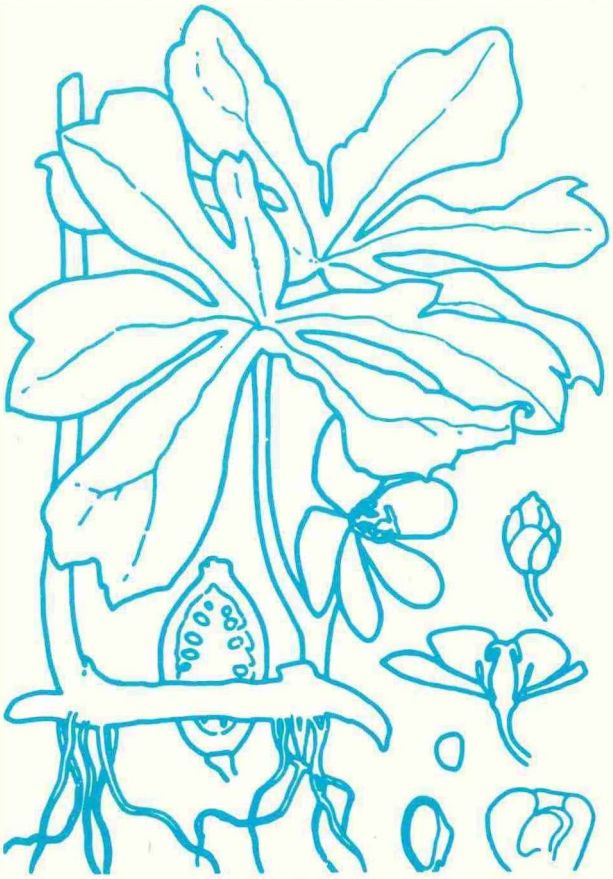
NEW APPROACHES IN SMALL CELL LUNG CANCER



P. E. POSTMUS

RIJKSUNIVERSITEIT TE GRONINGEN

NEW APPROACHES IN SMALL CELL LUNG CANCER

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op woensdag 13 november 1985
des namiddags te 2.45 uur precies

door

PIETER EDSGE POSTMUS

geboren te Groningen

krips repro meppel

STELLINGEN

I

Met de nu beschikbare cytostatica is hoge-dosis chemotherapie voor het kleincellig longcarcinoom misschien zinvol na het bereiken van een complete remissie.

II

Het effekt van hoge-dosis VP16-213 moet worden geëvalueerd in onbehandelde kleincellig longcarcinoom patiënten met hersenmetastasen.

III

Ondanks selectieve darm decontaminatie zijn infekties het belangrijkste probleem bij hoge-dosis chemotherapie.

IV

Na induktie chemotherapie is onderhoudstherapie voor het kleincellig longcarcinoom niet zinvol.

V

Bij inoperabele niet-kleincellige longtumoren is afwachten even goed als iedere andere electieve behandeling.

VI

Bij fase III studies is het vragen van "informed consent" aan de patiënt vóór randomisatie onethisch.

VII

Het is verantwoord bij stadium I en II longcarcinoom te volstaan met een segmentresectie.

VIII

Kleincellig longcarcinoom cellen bevatten keratines passend bij een epitheliale histogenese. De expressie van neurofilamenten in deze tumor moet beschouwd worden als ectopisch.

(V.P.Lehto e.a. Am. J. Pathol. 1983; 110: 113.)

IX

Benigne endobronchiale tumoren komen in aanmerking voor Nd-YAG-laser therapie.

X

Bevolkingsonderzoek voor longkanker is niet zinvol.

XI

Lobus vena azygos kan autosomaal dominant overerven.

XII

Behalve accijns dient op tabaksprodukten WAO-premie te worden geheven.

XIII

Het kinderrijke gezin van nu is de hoeksteen voor de vergrijzende samenleving samenleving van morgen.

XIV

Alle wetenschappelijke (hoofd)medewerkers zijn gelijk, maar sommigen zijn meer gelijk dan anderen.

STELLINGEN

behorende bij het proefschrift van

P.E. POSTMUS

NEW APPROACHES IN SMALL CELL LUNG CANCER

Groningen, 13 november 1985

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Referenten:

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aan ineke
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CONTENTS

VOORWOORD

Dit proefschrift is het resultaat van een prettige en intensieve samenwerking tussen de afdeling Longziekten (Hoofd: Prof.Dr.H.J.Sluiter) en de Werkgroep Interne Oncologie (Dr.D.Th.Sleijfer, Dr.N.H.Mulder) van de afdeling Inwendige Geneeskunde (Hoofd:Prof.Dr.E.Mandema) van het Academisch Ziekenhuis te Groningen.

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Het past mij om stil te staan bij de herinnering aan de in dit proefschrift beschreven patiënten. De vaak diepgaande gesprekken met hen zijn voor mij altijd een stimulans geweest om verder te gaan.

Ineke's steun, vermogen tot relativeren en kritiek waren en zijn voor mij onmisbaar.

INTRODUCTION

During the last decades, lung cancer has become the most important cause of death due to cancer in males in the technically developed world. The incidence in females is still increasing and within one or two decades it can be expected to be the most common cancer in females too (1).
In the Netherlands, central registration of cancer incidence is still not available; registration of causes of death, however, reveals a mortality of lung cancer of about 8000 a year (2).
Ninety-eight percent of the malignant lung tumors consists of the five major histologically different types (3): squamous cell lung cancer, large cell lung cancer, adenocarcinoma, adeno-squamous cell carcinoma and small cell lung cancer (SCLC). The origin of these tumors is unclear. SCLC-cells have animo precursor uptake and decarboxylation properties and were supposed to be related to the neuroendocrine cells that are derived from the neural crest. The other tumor types -commonly described as non-SCLC- were supposed to be related to the other cells of the bronchial mucosa and therefore of endodermal origin. Recent observations of changes in morphology in SCLC cell lines to non-SCLC, as well as the frequently found admixture in biopsies of SCLC tumors with morphologically non-SCLC cells, however, support the common stemcell hypothesis (4,5). SCLC accounts for 20-25% of all cases and differs from the other forms in prognosis, clinical presentation, biological behaviour, and available treatment. The prognosis of SCLC, if untreated, is unfavourable; the majority of the patients die within 3 months from the time of diagnosis (6). The clinical presentation of SCLC is frequently associated with signs of ectopic hormone production as for instance Cushing's syndrome, resulting from ACTH production, or inappropriate antidiuretic hormone secretion. Lymphogenic and hematogenic spread is present in almost all patients. Therefore these patients are only rarely eligible for curative surgery.
Despite this, the staging of SCLC is important for planning and evaluating therapy (7). The TNM classification, used for other lung tumors, is not useful for SCLC and therefore a new classification has been introduced: limited disease (LD) for patients with disease limited to one hemithorax plus supraclavicular nodes, and extensive disease (ED) for patients with signs of tumor outside this area (8).

In contrast to non-SCLC, treatment strategies directed only to the area of the primary, i.e. radiotherapy or surgery, have not been successful. Even initial dramatic responses to radiotherapy have only a minor effect on survival as a result of progression of the tumor elsewhere (9).

The prognosis, however, changed after the introduction of systemic treatment. In the late sixties, introduction of cyclophosphamide resulted in an improvement of survival (6). Since that time chemotherapy for SCLC has been under continued investigation. Combining two active drugs proved to be more effective than single agent therapy (10); subsequently three drug regimens resulted in a further improvement of response rate and survival (11). An other important step forward was that larger doses of cytostatic drugs in a combination regimen could be given (12); an increased knowledge of the handling of side effects permitted the acceptance of a higher degree of toxicity.

Since that time a definite, be it a small number of long-term disease-free survivors has been reported (13), leading to speculations about the possibility of cure in a much larger group of patients (14) within a few years. The results of therapy nowadays, however, are still on the same plateau as 5 years ago, despite new approaches. The role of radiotherapy in combination with chemotherapy in LD patients is probably very limited, it has no or only minor effects on survival but induces increased toxicity (15). Therefore, local radiotherapy is only indicated for palliation if chemotherapy fails. More important is the role of prophylactic cranial irradiation (PCI) for patients reaching a complete remission after standard chemotherapy. In these patients PCI leads to a significant reduction of the incidence of tumor relapses in the brain (16). Initial toxicity of PCI is minimal; unexpected neurological problems in long-term survivors, however, are currently a point of major concern (17).

In this thesis several aspects of new approaches in chemotherapy, especially for SCLC, are described. In Chapter 1, the results of a combination regimen, consisting of in vitro synergistic drugs, alternating with a standard, possibly non-cross resistant, combination are described. Based on the results of this study and of other investigations, we decided to investigate the role of high-dose chemotherapy for SCLC. The consequence of this treatment modality is that patients have to be protected against persistent marrow aplasia by bone marrow transplantation, preferably autologous marrow transplantation. In Chapter 2 we describe studies on the

bone marrow reserve capacity, measured by colony forming units in culture in the bone marrow, before and after standard induction chemotherapy. In Chapter 3, data of several studies of high-dose chemotherapy in a number of solid tumors have been compiled. Based on the activity and the toxicity at standard dose levels, two drugs, cyclophosphamide and VP16-213, seemed to be potentially useful for high-dose chemotherapy for SCLC. In Chapter 4 we describe the evaluation of the toxicity of high dose VP16-213, while pharmacokinetic studies of high-dose VP16-213 are mentioned in Chapter 5. Unexpected was the finding of penetration of VP16-213 into the central nervous sytem (CNS) and its effect on CNS-metastases (Chapter 6a and 6b). In Chapter 7 we describe a dose-finding study of the combination of high-dose cyclophosphamide and high-dose VP16-213 with autologous bone marrow transplantation. The problem of severe infection resulting from bone marrow aplasia in these patients is described in Chapter 8. The high-dose chemotherapy regimen, as found in the study of Chapter 7, was evaluated in patients with recurrent SCLC to define its activity (Chapter 9). The results of our studies, as well as data from the literature are summarized in Chapter 10, subsequently conclusions for further studies in SCLC are drawn.

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CHAPTER 1

PHASE II STUDY OF CYCLOPHOSPHAMIDE, VP16-213, AND CISPLATINUM ALTERNATING WITH VINCRISTINE, ADRIAMYCIN, AND PROCARBAZIN FOR UNTREATED SMALL CELL LUNG CANCER

Pieter E. Postmus , Dirk Th. Sleijfer , Aafke F. Meinesz , Huib A.M. Kerstjens , G. Tjong Lo , and Henk J. Sluiter .

SUMMARY.

In a phase II study in patients with small cell lung cancer (SCLC) the combination of cyclophosphamide, cisplatinum and VP16-213 was found to be active, the response rate was 91% (30% CR, 61% PR) in the whole group. In 40 limited disease patients 19 CR (48%) and 20 PR (50%) were seen, where as in 30 extensive disease patients only 2 CR (7%) and 23 PR (77%) were reached. Adding a second combination of adriamycin, vincristine and procarbazin resulted in only 2 patients in response improvement. Mean response duration was 71 weeks in CR patients and 37 in PR patients ($p < 0.01$). Mean survival was 97 in CR and 47 weeks in PR patients ($p < 0.002$). Performance score and disease stage were found to be good prognostic factors. Four patients (6%) are longterm disease-free. The value of cisplatinum, and of alternating chemotherapy for SCLC is probably minimal.

INTRODUCTION

Small cell lung cancer (SCLC) accounts for about 20% of all cases of lung cancer. It is characterized by early and wide-spread dissemination and at the time of diagnosis only few patients are eligible for surgical treatment. Even in apparently operable patients long-term disease-free survival is rare due to the development of distant metastases (1).

Without trèatment, survival beyond three months from the time of diagnosis is rare in the majority of the SCLC patients (2). The introduction of combination chemotherapy in the early seventies has considerably changed the prognosis and a survival up to one year is now possible, a small number, 5-10%, of the patients will even reach a long-term disease-free survival, i.e. over 2 1/2 years, (3). A prerequisite for long-term disease-free survival is the induction of a complete remission (CR) after chemotherapy.

Therefore the goal of several studies has been to increase the number of these CR's. Increasing the doses of the cytostatic drugs has caused an increase in the CR rate (4) and alternating so-called non-cross-resistant combinations of cytostatic drugs have resulted in a further increase (5). The introduction of two in-vitro synergistic drugs, cisplatinum and VP16-213 (6), resulted in a high response rate with a CR rate over 40%, independent of disease stage (7). Moreover, in-vitro a synergistic effect was also observed after combining cisplatinum with cyclophosphamide (6). Therefore we evaluated results of a combination of cyclophosphamide, cisplatinum, and VP16-213 alternated with a standard combination, consisting of vincristine, adriamycin, and procarbazin (8) in a group of newly diagnosed SCLC patients.

MATERIALS AND METHODS

Patients and staging.

From February 1980 to April 1982 70 newly diagnosed, histologically proven, inoperable SCLC patients were entered into the study. Entry criteria included age <70 years, creatinine clearance (CrCl) >60 ml/min, no previous chemo- and/or radiotherapy. Informed consent was obtained from all patients and the study was approved by the local medical ethical committee.

Before the therapy started, all patients underwent routine pretreatment investigations consisting of blood cell counts, liver and renal function tests, chest roentgenogram, chest tomography, and fiberoptic or rigid bronchoscopy with biopsy and/or brush. Staging procedures included isotope scintigrams of bone, liver, and brain, bilateral iliac crest biopsies and bone marrow smears, and a complete neurological investigation. Patients with disease limited to one hemithorax and supraclavicular nodes were staged as limited disease (LD), all other patients had extensive disease (ED).

Restaging procedures included roentgenograms and tomography of the chest, fiberoptic bronchoscopy and all initially abnormal investigations.

Therapy.

Chemotherapy consisted of 2 three-weekly courses of cyclophosphamide 750 mg/m^2 i.v. day 1, cisplatinum 75 mg/m^2 i.v. day 1, and VP16-213 100 mg/m^2 i.v. day 2, 5, 8 (CCV). After restaging, 2 three-weekly courses of

vincristine 1.4 mg/m i.v. day 1 and 8, adriamycin 60 mg/m^2 i.v. day 1, procarbazin 100 mg/m^2 orally day 1-10 (VAP) were given on day 50 and 71. Staging was repeated and maintenance therapy, CCNU 80 mg/m^2 orally day 1 and hexamethylmelamin 150 mg/m^2 orally day 1-7 and 22-28, was given at six weeks intervals during one year to all patients without disease progression. The dose of cisplatinum was reduced to 50% if CrCl decreased below 60 ml/min and was stopped below 40 ml/min. Cyclophosphamide and VP16-213 were given during both courses in full dose irrespective of blood cell counts.

The dose of adriamycin, procarbazin, CCNU, and hexamethylmelamin was reduced to 50% if leukocytes fell below 2.0 x 10^9/l and/or platelets <100 x 10^9/l.

The dose of vincristine and hexamethylmelamin was reduced to 50% if neurotoxicity grade 1-2 (WHO-grading) developed and stopped at grade 3-4.

Patients with a CR received prophylactic cranial irradiation (PCI) during the first maintenance course. Patients with LD, reaching only a PR received 10x3 Gy to the primary and the adjacent lymphnodes during the first maintenance course.

Response.

The response was evaluated 6 and 13 weeks after the start of treatment. Complete response (CR) was defined as disapperance of all known tumor lesions. Partial response (PR) was defined as a decrease of more than 50% of the product of the largest perpendicular diameters of all measurable lesions.

Stable disease (SD) was defined as a less than 50% regression without signs of progression. Progression was defined as an increase over 25% of a measurable lesion or appearance of new tumor lesions. Early death was defined as death due to progression of the tumor within 6 weeks. Toxic death (TD) was defined as death due to treatment-related toxicity.

RESULTS

Patients.

Of 70 patients 11 were female, 59 male. Mean age was 55 years (range 32-67 years). Performance score (PS) (ECOG) was 0 in 9 patients. 40 patients had PS 1, 8 PS 2, and 13 PS 3-4. Forty patients were staged as LD and 30 as ED.

Tumor response, response duration, survival, and relapse.

Response was evaluated after 2 courses of CCV and again after 2 courses of VAP. All patients were evaluable for response. After 2 courses of CCV a response was seen in 64 patients (91%) (CR 21 (30%), PR 43 (61%)). In the 40 LD patients 39 remissions were seen, 19 CR (48%) and 20 PR (50%). In the 30 ED patients, 25 responses were seen, 2 CR 7%) and 23 PR (77%). Stable disease was present in 1 LD and 1 ED patient; 4 patients died within 3 weeks due to toxicity (3) or tumor progression (1). After 2 courses of VAP the total response rate was unchanged, although two PR (1 LD, 1 ED) patients went into CR resulting in a CR rate of 33% and a PR rate of 59%. Progression was seen in 1 ED patient who had SD after 2 CCV. In 2 out of 19 LD patients with a PR radiotherapy resulted in further tumor regression. Maintenance therapy did not result in further tumor decrease in evaluable patients.

Mean response duration was 42 weeks (range 12-241+) (median 31). Mean CR duration was 71 weeks (range 20-241+) and was significantly longer than mean PR duration, 32 weeks (range 12-104) ($p < 0.01$) (median 41 versus 30). Median response duration in LD and ED patients was not different, 33 weeks versus 30 weeks.

Relapse pattern in limited and extensive disease patients was analyzed. Of the LD patients in CR (n=20), 12 (60%) had the first recurrence in the lung, 3 in the brain, and 1 in the liver. In LD patients in PR before radiotherapy (n=19) the first sign of tumor progression was in the region of the primary tumor in 5 cases, the other patients had progression outside the thorax, in 9 of them the brain was the first progression site. All ED patients had progression at the sites of tumor involvement at the time of diagnosis except 4 patients with initial progression in the brain. Survival (fig. 1) beyond two years was reached in 7 patients (10%), 4 patients (3 LD, 1 ED) are still alive with a minimum follow-up of two and a half year without signs of relapse. CR patients had a significantly longer survival than PR patients, mean 97 versus 47 weeks ($p < 0.002$) (median 66 versus 45) and LD patients longer than ED patients, mean 74 versus 43 weeks (median 55 versus 40) ($p < 0.01$). Patients with PS 0-1 had a significantly better prognosis than PS 2-4 ($p < 0.01$) (median 54 versus 39 weeks).

Toxicity.

Table 1

Response

	no pat(%)	CR(%)	PR(%)	SD(%)	Progr.	TD
after 2 CCV						
LD	40 (100)	19 (48)	20 (50)	1 (2)		
ED	30 (100)	2 (7)	23 (77)	1 (3)	1 (3)	3 (10)
Total	70 (100)	21 (30)	43 (61)	2 (3)		4 (6)
after 2 CCV + 2 VAP						
LD		20 (50)	19 (48)	1 (2)		
ED		3 (10)	22 (73)		1 (3)	
Total		23 (33)	41 (59)	1 (1)		

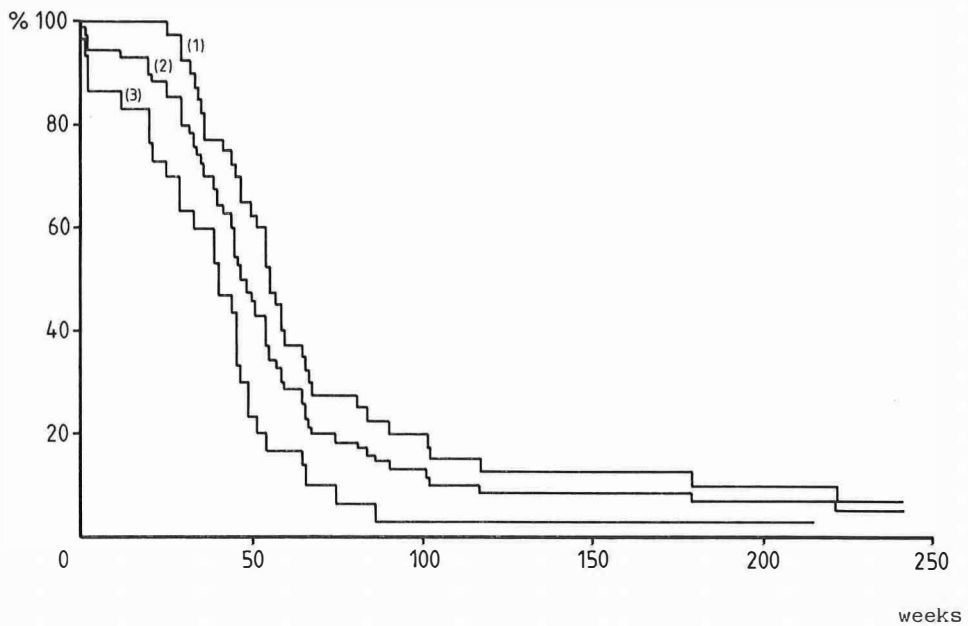

Fig. 1. Survival curves

1 = LD (n=40); 2 = ALL (n=70); 3 = ED (n=30)

All patients experienced nausea and vomiting during the CCV courses, in most patients diarrhea was seen shortly after the cisplatinum infusion. In general, renal toxicity was mild except in one patient with a very high tumor load and PS 4, who died of renal insufficiency after 3 days, probably due to hyperuricemia and cisplatinum; in all other patients cisplatinum was given two times in full dose. Temporary elevated serum creatinine levels normalized within three weeks and a decrease in CrCl below 60 ml/min has not been found. Myelotoxicity was moderate, in 40% of the courses leukocyte-count fell under $1.0 \times 10^9/l$ and thrombocytopenia ($<50 \times 10^9/l$) was present during 29% of the courses. Infections (i.e. axillary temperature $>38.5^{\circ}C$) were seen in 10% of the CCV courses. There were no bleeding episodes.

Two other patients died shortly after the first CCV course, 1 patient had a severe fungal infection with brain abcesses, the other patient died from pleural empyema.

Toxicity of VAP was in most instances nausea and vomiting; myelosuppression was minimal, in 5 patients dose reduction was necessary. Neurotoxicity was severe but reversible in 1 patient, all other patients received the full dose of vincristine.

Toxicity of the maintenance therapy was minimal except frequent longterm thrombocytopenia due to CCNU.

DISCUSSION

In this study the result of the induction chemotherapy regimen (CVV) regarding total response rate, is comparable to other regimens. Overall response rates of 80-90% have been reported several times (9). The number of CR in the ED group, however, is disappointingly low and in sharp contrast with the 41% CR rate reported by Sierocki after VP16-213 and cisplatinum (7).

Overall the median survival in this study is comparable to what has been reported after treatment with other regimens (9), the same can be said of the number of long-term disease free survivors. These results could only be achieved if regimens are used with a certain degree of toxicity. The regimen used in this study has been associated with more gastro-intestinal toxicity due to the use of cisplatinum; furthermore the necessity of adequate hydration to reduce renal toxicity is only possible on an in-patient

treatment base. These side effects and the results of therapy do not justify the use of cisplatinum for the treatment of SCLC as first-line therapy.

The introduction of a second non-cross-resistant chemotherapy regimen is theoretically attractive in destroying tumor cells resistant to the first regimen (10).

The response rate after the second combination (VAP), however, was only slightly better than after the initial treatment. The failure of the VAP combination to induce an increase in number of CR therefore suggests that this combination is not truly non-cross resistant.

The results of studies with the so-called non-cross resistant combinations initially seemed to be promising (5), however, controlled trials have not shown any survival benifit (11).

In this study maintenance therapy has probably not been of any importance considering the lack of activity of the maintenance regimen in patients in partial remission. It seems therefore to be justified to give short-term-chemotherapy to obtain the maximal profit for the patients. Results of other short term regimens support this view (12), but adequate phase III trials have not been reported yet.

This study illustrates once again the disappointing results obtained in the treatment of SCLC and it is therefore necessary to consider in what directions further studies should go. New active drugs are definitely necessary, however, since the introduction of VP16-213 into the clinic, no other promising drugs with activity against SCLC have been discovered. At present it seems therefore only possible to improve the quality of life during chemotherapy in the majority of the patients and probably the best way to do this is to define the minimal duration of treatment for these patients.

A small group of patients might benefit from the so-called "late intensification" according to the mathematical model of Norton and Simon (13).

The response rate of high-dose chemotherapy, with or without autologous bone marrow reinfusion, in relapsing patients with SCLC are in this respect promising (14). Application of this "late intensification" after maximum tumor response to standard treatment is therefore potentially useful and such approaches are currently under investigation.

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CHAPTER 2

Small Cell Lung Cancer and the Influence of Chemotherapy on CFU_cs in Bone Marrow

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To determine the optimal moment for the harvesting of bone marrow for autologous transplantation, the authors did serially colony forming units in culture (CFU_c) counts in a group of 42 patients with small cell lung cancer (SCLC) before and after remission induction chemotherapy and subsequent maintenance chemotherapy. Disease stage did not influence the CFU_c count except in patients with bone marrow metastases; this resulted in either abnormally low or abnormally high CFU_c counts, probably dependent on the degree of invasion. After 2 courses of induction chemotherapy, the number of CFU_cs was 3.1-fold higher than before therapy. After 4 courses, the CFU_cs number was comparable to the pretreatment value. An inverse correlation was found between the degree of hematologic toxicity (expressed as leucocytes count) and the increase of CFU_cs after induction chemotherapy. The number of CFU_cs decreased during prolonged chemotherapy to low levels after 1 year. Harvesting of bone marrow is probably done best shortly after induction chemotherapy.

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STANDARD DOSE CHEMOTHERAPY for small cell lung cancer (SCLC) results in a high remission rate, but only in a few patients in long-term disease-free survival.[1] These observations have led to a search for more promising therapeutic measures. One of the observations that could be important in this respect is the dose dependency of the tumor response in SCLC.[2] Therefore it is worthwhile to evaluate the efficacy of high-dose chemotherapy. Because of the side effects on bone marrow function, such treatment must be followed by rescue with bone marrow cells, usually from autologous marrow.[3]

Two problems are critical when autologous marrow is used in this situation: (1) the problem of tumor involvement of bone marrow; and (2) the ability of the reinfused marrow to reconstitute the patient.[4,5] Both are directly influenced by the moment chosen to procure marrow. Although other assays of stem cell function have been described recently, the determination of CFU_cs is as yet the most widely used.[6–10] Therefore we studied the number of CFU_cs in bone marrow of patients with SCLC before and after remission induction chemotherapy and subsequent maintenance chemotherapy. Furthermore, the relation was analyzed between these numbers of CFU_cs and the effects of chemotherapy on peripheral blood leucocytes.

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Materials and Methods

From June 1980 through December 1981, 42 consecutive previously untreated patients with a histologically proven diagnosis of SCLC were investigated. All patients underwent routine staging procedures: history, physical examination, hematologic evaluation, liver function, chest X ray, fiberoptic or rigid bronchoscopy, isotope scans of bone and liver, neurologic evaluation, and in some patients, liver biopsy. An unilateral posterior iliac crest bone marrow biopsy with a Yamshidi needle was performed in all patients. On that occasion bone marrow was also aspirated for a smearpreparation and for culturing purposes.

Patients with tumor limited to one hemithorax, mediastinal and/or supraclavicular lymph nodes, were designated as to have limited disease (LD); all other patients had extensive disease (ED), including those with bone marrow involvement.

All patients received intensive induction chemotherapy with cisplatin, 75 mg/m^2 intravenously (IV) day 1; cyclophosphamide, 750 mg/m^2 IV day 1; and VP-16-213, 100 mg/m^2 IV days 2, 5, 8 (CCV) for 2 courses at 3 week intervals without dose reduction for hematologic toxicity. This was followed by 2 courses consisting of vincristine, 1.4 mg/m^2 IV days 1 and 8; Adriamycin, 60 mg/m^2 IV day 1; and procarbazine, 100 mg/m^2 orally days 1–10

(VAP), at 3-week intervals. Response was evaluated after 6 and 12 weeks. In most patients this included also bone marrow investigation as described earlier. In responding patients, maintenance therapy was initiated at week 14: CCNU 80 mg/m^2 orally day 1 and hexamethylmelamine 150 mg/m^2 orally days 1–7 and 22–28, which was repeated every 6 weeks until 1 year from the start of treatment.

According to the staging procedure, 23 patients were shown to have LD and 19 ED, including 6 patients with bone marrow metastases. CFU_cs were measured in 37 patients before therapy (18 LD, 19 ED), in 20 after 2 courses of CCV (10 LD, 10 ED) and in 23 after 2 courses of both CCV and VAP (15 LD, 8 ED). In five patients, a CFU_c count was done after about 6 months, and in 6 patients at the end of treatment at 1 year.

Bone Marrow Culture Procedures (CFU_c)

Approximately 2 ml of the aspirated bone marrow was placed into a tissue tube containing 2 ml of Hanks balanced salt solution and 2000 U heparine. The culture procedure was as described by Pike and Robinson.[11] The leukocytes from normal healthy volunteers served as a source of colony stimulating factor. Throughout this

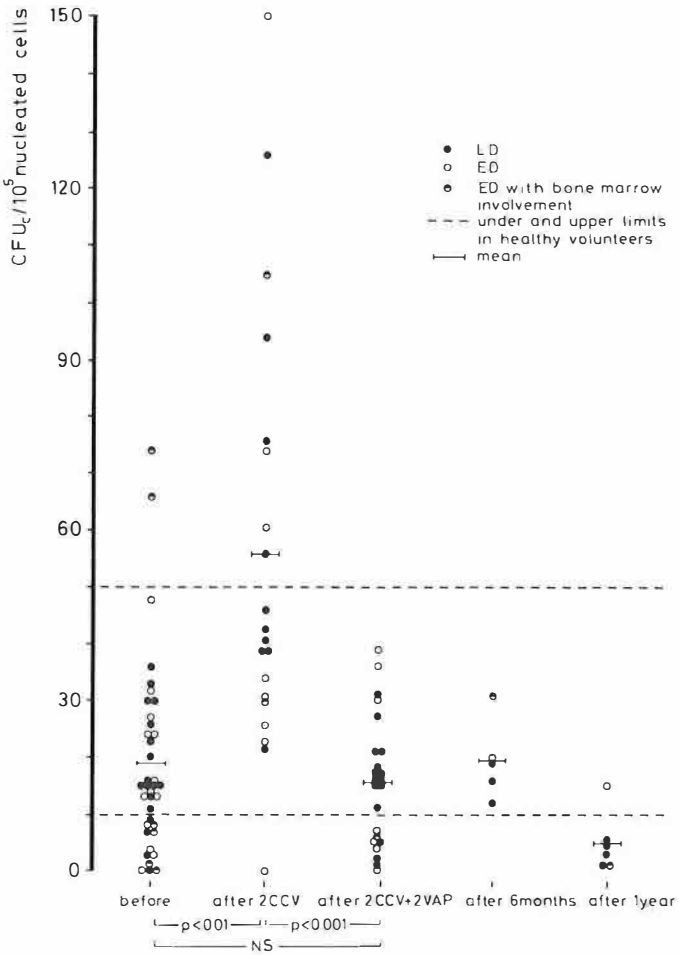

FIG. 1. CFU_c values before, during and after remission induction.

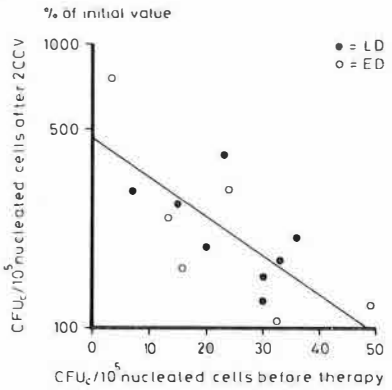

FIG. 2. The relation between CFU_c values before therapy and after 2 courses of cytostatic chemotherapy.

study, the same donor pool has been used. Alternating donors from this pool did not influence CFU_c counts in control experiments beyond the range of duplicate determinations. A total of 1×10^6 leukocytes per plate was used in the feederlayer and employed within 6 days.

The overlayer contained 1×10^5 nucleated bone marrow cells per plate. Cultures were scored at day 10 throughout this study, but were also examined on day 14. A dissecting microscope at ×10 magnification was

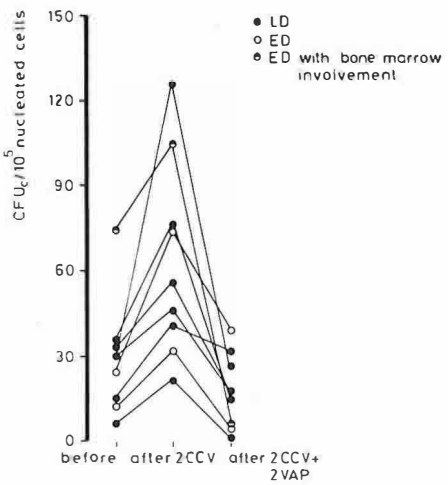

FIG. 3. CFU_c values in 9 patients before therapy, after the first 2 courses of chemotherapy and at the end of induction therapy.

used. Only groups of more than 40 cells were scored as colonies. Normal values have in our laboratory been determined in healthy volunteers. The range is 10 to 50 colonies per 10^5 cells (N = 11). This range is also found in patients without bone marrow related disease. All determinations have been performed in triplicate (SD = 2.10; N = 148).

Statistics

Differences in mean values were assessed with Student's *t* test. The statistical significance of the differences in CFU_c counts in patients who were evaluated both before and after therapy, was assessed by Wilcoxon rank sum test on paired samples. Correlation coefficients were determined and regression lines were calculated on the scatter diagrams of Figures 2 and 4.

Results

Patient Characteristics

The median age of the 42 patients studied is 56 years (LD, 57 years; ED, 54 years). Thirteen patients had a Karnofsky performance status < 60.

Pretreatment of CFU_c Levels

The pretreatment CFU_c values (Fig. 1) show no difference between LD and ED patients (mean 17.6 *versus* 20.1 per 10^5 cells). There is no relation between pretreatment CFU_c count and age, sex and performance status. In 12 patients, the CFU_c count was abnormally low (less than 10 per 10^5 cells). Four of these patients had histologically proven bone marrow involvement; in the smear of this aspirate there was a substantial number of tumor cells, at least 50%. As tumor cells were included in the plated cell numbers, these low CFU_c counts could be due to lack of hemopoetic cells.

In two patients, abnormally high CFU_c counts were found (above 50 per 10^5 cells); in the smears of these aspirates small clumps of tumor cells were found, but less than 5% of the total cell count.

CFU_c Count After Two Courses of CCV

In the 20 patients (10 LD, 10 ED), there is no difference in CFU_c counts between LD and ED (mean 58.2 and 54.4 colonies per 10^5 cells). In 17 of these 20 patients, a pretreatment value is known; in 15 patients (88.2%), the CFU_c value is increased after therapy. The increase in this group is significant (Wilcoxon rank sum test, $P < 0.01$). Also, the increase in the means of CFU_c values before (mean, 18) and after 2 courses (mean, 55) is significant ($P < 0.01$).

The three patients in this group with pretreatment bone marrow invasion had varying effects of chemotherapy on CFU_c count: from 74 to 105 after, from 66 before to 30 after, and from 1 to 0, respectively. There were no signs of metastases in the bone marrow biopsy specimen or aspirate in any of the 20 patients after 2 courses of CCV. In 14 patients without proven bone marrow involvement, we found a correlation between the pretreatment CFU_c value and the increase of the number of CFU_cs after 2 courses of chemotherapy (% of initial value): $r = 0.715$; $y = 459e^{-0.311x}$; $P < 0.025$ (Fig. 2).

CFU_c Counts After Two Courses of VAP (23 Patients)

Again there is no difference between LD (15 patients) and ED (8 patients) (mean, CFU_c 15.7 and 15.9 per 10^5 cells, respectively). These values are a little lower than the pretreatment values; the difference, however, is not significant (mean, 15.7 *versus* 18.9). The decrease between the values found after 2 and 4 chemotherapy courses is significant (mean, 56.3 *versus* 15.7; $P < 0.001$). CFU_c counts in 15 patients in whom cultures were performed both before and after these chemotherapy courses also showed a significant decrease ($P < 0.01$, Wilcoxon rank sum test on paired samples).

In nine patients, CFU_c counts were performed sequentially during induction therapy (Fig. 3). In these 9 patients, values after 2 courses of CCV differ significantly ($P < 0.01$) from the values before and after 2 courses of CCV + 2 courses of VAP. Before and after chemotherapy, the CFU_c counts do not differ significantly (Wilcoxon rank sum test on paired samples).

CFU_c Count During Maintenance Therapy

In five patients, after 6 months of chemotherapy, values in the normal range were found (12–31 per 10^5 cells); in 5 of 6 patients, an abnormally low value was found after 12 months of treatment (1–5 per 10^5 cells).

Hematologic Toxicity and Relation with CFU_c Count

While no difference was found in mean CFU_c count before therapy in patients with ED and LD, leukocytes counts in LD (mean, 7.1×10^9/l) were lower than in ED (mean, 10.7×10^9/l) ($P < 0.0025$). Granulocytes counts in LD (mean 4.6×10^9/l) were also lower than in ED (mean, 8.4×10^9/l) ($P < 0.0025$). Hemoglobin levels, 136.7 g/L in LD and 135.0 g/l in ED, and platelets 253.8×10^9/l and 276.5×10^9/l, were similar. There were no differences in leukocyte nadir (mean, 1.0×10^9/l in LD and 1.36×10^9/l in ED) and platelet nadir (mean 84.0×10^9/l and 82.4×10^9/l, respectively). A low leukocyte nadir during the first course of CCV is related to high

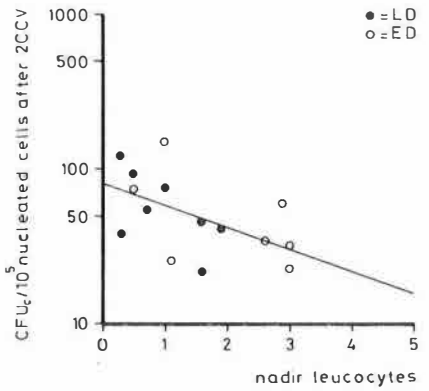

FIG. 4. Relation between leukocyte nadir during the first course of chemotherapy and the CFU_c count after 2 courses.

CFU_c counts after 2 CCV courses ($r = 0.544$; $y = 81.45e^{-0.325x}$ $P < 0.05$) (Fig. 4).

Discussion

In SCLC, high-dose chemotherapy with autologous bone marrow as a primary therapy has led to a high number of remissions.[12] Also, patients with refractory disease have shown responses of this form of treatment.[13,14] Current studies are underway in our and other institutes concerning the potential of this treatment form for patients who have reached complete remission.[15,16] The moment that is chosen to harvest the marrow might influence the outcome of the procedure. Harvesting bone marrow before therapy has started would have the advantage that stem cell numbers are not committed by cytostatics. However, SCLC is often, at the start of treatment, already metastasized, with a high risk of contamination with malignant cells if the marrow is then cryopreserved.[17]

This study shows that after 2 courses of chemotherapy, the mean CFU_c count is 3.1-fold higher than before therapy in 88% of the patients. Our results are comparable to those of Abrams *et al.*[18] in a comparable group of SCLC patients where the number of circulating CFU_cs was 6.7-fold higher after 1 course of induction chemotherapy in the majority of the patients.

CFU_c determinations only measure a part of the bone marrow function and other parameters could add worthwhile information. Erythroid precursors seem to be more susceptible to unfavourable storage conditions[19] and may therefore be of value in the quality control of preserved marrow. Potentially, assays that give rise to mixed colonies should more closely mirror the presence of stem cells.[20]

Spitzer *et al.*[9] found a good correlation between the number of CFU_cs infused and the time to hemopoietic recovery in leukemic and solid tumor patients treated by marrow ablative therapy. Ekert *et al.*[10] could not find this correlation, but as their patients received prophylactic co-trimoxazole, it is possible that this has caused a delay of hematopoietic recovery.[21]

In this study, a substantial increase was found in CFU_c counts following severe chemotherapy-induced leukopenia, the increase being related to the degree of leukopenia. This increase seems to be more overt when the pretreatment CFU_c counts are low. In our material, invasion of the bone marrow with tumor cells could lead to both abnormally low or abnormally high CFU_c counts, probably dependent on the degree of invasion. The increase in CFU_c count in patients with discrete marrow involvement could be due to production or stimulation of colony stimulating factor by tumor cells.[22] Interestingly, leukopenia due to chemotherapy in these patients did not lead to further increase in CFU_c count. The abnormal growth pattern reported by McCarthy and coworkers,[23] in two patients with SCLC was not seen in any of our patients.

It can be concluded from our study that soon after an attempt at remission induction might be an optimal moment to collect marrow if transplantation is considered. The main problem remains to exclude the possibility that tumor cells are also preserved. However, it can reasonably be assumed that the number of these cells is at a nadir shortly after a successful remission induction.

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CHAPTER 3

Intensive Chemotherapy and Autologous Bone Marrow Transplantation for Solid Tumors

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Studies in animal tumor systems show a dose-response relationship for a broad variety of cytostatic drugs (34,62,70). Data on the human situation are less evident (25,51), but in a number of tumors higher response rates are found after the use of increased doses of cytotoxic drugs (16,37,41). These observations have led to the investigation of chemotherapy at the maximally tolerated dose levels.

A considerable dose escalation can result in a rise in toxicity and for dose-escalation studies only cytostatics with primarily bone marrow toxicity are suitable. Thus, drugs such as the anthracyclines, bleomycin, cisplatinum, actinomycin-D, and the vinca alkaloids have a very limited potential for dose increments. The antimetabolites as a group are interesting candidates for dose escalation, however, their use as single agents, at any dose level, does not require bone marrow transplantation to accelerate hematopoietic recovery.

From the remaining agents, most attention has been given to the alkylating agents melphalan, mechlorethamine, cyclophosphamide, and BCNU. Recently, new drugs such as VP16-213 (etoposide), amsacrine (AMSA), and mitomycin C have been investigated in high-dose regimens with autologous bone marrow transplantation (ABMT). This chapter gives a summary of medullary and extramedullary toxicity and responses after high-dose single agent and combination chemotherapy with or without ABMT.

TOXICITY OF HIGH-DOSE CHEMOTHERAPY

Single Agents

Cyclophosphamide

Two important extramedullary side effects are reported. The urothelial toxicity, caused by one of the metabolites, acrolein, can result in severe hemorrhagic cystitis.

TABLE 1. *SCLC—phase II studies in heavily pretreated patients*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
9	CTX 1.5 g/m² × 4 VP 125 mg/m² × 4 BCNU 300 mg/m²	5 (1)	3	1	Mucositis 42%	3,4,5	66
1	CTX 50–60 mg/kg × 2–4 VLB 0.5 mg/kg × 1–2 TBI 8 Gy	1 (1)	—	—	Cardiorespiratory failure 50% Mucositis 57% Diarrhea 21%	2	20
1	MTX + CF 200 mg/kg ADM 150 mg/m² CTX 120 mg/kg TBI 8 Gy	1 (1)	—	—	NS	5	32
3	BCNU 600 mg/m²	0	3	—	—	—	67
5	BCNU 300 mg/m² PCZ 200 mg/m² × 4 L-PAM 140 mg/m²	4 (1)	—	1	NS	NS	54
5	CTX 7 g/m² VP 0.9–2.5 g/m²	1 (0)	2	2	Mucositis	10	58

Abbreviations for Tables 1–9: ADM, adriamycin; BCNU, carmustine; CDDP, cis platinum; CTX, cyclophosphamide; DTIC, dacarbazine; L-PAM, melphalan; MMC, mitomycin C; MTX, methotrexate; PCZ, procarbazine; TACC-1, 6-thioguanine 100 mg/m² day 1–4; VCR, vincristine; VLB, vinblastine; VP, VP16–213; NS, not significant.

Today this problem is virtually abolished, when 2-mercaptoethane sulfonate sodium (mesnum) is given in combination with cyclophosphamide. This water soluble sulfhydril containing compound inactivates acrolein (11).

High-dose cyclophosphamide appears to cause a unique form of hemorrhagic myocarditis, frequently with a fatal outcome. This complication is seen at a dose of 160 mg/kg in combination regimens. It is unlikely to occur below a dose of 240 mg/kg when used as single agent, although one fatal case was reported after 180 mg/kg (5,13,30).

Melphalan

One of the major advantages of melphalan is its short half-life (48), this makes rapid reinfusion of even nonfrozen autologous marrow possible and shortens the duration of aplasia.

Dose-limiting toxicity is located in the gastrointestinal tract and can be expected at doses of about 200 mg/m^2. The clinical significance of the development of autoantibodies against platelets and granulocytes after high-dose melphalan is uncertain (42).

Nitrosoureas

High-dose BCNU does result in several extramedullary toxic side effects. Most frequently encountered is pulmonary toxicity. This can be expected after a cumulative dose of 1.2 to 1.5 g/m^2. Also, the risk is increasing with the dose per single course. Pre-existing pulmonary disease is a major risk factor. Previous use of pulmonary toxic drugs and/or thoracic irradiation enhances the BCNU toxicity (46,84).

The development of encephalomyelopathy after high-dose BCNU is probably an effect of the drug on the cerebrospinal vessels. The lesions found in the central nervous system are comparable to those found after cranial radiotherapy or combined cranial radiotherapy and chemotherapy (14). Fatal liver toxicity was seen after 1.2 g/m^2 in 7 of 18 patients (53) and fatal venoocclusive disease of the liver was reported after high-dose BCNU employed as a single agent (49).

Mechlorethamine

Increasing the dose of mechlorethamine from 0.5 to 2.0 mg/kg is associated with a rapid increase of immediate as well as delayed neurotoxicity (73). In 2 of 3 melanoma patients receiving 0.8 mg/kg (33 mg/m^2), significant cardiotoxicity with persistent tachycardia, and functional or atrial ectopic beats were observed and did not allow further dose escalation (36).

TABLE 2. *SCLC–phase II studies in untreated patients*

No. patients	Therapy		No. (CR) response	NR
13	CTX 1.5 g/m² × 3 VP 0.2 g/m² × 3 VCR 2 mg × 2 ADM 80 mg/m² (7 patients)	× 2	13(7)	—
16	CTX 40–50 mg/kg × 4		13(7)	3
17	CTX 180 mg/kg		5(2)	12
7	CTX 180 mg/kg VP 1 g/m²		2(0)	5

See Table 1 footnote for abbreviations.

VP16-213 (Etoposide)

The most important side effect observed after high-dose VP16-213 is mucositis in the oropharyngeal region (86). This occurs in the majority of patients at 2.4 g/m² (79), however in a small number of patients treated with 3.0 g/m², the mucositis was still not dose limiting (57).

Amsacrine

In four of five courses of AMSA (250 mg/m², days 1, 2, and 3) severe and dose-limiting mucositis was observed, after a lower dose (200 mg/m², days 1, 2, and 3), only one episode of severe mucositis occurred in six courses (76).

Mitomycin C

Hepatic venoocclusive disease was reported in 4 patients after a dose of 60 to 75 mg/m² (31). Hemorrhagic colitis and pancreatic toxicity were considered to be too severe to permit doses over 60 mg/m² (61). However, lowering the infusion rate with lower peak serum levels seems to be associated with less toxicity (63).

Total Body Irradiation

Total body irradiation (TBI) has the advantage of killing tumor cells regardless of the cell cycle stage but it also effects the central nervous system. The classic treatment with a ^{60}Co unit gives dose-limiting toxicity in the lung at a level of 10 GY. Other toxicities are mostly in the gastrointestinal tract. A late side effect can be the development of cataracts (8). Fractionated TBI could be less toxic for normal tissue. Until now, results from a randomized study in acute leukemia and allogenic bone marrow transplantation (BMT) did not show a difference of pulmonary toxicity after fractionated TBI compared with standard TBI (78), but there was a better survival with fractionated TBI and allogenic BMT.

Treatment related death	Extramedullary toxicity	Response duration in months	Reference
—	Esophagitis (15%) Mucositis (61%) Cardiac (15%)	4–12 (maintenance therapy)	22
—	Mild diarrhea (31%)	1–16+ (also local radiotherapy)	65
—	NS	NS	15
—	NS	NS	15

Combination Chemotherapy

The dose-limiting toxicity of single agents is evaluated rather extensively, whereas the toxicity of high dose combination regimens is not studied sufficiently. Two phase I studies are in press. The dose-limiting toxicity for VP16-213 used in combination with cyclophosphamide at the highest tolerable dose (7 g/m^2) was reached at 2.5 g/m^2 (58). The combination of BCNU and melphalan did not give unacceptable toxicity at a dose of 800 mg/m^2 and 70 mg/m^2, respectively (79).

In general, the toxicity in other studies is severe with a high infection rate. Fatal cardiorespiratory problems were seen in 7 of 14 patients after high-dose cyclophosphamide, vinblastine, and TBI (20). The incidence of stomatitis was 42% in 18 courses consisting of cyclophosphamide 4.5 g/m^2, VP16-213 0.5 g/m^2, and BCNU 0.3 g/m^2, whereas diarrhea was seen less often (16%) (66). Dose-limiting mucositis was seen after a combination of cisplatinum 120 mg/m^2, VP16-213 1.08 g/m^2, and Adriamycin™ 135 mg/m^2 (43). Fatal carditis was seen in 3 of 14 patients treated for a pediatric Burkitt's lymphoma with BCNU, Ara-C, cyclophosphamide and 6-thioguanine (BACT) (3). Occasionally hepatic venoocclusive disease has been noted after high-dose therapy with or without TBI (83,87).

EFFECTIVENESS OF HIGH-DOSE CHEMOTHERAPY IN VARIOUS TUMORS

Small Cell Lung Cancer

Several studies with high-dose combination chemotherapy were carried out in heavily pretreated patients (Tables 1–3). Despite the resistance to conventional chemotherapy, a response was seen in the majority of the patients including a number of complete remissions. However, response duration is short. High-dose single agent or combination chemotherapy in patients with a partial remission after standard treatment resulted in an improvement of remission status (43,64,68,71,74). The results of high-dose combination chemotherapy as initial therapy are disap-

TABLE 3. *SCLC–high-dose therapy after remission induction with standard therapy*

No. patients	Therapy	No. responses before/after high-dose chemotherapy		Response duration in months	Reference
10	CDDP 120 mg/m²	CR → CR	(*N* = 3)	5–17+	43
	ADM 90 mg/m²	PR → PR	(*N* = 3)		
	VP 240 mg/m²	PR → CR	(*N* = 2)		
		PR → progr.	(*N* = 1)		
		toxic death	(*N* = 1)		
3	CDDP 120 mg/m²	CR → CR	(*N* = 1)	2–10+	43
	ADM 135 mg/m²	progr. → PR	(*N* = 1)		
	VP 360 mg/m² × 3	toxic death	(*N* = 1)		
3	ADM 135 mg/m²	CR → CR	(*N* = 3)	8–11+	64
	VP 360 mg/m² × 3				
	CTX 3 g/m²				
5	CTX 100–200 mg/kg	PR → PR	(*N* = 2)	5–10+	64
	VP 0.75–1 g/m²	PR → CR	(*N* = 2)		
		SD → PR	(*N* = 1)		
20	CTX 12 g	CR → CR	(*N* = 2)	NS	71
		NR → PR	(*N* = 2)		
		minor response	(*N* = 16)		
13	CTX 6 g/m²	CR → CR	(*N* = 3)	6+–25+	74
	BCNU 300 mg/m²	PR → CR	(*N* = 4)		
	VP 0.5 g/m²	PR → PR	(*N* = 3)		
		early death	(*N* = 3)		
10	CTX 1.5 g/m² × 3	CR → CR	(*N* = 4)	16+–20+	68
	VP 0.2 g/m² × 3	PR → CR	(*N* = 4)		
	VCR 1.5 mg/m² × 2	PR → PR	(*N* = 1)		
		NR → PR	(*N* = 1)		

See Table 1 footnote for all abbreviations.

pointing; response rate, response duration, and survival time are comparable to what can be achieved with standard dose therapy (22,68).

Recently, two single agent studies carried out in untreated patients gave support to a dose-response relationship for cyclophosphamide (65) and VP16-213 (40). In 81% ($N = 16$) a response was seen after cyclophosphamide 160 to 200 mg/kg, this is much higher than after standard doses (30 to 40%) (10). In another small study, 8 responses were noted in 10 patients with extensive disease after VP16-213 1.2 g/m², whereas after standard doses 40 to 50% response rate is seen (39). An important observation may be the response of central nervous system metastases after high-dose VP16-213 (1.0 and 1.5 g/m²) in 2 patients who had relapsed after cranial irradiation (55).

Germ Cell Tumors

High-dose VP16-213 (2.4 g/m²) results in a high response rate, 6 of 10 patients with a relapse responded, 2 of these patients were pretreated with standard dose VP16-213 (33) (Table 4).

TABLE 4. *Germ cell tumors*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
3	CTX 1.5 g/m² × 4 VP 125 mg/m² × 4 CCNU 300 mg/m²	2 (2)	1	—	Mucositis (42%) Diarrhea (16%)	1, 3.5	65
3	CTX 1.5 g/m² × 4 VP 125 mg/m² × 4	3 (1)	—	—	NS	1, 1, 2.5+	66
13	CTX 3.8–6.0 g/m² VP 450–750 mg/m² (some patient also BCNU, ADM or CDDP)	7 (4)	3	3	Stomatitis (14%) Diarrhea (21%)	1,1,1,3.5 4,5,5+	9
1	CTX 2 g/m² ADM 100 mg/m²	1 (0)	—	—	Mild mucositis	NS	81
2	CTX 50 mg/kg × 3 BCNU 400 mg/m² × 3	—	1	1	NS	—	7
6	CTX 50–60 mg/kg × 2–4 VLB 0.5 mg/kg × 1–2 TBI 8 Gy	1 (1)	—	5	Cardiorespiratory failure (50%) Mucositis (57%) Diarrhea (21%)	29+	20
2	MTX + CF 150–200 mg/kg CTX 100–180 mg/kg ADM 40–50 mg/m² VLB (1 patient) 10 mg/m²	1 (0)	1	—	NS	NS	27
6	CTX 7.0 g/m² VP 1.5–2.5 g/m²	6 (0)	—	—	Mucositis	1–2	58
4	L-PAM 140 mg/m²	4 (?)	—	—	NS	NS	47
1	VLB 1 mg/kg ADM 180 mg/m² CTX 200 mg/kg TBI 8 Gy	1 (1)	—	—	NS	10+	26
10	VP 0.8 g/m²	6 (1)	4	—	Mucositis	1–6	33

See Table 1 footnote for abbreviations.

TABLE 5. *Ovarian cancer*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extra-medullary toxicity	Response duration in months	Reference
6	CTX 2 g/m² ADM 100 mg/m²	4 (1)	2	—		NS	81
4	L-PAM 140 mg/m²	2 (0)	2	—	NS	NS	47
2	CTX 7 g/m² VP 0.9 g/m²	2 (2)	—	—	Mucositis	10+,12+	82
1	CTX 170 mg/kg ADM 50 mg/m² VM 26 100 mg/m²	1 (0)	—	—	NS	NS	27

See Table 1 footnote for abbreviations.

High response rates were found after high-dose combination chemotherapy, however, only a few patients had a long-term disease-free survival (20,26).

Ovarian Cancer

Data on high-dose chemotherapy in ovarian cancer are scarce (Table 5). A high response rate after 120 mg/kg cyclophosphamide (8 of 9 patients) is promising (12) and higher than after standard dose therapy (37,80). Two patients reached a long-term unmaintained complete remission after high-dose cyclophosphamide (7 g/m²) and high-dose VP16-213 (0.9 g/m²) (82).

Breast Cancer

Although several responses were seen after high-dose mitomycin C, it is still not clear if this treatment will be of value for clinical practice (19,72) (Table 6). The number of patients treated with high-dose chemotherapy is small and the results are inconclusive. A promising observation was the response in 3 of 4 patients after VP16-213, 1 g/m² (57).

Malignant Melanoma

Since the promising report of McElwain et al. (48), several studies with this rather resistant tumor were performed (Table 7). Both BCNU and melphalan were found to be active drugs with a number of complete remissions. In all cases, the complete response (CR) was of short duration. One long-term remission was seen after high-dose mechlorethamine; another one after a combination of high-dose melphalan and BCNU (79).

Childhood Tumors

High response rates were found in Ewing's sarcoma and neuroblastoma (Table 8) Also, a few long-term disease-free survivors were reported (6). Data on rhabdomyosarcoma are still inconclusive.

TABLE 6. *Breast cancer*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
11	MMC 40 mg/m²	2 (0)	9	—	NS	NS	19
5	CTX 50 mg/kg × 2 TBI 10 Gy	2 (2)	2	1	NS	5.6	66
1	CTX 2 g/m² ADM 100 mg/m²	1 (0)	—	—	Mild mucositis	NS	75
2	CTX 50–60 mg/kg × 2–4 VLB 0.5 mg/kg × 1–2 TBI 8 Gy	1 (1)	—	1	Cardiorespiratory failure (50%) Mucositis (57%) Diarrhea (21%)	5	20
3	L-PAM 120–225 mg/m²	2 (0)	1	—	NS	1+, 7+	42
3	L-PAM 140 mg/m²	2 (0)	1	—	NS	NS	47
16	AMSA 600–750 mg/m²	2 (0)	14	—	Mucositis (40%)	7,11	70

See Table 1 footnote for abbreviations.

TABLE 7. *Malignant melanoma*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
6	BCNU 400 mg/m² × 3	1 (0)	5	—	—	1	7
6	BCNU 600–750 mg/m²	1 (0)	5	—	—	1.5	67
8	BCNU 1,200–2,850 mg/m²	4 (1)	4	—	NS	NS	53
30	BCNU 1,050–1,200 mg/m²	14 (5)	13	3	Lung	2+–4+	23
27	L-PAM 140–260 mg/m²	12 (2)	15	—	Gut	NS	60
9	L-PAM 120–225 mg/m²	7 (3)	2	—	Mucositis Diarrhea	1+–6	45
2	L-PAM 140 mg/m²	2 (0)	—	—	NS	NS	47
5	HN_2 33 mg/m²	2 (1)	3	—	Heart	1.5,17+	79
8	L-PAM 35–70 mg/m² BCNU 400–800 mg/m²	6 (2)	2	—	—	1,2,4,6, 6+,14+	79
4	AMSA 600–1,000 mg/m²	0	4	—	Mucositis	—	88

See Table 1 footnote for abbreviations.

Colorectal Carcinoma

Five of 6 patients able to be evaluated showed a partial response after high-dose melphalan, 80 mg/m^2 (44).

Lymphoma

Even in heavily pretreated patients with high-grade malignant non-Hodgkin's lymphoma, a high response rate was obtained after a number of different high-dose regimens (Table 9). The most promising result is the number of long-term unmaintained complete remissions (3,27,29,77). A few authors reported on high-dose regimens in therapy resistant Hodgkin's disease (32,77), also in these patients the response rate is promising.

Brain Tumors

The penetration of BCNU through the blood-brain barrier may be useful for treatment of primary or secondary brain tumors. Responses of glioblastoma and several metastatic tumors have been reported (24).

DISCUSSION

Studies of high-dose chemotherapy are only just emerging. Although a large number of studies are already published, only very few answers to important questions have been obtained.

Many studies have evaluated the necessity of ABMT after high-dose chemotherapy. In two randomized studies (4,75), a beneficial effect of ABMT on aplasia duration was found; in some studies the period of aplasia was shorter after high-dose chemotherapy supported by ABMT compared to less chemotherapy without ABMT (48,61,81,88). Also, the number of infused colony forming units in cultures (CFU-c) and the quality of the cryopreserved bone marrow was found to influence hematopoietic recovery (1,28,69).

Despite ABMT, there is still a rather long period with severe granulocytopenia associated with a high frequency of infection. This is a major problem that has to be solved to reduce morbidity and mortality. One way of reducing the risk of developing viral infections related to transfusion of blood products may be the use of cryopreserved autologous platelets (38).

The key question to be answered is: is it worthwhile to perform this time and money consuming treatment which is associated with a high morbidity and mortality? Although *in vitro* studies are clearly supportive of high-dose chemotherapy, in clinical practice, this concept has not yet been unequivocally successful.

In general, two approaches are possible. The first is the use of the so-called "late-intensification" in patients with minimal residual disease after standard therapy. This method is supported by the mathematical model of Norton and Simon (50). Recently, a few studies of this kind were initiated in small cell lung cancer (SCLC); survival data are not yet available. A major advantage of intensification

TABLE 8A. *Ewing's sarcoma*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
5	L-PAM 120–225 mg/m²	5 (4)	—	—	NS	1,3+,5,14,14	17
3	L-PAM 60–225 mg/m²	1 (0)	2	—	Severe mucositis (>200 mg/m²)	NS	18
3	L-PAM 140–160 mg/m²	3 (2)	—	—	NS	NS	2
1	L-PAM ?	1 (1)	—	—	NS	9+	45
4	L-PAM 140 mg/m²	3 (2)	1	—	NS	9,12+,14+	85
1	CTX 50–60 mg/kg × 2–4 VLB 0.5 mg/kg × 1–2 TBI 8 Gy	1 (1)	—	—	Cardiorespiratory failure (50%) Mucositis (57%) Diarrhea (21%)	6	20

See Table 1 footnote for abbreviations.

TABLE 8B. *Neuroblastoma*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
14	L-PAM 140 mg/m²	10 (2)	4	—	Stomatitis (7%)	NS	59
6	L-PAM 140–225 mg/m²	4 (1)	2	—	NS	4.5+–6	45
7	L-PAM 60–225 mg/m²	7 (3)	—	—	Severe mucositis (> 200 mg/m²)	NS	18
4	DTIC, CTX, L-PAM, VCR (2 patients) ADM (2 patients) ARA-C (1 patient)	3 (1)	—	—	NS	2,3,4	21
4[a]	VM 180 mg/m² × 2 ADM 45 mg/m² × 2 (3 patients) L-PAM 140 + 70 mg/m² TBI 3 × 3.33 Gy	3 (3)	—	1	Severe mucositis	11+,12+,32+	6

[a]Two allogeneic bone marrow transplantations.
See Table 1 footnote for abbreviations.

TABLE 8C. *Rhabdomyosarcoma*

No. patients	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
4	CTX 1.2 g/m² × 4 DTIC 250–500 mg/m² × 4 ADM 20 mg/m² × 3	4 (2)	—	—	Severe diarrhea	1,3+, 6+,14	21
1	L-PAM 160 mg/m²	1 (1)	—	—	NS	NS	2
1	TACC-1	1 (0)	—	—	NS	NS	27

See Table 1 footnote for abbreviations.

TABLE 9. *Non-Hodgkin's lymphoma (including Burkitt's lymphoma) and Hodgkin's disease (HD)*

No. Patients	Disease	Therapy	No. (CR) response	NR	Treatment related death	Extramedullary toxicity	Response duration in months	Reference
4	NHL	TACC-1	4 (4)	—	—	—	9,18+,20,23+	
1	HD	TACC-1	1 (1)	—	—	—	1.5	27
1	HD	CCNU 200 mg/m² CTX 200 mg/kg ADM 60 mg/m²	1 (1)	—	—	—	3	
12	NHL	TACC-2	8 (8)	4	—	Pericarditis	3+–9+,11+, 11+,14+	29
2	NHL	CTX 50--60 mg/kg × 2--4 VLB 0.5 mg/kg × 1–2 TBI 8 Gy	2 (2)	—	—	Cardiorespiratory failure (50%) Mucositis (57%) Diarrhea (21%)	1,27+	20
14	NHL	BACT	10 (4)	—	4	Carditis	1–2,9+,19+, 29+	3
2	NHL	CTX 50 mg/kg × 3	2 (2)	—	—		24+,26+	
2	HD	BCNU 400 mg/m² × 3	2 (0)	—	—	NS	1,1	7
1	NHL	MMC 60 mg/m²	1 (0)	—	—	NS	1.5	61
18	NHL[a]	BACT (6 patients TBI 10 Gy)	7 (7)	—	4		NS	52
4	HD	BACT	4 (?)	—	—	NS	12+,12+	
3	NHL[b]	CTX 1.5 g/m² × 3	3 (1)	—	—	NS	2, 2, 4+	
2	HD	VP 150 mg/m² × 4 BCNU 300 mg/m²	2 (1)	—	—		5,18+	77
3	NHL[b]	CTX 2.4 g/m² × 3 TBI 1.7 Gy × 6	2 (2)	1	—	NS	4+,18+	
5	NHL	CTX 60 mg/kg × 2 TBI 10 Gy (1 patient ADM 80 mg/m²)	5 (5)	—	—	Hepatitis (20%) Carditis (20%)	1.7,6,12.9, 14.5,27+	35

[a] 11 patients in CR at the beginning of therapy.
[b] 1 patient allogeneic bone marrow transplantation.
See Table 1 footnote for abbreviations.

of the therapy after remission induction is the low risk of contamination of the bone marrow by tumor. The number of stem cells, determined by CFU-c is at that time still sufficient (56). Tumors suitable for this approach are SCLC, breast cancer, lymphoma, and ovarian cancer.

The second approach is the application of high-dose chemotherapy in the situation of primary or secondary tumor resistance. In this respect, it is interesting that a high response rate was found in melanoma (Table 7) and colon cancer (44). The response duration however is almost always short. Despite this, the susceptibility of tumors, always considered to be resistant to chemotherapy, warrants further studies with this treatment modality in patients in good condition.

Toxicity of single agent high-dose chemotherapy is rather well documented, but dose-limiting toxicity of combinations of suitable drugs, also in combination with TBI, has not been sufficiently evaluated. New phase I studies have to be performed to define this.

Can manipulation based on pharmacological data, noting that the toxicity of high-dose mitomycin C is related to the peak serum levels, lead for instance by a lower infusion rate to reduced toxicity (63)? In this respect new drugs should also be considered as candidates for high-dose chemotherapy.

The cleaning of bone marrow, for example, with monoclonal antibodies or incubation with cytostatic drugs, is fascinating but will not become of major importance for the treatment of solid tumors as long as long-term remissions are not yet the rule after intensive chemotherapy.

The use of intensive chemotherapy has a sound theoretical basis. The goal of its successful application in clinical oncology will require pharmacological studies, improvement of supportive therapy, and further studies on basic problems of tumor cell separation. However, the state of the art at this time suggests that this treatment modality could have some merit in the near future.

SUMMARY

This chapter extensively reviews in tabular form the up-to-date results of high-dose chemotherapy and ABMT in solid tumors. Antitumor responses have been clearly documented in small cell lung cancer, teratoma, ovarian cancer, breast cancer.. melanoma, colorectal cancer, lymphoma, gliomas, and various childhood tumors. Only a handful of randomized studies have been started and none is yet completed, so the eventual applications of the technique must be awaited.

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High-Dose Etoposide for Refractory Malignancies: A Phase I Study[1]

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Etoposide is active against a number of solid tumors when used in a standard dose. The toxicity at a standard dose level is mild myelosuppression without extramedullary toxicity. Recent studies in man support the dose-response relationship of etoposide. In a group of 22 patients with progressive disseminated malignancies, the dose of etoposide was escalated to define dose-limiting extramedullary toxicity, which was oropharyngeal mucositis at a dose level of 3.5 g/m². Bone marrow toxicity was completely reversible. No cumulative toxicity was seen. Partial responses were seen in nine patients. In two of three patients with CNS metastases, improvement was seen. Etoposide is a suitable drug for high-dose chemotherapy. [Cancer Treat Rep 68:1471–1474, 1984]

Etoposide, a semisynthetic podophyllotoxin derivative, is active against a number of solid tumors in a "standard" dose of 200-400 mg/m² (1). This standard dose was determined in phase I studies; dose-limiting toxicity in these studies was myelosuppression of modest degree and short duration (2,3).

Currently in clinical practice, much more severe hematologic toxicity is accepted (eg, in the treatment of acute leukemia and small cell lung cancer [SCLC]). This is due to the possibilities of improved supportive care, including prevention from infection (4), platelet transfusion, and feeding (5,6). From various experimental in vivo studies, there is evidence that for most cytostatic drugs, a positive dose-response relationship can occur at dose levels above the standard dose (7).

Etoposide is an ideal drug for a dose augmentation study, because increasing the dose up to 1.5 g/m² in patients with acute leukemia resulted in temporary myelosuppression and mild extramedullary toxicity (8). Furthermore, a dose-dependent activity against Lewis lung carcinoma and a human leukemic lymphoblast cell line was found in vitro (9,10), while in vivo data suggest a dose-response relationship in patients with germ cell malignancies (11) and SCLC (12).

At a standard dose, the activity of etoposide seems to be schedule-dependent (13); therefore, etoposide is generally given in a fractionated schedule. The optimal way of fractionation is unknown. To evaluate more extensively the dose-limiting toxicity, we started a phase I study of etoposide administered iv two times a day for 3 consecutive days.

MATERIALS AND METHODS

Patients

From November 1982 to September 1983, 22 patients with disseminated malignancies were entered in a phase I study with high-dose etoposide. Criteria for eligibility in the study were: age < 70 years, leukocyte count ≥ 3.0 × 10^9/L, platelet count ≥ 100 x 10^9/L, no signs of bone marrow involvement, Karnofsky score ≥ 50, normal renal function (serum creatinine ≤ 150 μmols/L), normal bilirubin (≤ 25 μmols/L), and no effective therapy available. Informed consent was obtained for all patients.

Etoposide Administration

Etoposide was diluted in normal saline to a maximum concentration of 0.8 mg/ml and administered iv in 1 hour. Single doses > 800 mg were given in 1.5 hours over 3 consecutive days with six infusions of equal amounts at 12-hour intervals.

The starting dose of this study was 1.0 g/m². Dose escalations were 0.5 g/m², and were to proceed if no unacceptable toxicity was seen in any of three patients at that level. Unacceptable toxicity, using the World Health Organization (WHO) grading system (14), was defined as oral

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TABLE 1.—Grading of observed toxic effects*

Toxic effect	Grade — 0	1	2	3	4
Oral	No change	Soreness/erythema	Erythema, ulcers, can eat solids	Ulcers, requiring liquid diet only	Alimentation not possible
Diarrhea	None	Transient, ≤ 2 days	Tolerable, but > 2 days	Intolerable, requiring therapy	Hemorrhagic dehydration
Cutaneous	No change	Erythema	Dry desquamation, vesiculation, pruritis	Moist desquamation, ulceration	Exfoliative dermatitis, necrosis requiring surgical intervention
Neurotoxicity	None	Paresthesias and/or decreased tendon reflexes	Severe paresthesias and/or mild weakness	Intolerable paresthesias and/or marked motor loss	Paralysis

* Grades are according to WHO criteria (ref 14).

mucositis (grade 4), diarrhea (grade 3), dermatitis (grade 4), and neurotoxicity (grade 3) (table 1). To be evaluable for toxicity, the patient had to survive for at least 4 weeks after the start of treatment.

All patients received etoposide on an inpatient basis. Selective decontamination of the digestive tract (4) with oral antibiotics and oral amphotericin B was used to prevent infection. Platelet transfusions were given when bleeding was present or prophylactically if the thrombocyte count was $< 15 \times 10^9$/L. Infection days were defined as days with fever ≥ 38.5 °C (axillary) and the presence of either clinically or bacteriologically documented infections.

If no disease progression was present and no unacceptable toxicity was seen, the patient was treated with another course in the same dosage.

Response

To be evaluable for response, the patient had to be followed up at least 4 weeks after the start of treatment.

Complete remission was defined as the disappearance of all signs and symptoms of detectable tumor. Partial remission was defined as a decrease in the size of at least 50% of the sum of the products of the largest perpendicular diameters of measurable lesions. If a decrease in the level of an established tumor marker (eg, $alpha_1$-fetoprotein or beta-human chorionic gonadotropin) of > 90% was seen in patients without measurable or evaluable lesions, this was also considered a partial response. Minor response was defined as a decrease of a measurable lesion between 25% and 50%. Stable disease was defined as a decrease or increase of a measurable lesion < 25%. Disease progression was defined as the growth of measurable lesions. Subjective response was defined as improvement of neurological symptoms of CNS metastases, improvement of dyspnea due to pulmonary lymphangitis, improvement of pain due to tumor involvement, and normalization of hypercalcemia.

RESULTS

Patients

Of 22 patients entered in the study, there were 15 males and seven females, with a mean age of 47 years (range, 21-69). Nineteen patients were heavily pretreated with combination chemotherapy with or without radiotherapy. In ten patients, the standard dose of etoposide was included in combination chemotherapy. Nine patients received more than one course of etoposide; six of these patients received more than two courses.

Nonhematologic Toxicity

Moderate nausea and vomiting, controllable by antiemetic therapy, was present in 11 patients (50%). Diarrhea did not occur. Mucositis seems to have been dose-related (table 2) and was dose-limiting in one patient after

TABLE 2.—Mucositis

Dose of etoposide (g/m^2)	No. of courses (No. of patients)	Grade* — 0	1	2	3	4
1.0	9 (5)	5	4	—	—	—
1.5	9 (4)	3	5	1	—	—
2.0	10 (4)	2	6	2	—	—
2.5	5 (4)	—	—	4	1	—
3.0	3 (3)	—	1	—	2	—
3.5	2 (2)	—	—	—	1	1

* Grades are according to WHO criteria (ref 14).

3.5 g/m². Mucositis was completely reversible within the period necessary for bone marrow recovery. Routine virological investigations revealed a herpes simplex infection in only three patients. Two of these patients had typical herpes lip lesions, which were different from the mucosal damage seen in the other patients. All patients without alopecia due to prior therapy had hair loss. No neurotoxicity occurred, nor did pre-existing neuropathy due to prior chemotherapy increase. In one patient after the first course of etoposide (2.0 g/m²), skin lesions developed on the neck, thorax, and arms comparable to the recently described Stevens-Johnson syndrome (15). There were no signs of acute or cumulative hepatic toxicity. Somnolence was not seen.

In the patients receiving more than one course, no signs of cumulative toxicity with respect to mucositis or bone marrow function were observed.

In one patient, hypotension and fever were seen shortly after the second infusion (3.0 g/m²); the following four infusions were given slower (2 hours), with no complications.

Hematologic Toxicity (table 3)

The duration and degree of leukopenia and thrombocytopenia did not increase with dose escalation. The moment of bone marrow recovery, defined as a leukocyte count $> 1.0 \times 10^9$/L and a thrombocyte count $> 50 \times 10^9$/L, is not postponed at the highest dose level, although the number of platelet transfusions was slightly increased. In ten of 38 courses, a clinically or bacteriologically documented infection was seen, which responded to antibiotic treatment.

Response (table 4)

Seventeen patients were evaluable for response after one course. There were no complete remissions. Nine patients had partial response, one had a minor response of cutaneous metastases of adenocarcinoma of the lung, and four had stable disease. Two patients had disease progression. One patient with a germ cell tumor had a good partial remission of systemic tumor, but progression of symptoms of meningeal carcinomatosis. In one patient with bone metastases, hypercalcemia normalized for a short period. In two patients without an evaluable tumor lesion, subjective improvement was seen: dyspnea due to pulmonary lymphangitis disappeared in one patient; the second patient had major improvement of pain and pareses due to meningeal carcinomatosis of SCLC for > 6 months.

DISCUSSION

In this study, the dose-limiting toxicity of high-dose etoposide, administered iv on 3 consecutive days, was oropharyngeal mucositis, occurring at 3.5 g/m². The severity of the mucositis seems dose-related, although there is a rather great difference between individuals. The mucositis of the oropharyngeal region could be influenced by a local effect of etoposide in the saliva (16). The influence of herpes simplex on the mucositis is uncertain; our data concerning this aspect seem to exclude an important influence. Wolff et al (17) found dose-limiting mucosal toxicity at a total dose of 2.7 g/m². This dose is lower than the total dose per cycle used in our study. This difference can possibly be explained by the different administration schedules used. Wolff et al used a schedule in which the total dose was divided into three infusions, probably leading to higher peak plasma and saliva levels of etoposide. Clinical signs of mucositis of the lower part of the digestive tract were not seen.

The hematologic toxicity was severe, but not dose-dependent. The duration of leukopenia and thrombocytopenia was not long enough to expect any benefit from autologous bone marrow transplantation (ABMT) as was done by Wolff et al (17). Moreover, the moment of bone marrow recovery in a comparable study using high-dose cyclophosphamide and high-dose etoposide with ABMT (18) was considerably postponed compared to the patients discussed in this study.

Several responses were seen. The assumption that a

TABLE 3.—Hematologic toxicity

Dose of etoposide (g/m²)	No. of courses (No. of patients)	Mean No. of days (range) with leukocyte count* — ⩽ 0.5	⩽ 0.1	Mean No. of days (range) with platelet count ⩽ 20*	Mean No. of platelet transfusions (range)	Mean cycle Day (range)† with leukocyte count > 1.0* and platelet count > 50*	No. of infection days‡
1.0	9 (5)	5.1 (0-10)	0.6 (0-3)	1.0 (0-3)	0.7 (0-2)	19 (17-21)	1.2 (0-4)
1.5	9 (4)	5.0 (0-8)	0	3.1 (1-7)	1.0 (0-2)	19 (19-21)	0.4 (0-3)
2.0	10 (4)	2.4 (0-9)	0.6 (0-6)	3.2 (1-8)	1.8 (1-4)	18 (16-18)	1.8 (1-4)
2.5	5 (4)	5 (1-10)	0.4 (0-1)	3.0 (1-4)	2.0 (1-3)	17 (16-18)	2.2 (0-6)
3.0	3 (3)	10.0 (9-11)	2.0 (1-5)	5.3 (4-7)	2.6 (2-3)	23 (21-26)	3.0 (2-4)
3.5	2 (2)	6.5 (6-7)	2.0 (1-3)	3.5 (3-4)	1.5 (1-2)	17	5.5 (4-7)

* × 10^9 cells/L.
† Day 1 = 1st day of chemotherapy.
‡ Axillary temperature ⩾ 38.5°C.

TABLE 4.—Response after 1 course

Diagnosis	No. of patients*						Duration of response (mos)
	Total	PR	MR	SD	DP	NE	
Breast cancer	4	1			1	2†	3
Adenocarcinoma, lung	1		1				2
Adenocarcinoma, unknown origin	1				1		—
Endometrium carcinoma	1			1			—
Squamous carcinoma, lung	1			1			—
SCLC	7	3		2		2	1, 2, 4
SCLC, CNS metastases only	2	1				1‡	3
Germ cell tumor	5	5§					1, 1, 1.5, 1.5, 2

* PR = partial remission; MR = minor response; SD = stable disease; DP = disease progression; NE = not evaluable.

† Improvement of dyspnea due to pulmonary lymphangitis, normalization of hypercalcemia.

‡ Improvement of symptoms due to meningeal carcinomatosis.

§ 1 patient with mixed response: progression of meningeal carcinomatosis, but regression of tumor elsewhere.

dose-response relationship exists for etoposide is supported by the short-term response seen in a patient with adenocarcinoma of the lung, since the tumor in this patient was progressive during standard-dose etoposide; a nearly partial remission was observed of several cutaneous metastases. Also, the effects seen in three breast cancer patients are promising; although the number of patients is small, the response rate is higher than that expected from standard-dose therapy (8% response rate) (1). An unexpected effect of high-dose etoposide was the response of CNS metastases in two patients with SCLC. Pharmacokinetic studies in these patients showed detectable levels of etoposide in the cerebrospinal fluid (19). In contrast with this is the progression of meningeal carcinomatosis in a patient with a germ cell tumor during a partial response of the tumor elsewhere. The assumed dose-response relationship and the effect against CNS metastases of SCLC make etoposide a suitable drug for incorporation in high-dose regimens. The dose suitable for further phase II studies seems to be 2.5 g/m^2. For studies on an outpatient basis or in combination regimens, 1.5 g/m^2 is probably the highest tolerable dose (18). Pharmacokinetic studies have to be performed to decide whether the administration scheme of etoposide in this study is optimal. Perhaps different schemes, such as continuous infusion, may be more effective.

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CHAPTER 5

PHARMACOKINETICS OF HIGH DOSE ETOPOSIDE (VP 16-213).

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SUMMARY.

This paper describes the pharmacokinetics of etoposide in cancer patients after high dose administration (up to 3.5 g/m^2). High performance liquid chromatography with electrochemical detection was used to determine etoposide, the trans hydroxy acid of etoposide, cis-etoposide and the glucuronide of etoposide in plasma, bile, cerebro-spinal fluid, urine, saliva and ascites. The plasma concentration-time curve shows a tri-phasic decay. The terminal phase is very slow. It was concluded that etoposide is strongly bound in the peripheral compartment. The volume of the central compartment varied from 7.4 to 20.1 liters/m^2 and the steady state volume of distribution from 3.1 to 7.8 liters/m^2. Relatively high concentrations of etoposide were found in saliva, bile, ascites and urine and low concentrations in cerebro-spinal fluid. The total body clearance varied from 12.0 to 26.8 ml/min/m^2. Of the administered dose 26.12 - 53.4% was excreted as unchanged etoposide into the urine and 8.3 - 17.3% as glucuronide into the urine. Very low amounts of the trans hydroxy acid of etoposide were detected in the urine. Glucuronides were found in urine and duodenal fluid but not in plasma.

INTRODUCTION.

Etoposide is a semi-synthetic podophyllotoxin derivative, active against a variety of solid tumors (1). Under physiological conditions the drug is neutral and has a high lipophylicity. The standard dose, 200-400 mg/m^2, was defined in phase I studies (2, 3). At this dose level pharmacokinetic studies showed an open two-compartment model (4-8).

The limited toxicity, primarily myelosuppression, of etoposide at the standard dose level makes it a suitable drug for much higher dosages. Dosages up to 2.5 g/m^2 are considered to be possible without serious

Table 1. Survey of patients.

Patient	Sex	Age Yrs	Body weight kg	Body surface m^2	Total dose g/m^2	Diagnosis
A.P.	M	53	72	1.8	0.5	TCC
O.L.	M	47	55	1.7	0.5	GC
G.F.	M	56	65	1.9	1.0	SqCLC
L.M.	M	55	98	2.4	1.5	SCLC
N.S.	M	53	60	1.7	1.5	SqCLC
R.D.	M	56	88	2.2	2.0	SCLC
H.S.	M	48	83	2.2	2.5	AC
H.H.	M	40	86	2.1	2.5	GCC
J.M.	M	41	76	1.8	2.5	GCC
J.J.	M	21	92	2.2	2.5	GCC
J.K.	M	50	95	2.2	3.0	SCLC
L.G.*	M	48	82	2.1	3.5	GCC
F.V.	F	71	62	1.8	1.0	OC
P.M.	M	21	88	2.0	3.5	GCC

SCLC : small cell lung carcinoma
TCC : transitional cell carcinoma
SqCLC : squamous cell lung cancer
GCC : germ cell carcinoma
AC : adeno carcinoma
GC : gastric cancer
OC : ovarian cancer
* : received diphantoine

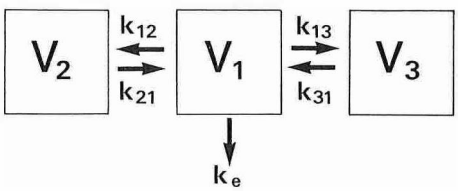

Fig. 1. Proposed pharmacokinetic model for etoposide after high dose i.v. administration to humans.

extra-medullary toxicity (9, 10). The pharmacological behaviour of the drug and its metabolites at this dose level are interesting, because if other myelotoxic drugs are combined with high dose etoposide, the use of autologous bone marrow transplantation may become necessary (11). The moment of bone marrow reinfusion depends in this situation on the level of drugs still present in the body.

In the only up till now reported study of the pharmacokinetics of high-dose etoposide, the distribution and excretion were apparently not influenced by the dose (12). In that study, however, data beyond 24 hours after the infusion were lacking and possible metabolites were not reported.

In this report we describe the results of a more extensive pharmacological study of high-dose etoposide.

MATERIALS AND METHODS.

Patients and therapy.

Fourteen patients with persistent or progressive malignant disease were studied (table 1). All patients had normal renal and liver functions, a Karnofsky score > 50, and gave informed consent.

All patients received etoposide on three consecutive days with six infusions at twelve hours interval. Etoposide was dissolved in normal saline, with a maximum concentration of 0.8 mg/ml, and administered i.v. in 1 hour. Single doses over 800 mg were given in 2 hours (10).

One patient (L.M.) also received cyclophosphamide in a total dose of 7 g/m^2.

Drugs and chemicals.

Etoposide (Vepesid^R) and teniposide (Vumon^R) were obtained from Bristol Myers Nederland B.V.. All other chemicals were of analytical grade and obtained from commercial sources and were used without further purification.

Sampling.

Blood plasma was collected by an in-dwelling heparine lock which was placed in an arm vein opposite to the infusion site, Samples of 5 ml were collected in heparinized tubes and plasma was removed after centrifugation for 10 minutes at 4^oC. The intention was to take blood

plasma samples at 0, 1, 1.25, 1.5, 2, 3, 5, and 12 hours after each administration and also at 24, 36, 60, 84, 108, 132, 156 and 180 hours after the last administration. There were a few slight deviations from this sampling scheme. The exact sampling times were used for pharmacokinetic evaluation.

The urine excreted within 3 to 6 hour periods was collected and after homogenizing and establishing of the volume, a 100 ml sample was stored. The urine was collected for up to 168 hours after the first administration of etoposide.

Saliva samples were collected from a few patients at the same time as the plasma samples. To collect saliva the patient was asked to chew on a tablet of teflon and 2 ml of the produced saliva was stored.

Duodenal fluid was aspirated from the proximal part of the duodenum via a polyvinyl chloride tube already inserted for nutritional purposes. The position of the tube was controlled by fluoroscopy.

Cerebro-spinal fluid (CSF) samples of 1.5 ml were taken from an Omaya reservoir.

Ascites fluid samples of 1.5 ml were taken during diagnostic or palliative percutane puncture.

All samples were stored at -20^{o}C until assayed.

Analysis.

Etoposide levels in biological fluids were measured by high performance liquid chromatography (HPLC) with electrochemical detection, as previously reported (13). At concentrations lower than 250 ng/ml, 1.0 ml of biological fluid was extracted with 2 ml 1,2-dichloroethane (DCE), at higher concentrations 0.1 ml of the biological fluid was sufficient for extraction with 1.0 ml DCE. Teniposide, a structure-analog of etoposide, was used as internal standard. The calibration samples were prepared by addition of etoposide to drug free biological fluids. These samples were analyzed simultaneously.

Etoposide glucuronide was determined by the analysis of etoposide after the enzymatic hydrolysis of the etoposide glucuronide. Prior to the hydrolysis with β-glucuronidase from bovine liver (Sigma Chemical Company), the urine (pH 7.0) was pre-extracted twice with a 6 fold amount of DCE. An amount of 100 ul of the pre-extracted urine was added to 1.0 ml 0.2 mM acetate buffer (pH 5.0) containing 2000 U β-glucuronidase per

ml. The mixture was incubated at 37°C during 16 hours. Etoposide which was liberated during the incubation was determined as described above. For the determination of the trans hydroxy acid of etoposide in urine, 25 µl of the pre-extracted urine is injected onto the HPLC system. Electrochemical detection at +750 mV was used. The mobile phase consisted of a mixture of water, methanol and acetic acid (73 : 25 : 2 w/w). An µBondapak phenyl column and a flow of 1.0 ml/min was used. The analytical methods used for the detection of these metabolites are extensively described in reference 14.

Pharmacokinetic data analysis.

Etoposide concentration-time curves are presented on a semi-logarithmic scale. The pharmacokinetic data analysis was performed with a NON-LIN computer program (15) extended by means of a Dirac Delta function to fit multiple dose data (16). It was assumed that the disposition of etoposide did not vary from administration to administration. The log plasma concentration-time curves were fitted to an open three compartment model with elimination from the central compartment (fig. 1). From the fitting procedure the following parameters were obtained: V_c, the volume of the central compartment; k_e apparent first order elimination rate constant from the central compartment; the apparent first order intercompartmental transfer rate constants, k_{12}, k_{21}, k_{13} and k_{31}. The rate constants α, β and γ and other pharmacokinetic parameters were calculated as described by Gibaldi and Perrier (17). The area under the plasma concentration-time curve (AUC) was calculated by numerical integration using the trapezoidal rule, from zero to the last measured concentration (< 0.2% of the peak concentration). The area under the moment curve (AUMC) (AUMC = (c x t) versus t) was calculated using the linear trapezoidal method. Total body clearance (Cl_{tot} in ml/min/m^2) for the multiple iv infusions was calculated by dividing the total dose (D in mg) by the total AUC (mg.hr/l) and by the body area (m^2) of the patient. The renal clearance (Cl_{ren}) was calculated by dividing the total excreted amount of unchanged etoposide (in mg) in the urine by the AUC. The clearance due to the renal excretion of etoposide glucuronide (Cl_{gluc}) was calculated by dividing the total amount of etoposide excreted as glucuronide, by the AUC. The

Table 2. Pharmacokinetic parameters obtained from the curve fitting of the plasma concentration time curves of each patient.

Patient	k_e hr^{-1}	k_{12} hr^{-1}	k_{21} hr^{-1}	k_{13} hr^{-1}	k_{31} $hr^{-1} \times 10^{-4}$	V_c l
A.P.	0.1798	0.7120	0.6833	0.2608	7.4742	7.4
O.L.	0.1283	0.1586	0.1552	0.0778	0.9415	10.9
G.F.	0.0675	0.0185	0.0173	0.0825	3.5280	13.2
L.M.	0.1573	0.0151	0.0509	0.1359	0.0554	12.6
N.S.	0.1010	0.0140	0.0515	0.0763	0.0008	11.9
R.D.	0.0981	0.0293	0.0439	0.0829	0.1216	12.7
H.S.	0.0660	0.0253	0.0863	0.0867	0.3170	12.6
H.H.	0.1877	0.0296	0.0471	0.1599	0.0017	9.6
J.M.	0.0896	0.0162	0.0543	0.0709	0.0015	13.8
J.J.	0.0906	0.5064	0.8523	0.0869	4.6550	10.5
J.K.	0.1108	0.0020	0.0067	0.0875	0.0095	14.7
L.G.	0.1341	0.0167	0.0327	0.0270	0.4316	20.1
mean ± s.d.						12.5 ± 3.1
median						12.6
range						7.4 - 20.1

Patient	α hr^{-1}	β hr^{-1}	γ $hr^{-1} \times 10^{-4}$	$V_d\ \beta$* l	$t_{½\,\alpha}$ hr	$t_{½\,\beta}$ hr
A.P.	1.6520	0.1826	3.0400	7.3	0.42	3.80
O.L.	0.4486	0.0713	0.5860	19.6	1.55	9.72
G.F.	0.1707	0.0152	1.58	56.0	4.06	45.59
L.M.	0.3113	0.0479	0.0297	41.4	2.23	14.47
N.S.	0.1962	0.0465	0.0005	25.9	3.53	14.90
R.D.	0.2176	0.0363	0.0659	34.2	3.18	19.09
H.S.	0.1975	0.0667	0.1370	12.5	3.51	10.39
H.H.	0.3813	0.0429	0.0009	42.0	1.82	16.15
J.M.	0.1835	0.0475	0.0009	26.0	3.78	14.59
J.J.	1.430	0.1060	2.37	9.0	0.49	6.54
J.K.	0.2003	0.0070	0.0053	246.3	3.46	99.00
L.G.	0.1814	0.0290	0.3590	92.9	3.82	23.90
mean ± s.d.					2.65±1.31	23.18±26.17
median					3.46	14.90
range					0.42-4.06	3.80-99.00

$$* V_d\,\beta = \frac{k_e \times V_c}{\beta}$$

model-independent steady state volume of distribution (Vd_{ss}) and the mean residence time (MRT) were calculated for multiple infusions, as described by Perrier et al. (18). MRT was corrected for the infusion time.

RESULTS.

Plasma concentrations of etoposide and metabolites.

In 12 patients it was possible to measure plasma levels during and after the six infusion. The peak levels after the six infusions were not different, suggesting that cumulation did not occur.

After the first five infusions a biphasic decay was seen, however, after the sixth infusion a triphasic decay was found. The third phase was detectable after approximately 24 hours after the sixth administration. The plasma concentrations after 168 hours from the start of the infusions varied between 10 and 250 ng/ml. A representative curve is shown in figure 2.

The first order mass transfer rate constants, the distribution and elimination rate constants are shown in table 2. A linear relation (fig. 3) was found between the total administered dose and the AUC up to a dose of 3 g/m^2 ($r = 0.9451$). The level of cis-etoposide in the plasma is less than 5% of the concentration of etoposide. Other metabolites, i.e. the glucuronide of etoposide and the transhydroxy acid derivative of etoposide, were not found.

Renal excretion of etoposide and its metabolites.

The total body clearance (Cl_{tot}), the clearance due to the renal excretion of etoposide (Cl_{ren}) and the glucuronide of etoposide (Cl_{gluc}) are shown in table 3. The amount of unchanged etoposide in the urine varied between 26.2 and 53.4% of the total administered dose, whereas the amount of the glucuronide of etoposide in the urine was 8.3-17.3% of the total dose. Together 34.5-66% of the amount of etoposide is excreted into the urine.

The amount of the transhydroxy acid derivative of etoposide in the urine is very small (table 4). The amount of the cis isomere is also < 1% of the administered dose.

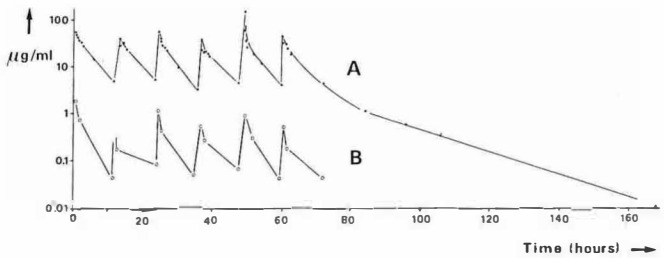

Fig. 2. Log plasma (A) and log saliva (B) concentrations vs. time curve after administration of 2 x 500 mg and 4 x 400 mg etoposide (Patient N.S.).

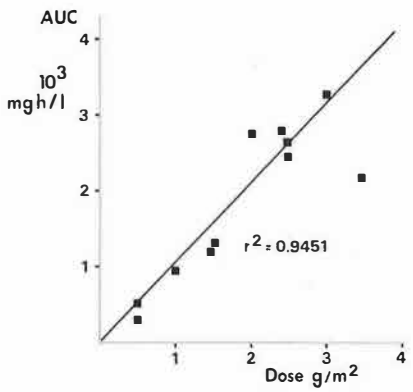

Fig. 3. Relation between the total area under the plasma concentrations vs. time curve (AUC) versus the total administered dose.

Table 3. Model-independent pharmacokinetic parameters.

Patient	AUC	Cl_{tot}	Cl_{ren}	Cl_{gluc}	Dose	MRT	Vd_{ss}
	mg.hr/l	ml/min/m²	ml/min/m²	ml/min/m²	g	hr	l/m²
A.P.	311	26.3	14.3	3.4	0.90	3.14	3.1
O.L.	510	16.2	6.0	2.8	0.84	4.63	4.5
G.F.	975	16.8	4.4	1.4	1.86	4.17	4.2
L.M.	1211	20.6	5.6	2.4	3.60	3.14	4.2
N.S.	1355	18.8	7.1	2.3	2.60	4.28	5.1
R.D.	2786	12.0	n.d.	n.d.	4.50	5.36	4.8
H.S.	2802	14.1	5.9	2.0	5.20	4.84	4.1
H.H.	2465	17.4	8.9	2.5	5.40	4.32	4.4
J.M.	2687	15.5	n.d.	n.d.	4.50	4.75	5.3
J.J.	2517	13.5	n.d.	n.d.	4.50	4.52	3.7
J.K.	3317	15.1	6.4	1.5	6.60	3.96	4.7
L.G.	2193	26.0	8.2	4.4	7.20	5.17	7.8
mean±s.d.	—	17.7± 4.7	7.4±2.9	2.5±0.9	—	4.36±0.70	4.7±1.2
median	—	16.8	6.4	2.4	—	4.42	4.5
range	—	12.0-26.8	4.4-14.3	1.4-4.4		3.14-5.17	3.1-7.8

$$Cl_{tot} = \frac{\text{Total D (mg)}}{\text{Total AUC x B.A.}} \qquad Cl_{ren} = \frac{X_u}{\text{Total AUC x B.A.}} \qquad Cl_{gluc} = \frac{X_u}{\text{Total AUC x B.A.}}$$

X_u = Amount (mg) of unchanged etoposide or etoposide glucuronide excreted into the urine
n.d. = not determined
B.A. = body area (m^2)

$$Vd_{ss} = \frac{D}{AUC}\left(\frac{AUMC}{AUC} - \frac{\int_0^\infty X.dt}{\Sigma D}\right) \qquad MRT = \frac{AUMC}{AUC} - \frac{\int_0^\infty X.dt}{\Sigma D}$$

$$\frac{\int_0^\infty X.dt}{\Sigma D} = \frac{(k_o.T^2/2)_1+(k_o.T^2/2)_2+(k_o.T^2/2)_3+(k_o.T^2/2)_4+(k_o.T^2/2)_5+(k_o.T^2/2)_6}{(k_o.T)_1+(k_o.T)_2+(k_o.T)_3+(k_o.T)_4+(k_o.T)_5+(k_o.T)_6}$$

The subscript refers to the infusion

T = infusion time (hr)
k_o = zero order infusion rate (mg/hr)
D = dose in mg = $k_o.T$

Table 4. Excretion of etoposide (% of the total dose) as the trans hydroxy acid derivative of etoposide.

Patient	% of total dose	Patient	% of total dose
A.P.	1.1	H.S.	0.7
O.L.	0.9	H.H.	1.2
G.F.	0.5	J.K.	0.2
N.S.	0.4	L.G.	2.2

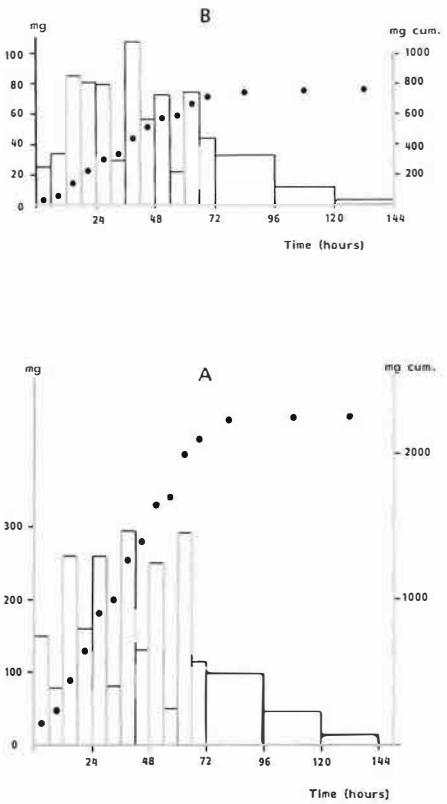

Fig. 4. Cumulative urinary excretion curve of unchanged etoposide (A) and its glucuronide (B) after administration of 4 x 900 mg and 2 x 800 mg etoposide (Patient H.S.).

In all patients the plateau of the cumulative excretion was reached within 12 hours of the last infusion. An example of this is shown in figure 4.

Excretion of etoposide into saliva.

The amount of etoposide in the saliva is small; in table 5 the saliva/plasma concentration ratios (s/p) are shown and in figure 2 an example of plasma and saliva curves is shown. These ratios were in the distribution and elimination phase not different. The curves of the saliva and plasma concentrations run in parallel (fig. 2). The amount of etoposide bound to plasma albumin has been calculated by multiplicating (1-s/p) by 100% (see table 5).

CSF concentrations of etoposide.

In patient L.G. CSF levels were measured several times during the different infusions (fig. 5). The amount of etoposide found in the CSF is small. The curves of figure 5 suggest that the penetration of etoposide into CSF is slower than the distribution into the central compartment, during the second phase the curves run in parallel.

Etoposide concentrations in bile.

In one patient concentrations of etoposide and its metabolites were measured in duodenal fluid during one infusion. The concentration of etoposide was 2-3 times higher than in plasma. The concentration of etoposide-glucuronide was about 1/10 of the etoposide concentration. The peak of etoposide-glucuronide appeared 45 minutes after the etoposide peak in the duodenal fluid. The curves are shown in fig. 6.

Penetration of etoposide into ascites.

In one patient etoposide concentrations were measured simultaneously in plasma and ascites. The distribution occurs at a lower rate than in plasma as is shown in figure 7. Regarding the somewhat higher levels in the ascites during and after the fifth etoposide infusion compared with the first infusion there is a slower clearance from the ascites than from plasma, resulting in cumulation.

Table 5. Saliva concentration/plasma concentration ratios of etoposide in different patients.

Patient	saliva/plasma ratio x 100%	s.d.	n	total dose g/m^2	"bound" %
A.P.	1.85	0.63	6	0.5	98.2
N.S.	1.60	0.64	16	1.5	98.4
H.H.	1.68	0.53	11	2.5	98.3
J.M.	0.65	0.34	14	2.5	99.4
J.J.	1.39	0.56	36	2.5	98.6
L.G.	2.48	1.39	33	3.5	97.5
P.M.	1.79	0.62	8	2.5	98.2

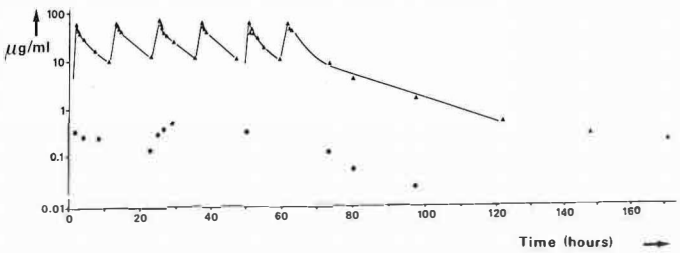

Fig. 5. Log plasma (▲) concentration and log CSF (●) concentration time curve after administration of 6 x 1200 mg etoposide (Patient L.G.).

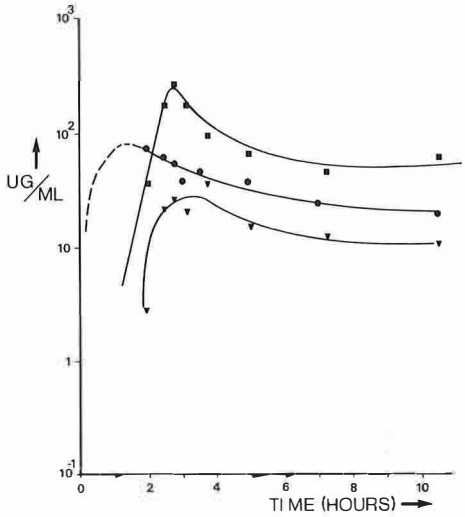

Fig. 6. Log plasma concentration (etoposide ●) and log duodenal fluid concentration (etoposide ■ and etoposide glucuronide ▼) vs. time curve after administration of 1200mg etoposide in a 2 hours' infusion (Patient P.M.).

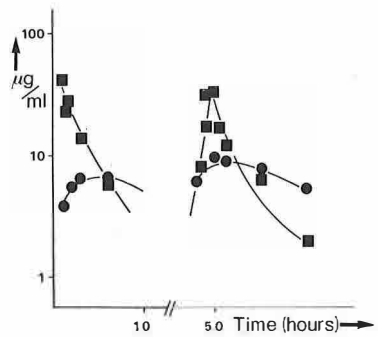

Fig. 7. Log plasma concentration (■) and log ascites (●) concentration time curve for etoposide after administration of 300 mg in a 1 hours' infusion (Patient F.V.) at 0 and 48 hr.

DISCUSSION.

In this study a biphasic decay of the plasma concentrations was seen during the first five infusions. After the sixth infusion a triphasic decay was found; the time between two infusions was too short to find this third phase. The obtained plasma concentration curves were fitted to an open three compartment model with elimination from the central compartment, using a computer program which enables multiple dose data to be fitted. The data from this fitting process indicate that the half life time of the third phase is very long, the small number of data obtained during this period makes it not realistic to calculate this half life time.

The found three compartment model is in contrast with data from the literature. Pharmacokinetic studies (4-8, 12) of oral, standard dose i.v. or high dose i.v. etoposide all show an open two compartment model, however, in all studies the sensitivity of the assay is too low or the study period too short to detect a third phase.

The rate of influx into the peripheral compartment during the third phase, represented by k_{13}, is very large compared with the rate of efflux back into the central compartment, k_{31}; this indicates that etoposide is almost irreversibly bound to the tissue in this compartment. This strong binding in the peripheral compartment partly explains the limited excretion of etoposide and metabolites into the urine; in a study using radio-labelled etoposide only 44% of the given amount was found in the urine within 72 hours (4, 5).

The total body clearance (Cl_{tot}) found in this study is lower than in the high dose study of Hande et al. (12). This is explained by the underestimation of the AUC caused by extrapolating the second phase to infinity. The MRT found is comparable to the MRT after low dose etoposide (19).

The amount of etoposide excreted into the urine indicates that this is a significant elimination pathway, although the interindividual variation is high. The amount of etoposide excreted after high dosages makes it probably necessary to adjust the dose in patients with renal impairment whereas with low dose etoposide this is not necessary (20).

Owing to the physico-chemical properties of etoposide, e.g. a lipophylic compound with some hydrophylic groups, its low solubility and

its high molecular weight, it is likely that another part will be eliminated by the liver and excreted into the bile. This is supported by the presence of etoposide in the faeces after parenteral administration (5); in rats etoposide and its glucuronide were found in the bile (21). In one patient we detected higher levels of etoposide in the duodenal fluid than in plasma, this reflects the concentration of etoposide in the bile and proves that etoposide is actively excreted by the liver into the bile. The amount of the glucuronide of etoposide found in the duodenal fluid was ten times lower than the etoposide concentration and its excretion was delayed compared to etoposide.

The excretion of etoposide into the duodenum could be the start of an entero-hepatic cycle, however, the plasma concentration curve does not indicate the absorption of etoposide in the period between two infusions. This is in contrast with the known absorption of orally administered etoposide (22), although the absorbed amount can be 40% of the given dose.

An important elimination pathway might be the metabolism of etoposide. Although the exact metabolic pattern of etoposide is unknown, in this study glucuronidation proved to be a major metabolic pattern, whereas the trans hydroxy acid derivative was found in small amounts in the urine. The importance of the metabolism for the elimination was even more striking in patient L.G.; the metabolism probably was enhanced by the concomittant use of diphantoine resulting in the increased synthesis of metabolising enzymes. In this patient the AUC was small and deviated from the linear relation between the total dose and the AUC found in other patients (fig. 3). The amount of etoposide glucuronide and the trans hydroxy acid derivative in the urine was large in comparison with the other patients.

The excretion of etoposide into the saliva can not be influenced by pH variations, it depends on the lipophylicity and the binding to plasma proteins. The excretion into the saliva is fast and the salivary glands probably belong to the central compartment. From the parallel plasma and saliva concentration curves one can assume that the saliva concentrations are a reflection of the amount of free etoposide, the major part (mean 98.4 $\pm$ 0.57%) is bound to protein. This is even higher than was found in vitro (94%) by Allen et al. (4).

The penetration of etoposide into the CSF is lower than was expected from the lipophylicity of etoposide, this is probably explained by the already mentioned high protein binding of etoposide. Despite the low levels in the CSF, the amount of etoposide in the brain is apparently in the active range regarding the responses seen in patients with brain metastases of small cell lung cancer (23, 24).

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Penetration of VP 16-213 into Cerebrospinal Fluid After High-Dose Intravenous Administration

By P. E. Postmus, J. J. M. Holthuis, H. Haaxma-Reiche, N. H. Mulder, L. M. Vencken, W. J. van Oort, D. T. Sleijfer, and H. J. Sluiter

VP 16-213 in standard doses is active against a number of solid tumors. Its penetration into the cerebrospinal fluid (CSF) is very limited at these dose levels. In 10 patients treated with high-dose VP 16-213 (0.9–2.5 g/m^2), CSF levels of up to 0.54 μg/mL were detected. In two patients with central nervous system (CNS) metastases of small cell lung cancer (SCLC) a response was seen after 1.0 and 1.5 g/m^2 intravenously. High-dose VP 16-213 can possibly play a role in the treatment of CNS metastases of SCLC. Its application in late intensification regimens as a form of prophylaxis of CNS metastases should be investigated.

VP 16-213 is a semisynthetic podophyllotoxin derivative that is active against a number of solid tumors.[1,2] Major therapeutic activity for the drug has been found in small cell lung cancer (SCLC) and germ-cell malignancies.[3-6] Dosages up to 400 mg/m^2 showed minor, primarily hematologic toxicity.[1,2] Pharmacokinetic studies at these conventional dose levels in humans showed hardly any penetration of VP 16-213 into the cerebrospinal fluid (CSF).[7] Increasing the dosage of VP 16-213 up to 1,500 mg/m^2 leads to severe reversible myelosuppression in humans but only minor extramedullary toxicity.[8]

In two phase I studies on the feasibility of high-dose VP 16-213 in patients with progressive solid tumors, we investigated CSF levels and the effect against central nervous system (CNS) metastases from SCLC.

MATERIALS AND METHODS

Patients

All patients who entered the phase I studies were pretreated with standard therapy and had progressive disease at the time of entrance into the study (Table 1). Normal renal and hepatic function were entrance criteria for the phase I studies. Histologic diagnoses were: SCLC (seven patients), adenocarcinoma of the lung (one patient), squamous cell carcinoma of the lung (one patient), and nonseminomatous testicular cancer (one patient). Cranial radiotherapy (30 Gy) had been given several months before study entry in patients no. 1, 2, 6, 7, 8, and 9. In patients no. 3 and 4 cranial radiotherapy was given between the first and second chemotherapy course with VP 16-213.

All patients were considered to be at high risk for CNS metastases; in all patients with signs and/or symptoms of cerebral metastases the diagnosis was confirmed by computer assisted tomography.

Infection prevention during granulocytopenia was given by selective decontamination of the digestive tract.[9]

Chemotherapy

All patients received VP 16-213 during three consecutive days, the total dose was divided into six one-hour infusions with 12-hour intervals. VP 16-213 (Vepesid®; Bristol Laboratories, Syracuse, NY) was dissolved in normal saline with a maximum concentration of 0.8 mg/mL. In patients no. 1, 2, 3, 4, and 5 more than one course was given.

In patients no. 6 and 9 cyclophosphamide was also given in a total dose of 7 g/m^2. These patients took part in a second phase I study and underwent autologous bone-marrow transplantation.

Sampling

CSF was procured by lumbar puncture within 24 hours after the last infusion of VP 16-213 (Table 2). In some patients samples were also obtained several days after the last infusion and before the following course of VP 16-213. Together with the CSF, blood samples were collected. CSF samples were placed in glass tubes and centrifuged at 10 *g* for 10 minutes and the supernatant CSF was removed. Blood was collected in heparinized tubes and plasma was removed after centrifugation at 10 *g* for 10 minutes. CSF and plasma were frozen immediately at −20°C and stored until assayed.

Analysis

VP 16-213 plasma levels and levels of the inactive cis isomer of VP 16-213 were measured by high-performance liquid chromotography with electrochemical detection as described elsewhere.[10] CSF levels were determined by extracting 1.0 mL CSF with 2.0 mL of 1,2-dichloroethane. Further sample treatment was identical to plasma.

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Table 1. Patient Characteristics

Patient No.	Age (years)	Histologic Diagnosis	VP 16-213 (g/m^2)*	CNS Metastases	Previous Cranial Radiotherapy
1	41	SCLC	1.5	Brain	Yes
2	70	SCLC	1.0	Meningeal	Yes
3	59	SCLC	1.5	Brain	No
4	62	SCLC	2.0	Brain	No
5	48	ACL	2.5	...	No
6	65	SCLC	0.9	...	Yes
7	52	SqCCL	1.5	Brain	Yes
8	48	SCLC	2.0	Brain	Yes
9	55	SCLC	1.5	...	Yes
10	40	NST	2.5	...	No

NOTE. SCLC = small cell lung cancer, ACL = adenocarcinoma of the lung, SqCCL = squamous cell carcinoma of the lung, and NST = nonseminomatous testicular cancer.

*Dosages were determined by the dosage step of the phase I study to which the patient was allocated.

Using this method VP 16-213 and its metabolites can be measured in plasma and CSF in the pmole range: the detection limit of VP 16-213 is 0.5 ng/mL and the quantitation limit is 2 ng VP 16-213/mL plasma.

RESULTS

CSF Levels

VP 16-213 was found in the CSF of all patients. The highest level was found in patient no 2. In patient no. 4 the levels before and after radiotherapy were not different. The levels found in patients with no previous radiotherapy of the brain were in the same range as in previously irradiated patients. In CSF a relatively high level of cis VP 16-213 was detected, compared to plasma levels.

Response

Patient no. 1 received cranial irradiation for asymptomatic multiple brain metastases of SCLC. Computerized tomography (CT) scanning two months after this treatment was normal (Fig. 1). Five months after completion of radiotherapy he had epileptic fits caused by new multiple brain metastases (Fig. 2). He was considered to be unsuitable for further radiotherapy, so treatment with high dose VP 16-213 was initiated. After one course, clinical improvement was evident and CT scanning of the brain after two courses showed a partial remission (Fig. 3). The primary tumor in the mediastinum and left upper lobe remained unchanged. Three months later the patient died of systemic tumor progression.

Patient no. 2 was still in complete remission after conventional chemotherapy and prophylactic cranial radiotherapy when signs of meningeal carcinomatosis were found (motor weakness, pain and sensory defects of the lower extremities in a radicular distribution, urinary retention, and fecal incontinence). Shortly after the first course of high-dose VP 16-213 the pain and pareses improved; after two courses the patient was continent and able to walk with help.

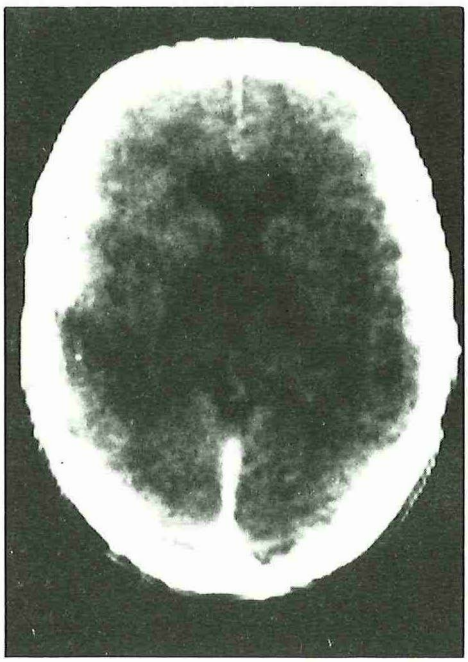
The other patients had either no evaluable CNS lesions or their clinical condition was

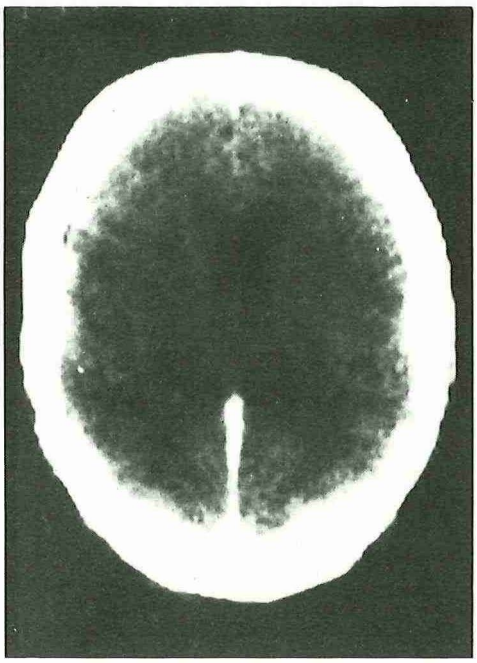
Table 2. CSF and Plasma Levels of VP 16-213 and cis-VP 16-213

Patient No., Course No.	Time After Last Infusion	VP 16-213		cis-VP 16-213	
		CSF Level (μg/mL)	Plasma Level (μg/mL)	CSF Level (ng/mL)	Plasma Level (ng/mL)
1					
1	4 hr	0.078	13.9	54	210
	22 days	<0.01	0.01	...*	...*
2	4 hr	0.14	14.3	...†	...
	20 days	...*	...*	...*	...*
3	5.5 hr	0.022	13.20	50	49
2					
1	4 hr	0.54	14.6	137	17
	2 days	0.023	4.07	19	...*
	9 days	<0.01	0.06	...*	...*
	35 days	...*	...*	...*	...*
2	5.5 hr	0.104	12.23	28	...*
3	2 hr	0.132	...	18	...
3					
1	18 hr	0.11	1.85	...†	...
2	4.5 hr	0.249	15.68	59	...*
4					
1	3 hr	0.28	30.1	...†	...
2	4.5 hr	0.282	15.02	76	95
3	12 hr	0.126	4.88	19	87
5					
1	14 hr	0.069	...	55	...
2	3.5 hr	0.246	...	66	33
6					
...	12 hr	0.103	5.61	38	87
7					
...	15 hr	0.153	3.00	65	110
8					
...	5.5 hr	0.137	19.41	40	95
9					
...	10 days	...*	0.06	...*	...*
10					
...	17 hr	0.158	7.44	85	57

*Below detection level.

†An insufficient amount of CSF was procured.





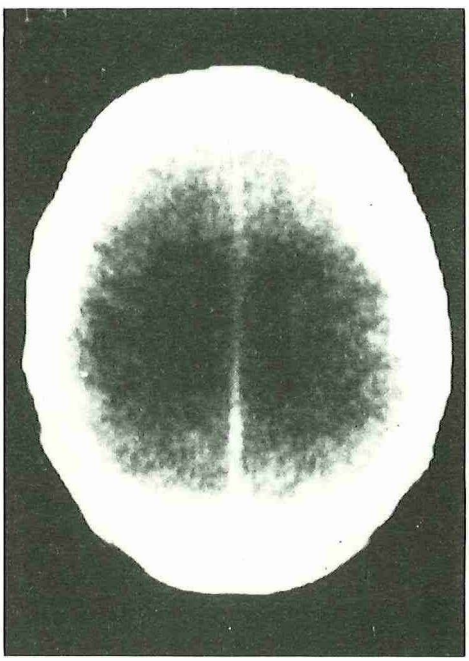


Fig. 1. Normal CT scan after radiotherapy (patient no. 1)

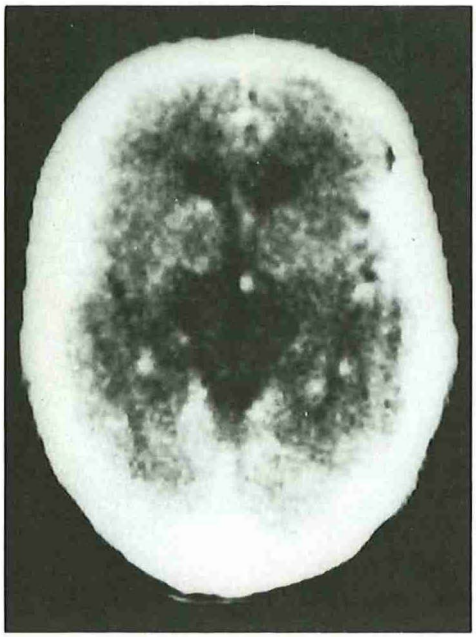
thought to require additional radiotherapy without delay.

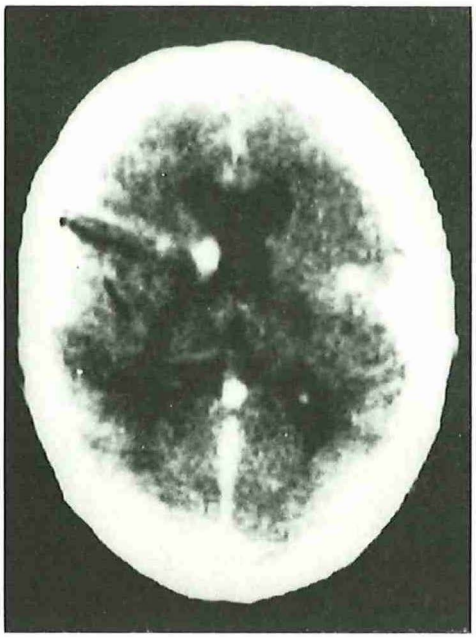
DISCUSSION

In this study we have found measurable levels of VP 16-213 in the CSF of all patients treated with high-dose intravenous VP 16-213.

The minimal concentration of VP 16-213 that can inhibit the growth of SCLC is unknown. Furthermore, it is unclear whether the levels found in the CSF accurately reflect the CNS tissue levels. For methotrexate and cisplatin it has already been shown that the CSF levels are not representative for CNS-tissue levels.[11, 12] Based on the poor water solubility this may also be the case for VP 16-213. From our data we cannot determine exactly the kinetics of VP 16-213 in CSF and CNS tissue. The high level found in patient no. 2 suggests that the existing meningeal carcinomatosis may have enhanced the penetration of VP 16-213 into the CSF.[13] Also the previously given cranial radiotherapy may have been of influence,[14] although we did not find a difference.

The relatively high level of cis-VP 16-213 in the CSF may be explained by a higher metaboliz-





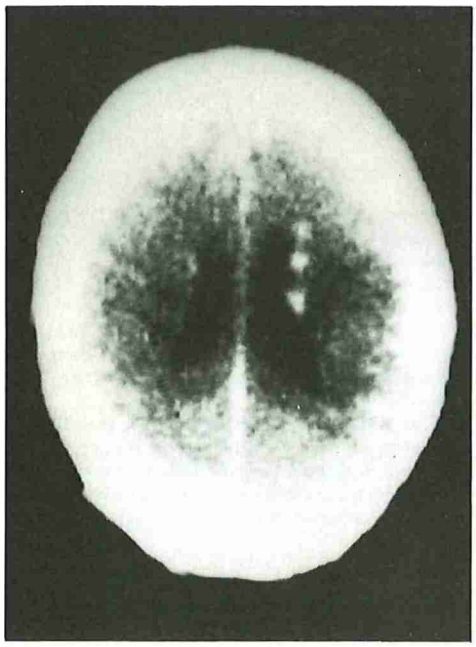
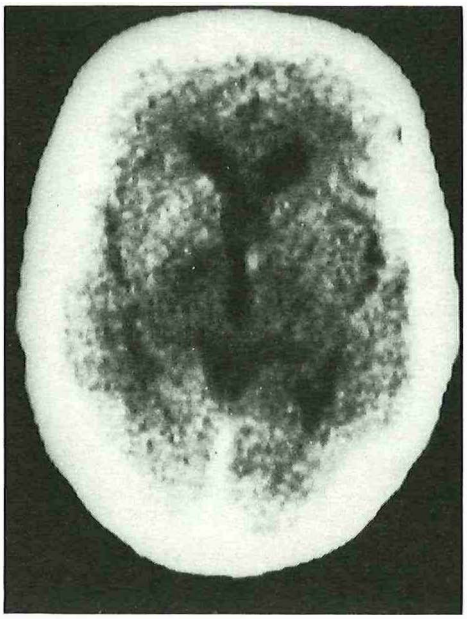


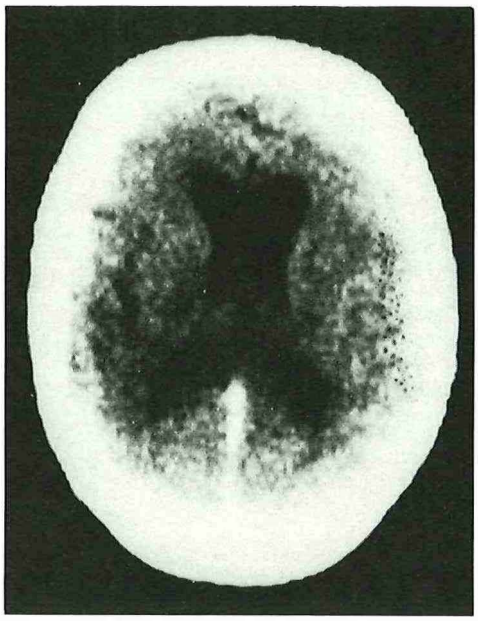
Fig. 2. Multiple brain metastases are now visible on CT scan of patient no. 1.

ing rate of VP 16-213 in the CNS, a decreased protein binding of cis-VP 16-213 in plasma, or an improved membrane passage of cis-VP 16-213 compared to VP 16-213.

Cis-VP 16-213 has cytostatic activity, although in in vitro studies in human leukemia cells it was about 100-fold less active than the parent form.[15] As no information is available concerning its activity in SCLC cells, the relevance of its presence in CSF remains undetermined.

In this study we observed clinical activity against CNS metastases in two patients. This observation may open new perspectives for the treatment of occult CNS metastases of SCLC. The high frequency of brain metastases in these patients can be reduced by prophylactic cranial irradiation.[16] The incidence of symptomatic CNS metastases outside this irradiation field is, however, increasing with the length of disease-free survival.[17] Until now effective prophylaxis for this sanctuary was not possible. The incorporation of high-dose VP 16-213 into standard chemotherapeutic regimens, for instance as late intensification after remission induction, may therefore be an interesting approach for improv-





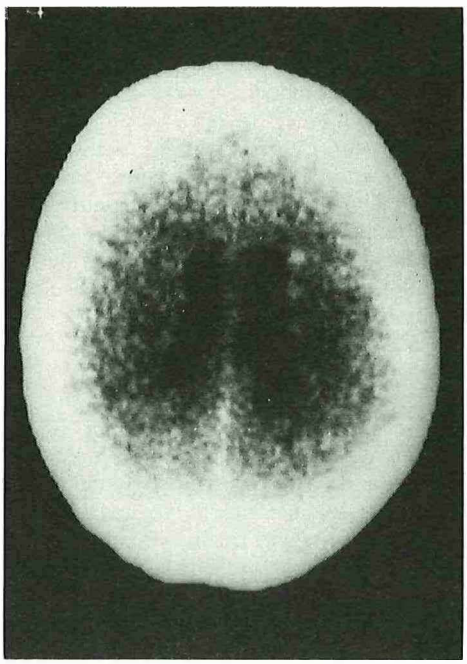


Fig. 3. Some metastases have disappeared altogether, some are less conspicuous after high dose VP 16-213 on CT scan of patient no. 1.

ing the results with respect to the CNS metastases in patients with SCLC. The contribution of disruption of the blood-brain barrier by radiotherapy in these patients, prior to administration of high-dose VP 16-213, should be evaluated further.

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REMISSION OF BRAIN METASTASES FROM SMALL-CELL LUNG CANCER AFTER HIGH-DOSE CHEMOTHERAPY.

TO THE EDITOR; The prognosis of patients with small-cell lung cancer has been improved by combination chemotherapy; however, central nervous sytem involvement is still an unfavorable prognostic factor (1), probably due to insufficient penetration of cytostatic drugs through the blood-brain barrier. Radiotherapy is at present the only treatment for brain metastases due to small-cell lung cancer. For brain metastases developing or recurring after radiotherapy, no effective therapy is available. In a phase I study (2) of etoposide (VP16-213), therapeutic effects on central nervous sytem metastases and detectable levels of etoposide in the cerebrospinal fluid were found (3). We report the case of a patient who achieved a complete remission after high-dose chemotherapy.

In December 1982 a 51-year old man presented with histologically proven small-cell lung cancer. Results of a neurologic evaluation were normal. After four courses of combination chemotherapy, a complete remission was achieved. A computed tomographic (CT) scan of the brain was normal, and prophylactic cranial irradiation was given. In November 1983, a local recurrence was found. Before beginning a phase II study of high-dose chemotherapy the patient underwent restaging; findings were normal with the exception of a CT scan of the brain that showed two small and one larger (asymptomatic) metastases. After one course of cyclophosphamide, 7 g/m^2 body surface area, and etoposide, 0.9 g/m^2 body surface area, with autologous bone marrow transplantation, a partial remission of the lung lesion occurred. On a repeated CT scan of the brain, no metastases were found. In addition, four monthly courses of high-dose etoposide (1.5 g/m^2 body surface area) were given and the CT scan of the brain remained unchanged. During treatment the patient kept working as a lawyer.

The frequency of brain relapses has become markedly reduced since prophylactic cranial irradiation has become a routine part of the treatment of small-cell lung cancer, but brain metastases still will develop in many patients. Until now no effective antitumor therapy was available for patients with brain metastases recurring after previous radio-therapy (4). As far as we know the complete remission seen in this patient is the first that has been reported. In a previous study (3) we found a partial remission in a similar patient. These observations are promising with respect to palliative treatment of recurrent brain metastases of small-cell lung cancer. Although this treatment is associated with severe short-term myelosuppression and mild mucositis (2), it can usually be given on an outpatient basis. A potential future application may be incorporation of high-dose etoposide in standard treatment as prophylaxis for central nervous system metastases, for instance as part of a "late intensification" regimen (5).

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CHAPTER 7

Cyclophosphamide and VP 16-213 with Autologous Bone Marrow Transplantation. A Dose Escalation Study

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Abstract—*In 13 patients with therapy-resistant solid tumors the feasibility of high-dose cyclophosphamide (7 g/m²) in combination with increasing doses of VP 16-213 with autologous bone marrow transplantation was studied. Dose-limiting extramedullary toxicity appeared to be mucositis and occurred after 2.5 g/m². Two toxic deaths were observed in patients older than 55 yr. Responses were seen in eight out of nine evaluable patients. Two patients with ovarian cancer still have no signs of disease progression after 12+ months. High-dose cyclophosphamide (7 g/m²) can be combined with VP 16-213 1.5 g/m² without important extramedullary toxicity. Age is probably a limiting factor for this kind of therapy.*

INTRODUCTION

IN ANIMAL studies a steep dose–response relationship has been found for cytostatic drugs against animal tumor cells [1, 2]. Studies in man suggest that this model is also valid for human tumors. The response rate in small cell lung cancer (SCLC), carcinoma of the ovary (OC) and germ cell tumor (GCT) increases with intensification of chemotherapy [3–6].

A considerable dose escalation of cytostatic drugs results in progressive damage of normal tissue. One of the most frequently encountered side-effects is myelosuppression. The use of autologous bone marrow transplantation (ABMT) can prevent persistent bone marrow aplasia [7]. Cyclophosphamide and VP 16-213 (etoposide) are active agents in the above-mentioned tumors. As single agents both can be administered in high doses without severe extramedullary toxicity. Dose-limiting extramedullary toxicity of cyclophosphamide is cardiac failure caused by a hemorrhagic myocarditis. This is unlikely to occur below a dose of 240 mg/kg, but has been reported after single-agent cyclophosphamide at a dose of 180 mg/kg [8]. To date only one dose escalation study of VP 16-213 with ABMT has been reported. In this study the tolerated dose was 2.4 g/m², as in the majority of the patients at this dose level severe mucositis was observed [9].

In order to establish the feasibility of the combination of high-dose cyclophosphamide and high-dose VP 16-213 we started a phase I study with escalating doses of VP 16-213.

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MATERIALS AND METHODS

Patients

Pertinent data on 13 patients entering the study are given in Table 1. Patients were eligible up to the age of 70 yr. Entry criteria were bilirubin levels $\leqslant$25 mmol/l, leucocytes $\geqslant 3.0 \times 10^9$/l, platelets $\geqslant 100 \times 10^9$/l, serum creatinine levels $\leqslant$150 μmol/l (normal $\leqslant$106 μmol/l) and no signs of bone marrow invasion with tumor in bone marrow biopsy and smear.

Patients had to have a Karnofsky performance score $\geqslant$60. All patients had persisting or progressive disease after conventional treatment programs. Informed consent was obtained from all patients and the study was approved by the local medical ethical committee.

Table 1. Patient characteristics

Patient	Age (yr)	Sex	Tumor type	Previous therapy	Karnofsky score	Dose Cy (g/m^2)	Dose VP (g/m^2)	Response	Response duration (months)
1	29	F	OC	Cy, HMM, ADR, CDDP	80	7	0.9	CR	16+
2	42	F	OC	Cy, HMM, ADR, CDDP	80	7	0.9	NE	*
3	65	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB, PCI	60	7	0.9	NE	†
4	24	F	GCT	CDDP, VBL, BL	60	7	1.5	PR	1
5	51	F	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	70	7	1.5	PR	9+
6	38	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	80	7	1.5	SD	1
7	55	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	90	7	1.5	ED	ED day 13
8	40	M	GCT	CDDP, VBL, BL	80	7	2.0	PR	1½
9	24	M	GCT	CDDP, VBL, BL	90	7	2.0	PR	2
10	41	M	GCT	CDDP, VBL, BL	90	7	2.0	PR	1
11	22	M	GCT	CDDP, VBL, BL	80	7	2.5	PR	2
12	36	M	GCT	CDDP, VBL, BL	60	7	2.5	PR	2
13	57	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	80	7	2.5	ED	ED day 22

ADR = adriamycin; BL = bleomycin; CDDP = *cis*-platinum; Cy = cyclophosphamide; VBL = vinblastine; VCR = vincristine; VP = VP 16-213; HMM = hexamethylmelamine; PCB = procarbazine; PCI = prophylactic cranial irradiation 30 Gy; SCLC = small cell lung cancer; GCT = germ cell tumor; OC = ovarian cancer; ED = early death; NE = non evaluable; CR = complete response; PR = partial response; SD = stable disease.
*No signs of progression after 12+ months.
†No signs of progression after 5+ months.

Bone marrow aspiration

Bone marrow was aspirated from the posterior iliac crests by multiple aspirations. The patients received mild sedation and general analgesia by diazepam 20 mg i.m. and meperidine 100 mg i.m. Lidocaine 1% was given as local anesthetic. A minimum of 2×10^8 nucleated cells/kg body wt was harvested in Hanks solution with HEPES buffer and Heparin, final concentration (FC) 150,000 IU/l. The marrow was centrifuged in an apheresis machine (Haemonetics Model 30S). The buffy coat was separated and resuspended in Hanks balanced solution (FC 200×10^6 nucleated cells/ml). This cell solution was dissolved (1:1) in 20% autologous plasma (FC 10%) and 20% DMSO (FC 10%) .nd placed in 5-ml freezing ampoules (Nunc). For freezing a Cryoson BV-4 liquid nitrogen controlled freezer was used at a rate of 1°C/min until -40°C. The ampoules were stored in liquid nitrogen.

Reinfusion was done after rapid thawing without washing. A blood filter was used to prevent infusion of possible clots.

Before marrow infusion the patients received prednisolone 60 mg i.v. and clemastine 2 mg i.v.

Cytostatic treatment

All patients received cyclophosphamide and VP 16-213. Cyclophosphamide was given as a 30-min infusion on three consecutive days. Mesna was given in a total dose of 4 g/m^2 in order to prevent hemorrhagic cystitis. VP 16-213 was dissolved in normal saline with a maximum concentration of 0.8 mg/ml. Two 1-hr infusions were given with a 12-hr interval on the same days as cyclophosphamide. The dose of VP 16-213 was increased if no dose-limiting extramedullary toxicity was seen in more than one out of three patients at that dose level (Table 1). The dose of cyclophosphamide given was 7 g/m^2, the highest dose that can be given without a high probability of cardiac toxicity [8].

The dose of VP 16-213 was escalated using a modified Fibonacci scheme [10], starting at 0.45 g/m^2 (unpublished observation).

The bone marrow was reinfused on day 7. At the moment of reinfusion of the bone marrow, VP 16-213 levels were measured. More extensive pharmacokinetic studies were done in two patients. VP 16-213 was determined by high-performance liquid chromatography with electrochemical detection [11].

Supportive care

Patients were treated in a single-person bedroom. Intravenous therapy was given either through a central venous Hickman catheter or through a needle inserted in an arteriovenous fistula [12].

All patients received prophylactic antibiotics directed against potential pathogenic intestinal flora [13]. All patients received amphotericin B lozenges and oral polymixin B; patients 3, 4, 8 and 9 also received intravenous temocillin. In case of an infection, defined as temperature $\geq$38.5°C (axillary) and clinical or bacteriological signs of infection, first-line antibiotic treatment consisted of cefuroxim and tobramycin. Nutritional support consisted of enteral tube feeding [14] and if necessary parental nutrition. Prophylactic

platelet transfusions were given at a platelet level below 15×10^9/l. A number of patients received cryopreserved autologous platelets [15], otherwise allogeneic single donor-platelets were used.

Toxicity

Physical examination and full blood cell counts were done daily, liver and renal functions three times a week. Neurological evaluation and electrocardiograms were performed weekly. Microscopic investigation of urine was done daily from day 1 to day 7, thereafter three times a week. Drug toxicity was graded according to WHO criteria (Table 2) [16]. Extramedullary grade 4 toxicity was considered to be dose-limiting.

Response

Complete response (CR) was defined as disappearance of all known tumor lesions and a return to normal of all relevant biochemical abnormalities over a period of at least 4 weeks. Partial response (PR) was defined as a decrease of more than 50% of the product of the largest perpendicular diameters of all measurable lesions for at least 4 weeks from the start of treatment. If in patients without an evaluable or measurable tumor lesion an established tumor marker (α-1-fetoprotein, human chorionic gonadotropin) decreased more than 90% this was also considered as a partial response [5]. Stable disease (SD) was defined as a less than 50% response without signs of progression. Progression was defined as an increase over 25% of a measurable lesion or appearance of new tumor lesions.

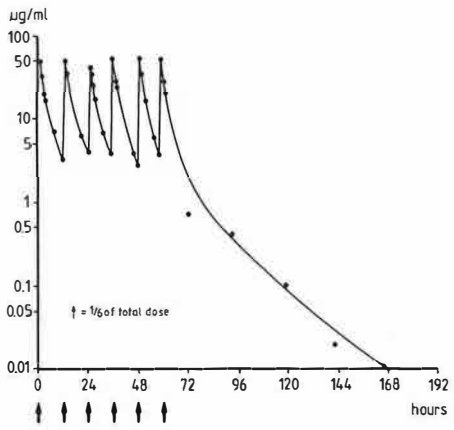

Fig. 1. Plasma levels of VP 16-213 after 1.5 g/m² VP 16-213 administration.

RESULTS

Pharmacokinetics

Low plasma levels of VP 16-213 could still be found on day 7 (day of marrow infusion). Detailed information on pharmacokinetic data will be published separately. A complete pharmacokinetic study is shown in one representative patient (Fig. 1).

Toxicity

During chemotherapy all patients experienced nausea and vomiting, grades 2–3. Some had moderate self-limiting diarrhea, grade 2. No bladder toxicity occurred. No hypertension, chills or fever were seen during the infusion of VP 16-213.

In one patient an anaphylactoid reaction occurred after infusion of about 80% of the stored bone marrow. Cardiac and pulmonary resuscitation was successful.

From day 4 until bone marrow recovery mucositis was severe and dose-limiting at 2.5 g/m² VP 16-213 (Table 3). Mucositis became evident at the 1.5 g/m² VP 16-213 dose level and therefore further dose escalations were modified to 2.0 and 2.5 g/m². Usually oropharyngeal erythema and soreness started on day 9 and recovered within 10 days. No other signs of digestive tract involvement were seen. Dermatitis was minimal (grade 0–2). There were no other important toxic side-effects. Neuropathy was not seen and no hemorrhagic complications occurred. Neither ECG abnormalities nor signs of cardiac failure were noted. All patients received platelet transfusions. Duration of granulocytopenia is

Table 2. Grading of observed toxic side-effects [16]

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Oral	no change	soreness/erythema	erythema, ulcers, can eat solids	ulcers, requires liquid diet only	alimentation not possible
Diarrhea	none	transient ≤2 days	tolerable, but >2 days	intolerable, requiring therapy	hemorrhagic dehydration
Cutaneous	no change	erythema	dry desquamation, vesculation, pruritis	moist desquamation, ulceration	exfoliative dermatitis, necrosis requiring surgical intervention

shown in Table 3. Fever due to infection was seen in ten patients (Table 4). In eight patients a causative microorganism was found, and two patients had clinical signs of infection. Two patients died during the cytopenic phase. In both patients high-grade fever developed during cytopenia, coinciding with erythematous dermatitis. In the following days fluid retention developed, probably due to a 'capillary leak syndrome'. Antibiotic treatment did not lead to improvement. Several cultures, including blood cultures, gave no support for an infectious origin of the fever. The patients gradually became confused and died on days 13 and 22 respectively. At autopsy no explanation for the fever was found.

Tumor response

In 9 patients response could be evaluated. In patients 1 and 2 before treatment macroscopic tumor was documented at laparotomy. After 10 months laparotomy in patient 1 did not reveal microscopic tumor. In patient 2 no clinical signs of tumor progression were seen, and a CT scan of the abdomen did not show any abnormalities. In patients 4, 9, 10, 11 and 12 α-1-fetoprotein levels decreased more than 90%. In patients 8 and 12 lung metastases showed a PR. In patient 5 mediastinal lymph nodes showed a PR on CT scan for more than 9 months without therapy. Hilar lymph nodes and primary tumor were unchanged in patient 6. In patient 3 routine investigations during follow-up did not reveal any signs of tumor progression anywhere for more than 5 months. At autopsy tumor was still present in patients 7 and 13.

DISCUSSION

In this study with high-dose cyclophosphamide and escalating doses of VP 16-213 the

Table 3. Toxicity

Patient	Mucositis grade	No. of platelet tranfusions	No. of days with granulocytes $\geq 0.5 \times 10^9$/l	First day post-transplant with granulocytes $\geq 0.5 \times 10^9$/l	No. of infused nucleated marrow cells $\times 10^8$/l
1	1	3	13	12	1.69
2	1	4	18	18	1.25
3	1	3	14	14	1.11
4	2	10	28	27	1.42
5	2	4	16	17	2.16
6	3	4	15	15	1.14
7	2	2*	*	*	1.63
8	4	4	12	10	0.92
9	3	4	17	16	1.3
10	3	3	13	12	1.2
11	2	4	22	20	1.5
12	4	5	13	13	1.4
13	4	5†	†	†	0.73

*Patient died on day 13, granulocytes 0, platelets 15 $\times 10^9$/l.
†Patient died on day 22, granulocytes 0, platelets 15 $\times 10^9$/l.

Table 4.

Patient	Infection days	Site of infection	Causative microorganism
1	0	-	-
2	10	esophagus	*Candida* spp.
3	3	skin at insertion of Hickman catheter	*Staph. epidermis*
4	3	skin	*Ps. fluorescens*
5	12	septicemia	*Strept. viridans*
6	6	skin of nose	Not found
7	0*	-	-
8	8	septicemia	*Strept. viridans*
9	5	sinusitis maxillaris	*Staph. epidermidis*
10	6	septicemia	*Strept. viridans* *Staph. epidermidis*
11	16	lung	*H. influenzae* *Strept. pneumoniae*
12	9	skin	*Serratia* spp.
13	0*	-	-

*Patient died, fever >40°C, no infection found.

dose limit of VP 16-213 appeared to be 2.5 g/m², due to mucositis of the oropharyngeal region. It is interesting to note that this mucositis was limited to the upper part of the digestive tract and that diarrhea was not seen.

The main and life-threatening toxicity in this study was infection. Its chief cause was the prolonged period of severe granulocytopenia. Earlier reinfusion of bone marrow was hampered by the slow elimination of VP 16-213. It is conceivable that isolation and gut sterilization would reduce the infection rate; however, its financial consequences would limit the applicability of this form of treatment to only a small percentage of all patients with solid tumors. The high infection rate could be influenced by the damage to the mucosal barrier of the oropharynx. In view of the limited toxicity to the lower digestive tract, it is tempting to speculate on a role of high saliva concentration of VP 16-213 as an explanation for the oropharyngeal mucositis [17].

Another major toxicity occurring in this study was the death of two older patients from a syndrome consisting of erythematous dermatitis, fever and fluid retention, finally leading to multiple organ failure. Although none of these other two signs occurred in the patients of this age group, we cannot recommend any of the tested dose regimens for patients of this age group. A syndrome comparable to this toxicity was recently described as 'the capillary leak syndrome' in mismatched as well as matched allogeneic bone marrow transplant patients [18]. Its incidence might be triggered by the radiomimetic effect of the alkylating agent, supposedly increased by age and the addition of VP 16-213.

Cyclophosphamide and VP 16-213 administered as single agents are not absolutely marrow ablative [9, 20]. Although no increase in the time until recovery was noted at the escalating dose levels, it is impossible to conclude whether the combination of the drugs necessitates the use of autologous bone marrow transplantation, as has been done in this study. We can conclude that cyclophosphamide at 7 g/m² and VP 16-213 at 1.5 g/m² can be given without major extramedullary toxicity when combined with ABMT. However, infectious complications are still an important problem and perhaps an age limit should be introduced because of the above-mentioned 'capillary leak syndrome'.

The response rate and duration, however, make this approach worthwhile for study in the tumor types treated in this report. Especially for SCLC, this combination of cyclophosphamide and VP 16-213 may be important for late intensification studies. Bone marrow harvesting after remission induction with standard dose therapy results in a minimal risk of bone marrow contamination, while marrow reserve as measured by granulocyte colony-forming units is still sufficient [21]. VP 16-213 in this combination can be administered at a dose of 1.5 g/m² without major extramedullary toxicity. At this dose a sanctuary site, the central nervous system (CNS), can be reached in apparently effective concentrations [22]. This can be important for prophylaxis of CNS metastases. The two patients with ovarian cancer have clearly benefited from this treatment and more patients with minimal residual disease will be treated with this regimen. Furthermore, the addition of drugs with dose-limiting toxicity other than encountered in this regimen, for instance *cis*-platinum, might increase the therapeutic potential.

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CHAPTER 8

Failure of Selective Gut Decontamination with Nonabsorbable Antibiotics in Preventing Infections in Patients Treated with High-Dose Chemotherapy and Autologous Bone Marrow Transplantation

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Increasing the dosages of anticancer drugs can result in higher response rates in patients with solid tumours (5,11). However, this increase could lead to unacceptable haematological toxicity unless autologous bone marrow transplantation (ABMT) is performed. By this technique, it is possible to prevent persistent bone marrow aplasia, although temporarily there will still be severe granulocytopenia ($<0.1 \times 10^9$/liter). The major complication of granulocytopenia is infection (1,2,4,13). The causative bacteria in this situation are usually the gram-negative organisms already present in the patient's digestive tract (3,17) or acquired in the hospital (14). Elimination of all endogeneous bacteria by gut sterilisation with oral nonabsorbable antibiotics results in diminished infection rates when this treatment is performed in a germ-free environment (12). However, this procedure is associated with high costs and patient discomfort. If "gut sterilisation" is used outside a protective environment, overgrowth with resistant aerobic microorganisms can occur (10).

Prophylactic treatment with antibiotics, directed selectively against aerobic gram-negatives and yeasts in the digestive tract, reduces the infection frequency in granulocytopenic patients even outside a protective environment (15). The resistant anaerobic flora remain, and prevent colonization with contaminating aerobic gram-negatives (18). This procedure has been called selective decontamination (19). The drugs suitable for this infection prevention are cotrimoxazole, nalidixic acid, low-dose neomycin, polymyxin, and tobramycin (3,6-8,15,20). All these drugs have to be given orally.

Nalidixic acid is associated with nausea and vomiting in a high percentage of patients. Cotrimoxazole can possibly result in a delay of hematopoietic recovery

(6), which is particularly undesirable in ABMT. Therefore, we decided to use only nonabsorbable drugs, which leave the anaerobic flora intact, in a group of granulocytopenic patients who were treated with high-dose chemotherapy and ABMT, without isolation measures.

MATERIALS AND METHODS

Patients

From January 2, 1982 until January 11, 1983, 10 patients (7 males, 3 females), mean age 37.2 years (18 to 56 years), were entered into a phase I study of high-dose chemotherapy with ABMT. All patients had widely disseminated solid tumours, which were progressive during, or unresponsive to standard dose chemotherapy at the time of admission. Histological diagnoses were: small cell lung cancer (5 patients), ovarian cancer (2 patients), and nonseminomatous testicular cancer (3 patients). The study period during which infection prevention was evaluated started on the 1st day of administration of high-dose chemotherapy and was terminated upon discharge from the hospital or when the granulocyte count was above 0.5×10^9/liter for 3 days, or at death.

Chemotherapy and Bone Marrow Transplantation

Nine patients received increasing doses of cyclophosphamide and VP16-213 (Table 1); one patient received standard doses of cisplatin, VP16-213, and actinomycin D. The bone marrow was procured and cryopreserved about 1 week before the start of chemotherapy and reinfused 4 days after the last infusion of cytostatic drugs.

Selective Decontamination

In all patients, polymyxin B (PMB) 200 mg four times a day was given before granulocytopenia was established, and in eight patients neomycin 250 mg four times a day started at the same time (Table 2). If the cultured gram-negative bacteria were resistant to these drugs, tobramycin 100 mg four times daily was added. For elimination of yeasts, amphotericin B suspension or tablets were used (500 mg two times a day). For elimination of yeasts from the oropharynx, amphotericin B lozenges (each containing 10 mg of the active substance) were administered four times a day.

Microbiological Surveillance

To control the effect of oral administration of the drugs, cultures from the throat and faeces were performed three times per week; cultures were made from stools or occasionally from an anal swab. Only an aerobic culture was made on the specimens, with special attention given to potential pathogens such as aerobic gram-negative rods (MacConkey agar, Merck), yeasts and fungi (Sabourad agar,

TABLE 1. *Chemotherapy and toxicity*

Patient	CF g/m²	VP g/m²	Study days	Days granulocytes (× 10⁹/liter) 0.5	0.1	0.05	Fever days MDI	CDI	FUO	NIF	Localisation of infection	Mucositis grade	Dermatitis grade
1	3	0.6	18	11	7	5	—	—	—	—	—	0	0
2	4.5	0.9	30	22	18	13	—	2	—	—	Tonsils	1	1
3	7	0.9	21	13	9	9	—	—	4	—	—	1	1
4	7	0.9	26	19	14	13	10	—	—	—	Esophagus	3	2
5	7	1.5	23	15	10	9	—	5	—	1	Skin	4	1
6	7	1.5	25	17	13	12	15	—	—	—	Skin/soft tissue	2	2
7	7	2.5	21	15	15	11	—	—	14	—	—	4	2
8	7	2.5	22	15	14	10	8	—	—	—	Lung	4	1
9	7	2.5	30	23	16	10	11	—	—	—	Skin/soft tissue	2	1
10	Actinomycin D Cisplatinum VP		20	11	9	8		13	—	—	Lung	4	3
Total			236	161	125	100	44	20	18	1			
								64					

CF, cyclophosphamide; VP, VP16–213.

TABLE 2.

Patient	Nonabsorbable antibiotics	Starting day[a]	Decontamination day[b]	First day ≤ $0,5 \times 10^9$ granulocytes/liter
1	PMB	1	1	7
2	PMB	1	3	8
	Neo	1		
3	PMB	5	7	7
	Neo	5		
4	PMB	−6	−4	7
	Neo	−6		
5	PMB	−2	−1	7
	Neo	−2		
6	PMB	−2	2	7
	Neo	−2		
7	PMB	1	2	7
	Neo	1		
8	PMB	1	2	7
	Neo	1		
9	PMB	−2	1	7
	Neo	−2		
	Tobra	10		
10	PMB	8	14	9
	Tobra	12		

[a]Day study period started.
[b]First day of effective decontamination.
Neo, neomycin; Tobra, tobramycin; (−), started before initiation of study period.
All patients received oral amphotericin B.

Merck, Darmstadt, West Germany). *Staphylococci* and *Streptococci* (blood, agar, Oxoid, Basingstoke, England) and *Haemophilus influenzae* (Levinthal agar, Oxoid). Gram-negative rods were identified and biotyped with API 20 E system (API System SA, La Balme les Grottes, France).

From all cultured biotypes, as well as from *Staphylococci*, *Streptococci*, and *Haemophilus*, the sensitivity pattern was determined by a standard series of antibiotics currently in use for the treatment of infections; this included the determination of the sensitivity for neomycin, polymyxin, and tobramycin.

Haematological Surveillance

White blood cells were counted by the Coulter counter (>3,000 cells/mm^3) or in a counting chamber (≤3,000 cells/mm^3). Absolute levels of granulocytes were measured in the hemocytometer daily until discharge.

Clinical Surveillance and Treatment

Physical examination was done daily from the start of chemotherapy until discharge or death. In case of fever (axillary temperature above 38.5°C) cultures of

blood, urine, and sputum (if possible) were performed. The patients were treated in a conventional one-bed hospital room on an open ward under standard conditions. Standard nonsterile hospital food was provided. Three patients received enteral feeding via a nasogastric tube; this tube-feeding was sterile (Nutricia N.V., Zoetermeer, Holland). When indicated, supportive treatment was given; this included leukocyte-free packed cells, platelet transfusions, as well as i.v. broad spectrum antibiotics and antimycotic therapy. First-line antibiotic treatment consisted of cefuroxime and tobramycin. First-line antimycotic therapy was amphotericin B and ketoconazole.

Registration of Acquired Infections

The following descriptions were used. Fever day: registered when axillary temperature was above 38.5°C. Microbiologically documented infection (MDI): defined as the presence of definite signs and symptoms of infection plus the isolation and identification of pathogenic microorganisms from blood, urine, sputum, local sites, or tissue at autopsy. Clinically documented infection (CDI): defined as the presence of definite signs and symptoms of infection with negative cultures. Noninfectious, "allergic fever" (NIF): fever associated with a noninfectious cause such as blood transfusion, administration of cytostatics, allergic reactions to drugs, or fever associated with bone marrow infusion. Fever of unknown origin (FUO): defined as fever not associated with signs or symptoms of infection, nor with allergy.

RESULTS

Chemotherapy Toxicity

In the nine patients who received increasing doses of cyclophosphamide and VP16-213, mucositis of the oral cavity and pharynx was the major extramedullary toxic side effect (range 1 to 4, WHO toxicity grade) (19) (Table 1). Stomatitis was seen in nine patients (range 1 to 4).

Granulocytopenia was severe as is shown in Table 1; in all patients, granulocytes were less than 0.1×10^9/liter for at least 7 days. Of the 236 study days, granulocytes were less than 0.5×10^9/liter on 161 (68%) days, including 125 (78%) days less than 0.1×10^9/liter and 100 (62%) days with less than 0.05×10^9/liter.

Bacteriological Evaluation

Faecal cultures revealed growth of gram-negative bacteria in all patients on admission, before antibiotic treatment was started.

In nine patients, decontamination was effective within 2 or 3 days after starting nonabsorbable antibiotics. These patients therefore were decontaminated before granulocytes were below 0.5×10^9/liter. In patient 10, decontamination failed because of persistence of a resistant gram-negative rod (Proteus). In this case, however, the addition of oral tobramycin was effective.

During study days on which patients received nonabsorbable antibiotics, 94 faecal cultures (Table 3) were done, 18 (19%) contained gram-negative rods. In two patients (5 and 6), these microorganisms were found on one occasion and were sensitive to the antibiotics used. In two other patients (9 and 10) resistant gram-negative rods were cultured. In all four patients, the cultured microorganisms were absent initially. In 18 (19%) cultures, yeasts were found occasionally in low concentrations in seven patients.

Ninety throat swab cultures (Table 4) were done after the start of nonabsorbable antibiotic treatment. In 5 patients, gram-negative rods were found in 10 (11%)

TABLE 3. *Faecal cultures during study period*

	Nonabsorbable antibiotics[a]			Granulocytes $< 0.5 \times 10^9$/liter[b]		
Patient	No.	Gram-neg rods	Yeasts	No.	Gram-neg rods	Yeasts
1	6	0	3	4	0	2
2	11	0	4	8	0	4
3	5	0	1	4	0	1
4	10	0	1	7	0	1
5	11	1S	0	9	1S	0
6	10	1S	0	6	0	0
7	10	0	3	7	0	1
8	9	0	0	6	0	0
9	15	13R	3	12	11R	1
10	7	3R	3	7	3R	3
Total	94	18	18	70	15	13

[a]After starting antibiotics.
[b]During granulocytes.
R, resistant; S, sensitive.

TABLE 4. *Cultures of throat swabs during study period*

	Nonabsorbable antibiotics[a]				Granulocytes $< 0.5 \times 10^9$/liter[b]			
Patient	No.	Gram-neg rods	Yeasts	*S. aureus*	No.	Gram-neg rods	Yeasts	*S. aureus*
1	7	0	3	0	5	0	1	0
2	13	0	9	2	11	0	7	1
3	6	0	0	0	6	0	0	0
4	9	1S	1	0	7	1S	1	0
5	9	2S	0	0	7	1S	0	0
6	10	3S	1	0	8	1S	1	0
7	10	0	1	0	8	0	1	0
8	8	3S	1	1	8	3S	1	0
9	13	1S	4	0	6	1S	2	0
10	5	0	0	0	11	0	0	0
Total	90	10	20	3	74	7	14	1

[a]After starting antibiotics.
[b]During granulocytes.
S, Sensitive.

cultures. These bacteria were not cultured at the initial inventarisation, except for patient 5. All bacteria were transiently present and sensitive to the drugs used. Yeasts were present in nine patients on admission. In 20 (22%) cultures yeasts were found during the treatment. In all patients, these were low concentration and transient. In two patients, *Staphylococcus aureus* were found in three cultures in low concentrations.

Clinical Evaluation

Fever

Nine patients had 83 days with fever (axillary temperature $\geq 38.5°C$); 81 fever days were associated with granulocytopenia $\leq 0.1 \times 10^9$/liter. In all, the blood cultures of nine patients ($N = 26$) were performed, but they never revealed growth of microorganisms ($N = 26$).

Infections

In four patients, four MDI were registered. In patient 4, *Candida esophagitis* was found on esophagoscopy, performed soon after marrow recovery. Patient 6 developed a lesion in the left groin with blisters, while still severely granulocytopenic. Cultures of the aspirate revealed *Pseudomonas fluorescens*. This microorganism was cultured only once from an anal swab before granulocytopenia was present. In patient 8, fever and signs and symptoms suggestive of pneumonia occurred soon after marrow infusion. Sputum cultures were positive for *Haemophilus influenzae* and *Streptococcus pneumoniae*. Patient 9 developed an ulcerating skin lesion, infected with a *Serratia* species. This microorganism was also found in the faecal cultures and could not be eliminated from the digestive tract by the applied regimen of decontamination because of resistance.

In three patients, a clinically but not bacteriologically documented infection (CDI) occurred. Patient 2 had signs of tonsillitis with submandibular lymphadenopathy. Patient 5 developed a lesion in the skin of the nose, with fever and local symptoms of infection. Patient 10 developed pneumonia shortly before death. In patients 3 and 7, fever developed without definite signs of infection or allergy.

DISCUSSION

In this study, we describe 10 patients who were treated with intensive chemotherapy resulting in 125 study days with a granulocyte count below 0.1×10^9/liter. Infection prevention was attempted with oral nonabsorbable drugs. However, we registered infections on 64 days (51.2%) (Table 1). Therefore, this regimen did not seem particularly effective.

In contrast, we recently demonstrated an infection frequency during intensive chemotherapy of 11.4% (16). In this study, cotrimoxazole, nalidixic acid, and polymyxin were applied as infection prevention. This rate of infection was also

found in a study that used cotrimoxazole in severely granulocytopenic patients (9). There are a number of ways to explain why the present scheme of infection prophylaxis failed.

First, experimental and some clinical evidence indicates that during decontamination of the digestive tract, the anaerobic flora should be kept intact. We did not actually test the integrity of the anaerobic flora, and this barrier against infection might have been lost. However, the consequence of disturbing the anaerobic flora would have been overgrowth with resistant microorganisms. This happened only once and can therefore probably not explain the high infection frequency.

Another important factor might be the lack of a systemic effect with the use of nonabsorbable drugs as compared to cotrimoxazole and to some extent nalidixic acid. The infection rate for absorbable drugs was 6.8% and 5.3%, and 11.2% for nonabsorbable drugs in a group of patients with granulocytopenia as a result of antileukemic therapy (16), indicating the importance of the systemic effect. Furthermore, following the ablative chemotherapy used in this study, more severe granulocytopenia of longer duration was seen than after standard antileukemic therapy. This could also account for an increase in infection rate.

The infecting microorganisms could have two sources; first, small numbers of bacteria in the gut, which could have been missed in routine cultures, can, after invasion, lead to infection due to the absolute lack of the granulocyte barrier; second, environment-related contamination with bacteria (as in patient 9) can easily lead to a systemic infection due to the same cause. The invasion of microorganisms from the gut flora or by exogenous contamination can possibly be enhanced by mucositis. In the regimen used in most patients in this study, this mucositis is limited to the upper part of the digestive tract, especially mouth, pharynx, and esophagus. The role of directly invading microorganisms from the nonsterile environment of the patient can be more important in the patients with dermatitis and skin laceration because of intensive chemotherapy. In patients 5 and 9, this problem could have resulted in the skin infection.

Improvement of infection prevention in these patients can probably be realised by the use of a protective environment and gut sterilisation. However, this would limit accessibility to ABMT to a small percentage of the vast number of patients with solid tumours. We therefore plan to investigate further methods of improving selective decontamination of the digestive tract. Important measures could be the use of gut decontamination with absorbable drugs and of additional protection of the upper digestive tract.

SUMMARY

Ten patients were treated for progressive disseminated solid tumours with high-dose chemotherapy and ABMT. Selective decontamination of the digestive tract was used as infection prevention. Patients were treated on an open ward, and only nonabsorbable antibiotics were used. In these patients, the infection frequency was 51.2% and was much higher than in a previous study in patients treated with

aggressive chemotherapy for acute leukemia. The causes of this failure of infection prevention are probably the lack of a systemic effect, the severity of granulocytopenia, and the mucositis or stomatitis due to chemotherapy toxicity.

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CHAPTER 9

HIGH DOSE CYCLOPHOSPHAMIDE AND HIGH DOSE VP 16-213 FOR RECURRENT OR REFRACTORY SMALL CELL LUNG CANCER. A PHASE II STUDY.

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SUMMARY.

In 9 patients with recurrent or refractory small cell lung cancer a phase II study with high dose cyclophosphamide and high dose VP 16-213 with autologous bone marrow transplantation was performed. The used regimen was based on a previously reported phase I study. In 8 out of 9 evaluable patients a response was seen (6 PR, 2 CR). One patient died of treatment related toxicity. Infection is the most important toxicity. The response duration was short. This combination is a suitable "late intensification" regimen for patients with minimal residual disease after standard dose induction chemotherapy.

INTRODUCTION.

Studies in animal tumor systems have shown a steep dose response relationship for a variety of cytostatic drugs (1, 2). The clinical equivalent of these experiments is found in the increased cure rate since the introduction of high dose chemotherapy for lymphoma and leukemia (3, 4). Also for small cell lung cancer (SCLC) there seems to be a dose response relationship for some drugs. Cohen (5) found a considerable improvement of both response rate and median survival after increasing the doses of drugs used in a combination regimen, although the initial as well as the increased dose levels in this study now fall in the low to standard dose range. Two of the most active drugs against SCLC, cyclophosphamide and VP 16-213, have at standard dose levels a response rate of approximately 40% (6, 7). At much higher dose levels the response rates increase to 84% for cyclophosphamide (8) and 80% for VP 16-213 (9). These observations support the existence of a dose response relationship for these drugs over a wide range of dosages in patients with SCLC.

Table 1. Patient characteristics.

No.	Age/sex	Previous therapy (total dose in mg).	Response to previous therapy	Time between end of initial therapy and relapse (in months)
1	55/M	CTX (3000), CDDP (300), VP (6600), ADM (240), VCR (8), PCZ (4000), PCI 30Gy, XRT 30Gy	1. CR	12
2	59/M	CTX (5200), CDDP (520), VP (2400)	2. CR	1
3	52/M	CTX (6800), CDDP (680), VP (2400), PCI 30Gy	3. CR	6
4	39/M	CTX (5600), CDDP (560), VP (2400)	4. PR	2
5	47/M	CTX (6400), CDDP (640), VP (2400), VDS (11.1)	5. PR	1
6	37/F	CTX (5600), CDDP (560), VP (2400), PCI 30Gy	6. CR	3.5
7	52/M	CTX (6000), CDDP (600), VP (7800), PCI 30Gy	7. CR	11
8	56/M	CTX (8500), ADM (375), VP (3000), PCI 30Gy	8. CR	4.5
9	35/M	CTX (5600), CDDP (560), VP (2400), PCI 30Gy	9. CR	9

Abbreviations used: CTX = cyclophosphamide; CDDP = cisplatinum; VP = VP 16-213; ADM = adriamycin; VCR = vincristine; PCZ = procarbazin; PCI = prophylactic cranial irradiation; XRT = radiotherapy to primary tumor; Gy = Gray; VDS = vindesine.

Previously we described the results of a phase I study with high dose cyclophosphamide together with increasing doses of VP 16-213 and autologous bone marrow transplantation (ABMT) in patients with solid tumors (10). The dose limiting toxicity of this combination was mucositis of the oropharyngeal region at dosages of cyclophosphamide 7 g/m^2 and VP 16-213 2.5 g/m^2. Acceptable toxicity was seen at dosages of 7 g/m^2 and 1.5 g/m^2 respectively, for older patients (> 50 yrs) a VP 16-213 dose of 0.9 g/m^2 was considered to be tolerable.

In this report we describe the results of a phase II study with this combination regimen and ABMT in patients with SCLC.

MATERIALS AND METHODS.

Patients.

Pertinent data on 9 patients with recurrent of refractory SCLC entering the study are given in table 1. Entry criteria were age $\leq$ 65 yrs, Karnofsky score $\geq$ 60, bilirubin levels $\leq$ 25 mmol/l, serum creatinine levels $\leq$ 150 µmol/l, leucocytes $\geq$ 3.0 x 10^9/l, platelets $\geq$ 100 x 10^9/l and no signs of tumor invasion in bilateral iliac crest biopsies and bone marrow smears.

All patients had measurable tumor localizations, not previously irradiated.

Informed consent was obtained from all patients and the study was approved by the local medical ethical committee.

Bone marrow aspiration.

Bone marrow aspiration, storage and reinfusion was performed as described previously (10, 11).

Cytostatic treatment.

All patients received cyclophosphamide 7 g/m^2. One third of the total dose, dissolved in 500 ml normal saline, was given as a 30-min infusion on three consecutive days. Mesna was given on the same days in a total dose of 4 g/m^2 in order to prevent hemorrhagic cystitis.

VP 16-213 was dissolved in 500 ml normal saline and given in two, one hour infusions with 12 hours interval on the same days as cyclophosphamide. Patients $\leq$ 50 yrs received 1.5 g/m^2 total dose, older patients 0.9 g/m^2.

After evaluation of the response on high dose chemotherapy the patients 2, 4, 5, 6, 7, 8, 9 received radiotherapy on the site of the major tumor bulk. Patient 3 subsequently received four courses of high dose VP 16-213 (1.5 g/m^2).

Toxicity was graded according to WHO criteria (12).

Supportive care.

Patients were treated in a single person bedroom. Intravenous therapy was given through a central venous Hickman catheter. Nutritional support consisted of 4000 kcal/day given either totally parenteral or a combination of oral and parenteral feeding. All patients received prophylactic antibiotics directed against potential pathogenic flora in the digestive tract (13). This regimen consisted of amphotericin B 4 x 500 mg orally and lozenges (6 x 10 mg), polymyxin B 4 x 200 mg and cotrimoxazole 3 x 2 tablets (1 tablet contains 80 mg trimethoprim and 400 mg sulfamethoxazole).

Prophylactic platelet transfusions were given at a thrombocyte level of $\leq 15 \times 10^9/l$.

In case of an infection, defined as temperature $\geq 38^{o}C$ (axillary) and clinical or bacteriological signs of an infection, first line antibiotic treatment consisted of a combination of cefuroxim and tobramycin.

Response.

The response was evaluated 4 weeks after the start of treatment.

Complete response (CR) was defined as disappearance of all known tumor lesions.

Partial response (PR) was defined as a decrease of more than 50% of the product of the largest perpendicular diameters of all measurable lesions.

Stable disease (SD) was defined as a less than 50% regression without signs of progression. Progression was defined as an increase over 25% of a measurable lesion or appearance of new tumor lesions. Toxic death (TD) was defined as death due to treatment related toxicity.

Response duration and survival time were measured from the first day of high dose chemotherapy.

RESULTS.

Tumor response.

Eight out of 9 patients were evaluable for response. Two patients had a CR, 6 a PR (Table II). One patient died on day 15 due to treatment related toxicity, at autopsy no tumor was found. In one of the PR patients a CR of the brain metastases was seen.

The median response duration in the 8 responding patients was 5 months (range 2-8+). The median survival was 6 months (range 2+-12). Two patients are still alive without tumor progression.

Toxicity.

All patients developed leuco- and thrombocytopenia (table III). There were no bleeding episodes, except one small gastro- intestinal tract bleeding. In all patients fever due to an infection developed, in 8 patients a causative microorganism was found, in patient 1 this was found at autopsy; in 1 patient herpes simplex infection was suspected but not confined. During the 3 days of chemotherapy infusion all patients experienced nausea and vomiting, WHO grade 2-3. Some had at that time diarrhea grade 2; during cytopenia 5 patients had diarrhea, grade 2-4 (table 3), in patient 9 candida overgrowth was found in the gastro-intestinal tract and in patient 4 Clostridium difficile toxin was present in the faeces. Mucositis of the oropharyngeal region developed in all patients (table III). Skin toxicity was mild, grade 1. There were no signs of bladder toxicity.

DISCUSSION.

Although SCLC is a tumor with a high response rate, after standard dose chemotherapy often resulting in complete clinical remissions, only a minority of the patients will have a long term disease free survival. The response rate of second line chemotherapy for progressive or relapsing SCLC is low, therefore new treatment modalities, as for instance high dose chemotherapy, have to be investigated.

In this study we used a combination of high dose cyclophosphamide and high dose VP 16-213 with ABMT based on the results of a previously described phase I study (10). Both drugs are among the most active against SCLC at standard dose levels, and furthermore for both drugs a dose response relationship for SCLC exists.

Table 2. Results of high dose chemotherapy.

Pat.	Response	Response duration (months)	Survival (months)
1	TD	-	-
2	PR	3.5	5
3	PR/CR	6	12
4	PR	7	9+
5	CR	6	8
6	CR	6	8
7	PR	2	3
8	PR	4+	4+
9	PR	3+	3+

Table 3. Toxicity.

Pat.	No. days with leuc. $\leq 0.5 \times 10^9/l$	No. days with leuc. ≤ 1.0	No. platelet transfusions	Mucositis WHO-grading (12)	Diarrhea during cytopenic phase WHO-grading (12)
1	12+	12+	3	1	1
2	10	12	3	0	0-1
3	13	14	5	1	3-4
4	13	14	5	1	2
5	15	17	5	2	3
6	13	14	8	3	3-4
7	20	21	4	1	0
8	14	15	4	1	0
9	13	14	4	4	4

This is an aggressive regimen with considerable toxicity. Overall the treatment was tolerated well, which might have been influenced by the relatively young patients. Previously in the phase I study we already described severe toxicity of this regimen, especially in older patients. The major disadvantage of this treatment modality is the high incidence of serious, mainly grampositive, infections. In the study presented now a response rate of 100% is seen and although it is only in a small group of patients, it can be predicted that it will be an active combination with a > 50% response rate in a larger group of comparable patients within 95% confidence limits.

The high response rate in this group of patients might have been influenced by the selection of the patients, because 7 patients had apparently very sensitive tumors regarding the CR after standard chemotherapy. On the other hand the response improvement in the 2 patients with only a partial remission after the induction regimen supports the presumed dose response relationship for both drugs. In this way it might be possible to overcome to some degree the presumed existence of either primary or induced drug resistance. The latter might have been the case since both drugs were used in the initial treatment.

The main potential for the application of this regimen is its use as a "late intensification" to eliminate minimal residual disease. The patients who could with this treatment strategy possibly become curable are those who are in complete clinical remission after standard chemotherapy. At that moment marrow is harvested and reinfused after ablative chemotherapy.

This strategy is supported by the mathematical model of Norton and Simon (14). In this situation the bone marrow reserve capacity is still sufficient with respect to the number of bone marrow CFU_C's (15). Furthermore, the risk of bone marrow involvement by tumor cells is minimal due to the "clean-up" by the standard therapy.

The treatment of SCLC patients with persistent or progressive tumors with this high dose regimen is not justifiable considering its high morbidity and short response duration. The addition of new active myelotoxic drugs to cyclophosphamide and VP 16-213 might however yet improve the response duration in these patients.

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CHAPTER 10

HIGH-DOSE CHEMOTHERAPY FOR SMALL CELL LUNG CANCER

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INTRODUCTION

Initially, the introduction of chemotherapy in the treatment of small cell lung cancer (SCLC) has considerably improved the bad prognosis of the patients with this highly malignant tumor (1). The change of a median survival of less than 3 months before chemotherapy into more than one year as a result of its introduction, and the observation of the occurrence of two year disease free survival, be it only in a small number of patients, led half a decade ago to speculations that the goal of cure in a larger number of these patients might be within reach (2). Since that time however, no further progress has been made and the therapeutic results are still on the same plateau as five years ago. This disappointing reality necessitates the investigation of other therapeutic approaches and in this chapter the results of high-dose chemotherapy (HDCT) will be summarised and discussed.

RATIONALE OF HIGH-DOSE CHEMOTHERAPY

The use of HDCT is based upon the assumption of a dose-response relationship for cytostatic drugs used in clinical practice. The cause of this dose response relation could be the overcoming by high drug concentrations of mechanisms that protect cells against the action of cytostatic drugs in normal concentrations. In this way the effect of high-dose methotrexate (HDMTX) in cells resistant to standard doses of MTX could be explained by the neutralization of increased dihydrofolate reductase levels in MTX-resistant cells. In the clinical situation, high drug concentrations could surmount physiological barriers such as the blood-brain-barrier or those brought about by hypovascularization of a tumor. In this respect the recent successful application of high-dose VP16-213 in patients with Central Nervous System (CNS) metastases of SCLC is interesting (3,4). Also the effect of high-dose BCNU on metastatic disease in the CNS in a number of malignancies is noteworthy (5).

Several studies in animal tumor systems have shown a dose-response relationship for a broad variety of cytostatic drugs (6,7,8). The clinical equivalent of these experiments is found in the increased cure rate since the introduction of high-dose therapy in lymphoma and leukemia (9,10,11).
In SCLC a relation between dose and response seems to exist over a wide range of dosages for a variety of drugs. Cohen found a considerable improvement in both the response rate and median survival after doubling the doses used in a combination regimen, although the initial as well as the increased dose levels in this study fall in the low to standard dose range (12). Response rates of standard dose cyclophosphamide (13) and VP16-213 (14) are 30-40% and 40-50%. The much higher response rates seen in previously untreated patients after high-dose cyclophosphamide (84%) (15) and high-dose VP16-213 (80%) (16) indicate that this relationship also exists at much higher dose levels.
It is generally assumed that combination chemotherapy is superior to sequential therapy with the same drugs. In sofar as this hypothesis is valid for standard dose therapy, it is conceivably also true for high-dose treatment.
A prerequisite for this will be the existence of a dose-response relationship for the different agents forming the combination regimen. In the situation of HDCT the effect of the increased or cumulative toxicity could however be much more devastating.

TOXICITY OF HIGH-DOSE CHEMOTHERAPY

A considerable dose escalation of cytostatic drugs is limited by the increase of haematological and extramedullary toxicity of these drugs. The anthracyclines, cisplatinum and the vinca alkaloids frequently induce extramedullary toxicity already at conventional dosages and are therefore not suitable candidates for high-dose regimens. For some drugs, toxicity can be reduced or prevented by pharmacological rescue methods as for instance leucovorin for methotrexate (17) and mesnum for cyclophosphamide (18).
Escalation of the dose of cytostatics without important extramedullary toxicity at standard dose levels, such as the alkylating agents cyclophosphamide, melphalan, and BCNU, and the podophyllotoxin derivatives VP16-213 and VM26, will lead to an increase in bone marrow toxicity. Supportive measures such as infection prevention (19) and platelet transfusions, can

be used to ameliorate the expression of this toxicity. Furthermore administration of combinations of these drugs in dosages that are more or less completely marrow ablative is also possible if autologous bone marrow transplantation (ABMT) is used to reconstitute the bone marrow, or at least shorten the period with cytopenia (20).
ABMT was already introduced in the 1950's (21) but was not widely applicated till late in the 1970's. In most centres bone marrow aspiration is performed under general or epidural anesthesia (22,23) but it can also be done under mild analgesia with local anesthesia (24). Of the aspirated marrow the buffy coat is separated and stored after controlled freezing in liquid nitrogen. If reinfusion is planned within 48-72 hours it is also possible to store the whole marrow at $4^{o}C$ (25). Procedures aimed at elimination of malignant cells from the marrow have not yet found wide application in solid tumors.

EXTRAMEDULLARY TOXICITY OF CYTOSTATIC DRUGS SUITABLE FOR HIGH-DOSE CHEMOTHERAPY

Several drugs underwent investigation in dose escalation studies. Of these drugs only cyclophosphamide, the nitrosureas, VP16-213, VM26 and MTX have shown some activity at standard dose levels against SCLC and are therefore first choice for use in high-dose regimens in patients with this tumor.

Cyclophosphamide.

Two important extramedullary side effects have been reported. The urothelial toxicity, caused by one of its metabolites, acrolein, can result in severe hemorrhagic cystitis. This complication is seen in 8% of patients treated with weekly dosages of 200 mg/m^2 after a medium cummulative dose of 10.1 g/m^2 (26), but occurs in 32% of the patients after single doses of 200 mg/kg (27). Today this problem is virtually abolished, when 2-mercaptoethane sulfonate (mesnum) is given in combination with cyclophosphamide. This water soluble sulphydril containing compound inactivates acrolein (18) and prevents the epithelial toxicity. Furthermore, high-dose cyclophosphamide appears to cause a unique form of hemorrhagic myocarditis, frequently with a fatal outcome in combination regimens. This complication is then seen at a dose of 160 mg/kg or higher (28). When cyclophosphamide is used as a single agent up to dosages of 240 mg/kg (29) this complication is unlikely to occur, although one fatal case has been

reported after 180 mg/kg (30). Although myelosuppression at the highest dose level as single agent is severe, there is no benificial effect of ABMT on the nadir of the granulocytes or the time of recovery of the bone marrow aplasia (31).

Nitrosureas.

High-dose BCNU does result in several extramedullary toxic side effects. Most frequently encountered is the pulmonary toxicity. This was fatal in 9.5% of the patients at a dose of 1.2 g/m^2 (5). Pre-existing pulmonary disease is a major risk factor, while previous use of pulmonary toxic drugs and/or thoracic irradiation enhances the BCNU toxicity (32,33).

At a dose of 1.2 g/m^2 fatal hepatotoxicity occurs in 3% of the patients doses over 1.5 g/m^2 even have a 35% fatal outcome due to hepatic necrosis (5). ABMT has been found to shorten the period of marrow aplasia after high dose BCNU (34).

VP16-213.

Mucositis of the oropharyngeal region is the most important extramedullary toxicity of high-dose VP16-213. Increasing the doses over 1 g/m^2 leads to some mucositis in the majority of the patients. This could be caused by a local effect of the VP16-213 present in the saliva (36).

Wolff et al found dose limiting mucositis at 2.7 g/m^2, the drug was given i.v. on three consecutive days by one infusion per day (36). In our own institution dose limiting mucositis was seen at a dose level of 3.5 g/m^2, in this study 6 infusions were given with 12 hours interval (37). Bone marrow toxicity was serious but recovery occurred within three weeks after the infusions of VP16-213, and ABMT will probably not have any benificial effect on the bone marrow recovery at maximally tolerated dosages of VP16-213.

VM26.

In a dose escalation study of VM26 (38) bone marrow toxicity was substantial, although not dose-limiting. At a cumulative dose level of 1 g/m^2, given i.v. during three consecutive days, dermatologic toxicity was considered to be dose limiting. This dermatologic toxicity is comparable to the Stevens-Johnson syndrome which has been described after VP16-213 administration (39).

Methotrexate.

Haematological and extramedullary toxicity induced by HDMTX can be prevented completely by the use of leucovorin rescue (17). The renal toxicity of HDMTX, due to precipitation of the drug in the renal tubules and collecting ducts, can be prevented by adequate hydration and alkalinization of the urine during HDMTX administration.

TOXICITY OF COMBINATION REGIMENS

The development of high-dose combination regimens has not been extensively studied. Just as for single agents, defining useful combinations can only be done by following the traditional scheme of drug testing i.e. first evaluating single agent toxicity at high-dose levels, thereafter the toxicity of the combination of two or more antineoplastic drugs. At that time the combination can be used in phase II and phase III studies. Until now in only one study this methodical approach regarding the evaluation of the toxicity of high-dose cyclophosphamide and high-dose VP16-213 in combination with ABMT has been followed (40). Mucositis of the oropharyngeal region was dose-limiting after administration of 7 g/m^2 cyclophosphamide plus 2.5 g/m^2 VP16-213.

TOXICITY OF HDCT WITH TOTAL BODY IRRADIATION

Adding total body irradiation (TBI) to HDCT regimen might be useful because SCLC is very sensitive for radiotherapy. Furthermore the penetration of TBI into the CNS is not hampered by physiological barriers and may therefore be of value for the treatment of possibly present CNS metastases.

A major disadvantage of TBI in combination with HDCT is the very severe toxicity; besides the severe mucositis in most patients, cardiorespiratory failure was fatal in 50% of the patients after TBI 8 Gy, cyclophosphamide 100-240 mg/kg and vinblastine (41). Also combining nitrosureas and TBI is dangerous, two out of 6 patients developed fatal interstitional pneumonitis, when CCNU or BCNU was added to the less toxic combination of TBI and high-dose cyclophosphamide (42).

INDICATIONS FOR ABMT

Only two randomized studies have shown a beneficial effect of ABMT after HDCT (20,34). Other studies showed a shorter period of aplasia after HDCT

Table 1

Phase II studies in SCLC patients with progressive disease

therapy			CR	PR	NR	treatment related death	response duration in months	reference
CTX	6	g/m^2 **	1	4	3	1	3,4,5,7*,12+*	56
VP	0.5	g/m^2						
BCNU	300	mg/m^2						
BCNU	300	mg/m^2 **	2	3	0	0	not specified	57
PCZ	800	mg/m^2						
L-PAM	140	mg/m^2						
CTX	7	g/m^2 **	3	5	0	1	1+*,2+*,2*,3*,4*,4*,7*,9*	58
VP	0.9-1.5	g/m^2						
BCNU	0.6-1	g/m^2 **	1	3	4	0	not specified	59
BCNU	0.6-2.85	g/m^2 **	0	4	0	0	1.5	5
BCNU	0.6	g/m^2 **	0	0	3	0	not specified	60
MTX	1.5	g/m^2	0	0	17	0	-	54

* received further radio and/or chemotherapy

** received ABMT

with ABMT compared to less chemotherapy without ABMT (43,44). Also, the number of infused colony forming units in culture (CFU_c's) and the quality of the cryopreserved bone marrow was found to influence hematopoietic recovery (45,46,47). Certainly the aplastic period will be shortened by ABMT if TBI forms a part of the treatment. In other situations it is not certain if ABMT is necesary. The relative mild additional burden to the patients from the harvesting and reinfusion of bone marrow however, makes it difficult to perform controlled studies and therefore combining HDCT with ABMT is probably preferable.

STRATEGY OF HIGH DOSE THERAPY

Two different approaches for HDCT for SCLC are possible and both are currently investigated.

The first approach is the application of "up-front" HDCT in untreated patients. This approach can be regarded as a very intensive induction chemotherapy. The major advantage is the treatment of a tumor in which no resistance to cytostatic drugs has been induced by previous chemotherapy. A problem is what to do after this intensive induction if cure is not achieved after one course of therapy, as is with the current available drugs the most likely outcome. A repeated course of this intensive chemotherapy will hardly be possible because most patients are relatively old and recover slowly after the initial intensive treatment.

On the other hand treatment with standard dose therapy will also be difficult due to the limited marrow reserve after the induction treatment. Furthermore the value of "maintenance" standard therapy in this situation is questionable. The use of HDCT with ABMT "up-front" implicates that bone-marrow is reinfused which is potentially contaminated with tumor cells. In SCLC bone marrow metastases are already found in 20% of the patients at the time of the initial diagnosis (48) and regarding its early and wide spread dissemination the real number of patients with bone marrow involvement will be even much higher. Although it is uncertain if reinfusion of a small number of tumor cells will really lead to regrowth of tumor, it is not an attractive risk. This problem could become solvable as soon as specific monoclonal antibodies become available to purge the marrow in vitro with these antibodies. Because SCLC is a heterogenous tumor (49) it is probably indicated to use a panel of monoclonals to eliminate all contaminating tumor cells.

Table 2

"up-front" high dose chemotherapy *

Therapy			CR	PR	NR	treatment related death	response duration months	reference
CTX	4.5	g/m² **	7	6	0	0	med. 10 range 5-28+	61
VP	0.6	g/m²						
VCR	2x2	mg						
ADM	80	mg/m² (7 pat)						
VP	1.2	g/m²	4	4	2	0	not specified	16
CTX	160-200	mg/kg**	14	7	4	0	med. 10	15
CTX	100	mg/kg	3	10	1	0	med. 4+ range 2-11	62
VP	1.2	g/m²						

* all patients received further therapy

** with ABMT

Table 3 continued

CTX, ADM, VP +/- CCDP	CTX 100-200 mg/kg** VP 0.75-2.5 g/m²	6 PR, 3 NR → all further regression	not specified	66
VCR, ADM and IFO or CTX, VP	CTX 4.5 g/m² ** VP 0.6 g/m² VCR 2 mg +/- MTX 0.5-1.2 g/m² +/- ADM 40-80 mg/m²	10 CR → 10 CR 18 PR → 11 CR, 7 PR 1 NR → 1 PR	4 pat 24+	67
CTX, ADM, VP VCR, MTX	CTX 120 mg/kg** TBJ 10 Gy +/- CCNU/BCNU	3 CR → 3 CR 6 PR → 2 CR, 2 PR, 2 NE 1 NR → PR	survival 2-13 1 pat 2 yr	42
VCR, ADM, VP	CTX 7 g/m²	12 CR → 12 CR 15 PR → 4 CR, 11 PR	not specified	31
CTX, ADM, VCR, MTX	CTX 120 mg/kg** BCNU 400 mg/m² VP 1 g/m²	2 PR → 2 CR 1 NE 1 early death	3+,6	68
MTX, VCR, CTX, ADM VP, CDDP	CTX 6 g m² ** VP 0.5 g/m² BCNU 300 mg/m²	3 CR → 3 CR 10 PR → 4 CR, 3 CR, 3 ED	6+,25+	55

* received subsequent therapy; ** with ABMT

Table 3

Intensification after remission induction by standard dose chemotherapy

previous therapy	intensification regimen	intensification: response before → after	time till relapse (months)	reference
CTX, MTX, CCNU	CTX 120 mg/kg**	3 CR → 3 CR	4,8,15	63
VCR, PCZ, ADM	VP 0.6 g/m²	5 PR → 1 CR	3	
	20 Gy to tumor sites	2 PR	2,4	
		2 TD		
CTX, ADM, VCR	CTX 200 mg/kg**	5 CR → 5 CR	18	64
pred.		18 further regression but no CR	9	
CTX, ADM, VP	CTX 200 mg/kg**	11 CR → 11 CR	not specified	64
VCR, MTX	VP 1 g/m²	11 PR → 2 CR		
		9 PR		
CDDP, ADM, VP	CDDP 120 mg/m² **	3 CR → 3 CR		
	ADM 90 mg/m²	6 PR → 2 CR	5-17+	65
	VP 240 mg/m²	3 PR		
		3 Progr.		
		1 PR → 1 TD		
CDDP, ADM, VP	CDDP 120 mg/m² **	1 CR → CR	10+	65
	ADM 135 mg/m²	Progr. → PR	2	
	VP 360 mg/m²	1 CR → TD		

The second approach is the application of HDCT after remission induction. This second approach is based upon the mathematical model described by Norton and Simon (50); this model suggests that in treating minimal residual disease, only increased doses of chemotherapy could lead to cure, as it is obligatory to overcome relative drug resistance. Until now, this concept of "late intensification" has not been tested adequately in solid tumors. The results of allogenic bone marrow transplantation in leukemia, however, support this model. An advantage of this approach is the in vivo "clean up" of the bone marrow by the standard chemotherapy used for primary remission induction and in this way the risk of tumor cell infusion at the time of ABMT will be minimal. Furthermore by reducing the tumor load by standard dose therapy, a number of patients will have an improvement of their performance score and HDCT with ABMT will probably be tolerated easier than at the time of diagnosis. A potential risk is the diminished reserve capacity of the bone marrow that has to be reinfused at the time of intensification. The number of CFU_cs in the bone marrow after 4 courses of standard therapy in a group of SCLC patients, however, was not significantly different from the pretreatment number and harvesting of adequate numbers of stem cells for ABMT therefore seems to be no problem (51).

HIGH-DOSE CHEMOTHERAPY STUDIES IN SCLC

In the studies describing the effects of HDCT in SCLC up till now, arbitrary dosages of arbitrary combinations of cytostatic drugs have been given.

Furthermore only for cyclophosphamide and VP16-213 the existence of a dose-response relationship in SCLC has been made plausible. VM26 and BCNU have not been investigated sufficiently. HDMTX, however, does not show much more activity in SCLC than at standard doses (52), furthermore the addition of HDMTX to a combination regimen did not make a major difference in both response rate and survival (53). Also its activity in pretreated patients is minimal (54).

Probably only the methodical testing of single agents and combination regimens in phase I and II studies will result in the administration of the optimally dosed drug combinations.

The studies can be distinguished into three groups:

1. high-dose therapy in pretreated patients with recurrent or progressive disease (table 1),
2. high-dose therapy "up front" (table 2),
3. high-dose therapy after successful remission induction, the so called "late intensification" (table 3).

DISCUSSION

The evaluation of HDCT with or without ABMT has been started with a lot of optimism but the enthousiasm is lessening after what has been reported during the last two years. It is necessary to evaluate this time and money consuming therapy and to define at which point we are now. HDCT is undoubtedly an effective second line therapy for SCLC considering its high response rate in heavily pretreated patients, but application of HDCT as the initial treatment is not unequivocally successful regarding the survival time and the number of long term disease free survivors; both are comparable to what can be reached by standard dose chemotherapy. Although the regimens consist of either single agents at an optimal dose or combinations of submaximally dosed drugs, even a slight improvement has not been seen. This seems not to fit with the assumed dose response relationship and probably the application of a regimen consisting of presently available drugs at maximally tolerated dose levels will not result in a dramatic improvement. This makes high-dose induction chemotherapy not a promising approach.

The situation is more or less the same for "late intensification" regimens in partial or non responders. In almost every published study activity of the used combination is shown, regarding the improved responses in patients with still evaluable tumors after standard chemotherapy. Its effect in patients in complete remission is much more difficult to evaluate, due to the short follow up in the reported studies; besides that the number of long term survivors is frequently not mentioned.

Until now the preliminary results of only one phase III study have been reported, the results in patients with limited disease seem to be promising (55).

Theoretically the "late intensification" approach should be limited to patients with minimal residual disease, which means that only patients in a complete remission are suitable candidates. To answer the question if

this "late intensification" hypothesis is really of any value it has to be tested in this group of patients either in a phase II or phase III study. It is not clear at what time the "late intensification" has to be given; regarding the pattern of response in SCLC it should be rather early, for instance after 3 courses of standard chemotherapy because the maximal response is in most patients reached after 2 courses (12). Furthermore prolonged standard dose chemotherapy might give rise to drug induced tumor cell resistance in these initially very sensitive tumors.

As this moment the drugs suitable for dose escalation are only cyclophosphamide and VP16-213. HDMTX has not shown any increased effect in SCLC in comparison with standard doses. The widely applicated high-dose BCNU is probably not a very good choice considering its low activity at standard dose levels and its very severe extramedullary toxicity at high-dose levels. Also adding TBI results in an increase in toxicity and is therefore not an opportune choice. In conclusion HDCT is active as far as the response rate in patients with SCLC is increased, but a survival benefit has not been shown until now. Further phase II and III studies are necessary to decide if "late intensification" regimens have to be used on a larger scale. Improvement of the regimens used has to be pursued by adding new active drugs with limited extramedullary toxicity to the currently available combination regimen.

Abbreviations used:

CTX	= cyclophosphamide	VP	= VP16-213 or etoposide
BCNU	= carmustine	PCZ	= procarbazin
L-PAM	= melphalan	MTX	= methotrexate
CCNU	= lomustine	TBI	= total body irradiation
Pred	= prednisolone	CDDP	= cisplatinum
IFO	= ifosfamide	VCR	= vincristine
CR	= complete remission	PR	= partial remission
NR	= no response	TD	= toxic death
ED	= early death		

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SUMMARY AND CONCLUSIONS

This thesis deals with several aspects of experimental chemotherapy for SCLC. The development of chemotherapeutic intervention in patients with SCLC has been summarized in the introduction.

In Chapter I, the effects of a new combination of drugs that are synergistic in vitro, alternating with a potentially non-cross resistant standard-dose-regimen are described. The results of this treatment are comparable to several others, as published in the literature, with regard to response rate, response duration, median survival, and long term survival. The role of alternating regimens seems to be neglectable, probably because of not being non-cross resistant.

Other approaches for SCLC are necessary, one of these could be further intensification by means of dose escalation of chemotherapy. Only drugs, that have mainly myelotoxicity as side effect, are suitable for a considerable dose escalation, because in this situation rescue by autologous bone marrow transplantation is possible. Therefore the bone marrow reserve capacity of SCLC patients before and during chemotherapy was evaluated (Chapter 2). In a group of SCLC patients the number of colony forming units in culture (CFU_c's) in bone marrow was determined at different times.

Although CFU_c's do not really measure the number of stem cells, there is a relation between CFU_c's and the capability to restore hemopoiesis after potentially bone-marrow-ablative chemotherapy. The number of CFU_c's after induction chemotherapy was considered to be sufficient to aspirate and store the bone marrow at that time. A major advantage of aspiration after remission induction is the reduced risk of contamination with tumor cells.

In Chapter 3, the results of high-dose chemotherapy for solid tumors in the literature, have been compiled. In general there has not been a systematic approach in this area which has led to fragmentary data and investigations.

Toxicity data of single agents are usually available, but sufficient evaluation of combination regimens has not been done. Based on these data, two drugs were selected for high-dose chemotherapy in patients with SCLC: Cyclophosphamide and VP16-213. Toxicity data of high-dose cyclophosphamide are available from the literature, but the toxicity of high-dose VP16-213 has not been evaluated extensively, therefore we have performed a phase I

study of high-dose VP16-213 (Chapter 4). The dose-limiting toxicity was mucositis of the oropharyngeal region and occurred after 3.5 g/m^2. Hematologic toxicity was severe but does not seem to be dose-dependent. The possible existence of a dose-response relationship was supported by the response in patients with breast cancer and in a patient with adenocarcinoma resistant to standard dose VP16-213.

In Chapter 5, pharmacokinetics of high-dose VP16-213 were studied. VP16-213 was measured by high performance liquid chromatography with electrochemical detection. The plasma concentration versus time curve shows a tri-phasic decay with a very slow terminal phase. VP16-213 is strongly bound in the peripheral compartment. The volume of the central compartment varied from 7.4 to 20.1 l/m^2 and the steady state volume of distribution from 3.1 to 7.8 l/m^2. Relatively high concentrations were found in saliva, bile, ascites and urine. The total body clearance varied from 12.0 to 26.8 ml/min/m^2 and 26.2 to 53.4% was excreted unchanged into the urine, 8.3 to 17.3% as glucuronide.

The responses in two patients with CNS metastases of SCLC after high-dose VP16-213 prompted us to investigate the penetration of VP16-213 into the cerebrospinal fluid (CSF) (Chapter 6a). In all patients VP16-213 was found in the CSF, concentrations were low but apparently in the effective range for SCLC.

Interesting was the detection of a cis-isomere of the parent drug in CSF in a much higher concentration than in plasma. The role of this compound is still uncertain. The potential role of high-dose VP16-213 for the frequently occurring CNS metastases is illustrated in Chapter 6b describing a radiologically complete remission of brain metastases lasting up to six months.

In Chapter 7, the toxicity of the combination of the two most suitable drugs for high-dose chemotherapy for SCLC was evaluated. Combining cyclophosphamide, 7 g/m^2, and VP16-213, 2.5 g/m^2 led to unacceptable toxicity. WHO-grade 4 mucositis occurred in 2 out of 3 patients. Furthermore two older patients died during aplasia without a clear cause of death. The value of this treatment in older patients seems therefore questionable. In the 11 surviving patients bone marrow recovery was reached within 4 weeks following bone marrow infusion. Two patients with minimal residual disease of ovarian cancer had a long-term disease-free survival after this treatment. An important complication of high-dose

chemotherapy is infection during aplasia. Several aspects of selective decontamination of the digestive tract (SDD) in these patients are described in Chapter 8. Despite adequate decontamination by non-absorbable drugs, the infection incidence is high. This could be due to the intensity of the cytostatic treatment destroying natural barriers, such as skin and mucosa. To improve these disappointing results it would be necessary to use for SDD drugs with a systemic effect.

In Chapter 9, the regimen defined in Chapter 7, was evaluated regarding efficacy. Out of 9 patients with progressive SCLC 8 responded, 1 patient died during aplasia. The response rate of this regimen is >50% and might therefore be an interesting regimen for "late intensification" in patients in complete remission after standard dose chemotherapy.

In Chapter 10 all high-dose chemotherapy studies for SCLC from the literature have been compiled. Based on these data some conclusions can be drawn:

1. high-dose induction chemotherapy is not better than standard chemotherapy,
2. only two drugs, cyclophosphamide and VP16-213, are suitable for high-dose chemotherapy for SCLC,
3. high-dose chemotherapy after standard dose induction chemotherapy ("late intensification") in patients in complete remission, seems at present to be the only promising approach.

GENERAL CONCLUSION

SCLC has been treated by chemotherapy since the early seventies. The initial promising results have not been followed by a dramatic improvement in the prognosis. Overall treatment results have not changed during the last decade and are poor in most patients. The studies described in this thesis confirm this general trend. The results of the high-dose chemotherapy regimen are promising but this treatment can only be considered in a small group of patients with minimal residual disease. Although the role of high-dose VP16-213 for CNS relapses may be important, the number of patients with late CNS-relapse only is small, and therefore it will probably have only minor influence on long term survival.

In general, high-dose chemotherapy seems to be potentially useful in the small group of patients reaching a complete remission after standard dose

chemotherapy. For the great majority of the SCLC patients there are still no new therapies and the outlook is grim. Further understanding of the nature of this highly malignant tumor is needed. Application of new diagnostic techniques, as for instance monoclonal antibodies directed to SCLC, and the study of the in vitro behaviour of SCLC will probably shed new light on the nature of this tumor. Understanding of the factors influencing growth, development of resistance, and metastases may have implications for future treatment policies. In the mean time every effort has to be made to curtail or even abandon smoking habits especially in the younger age group. For the large group of already tumor-bearing patients the main goal of therapy has to be improvement of the quality of life.

SAMENVATTING EN CONCLUSIES.

In dit proefschrift worden verschillende aspecten van experimentele chemotherapie voor het kleincellig longcarcinoom beschreven.

De ontwikkeling van de chemotherapeutische behandeling voor patiënten met een kleincellig longcarcinoom is samengevat in de inleiding.

In hoofdstuk 1 worden de effecten van een in vitro synergistische combinatie van verschillene cytostatica, gealterneerd met een potentieel niet kruis-resistent, standaard-regime, beschreven. De resultaten van deze behandeling zijn vergelijkbaar met verschillende andere, zoals gepubliceerd in de literatuur, wat betreft responsfrequentie, responsduur, mediane overleving en langdurige overleving. Het nut van alternerende regimes lijkt nihil, waarschijnlijk doordat ze niet echt kruis-resistent zijn.

Andere benaderingen voor het kleincellig longcarcinoom zijn noodzakelijk. Een van de mogelijkheden is verdere intensivering van de behandeling door een dosisescalatie van de chemotherapie. Hiervoor zijn alleen cytostatica geschikt die als belangrijkste bijwerking beenmergsuppressie hebben, omdat in deze situatie door autologe beenmergtransplantatie persisterende aplasie kan worden voorkomen. Daarom werd de beenmergreservecapaciteit van patiënten met een kleincellig longcarcinoom voor en tijdens chemotherapie geëvalueerd (hoofdstuk 2). In een groep patiënten met een kleincellig longcarcinoom werd het aantal kolonievormende eenheden (CFU_c's) in beenmerg bepaald op verschillende tijdstippen. Hoewel CFU_c's niet echt het aantal stamcellen meten, is er wel een relatie tussen het aantal CFU 's in het beenmerg en de mogelijkheid om door dat beenmerg herstel van de hematopoiese te verkrijgen na potentieel beenmerg-ablatieve chemotherapie. Het aantal CFU_c's na de inductiechemotherapie was voldoende om op dat tijdstip beenmerg af te nemen en zonodig in te vriezen. Een groot voordeel van beenmergafname na de remissie-inductie is het verminderde risico van contaminatie met tumorcellen.

In hoofdstuk 3 worden de resultaten samengevat van hoge-dosis chemotherapie voor solide tumoren. In zijn algemeenheid is er niet een systematische benadering geweest op dit gebied wat ertoe geleid heeft dat er slechts weinig gegevens uit de literatuur bekend zijn. Gegevens over bijwerkingen van de verschillende middelen afzonderlijk zijn meestal wel bekend, maar combinatiebehandelingen zijn onvoldoende geevaluëerd. Op

grond van de literatuurgegevens werden 2 middelen geselecteerd voor hoge-dosis chemotherapie bij patiënten met een kleincellig longcarcinoom: cyclofosfamide en VP16-213. De bijwerkingen van hoge doses cyclofosfamide zijn bekend uit de literatuur; de bijwerkingen van hoge doses VP16-213 zijn veel minder bekend en daarom werd er een fase-1-studie van hoge doses VP16-213 verricht (hoofdstuk 4). De dosis limiterende toxiciteit van deze behandeling was mucositis van de oropharynx; deze trad op na 3,5 g/m^2.
De hematologische toxiciteit was ernstig, maar niet dosisafhankelijk. De mogelijk bestaande dosis-respons relatie van VP16-213 wordt ondersteund door de respons gevonden bij patiënten met mammatumoren en bij een patiënt met een adenocarcinoom. Dit adenocarcinoom was tevoren resistent geworden tegen standaard-dosis VP16-213.
In hoofdstuk 5 wordt de farmacokinetiek van hoge dosis VP16-213 beschreven. VP16-213 werd gemeten door middel van HPLC met electrochemische detectie. De plasmaconcentratiecurve toonde een driefasisch beloop in de tijd met een erg langzame terminale fase. VP16-213 wordt zeer sterk gebonden in het perifere compartiment. Het volume van het centrale compartiment varieerde van 7,4-20,1 l/m^2 en het distributievolume op het moment van de steady state varieerde van 3,1-7,8 l/m^2. Relatief hoge concentraties werden gevonden in speeksel, gal, ascites en urine. De totale lichaamsklaring varieerde van 12,0-26,7 ml/min/m^2 en 26,2-53,4% werd onveranderd uitgescheiden in de urine, 8,3-17,3% als glucuronide.
De respons, gezien in 2 patiënten met kleincellig longcarcinoom met metastasen in het centrale zenuwstelsel, na hoge doses VP16-213 waren aanleiding om de penetratie van VP16-213 in de liquor te onderzoeken (hoofdstuk 6a). Bij alle patiënten werd VP16-213 gevonden in de liquor, de concentraties waren laag, maar klaarblijkelijk wel hoog genoeg om effekt te sorteren tegen het kleincellig longcarcinoom. Een cis-isomeer van VP16-213 werd gevonden in de liquor in een veel hogere concentratie dan in plasma, de betekenis hiervan is onduidelijk. De potentiële waarde van hoge dosis VP16-213 voor de zeer frequent voorkomende hersenmetastasen is geïllustreerd in hoofdstuk 6b, hierin wordt een radiologisch complete remissie van hersenmetastasen gedurende 6 maanden beschreven.
In hoofdstuk 7 worden de bijwerkingen van de combinatie van de twee meest geschikte middelen voor hoge dosis chemotherapie voor het kleincellig longcarcinoom geëvalueerd. Het combineren van cyclofosfamide, 7 g/m^2, en

VP16-213, 2,5 g/m², leidde tot onacceptabele toxiciteit, bij 2 van de 3 patiënten werd er ernstige mucositis gevonden. Bovendien overleden 2 van de oudere patiënten gedurende de aplastische fase zonder een duidelijke verklaring voor hun dood. Het nut van deze behandeling voor met name oudere patiënten is dan ook twijfelachtig. Bij de 11 overlevende patiënten was er beenmergherstel binnen 4 weken na de beenmerginfusie. Twee patiënten met minimale restanten van een ovariumcarcinoom waren langdurig ziektevrij na deze behandeling.

Een belangrijke complicatie van hoge dosis chemotherapie is infectie gedurende de aplastische fase. Enkele aspecten van selectieve decontaminatie van de tractus digestivus (SDD) bij deze patienten worden beschreven in hoofdstuk 8. Ondanks adequate decontaminatie door niet-resorbeerbare middelen, was de infectie-frequentie hoog. Dit is mogelijk het gevolg van de intensiteit van de chemotherapie waardoor natuurlijke barrieres,zoals huid en slijmvlies, worden vernietigd. Om deze teleurstellende resultaten te verbeteren zal het nodig zijn SDD te geven door middelen met een meer systeem effect.

In hoofdstuk 9 wordt de effectiviteit van de combinatie zoals beschreven in hoofdstuk 7 geëvalueerd. Van 9 patiënten met een progressief kleincellig longcarcinoom toonden 8 een respons, 1 patiënt overleed gedurende de aplastische fase. De respons-frequentie van dit regime is >50% en is mogelijk een waardevolle behandeling voor patiënten in complete remissie na standaard-dosering chemotherapie.

In hoofdstuk 10 zijn alle studies betreffende hoge dosis chemotherapie voor het kleincellig longcarcinoom, zoals tot nu toe gepubliceerd, samengevat. Gebaseerd op deze gegevens kunnen enkele konklusies worden getrokken:

1. Hoge-dosis chemotherapie is niet beter dan standaard-chemotherapie.
2. Tot nu toe zijn slechts 2 middelen, cyclofosfamide en VP16-213, geschikt voor hoge-dosis chemotherapie voor het kleincellig longcarcinoom.
3. Hoge-dosis chemotherapie, na standaardinductie-chemotherapie ("late intensification") lijkt op dit moment de enige potentieel waardevolle benadering.

ALGEMENE CONCLUSIE.

Het kleincellig longcarcinoom wordt sinds de beginjaren '70 behandeld met chemotherapie. De aanvankelijk zeer veel belovende resultaten hebben helaas nog niet geleid tot een drastische verbetering van de prognose. De laatste 10 jaar zijn de resultaten van de behandeling niet veranderd en nog steeds erg slecht bij het merendeel van de patiënten. De onderzoekingen beschreven in dit proefschrift bevestigen deze algemene trend. De resultaten van hoge-dosis chemotherapie zijn mogelijk hoopvol, maar deze behandeling is waarschijnlijk alleen zinvol bij een kleine groep patiënten met minimale resttumor. Hoewel de behandeling met hoge-dosis VP16-213 voor centraal zenuwstelsel recidieven belangrijk kan zijn, is het aantal patiënten dat alleen daar recidiveert klein en zal het effect op de langdurige overleving van de grote groep patiënten waarschijnlijk zeer gering zijn

Voor het merendeel van de patiënten met een kleincellig longcarcinoom zijn er nog geen nieuwe therapieën en de vooruitzichten zijn tot nu toe zeer somber. Toepassing van nieuwe diagnostische technieken zoals bijvoorbeeld monoclonale antilichamen gericht tegen het kleincellig longcarcinoom, en de studie van het in vitro gedrag van kleincellig longcarcinoom, kunnen mogelijk nieuw licht werpen op de aard van deze tumor. Het begrijpen van factoren die de groei, de ontwikkeling van resistentie en de metastasering beïnvloeden zal consequenties hebben voor toekomstige behandelingsstrategieën. In de tussentijd moet alles in het werk gesteld worden om de rookgewoonten van met name de jongere bevolkingsgroep te veranderen. Voor de grote groep patiënten die al een kleincellig longcarcinoom heeft is het belangrijkste doel van de behandeling op dit moment verbetering van de levenskwaliteit.