Europ. J. Cancer Vol. 15, pp. 499–507.

0014–2964/79/0401–0499 $02.00/0

Lack of Vincristine–Cyclophosphamide Potentiation in Different Experimental Tumour Lines*

J. H. MULDER,†‡§ P. LELIEVELD† and L. M. van PUTTEN†

†*Radiobiological Institute, Rijswijk, The Netherlands*
‡*Department of Internal Medicine, Rotterdam Radiotherapeutic Institute, Rotterdam, The Netherlands*

Abstract—*Combination chemotherapy with vincristine and cyclophosphamide was investigated in the L1210 leukaemia, the Lewis lung carcinoma, the mouse C22LR osteosarcoma and in two experimental colon tumour lines. Therapeutic synergism could not be demonstrated in any of the tumour cell lines. In the L1210 experiments, simultaneous treatment resulted in therapeutic antagonism. This less than additive effect could be avoided by using scheduling with a time interval of at least 12 hr. The sequence in which the two drugs were administered did not influence the results. Studies on haemopoietic stem cells showed an additive effect in all of the schedules investigated. It seems preferable to treat L1210 leukaemia and possibly other rapidly proliferating neoplastic lines by a sequential application of vincristine and cyclophosphamide, not because of any potentiating effect but because drug antagonism can be circumvented in this way.*

INTRODUCTION

After vincristine (VCR) application, cycling cells are arrested in the metaphase of the cell cycle and therefore a partially synchronized cell population can be obtained. Klein and co-workers have used, among other drugs, VCR as a synchronizing agent in the treatment of mice inoculated with the Ehrlich ascites tumour [1]. In their clinically used so-called synchronization treatment schedule. VCR administration is followed within a short time interval by cyclophosphamide (Cyclo). Although this approach may eventually lead to an improvement in therapeutic results [2], the explanation of the underlying mechanism for their observations has been questioned [3–5].

Any drug interaction resulting in therapeutic synergism or antagonism should be investigated in a variety of tumour cell lines because extrapolation of promising results found in one rapidly proliferating experimental tumour system to human solid tumours can be misleading. In addition, investigations on drug interaction must take into account the effect of treatment on critical normal tissues [6, 7].

The work in this paper is concerned with the question of selective potentiation. The effect of sequential treatment with VCR and Cyclo was investigated in the L1210 leukaemia and in various experimental solid tumour lines. The survival of haemopoietic bone marrow stem cells was chosen as a parameter of the effect of treatment on critical normal tissue.

MATERIALS AND METHODS

Materials

For our L1210 leukaemia investigations, (BALB/c × DBA 2)F1 hybrids (hereafter called CD2 F1) were used. The Lewis lung carcinoma was maintained by s.c. serial transplantation in C57BL/Ka mice and transplanted into (C57BL/Rij × CBA/Rij)F1 mice (hereafter called BCBA F1) for experiments. The C22LR osteosarcoma studies were per-

Accepted 5 September 1978.
*Supported by the "Koningin Wilhelmina Fonds" of the National Cancer League.
§Correspondence and reprint requests: J. H. Mulder, Radiobiological Institute TNO, 151 Lange Kleiweg, 2288 GJ Rijswijk, The Netherlands.

formed in BCBA F1 mice [6]. Two colon tumour lines were transplanted into CD2 F1 mice, details of which have been recently described [8]. Vincristine sulphate was obtained from Eli Lilly & Co. Cyclophosphamide was kindly supplied by Asta-Werke, Brackwede, Germany.

Methods

From an exponentially growing L1210 *in vitro* culture, 10^5 cells were injected i.p. into CD2F1 mice. The endpoint of treatment was the mean survival time (MST). The effect of treatment on L1210 leukaemia was also investigated by a combined *in vivo* and *in vitro* technique [6]. In treated leukaemic mice, the number of surviving L1210 cells was determined by cloning *in vitro*. The surviving fraction was calculated as the ratio of the number of donor leukaemic colony forming units (CFU) per femur of a treated group to that of a control group.

The spleen colony technique of Till and McCulloch was used to assess the effect of treatment on normal resting haemopoietic stem cells [9]. We also investigated the effect of treatment on recruited, rapidly proliferating normal bone marrow stem cells [10]. The survival of resting and proliferating CFU was calculated as the ratio of the mean number of CFU per donor femur and per spleen, respectively, of treated to that of simultaneously assayed control mice.

A single-cell suspension was made from the solid tumours. Cells were inoculated s.c. or i.m. into mice and the volume of the tumours was measured twice weekly. The endpoint of treatment was growth delay in days: the displacement in time between the growth curve of the control group and the growth curve of the tumours recurring after treatment. If no original tumour volume was known (e.g., for tumour inocula exposed to treatment before a tumour was palpable), the delay in comparison with the control group to reach an arbitrary volume between 400 and 800 mm^3 was estimated.

For the assessment of the effect of treatment on artificially induced lung metastases, Lewis lung tumour cells were injected i.v. into one of the tail veins. Two weeks later, the number of macroscopic tumour nodules on the lung surface was counted.

Treatment with VCR and Cyclo was either simultaneously or sequentially: VCR before (VCR ↳ Cyclo) or after Cyclo administration (Cyclo ↳ VCR). The length of the time interval in hours between the two drug administrations is indicated in each particular schedule. If the two agents were given sequentially in the L1210 experiments, the days of treatment varied, e.g., VCR on day 2 and Cyclo on day 3 or Cyclo on day 2 and VCR on day 3. In the solid tumour investigations, the day of Cyclo administration was kept constant. The time of the last drug administration and of assay were kept constant in the stem cell experiments. The Student's *t*-test was used for statistical evaluation.

RESULTS

The effect of sequential treatment on L1210 is shown in Tables 1, 2 and 3. The results of the *in vivo–in vitro* experiments in Table 1 are expressed in surviving fractions. Note that the observed surviving fractions for the simultaneous treatment were at least two times greater than the calculated surviving

Table 1. Surviving fractions of L1210 cells treated with vincristine and cyclophosphamide in various schedules

	Surviving fraction	
Treatment schedule	Exp. I	Exp. II
VCR 0.5 mg/kg s.c.	0.47	0.31
Cyclo 30 mg/kg s.c.	0.13	0.16
VCR $\xrightarrow{24\,hr}$ Cyclo	0.08	0.09
VCR + Cyclo	0.15	0.12
Cyclo $\xrightarrow{24\,hr}$ VCR	0.05	0.07
Expected effect of combination	0.06	0.05

Antagonism is evident when the observed fraction is greater than the expected surviving fraction calculated by multiplying the individual surviving fractions.

Table 2. The influence of combination chemotherapy with vincristine and cyclophosphamide on the life-span of leukaemic mice

Treatment schedule	Mean survival time ± S.E. Exp. No. C133	C137	C145
Control	9.2 ± 0.1	10.0 ± 0.6	10.6 ± 0.5
VCR	10.3 ± 0.3	10.2 ± 0.3	11.8 ± 0.2
Cyclo	13.6 ± 0.2	16.2 ± 1.0	17.6 ± 1.4
VCR $\xrightarrow{24\,hr}$ Cyclo	15.2 ± 0.5*	16.1 ± 0.2	15.5 ± 1.1
VCR $\xrightarrow{12\,hr}$ Cyclo	15.8 ± 0.4*	17.2 ± 0.3	19.1 ± 0.5
VCR + Cyclo	13.0 ± 0*	15.1 ± 0.3	14.9 ± 0.3*
Cyclo $\xrightarrow{12\,hr}$ VCR	n.d.	n.d.	19.3 ± 0.8
Cyclo $\xrightarrow{24\,hr}$ VCR	16.7 ± 1.1*	17.3 ± 1.1	18.4 ± 0.5
Expected effect of combination	13.8 ± 0.3	16.3 ± 0.9	18.2 ± 1.0

One hundred thousand L1210 cells were injected i.p. into groups of 10 CD2F1 mice (single drug treatment in groups of 5 mice). Treatment was given on days 2 and 3 or on days 3 and 4. A dose of 0.075 mg/kg VCR was given i.p. in combination with a dose of Cyclo which varied in the different experiments between 150 and 200 mg/kg i.p. Drug induced early toxic death was not observed. The expected effect of the combination was calculated from the mean survival times of the VCR- and the Cyclo-treated animals. An asterisk (*) indicates a significant difference between observed and expected effect of the combination, n.d. indicates the investigation was not done.

Table 3. The influence of drug scheduling with vincristine and cyclophosphamide on the life-span of leukaemic mice

Treatment schedule	Mean survival time ± S.E. Exp. No. C141	C142
VCR $\xrightarrow{24\,hr}$ Cyclo	19.1 ± 0.8*	22.2 ± 1.0*
VCR $\xrightarrow{12\,hr}$ Cyclo	18.6 ± 0.7	22.0 ± 1.6*
VCR + Cyclo	16.7 ± 0.5	14.6 ± 0.7
Cyclo $\xrightarrow{24\,hr}$ VCR	17.4 ± 1.4	19.7 ± 0.6*

Groups of 10 L1210 leukaemic mice were treated according to details given in Table 2. The effect of single drug treatment was not investigated. An asterisk (*) indicates a significant difference in the results of sequential treatment as compared to simultaneous administration of the drugs.

fractions in both experiments. This less than additive effect of treatment was also found in the L1210 survival experiments. In Table 2, the expected MST of the combination is calculated from the data of the single drug treatments.

The observed MST was always lower than the expected MST, indicating drug antagonism. A more than additive effect was sometimes observed when the two drugs were given in sequence. Table 3 shows the effect of drug scheduling: sequential treatment with an interval of at least 12 hr was more effective than simultaneous treatment. The sequence in which the drugs are given seems not to influence the therapeutic effect of treatment.

The results of sequential treatment in the Lewis lung carcinoma are shown in Table 4. The animals died of lung metastases and therefore the survival time could be used as an endpoint of treatment. However, the presence of the primary tumour in the preterminal stage caused great discomfort to the mice and instead of repeating this type of experiment the animals were sacrificed at day 22 after inoculation. Table 5 shows the effect of treatment on the volume of the primary tumour and on the number of lung metastases. The results of a lung colony assay are presented in Table 6. From Tables 4 to 6, we conclude that there are no significant differences in effects of treatment between the different drug schedules. This lack of schedule

Table 4. The effect of sequential treatment with vincristine and cyclophosphamide in Lewis lung carcinoma

Treatment schedule	Growth delay (days)	Median survival time and range (days)
Control	0±0.1	32 (27–43)
VCR 0.05 mg/kg i.p.	1.8±0.2	33 (33–40)
Cyclo 50 mg/kg i.p.	2.3±0.2	34 (28–41)
VCR $\xrightarrow{24\,hr}$ Cyclo	3.4±0.2	38 (35–39)
VCR $\xrightarrow{16\,hr}$ Cyclo	4.5±0.5	37 (30–41)
VCR + Cyclo	3.2±0.4	36 (36–44)
Cyclo $\xrightarrow{24\,hr}$ VCR	3.2±0.4	38 (32–40)

Groups of 5 C57BL/Ka mice were inoculated i.m. with 10^6 Lewis lung tumour cells on day 0. Cyclo treatment was given on day 8. The mean growth delay and standard error is given. The differences between the results of the various treatment schedules are not statistically significant.

Table 5. The effect of sequential treatment with vincristine and cyclophosphamide in Lewis lung carcinoma

Treatment schedule	Growth delay (days)	Number of lung metastases per mouse
Control	0±0.2	33.9±3.4
VCR 1 mg/kg i.p.	1.3±0.4	26.4±2.7
Cyclo 100 mg/kg i.p.	3.5±0.3	10.7±2.3
VCR $\xrightarrow{24\,hr}$ Cyclo	3.5±0.3	6.4±1.6
VCR $\xrightarrow{16\,hr}$ Cyclo	2.8±0.5	9.5±2.6
VCR + Cyclo	4.3±0.3	7.7±1.1

Groups of 10 BCBA F1 mice were inoculated i.m. with 10^6 Lewis lung tumour cells on day 0. Cyclo was given on day 8. Lung metastases were counted on day 22. The means with standard errors are given. Except for the 16 hr time interval, the differences in number of lung metastases or growth delay between simultaneous and sequential treatment are not statistically significant.

Table 6. The effect of sequential treatment with vincristine and cyclophosphamide on artificially induced lung colonies of the Lewis lung carcinoma

Treatment schedule	Number of lung colonies per mouse Mean±S.E.
Control	251±16
VCR 0.05 mg/kg i.p.	262±11
Cyclo 50 mg/kg i.p.	194±11
VCR $\xrightarrow{24\,hr}$ Cyclo	154±28
VCR $\xrightarrow{16\,hr}$ Cyclo	164±30
VCR + Cyclo	197+32

Lewis lung tumour cells, 0.6 10^5 in 0.5 ml Hanks' balanced salt solution, mixed with 10^6 microspheres were injected into one of the tail veins of C57BL/Ka mice. Groups of 5 mice were treated with Cyclo on day 3. Lung colonies were counted on day 14. There are no statistically significant differences between the effects of the different combination treatment schedules.

dependency is also observed in the C22LR osteosarcoma, as shown in Table 7. Combination chemotherapy in which either a low or a high dosage of VCR is administered never resulted in a potentiation of the effect of Cyclo treatment. The results of the colon 26 and colon 51 investigations shown in Table 8 were comparable with the Lewis lung and osteosarcoma experiments: a VCR-induced Cyclo potentiation could not be demonstrated.

Bone marrow stem cell data are presented in Table 9. The results of simultaneous treatment were always additive and no effect of drug scheduling was observed.

Table 7. The effect of sequential treatment with vincristine and cyclophosphamide in C22LR osteosarcoma

Treatment schedule	Mean growth delay and standard errors (days) Exp. I	Exp. II	Exp. III
Control	0±0.5	0±0.3	0±0.3
VCR	−1.0±0.4	0.6±0.4	−0.6±0.4
Cyclo	5.2±0.7	4.3±0.4	5.0±0.8
VCR $\xrightarrow{24\,hr}$ Cyclo	4.4±0.5	5.9±0.4	3.7±0.4
VCR $\xrightarrow{16\,hr}$ Cyclo	n.d.	5.6±0.2	4.0±0.5
VCR + Cyclo	6.8±0.6	5.0±0.3	4.7±0.5
Cyclo $\xrightarrow{24\,hr}$ VCR	5.4±0.4	4.2±0.3	5.1±0.4

One million osteosarcoma cells were injected s.c. bilaterally into groups of 5 BCBA F1 mice on day 0. In experiment I, the dosages were Cyclo 100 mg/kg i.p. on day 4 and VCR 0.05 mg/kg i.p. In experiment II, the dosages were Cyclo 50 mg/kg i.p. on day 4 and VCR 1 mg/kg i.p. In experiment III, the dosages were Cyclo 50 mg/kg i.p. on day 11 and VCR 1 mg/kg i.p.; n.d. indicates the investigation was not done. The differences between the results of the various treatment schedules are not statistically significant.

Table 8. The effect of sequential treatment with vincristine and cyclophosphamide in two experimental colon tumour lines

Treatment schedule	Mean growth delay and standard errors (days) Colon 26		Colon 51	
Control	0±0.6		0±0.3	
VCR	0.4±1.1		1.1±0.4	
Cyclo	5.1±1.1		14.1±0.2	
VCR $\xrightarrow{24\,hr}$ Cyclo	3.1±0.8	n.s.	13.1±0.7	n.s.
VCR + Cyclo	5.2±0.5		14.4±0.9	
Cyclo $\xrightarrow{24\,hr}$ VCR	2.3±0.8	$P<0.05$	14.1±0.7	n.s.

Approximately one million tumour cells were inoculated s.c. into CD2 mice. In the colon 26 experiment, treatment was given on days 10 and 11. The dosages were VCR 1 mg/kg i.p. and Cyclo 100 mg/kg i.p. In the colon 51 experiment, the days of treatment were 8 and 9. The dosages were VCR 1 mg/kg i.p. and Cyclo 200 mg/kg i.p.

Table 9. Surviving fractions of normal haemopoietic bone marrow stem cells per femur and repopulating spleen in comparison with untreated control mice

Treatment	Resting stem cells Exp. I	Exp. II	Exp. III	Proliferating stem cells Exp. I	Exp. II
VCR 0.5 mg/kg i.p.	1.01	0.97	n.d.	0.77	0.59
Cyclo 100 mg/kg i.p.	0.23	0.27	n.d.	0.09	0.17
VCR $\xrightarrow{24\,hr}$ Cyclo	0.11	0.38	0.28	0.06	0.05
VCR + Cyclo	0.13	0.28	0.40	0.06	0.09
Cyclo $\xrightarrow{24\,hr}$ VCR	—	0.22	0.28	0.04	0.07
Calculated fraction	0.23	0.26	—	0.07	0.10

DISCUSSION

We explored the effect of VCR–Cyclo treatment on rapidly and slowly proliferating tumour cell lines and on normal bone marrow. Two sets of results will be analyzed. One concerns data of the effect of combination chemotherapy with VCR and Cyclo when given concomittantly. The question to be answered is whether a more or less than expected effect was observed. Secondly, we will discuss the effect of drug scheduling. The point to be made here is whether giving VCR before Cyclo resulted in a potentiation of the effect of Cyclo treatment.

Simultaneous treatment

The data of the L1210 cell survival experiments as well as of the leukaemic mice survival studies showed a less than additive effect when VCR and Cyclo were administered simultaneously. Results of the bone marrow investigations showed no discrepancy between observed cell survival and calculated cell survival. Therefore, the effect of VCR and Cyclo treatment on haemopoietic stem cells is additive. As a consequence of this differential effect, the therapeutic index (ratio of effect on tumour and effect on normal tissue) will decrease. Simultaneous administration of VCR and Cyclo in this experimental system is disadvantageous. To draw this conclusion from the data of the solid tumour investigations seems less warranted. Never was a significant difference from what should be expected from single drug data observed.

Sequential treatment

In only one of the two types of L1210 experiments performed, (C133 in Table 2). was a significant increase in efficacy demonstrated by drug scheduling. In each L1210 experiment, the least effective schedule was found when the two drugs were given simultaneously. No sign of drug potentiation was observed in any of the solid tumour investigations. No effect of VCR–Cyclo scheduling was observed in the bone marrow experiments.

Razek [11] studied the effect of VCR and Cyclo interaction in L1210 leukaemia, AKR lymphoma and in resting bone marrow stem cells. Our results are similar with his findings, except that we did not repeatedly observe a statistically significant synergistic effect in the L1210 leukaemia when VCR was given before or after Cyclo treatment. Klein [12, 13] investigated a treatment schedule in which VCR (with or without hydroxyurea) preceded Cyclo administration. Based on ^{3}H-TdR and fluorometric studies, he suggested a tumour cell synchronizing effect of VCR. A simultaneous application of VCR and Cyclo caused no significant necrobiotic effect on the Ehrlich ascites tumour. This observation was in marked contrast with Cyclo treatment alone and with sequentially administering VCR followed by Cyclo. The survival rate of Ehrlich ascites tumour bearing animals was also better when Cyclo was given during the S phase of the cell cycle of the partially synchronized cells than when the drugs were given simultaneously. Klein's investigations show schedule dependency and a marked antagonism of simultaneous treatment can sometimes be noted in his text figures. Suggestions on therapeutic synergism as a result of drug scheduling can not be made. For this, more extensive data on the effect of single drug treatment and on the various combinations are needed. From experimental data on the solid form of the Ehrlich tumour, VCR→Cyclo seems to be more effective than VCR + Cyclo treatment. Selective drug potentiation, caused by a VCR induced tumour cell synchronization, can more convincingly be claimed when the opposite sequence Cyclo →VCR proves to be less effective than the so-called synchronizing schedule VCR→ Cyclo. Unfortunately, Cyclo→VCR investigations have not been performed. In L1210 experiments, Pouillart [14] showed as we did, the effect of sequential administration (VCR →Cyclo or Cyclo→VCR) to be more effective than simultaneously given treatment. In another leukaemia model, Zeller [15] described VCR→Cyclo synergism. However, to draw a conclusion on synergism from the presented data seems rather optimistic. In particular when the experimental data is used to suggest clinical applicability of the findings. Two papers have been published recently on experimental solid tumours treated with VCR and Cyclo. Schiffer suggests a cell kinetic relationship between the effect of VCR administration and early Cyclo treatment or late Cyclo treatment on C3H/He spontaneous mammary tumours. According to Schiffer, to increase the therapeutic effect, the time interval between VCR and Cyclo administration should be at least 2 days instead of 24 hr [16, 17]. More tumour volume response data is needed before this statement can be considered generally valid. Another report on VCR–Cyclo interaction was that of

Table 10. Vincristine–cyclophosphamide combination chemotherapy in experimental tumour lines

Author (ref.)	Tumour line	Author's conclusion	Comment (see text)
Razek [11]	L1210 and AKR lymphoma	Schedule dependent synergism	Synergism based on spleen colony assay. VCR + Cyclo less effective than VCR→ Cyclo or Cyclo→VCR
Klein [1, 2, 12]	Ehrlich ascites tumour	Potentiation as a result of synchronization	Potentiation not proven. VCR + Cyclo less effective than VCR→Cyclo. Cyclo→VCR not investigated.
Pouillart [14]	L1210	More than additive effect after sequential therapy.	Conclusions based on survival studies. VCR + Cyclo less effective than both sequences
Zeller [15]	L5222 leukaemia	VCR→Cyclo synergism	Results based on survival data. VCR + Cyclo less effective than VCR→Cyclo. No information on Cyclo→VCR
Schiffer [16, 17]	C3H/He mammary tumour	Schedule dependency based on tumour cytokinetics	Conclusions on synergism difficult to make. VCR + Cyclo seems less effective than VCR→Cyclo
Stephens [18]	B16 melanoma	No schedule dependency and lack of potentiation	Cell survival assay
Mulder [this paper]	L1210, Lewis lung tumour osteosarcoma and colon carcinoma	Schedule dependent antagonism in L1210, no potentiation in solid tumour lines	Survival and growth delay studies

Stephens [18]. He was unable to demonstrate any VCR→Cyclo potentiation in the B16 melanoma. From our data and those summarized in Table 10, it seems preferable to treat L1210 and possibly other rapidly proliferating neoplastic tissues as well by a sequential application of VCR and Cyclo in order to avoid drug antagonism. A VCR induced potentiation of the Cyclo effect should not generally be anticipated.

The possible causes for the observed therapeutic antagonism are complex. When a subpopulation of tumour cells is "twice killed" by two different agents, a less than additive effect should be expected. L1210, however, is relatively insensitive to VCR treatment and the observed VCR and Cyclo antagonism cannot be explained therefore according to this "over kill" theory. There is possibly some interference with the mode of action of both drugs, although VCR and Cyclo are effective in different phases of the cell style. If this was indeed the case, we should expect drug antagonism of VCR and Cyclo treatment not only in L1210 but also in rapidly proliferating haemopoietic stem cells.

The clinical relevance of our findings is self-evident. For the clinical management of solid tumours, there seems to be no support to suggest sequential treatment with VCR followed by Cyclo, unless more convincing experimental data on solid tumours can be presented. In combination chemotherapy of rapidly proliferating tumours with VCR and Cyclo, it may be advantageous to split the administration of the two drugs. Klein and co-workers have organized a randomized clinical trial in Hodgkin's disease and non-Hodgkin lymphoma in which the effect of COPP therapy (VCR, Cyclo, procarbazine and prednisone) will be compared with the effect of their synchronization schedule, VCR followed by Cyclo administration [2]. Since the two treatment arms of the trial are not fully comparable, the significance of cell kinetic manipulation will be difficult to evaluate. In an EORTC non-Hodgkin trial, simultaneously administered Cyclo + VCR + prednisone maintenance therapy is compared with a sequential VCR→Cyclo + prednisone schedule [19]. At the time of writing of that particular protocol, this seemed the correct approach: testing an attractive hypothesis in a clinical trial. If ultimately no significant differences in therapeutic results can be demonstrated in the EORTC trial we should, at least for the time being, forget about VCR–Cyclo potentiation.

Acknowledgements—Grateful appreciation is expressed to Marjolein Huurman, Wil Akkerman and Hetty Jense.

REFERENCES

1. H. O. Klein, K. J. Lennartz, W. Habicht, M. Eder and R. Gross, Synchronization von Ehrlich-ascites-Tumorzellen und ihre Bedeutung bei der Anwendung eines alkylierenden Cytostaticum. *Klin. Wochenschr.* **48,** 1001 (1970).
2. H. O. Klein. N. Brock, H. Burkert, V. Diehl, U. Feldmann, R. Fischer, A. Georgii, D. Gerecke, R. Gross, H. Hoefer-Janker, K. J. Lennartz, W. Scheef, B. Schneider and J. Schnitker, Interim data of a randomized trial in Hodgkin's disease and non-Hodgkin's lymphoma. A comparison between the COPP and the so-called synchronization therapy. Submitted for publication.
3. M. Volm, L. Krieg, J. Mattern and K. Wayss, Effect of synchronization on chemotherapy of solid transplanted tumours. *Europ. J. Cancer* **13,** 1099 (1977).
4. W. Jellinghaus, B. Schultz and W. Maurer, The effect of vincristine on mouse jejunal crypt cells of differing cell age: double labelling autoradiographic studies using 3H- and 14C-TdR. *Cell Tiss. Kinet.* **10,** 147 (1977).
5. L. M. van Putten, H. J. Keizer and J. H. Mulder, Synchronization in tumour chemotherapy. *Europ. J. Cancer* **12,** 79 (1976).
6. J. H. Mulder, T. Smink and L. M. van Putten, Schedule dependent effectiveness of CCNU and 5-fluorouracil in experimental chemotherapy. *Europ. J. Cancer* **13,** 1123 (1977).
7. J. H. Mulder, M. B. Edelstein, P. Lelieveld and L. M. van Putten, Synergism and schedule dependent cytotoxicity of cyclophosphamide and CCNU in experimental cancer chemotherapy. *Europ. J. Cancer* **14,** 537 (1978).
8. P. J. van Kranenburg-Voogd, H. J. Keizer and L. M. van Putten, Experimental chemotherapy of transplantable mouse colon tumours. Submitted for publication.

9. J. E. Till and E. A. McCulloch, A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* **14,** 213 (1961).
10. P. Lelieveld and L. M. van Putten, Biological activity of two derivatives and six possible metabolites of cyclophosphamide (NSC-26271). *Cancer Treat. Rep.* **60,** 373 (1976).
11. A. Razek, T. Vietti and F. Valeriote, Optimum time sequence for administration of vincristine and cyclophosphamide *in vivo*. *Cancer Res.* **34,** 1857 (1974).
12. H. O. Klein and K. J. Lennartz, Chemotherapy after synchronization of tumour cells. *Semin. Hematol.* **11,** 203 (1974).
13. H. O. Klein, D. Adler, M. Doering, P. J. Klein and K. J. Lennartz, Investigations on pharmacologic induction of partial synchronization of tumour cell proliferation: its relevance for cytostatic therapy. *Cancer Treat. Rep.* **60,** 1959 (1976).
14. P. Pouillart, T. Hoan Thy Huong, E. Brugerie and J. Lheritier, Sequential administration of two oncostatic drugs: study of modalities for pharmacodynamic potentiation. *Biomedicine* **21,** 471 (1974).
15. W. J. Zeller, S. Ivankovic and D. Schmähl, Chemotherapy of the transplantable acute leukemia L5222 in rats. *Cancer Res.* **35,** 1168 (1975).
16. L. M. Schiffer, P. G. Braunschweiger and L. Poulakos, Rapid methods for utilizing cell kinetics for treatment in the C3H/He spontaneous mammary tumour: effects of vincristine. *Cancer Treat. Rep.* **60,** 1913 (1976).
17. P. G. Braunschweiger and L. M. Schiffer, Cell kinetics after vincristine treatment of C3H/He spontaneous mammary tumors: implications for therapy. *J. nat. Cancer Inst.* **60,** 1043 (1978).
18. T. C. Stephens, J. H. Peacock and G. G. Steel, Cell survival in B16 melanoma after treatment with combinations of cytostatic agents: lack of potentiation. *Brit. J. Cancer* **36,** 84 (1977).
19. Protocol 20751, Clinical trial using radiotherapy and chemotherapy in non-Hodgkin lymphoma (LR2) of the EORTC Radiochemotherapy Cooperative Group. In *Current Research of the EORTC Cooperative Groups, Project Groups, Clubs, Task Forces and Working Parties*. M. Staquet (1978).