and cyclophosphamide for the maintenance of remissions in 31 children with acute leukaemia has been investigated. The median duration of the first remission was 60 to 65 weeks (14 to 15 months). Relapses were treated with prednisolone and weekly intravenous vincristine, second remissions being obtained in 12 out of 13 patients. Thereafter, cyclic therapy was reinstituted, resulting in a median survival from diagnosis of over 82 weeks (19 months).

The level of clinical toxicity was entirely acceptable, the children being free from the effects of hypercorticism for the greater part of this period and leading normal lives at home and at school.

We should like to acknowledge the co-operation of Professor J. H. Hutchison and Drs. R. A. Shanks, D. H. Wallace, G. C. Arneil, J. O. Craig, E. N. Coleman, W. Hamilton, and J. A. Inall under whose care these patients were admitted. The cytochemical tests were performed by Mr. F. G. Jewell, F.I.M.L.T., and the continuous graphical representation of haematological and other data by Mrs. M. Shanks.

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