Eur J Cancer, Vol. 28A, No. 8/9, pp. 1311–1315, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00
Pergamon Press Ltd

Pharmacokinetically Guided Dosing for Intravenous Melphalan: a Pilot Study in Patients with Advanced Ovarian Adenocarcinoma

Dominique Y. Ploin, Brigitte Tranchand, Jean-Paul Guastalla, Paul Rebattu, Franck Chauvin, Michel Clavel and Claude Ardiet

Pharmacokinetically guided administration of melphalan was investigated during a pilot study in patients with advanced ovarian adenocarcinoma. The schedule involved a fixed dose on day 1 (7.9 mg) followed by a second dose on day 2, calculated on the basis of pharmacokinetic data to achieve a target area under the concentration–time curve (AUC). 20 courses of intravenous melphalan were administered to 7 patients. AUC, standardised to 1 mg/m², ranged between 4.3 and 8.9 (mg/l) min. In 12 fully evaluable courses, less than 15% deviation from the target AUC was found, showing that AUC monitoring was possible by means of the test dose. Pharmacodynamic effects showed a positive correlation with melphalan AUC. Myelosuppression appeared at 47 (mg/l) min and grade 3 or 4 haematological toxicities were observed in 4 cycles, associated with AUC values ranging between 86 and 112 (mg/l) min. Relative leucocyte decreases were well correlated with AUC values.
Eur J Cancer, Vol. 28A, No. 8/9, pp. 1311–1315, 1992.

INTRODUCTION

SYNTHETISED IN 1953, melphalan is still a widely used anticancer drug. This alkylating agent has been extensively studied and its chemical, kinetic, therapeutic and toxic properties are well defined: the main dose-limiting side-effect is myelosuppression, which is observed even after conventional doses [1–4].

Many pharmacokinetic studies have been undertaken in patients receiving high-dose melphalan therapy [5–9]. When doses of 60 mg/m² or more were employed, no evidence for a relationship between pharmacokinetic parameters and the intensity of myelosuppression could be established since all patients were in total aplasia within a few days after treatment [10–14]. At conventional doses, a very wide range of pharmacokinetic parameters have been reported [9, 15–19]. On the whole, no relationship between myelosuppression and pharmacokinetic data has been found. Since the use of area under the plasma concentration–time curve (AUC) as a pharmacokinetic guide to escalate doses is advocated for phase I studies [20–22], the objective of the present study was to establish a relationship between haematological toxicities in patients and melphalan AUC.

Knowledge of the relationship between melphalan AUC and toxicity might allow the control of dose-dependent toxicity as with, for example, methotrexate [23, 24] and 5 fluorouracil [25].

The present report gives our experience of the relation between melphalan toxicity and AUC (units in (mg/l)min) as compared to the dose (mg or mg/m²).

Correspondence to D.Y. Ploin.
D.Y. Ploin, B. Tranchand, J.-P. Guastalla, P. Rebattu, M. Clavel and C. Ardiet are at the Département de Médecine, and F. Chauvin is at the Service de Biostatistiques, Centre Léon Bérard, 28 Rue Laënnec, F-69373 Lyon Cedex 08, France.
Revised 22 Jan. 1992; accepted 17 Mar. 1992.

PATIENTS AND METHODS

Methods

This study was a dose escalation trial using AUC in place of usual dose units. The design was that of an open trial without any control population. Interpatient AUC escalation was carried out between cohorts of 3 patients. Intrapatient dose modifications were carried out according to the toxicity observed in the previous cycle. The dosing strategy was based on the observation that, for individual patients, there is a linear relationship between dose and AUC within a 24-h interval [8].

The entry target AUC was defined as follows: referring to the study conducted by Zucchetti *et al.* in a similar patient population [26]; referring to our historical population treated with 140 mg/m² of melphalan, with an observed mean AUC of 427 (154) (mg/l)min [mean (S.D.)] [5]. Since 12 mg/m² was grossly equivalent to 30 (mg/l) min, this value was chosen for the entry AUC level. For dose escalation, a step fixed to 15 (mg/l)min was chosen, corresponding to half the entry AUC level.

Thus the entry target AUC was 30 (mg/l) min for 3 patients, 45 for 3 patients and 60 for 1 patient. Furthermore, the target AUC in successive cycles in individual patients (intrapatient modification) was modified according to the degree of myelosuppression encountered after the previous cycle; this involved the leucocyte, neutrophil and platelet nadirs scored on the WHO scale [27] with modifications as follows: reduction by 30 AUC units when a grade 4 toxicity was observed, reduction by 15 AUC units when a grade 3 toxicity was observed, no change when a grade 2 toxicity was observed and escalation by 15 AUC units when a grade 1 or 0 toxicity was observed.

Written informed consent was obtained from each patient before treatment. The protocol was activated when the local ethical committee gave its agreement. The study was stopped when the feasibility of AUC monitoring was shown.

Patients' characteristics

7 women with advanced ovarian adenocarcinoma histologically proven and refractory to prior chemotherapy or radio-

Table 1. Characteristics of the patients when enrolled

Patient no.	Age* (years)	PS†	Body area (m^2)	GRF‡ (ml/s)
1	62	2	1.68	0.71
2	62	2	1.68	0.71
3	63	2	1.46	0.82
4	67	2	1.60	0.73
5	60	1	1.77	0.66
6	68	0	1.48	0.59
7	48	1	1.58	0.76

*Age when enrolled.
†WHO scale for performance status [27].
‡Calculated creatinine clearance [28].

therapy were enrolled from March to June 1989 and treated in the Centre Léon Bérard (Lyon, France). Inclusion criteria were: predicted life expectancy of at least 2 months at time of entry, a WHO performance status less than 2, more than a 1-month interval since prior chemotherapy, previous radiotherapy involving less than 30% of the skeleton, and no evidence for pulmonary fibrosis. Baseline serum creatinine, aspartate and alanine transaminase, bilirubin less than 125% of the upper limit of normal range, normal peripheral blood counts (leucocytes > 4000/μl, neutrophils > 1500/μl and platelets > 150 000/μl) were taken as biological inclusion criteria.

Patients were 48 to 68 years old. They were enrolled between 8 and 114 months after diagnosis of the disease. All patients received prior chemotherapy including an association of an alkylating agent and cisplatin. 5 patients had a history of myelosuppression related to prior chemotherapy. 4 patients had polyneuritis related to prior platinum treatment. Additional characteristics of the patients are given in Table 1; creatinine clearance calculated according to Lott and Hayton [28] ranged between 0.59 and 0.82 ml/s; 5 patients had pleural or abdominal effusion. There was evidence for progressive disease for 6 patients: 3 patients (1, 3, 4) had measurable disease, 3 patients had appreciable disease and 1 patient did not present any evidence of disease after surgery for a retrocrural metastatic lymph node. Serum carbohydrate antigen 125 (CA-125) assay was positive for 6 patients, ranging between 278 and 3683 U/ml when enrolled.

Treatments

Each cycle consisted of two injections. Melphalan was administered by a 5-min regular intravenous infusion. On day 1, each patient was given a test dose of melphalan (7.9 mg corresponding to 0.85 ml of melphalan-reconstituted solution measured with an insulin syringe), after which the AUC was determined ($AUC_{day\ 1}$). On day 2, a second injection of melphalan was given to achieve $AUC_{day\ 2}$ ($AUC_{day\ 2} = AUC_{target} - AUC_{day\ 1}$). The actual $AUC_{day\ 2}$ was measured whenever possible or estimated from the dose administered, assuming linear pharmacokinetics [8]. Each cycle of two injections was repeated at 28-day intervals for a total of two to five cycles, depending on toxicity and tumour response. The interval between cycles could be extended from 28 days (4 weeks) to a maximum of 49 days (7 weeks) to allow recovery of normal blood counts. Patients whose blood counts were not normalised by day 49 were withdrawn from the study.

Pharmacokinetic data were obtained by blood sampling 5 min before melphalan infusion and at 5, 10, 15, 30, 45, 60, 90 and 120 min after each injection. Melphalan assay was performed following a method adapted from Woodhouse and Henderson [29]. Briefly, 500 μl of plasma were vortexed for 1 min with 1 ml isopropanol containing 200 ng/ml of *N*-acetyl procainamide as internal standard. The protein precipitate was removed by centrifugation and the supernatant was extracted with 1 ml dichloromethane. The aqueous layer was discarded and the organic layer was evaporated under a stream of nitrogen. The residue was then reconstituted with 150 μl of mobile phase and 50 μl injected in the high-performance liquid chromatography (HPLC) apparatus. This consisted in a Waters 510 pump delivering the mobile phase (methanol–water 50:50, with sodium heptane–sulphonate 5 mmol/l) at a rate of 1.2 ml/min through a C18, 5 μm, 4.1 mm internal diameter, 15 cm column. The effluent was monitored at the maximum ultraviolet absorbance (260 nm) and peak height integration was performed for *N*-acetyl procainamide and melphalan by means of a Kontron Anacomp 220 computer. Pharmacokinetic data were treated using PHARM program (Simed, Creteil).

Toxicity and response evaluation

Patients were evaluated for response 4 weeks after the third and the fifth cycle or after withdrawal from the study. This evaluation included general physical examination, chest X-ray, abdominoperitoneal sonography or computerised tomographic scan, carcinoembryonic antigen (CEA) and CA-125 assay, clinical chemistry including blood counts, renal and hepatic function assessment; these were done comparatively to evaluation at enrollment.

Toxicities of each cycle were evaluated by physical examination on days 0, 1, 2 for immediate side-effects. Blood counts including differential and serum creatinine measurement were repeated on days 0, 1, 2, 7, 14, 21, 28 and reconducted each week when necessary.

RESULTS

34 infusions of melphalan were administered during 20 cycles of treatments. AUC values are presented for each cycle in Table 2. The target AUC ranged between 30 and 75 (mg/l)min. Total doses (7.9 mg of the test dose + complementary dose given on day 2) ranged from 7.9 to 30.2 mg. We succeeded in determining the AUC values for 31 infusions. AUC values are detailed for days 1 and 2 in Table 2. The observed AUC ranged from 31 to 111.8 (mg/l) min. AUC values, standardised to 1 mg/m^2, are presented in Table 3; they ranged between 4.3 and 8.9 (mg/l)min. Figure 1 shows standardised AUC values observed in this study {5.8 (1.3) [(mg/l)min]/(mg/m^2):mean (S.D.)}, compared to our historical reference population [5] {3.1 (1.1) [(mg/l)min]/(mg/m^2)}. There was a significant difference between the two data sets with a *P*-value less than 2.10^{-6} (Mann–Whitney test).

Feasibility of the monitoring

14 cycles were monitored by the method of the test dose. The target AUC was reached for 12 cycles with a measured error less than 10% in 11 cases and less than 15% in 1 case, compared to the target AUC (Table 2). In those cases, the monitoring was considered as successful. Two cycles showed an observed AUC with a deviation about 45%; they were considered as monitoring failures. Six cycles were not monitored. For patient 4, cycle 2, the patient refused blood sampling on day 2. For patient 2, cycle 1, the patient was accidentally overdosed. In 4 cases, pharmacokinetics could not be monitored because endogenous

Table 2. AUC Target, doses received at days 1 and 2, and AUC observed for days 1 and 2

Patient	Cycle	Target AUC [(mg/l)min]	$Dose_{day\ 1/day\ 2}$ (mg)	$AUC_{day\ 1/day\ 2}$ [(mg/l)min]	AUC Deviation (%)
1	1	30	7.9 / 2.4	23.8 / 7.3*	3.4
	2	45	9.2†/ 2.3	38.1 / 9.5*	5.7
	3	60	7.9 / 6.2	33.8 /30.7	7.5
2	1	30	7.9 /15.7	40.1 /71.7	‡
	2	45	7.9 /—	45.6 /—	1.3
3	1	30	7.9 /—	31.6 /—	5.2
	2	45	7.9 / 6.0	24.8 /19.6	1.4
	3	60	7.9 /10.3	26.0 /30.8	3.5
4	1	45	7.9 / 3.9	33.0 /16.3§	9.6
	2	60	7.9 /14.4	22.6 /—§	NE
5	1	45	7.9 / 5.3	27.1 /18.1	0.4
	2	60	7.9 /22.3	15.7 /72.1	46.3
	3	45	7.9 / 6.3	25.5 /15.7	2.5
	4	60	7.9 /—	59.7‖/—	NE
	5	60	7.9 /—	35.4‖/—	NE
6	1	45	7.9 / 5.5	26.5 /20.5	4.4
	2	60	7.9 /21.8	16.0 /70.1	43.3
7	1	60	7.9 /—	NE¶ /—	NE
	2	75	7.9 /19.9	17.1 /68.8	14.6
	3	60	7.9 /—	NE¶ /—	NE

* Concentrations to low for AUC measurement.
† 1 ml of drug administered in place of 0.85 ml usually.
‡ Accidentally overdosed.
§ Patient's refusal of sampling at day 2.
‖ Delayed AUC results (> 24 h).
¶ HPLC interference in melphalan assay.
NE= not evaluable.

Table 3. Doses of melphalan administered and haematological toxicities scored according to the WHO scale for evaluable cycles

Patient no.	Cycle	Doses of melphalan: Usual units (mg)	Usual units (mg/m^2)	Observed AUC [(mg/l)min]	Observed AUC (standardised to 1 mg/m^2)	Haematological toxicities: WHO grading* L/N/P
1	1	10.3	6.1	31.0	5.1	0/0/0
	2	11.6	6.9	47.6	6.9	0/0/0
	3	14.0	8.6	64.5	7.5	NE
2	1	23.6	15.2	111.8	7.4	3/3/4
	2	7.9	5.1	45.6	8.9	NE
3	1	7.9	5.4	31.6	5.8	0/0/0/
	2	13.9	9.5	44.4	4.7	0/0/0
	3	18.2	12.5	56.8	4.5	1/0/0
4	1	11.8	7.4	49.3	6.7	1/0/0
5	1	13.1	7.4	45.1	6.1	0/0/0
	2	30.2	17.0	87.8	5.2	3/3/2
	3	14.2	8.0	41.2	5.2	0/0/0
6	1	13.4	9.0	47.0	5.2	1/1/0
	2	29.6	20.0	86.0	4.3	3/3/2
7	2	27.8	17.6	86.0	4.9	3/3/2

*L = leucocyte nadir, N = neutrophil nadir, P = platelet nadir [27].
NE = not evaluable.

or drug-associated plasma peaks interfered during melphalan assay; this resulted either in non-evaluable kinetics (patient 6, cycles 1 and 3) or in delayed AUC determination (patient 5, cycles 4 and 5). In these cases, the AUC of the test dose ($AUC_{day\ 1}$) could not be measured within 24 h after infusion on day 1 and the corresponding cycles could not be monitored: melphalan could be separated thereafter with either modification of the mobile phase (e.g. adding 3% tetrahydrofuran) or by using a C8 column in place of the C18 phase usually employed. For patient 6, cycles 1 and 2, sufficient plasma was not collected to find adequate chromatographic conditions.

Toxicity

In the 15 courses fully evaluable for AUC measurements, blood counts could be completely investigated in 13 (two deaths related to progressive disease). Values for the nadirs, scored on the WHO scale, are given in Table 3 as well as the melphalan dose expressed as mg, mg/m^2 or AUC units. Haematological toxicity appeared at 47 (mg/l)min. Grade 3 or 4 neutrophil or platelet toxicities were observed between 86 and 111.8 (mg.l)min. Relative leucocyte decrease percentages: 100 × (pretreatment leucocyte count − nadir leucocyte count)/(pretreatment leucocyte count), showed a positive correlation with AUC values: $r=0.854$ (see Fig. 2).

The only clinical consequence of haematological toxicity was observed when patient 2 was accidentally overdosed with 23.6 mg (15.2 mg/m^2) melphalan, leading to an AUC level of 111.8 (mg/l)min. Myelosuppression resulted in a septicaemic episode, with a neutrophil nadir of 825/μl and a platelet nadir of 18000/μl. Septic signs were controlled within 4 days after usual antibiotherapy and hydration; platelet transfusions were performed until platelet counts were greater than 50 000/μl and no haemorrhage was observed. Normalisation of platelet counts was delayed: > 50 000/μl at day 30 and > 100 000/μl only at day 44; this cycle was not considered as monitored. No incident was encountered during the monitored cycles.

Nausea and vomiting were the only non-haematological side-effects. They were always moderate, never exceeding 2 on the WHO scale [27].

Evaluation of patients

A total of four progressive diseases were observed (patients 1, 3, 4, 7) and three disease-related deaths occurred during the

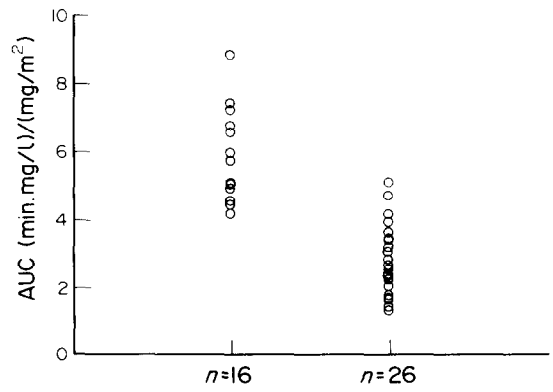

Fig. 1. Distribution of AUC standardised to 1 mg/m^2. Left side: 16 courses of the present study; right side: 26 courses in the historical reference population [5].

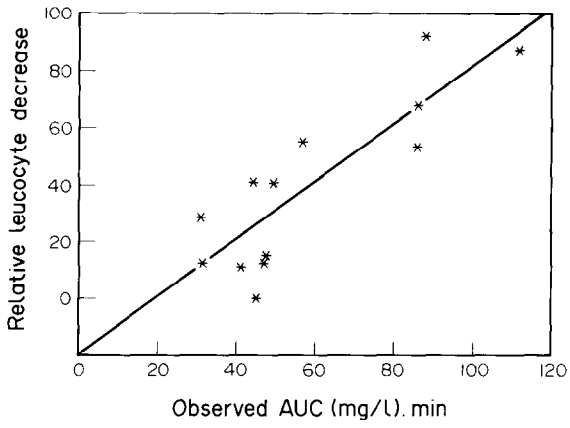

Fig. 2. Percentage of decrease in leucocyte count vs. AUC values.

trial (patients 1, 2, 7). No new lesion appeared during the trial. For patient 5, a minor response was observed. For patient 6, no evidence of relapse was found after 19 months of follow-up.

DISCUSSION

The purpose of the present pilot study was to evaluate the feasibility of administering melphalan at predefined AUC levels, and to assess the relationship between pharmacokinetic parameters (AUC) and haematological side-effects.

We observed a wide range of AUC values, even when AUC values were standardised to a 1 mg/m^2 dose for comparative purposes. In a previous study of high-dose melphalan administration followed by autologous bone marrow graft, a broad range of values was also found [5]. However, the mean value in our high-dose melphalan patients was found to be 3.1 (1.1) (mg/l)min [mean (S.D.) standardised to a 1 mg/m^2 dose], which is a significantly lower value than in the present study [5.8 (1.3) (mg/l)min]: P-value less than 2×10^{-6}. The main apparent difference between the two sets of patients is a high hydration regimen with forced diuresis which was used during high-dose melphalan for bone marrow conditioning. In the present study, our group of patients was not given any forced diuresis; the calculated GFR was low in every patient as shown in Table 1, and according to the previous treatments including platinum derivatives their renal function could have been damaged at some extent. Furthermore, most of ovarian cancer patients in this study suffered from either abdominal or pleural effusion, or both. This may explain, at least in part, the particular feature of the drug disposition in these patients.

In 2 cases, pharmacokinetic monitoring failed. For patient 5, cycle 2, a parenteral injection of lidocaine for local anesthesia in order to insert a subclavian intravenous line a few hours before the test dose infusion might have led to a possible drug interaction. For patient 6, cycle 2, no drug-associated interaction was apparent; failure of melphalan assay was possible since many plasma components were found on the HPLC plot. In fact, the presence of plasma components interfering in the HPLC assay may represent a difficulty, which was controllable in most cases in the present study. In our subsequent experience, a check sample of plasma, drawn even several days before treatment, was found very useful for detecting endogenous, potentially interfering, compounds in order to identify adequate chromatographic conditions for individual patients.

Previously, no relationship has been established in the literature between the degree of myelosuppression and the occurrence of low or high values of AUC for melphalan. Due to the properties of this alkylating agent, haematological side-effects are likely to depend on pharmacokinetic parameters, in particular the AUC value. Our hypothesis was that toxicity should increase with AUC levels, which appears to be confirmed by the fact that the four highest AUC levels led to toxicities scored 3 or 4. Patient 2, cycle 1 was particularly remarkable: a moderate dose (23 mg; 15 mg/m^2) resulted in an important toxicity with aplasia scored 3 on leucocytes and 4 on platelet counts simultaneously with septic signs and positive bacteriaemia. The moderate dose given to this patient gave a high AUC level of 111.8 (mg/l)min; it might be explained by a particular disposition of melphalan in this patient. Furthermore, the patient was heavily pretreated before enrollment. From Table 3, it appears that increasing AUC leads to increased haematological toxicity. This is not only true between patients, but is also found during intrapatient dose modifications. Pharmacokinetic differences between patients may explain, at least in part, the variability in the occurrence of haematological side-effects. This unwanted part of the pharmacodynamic effects must be kept within acceptable limits during conventional chemotherapeutic treatments, keeping in mind the beneficial dose effect demonstrated by some authors [12].

In conclusion, the feasibility of melphalan monitoring was demonstrated as well as the positive correlation between AUC values and haematological toxicities. A new study is ongoing to specify the relationship between pharmacokinetic and pharmacodynamic data in order to define the optimal target AUC in individual patients.

1. Sarosy G, Leyland-Jones B, Soochan P, Cheson BD. The systemic administration of intravenous melphalan. *J Clin Oncol* 1988,**6**, 1768–1782.
2. Cornwell GG III, Pajak TF, McIntyre OR, Kochwa S, Dosik H. Influence of renal failure on myelosuppressive effects of melphalan: cancer and leukemia group B experience. *Cancer Treat Rep* 1982, **66**, 475–481.
3. Smith DB, Kenny JB, Scarffe JH, Maley WV. Phase II evaluation of melphalan in adenocarcinoma of the pancreas. *Cancer Treat Rep* 1985, **69**, 917–918.
4. Spitzer G, Valdivieso M, Farha P, *et al.* IV melphalan in carcinoma of the lung: effect of cyclophosphamide priming on hematopoeitic toxicity. *Cancer Treat Rep* 1986, **70**, 449–453.
5. Ardiet C, Tranchand B, Biron P, Rebattu P, Philip T. Pharmacokinetics of high-dose intravenous melphalan in children with forced diuresis. *Cancer Chemother Pharmacol* 1986, **16**, 300–305.
6. Gouyette A, Hartmann O, Pico JL. Pharmacokinetics of high-dose melphalan in children and adults. *Cancer Chemother Pharmacol* 1986, **16**, 184–189.
7. Taha IAK, Ahmad RA, Rogers D, Pritchard J, Rogers HJ. Pharmacokinetics of melphalan in children following high-dose intravenous injection. *Cancer Chemother Pharmacol* 1983, **10**, 212–216.
8. Tranchand B, Ploin YD, Minuit MP. *et al.* High-dose melphalan dosage adjustment: possibility of using a test-dose. *Cancer Chemother Pharmacol* 1989, **23**, 95–100.
9. Reece PA, Hill HS, Green RM. *et al.* Renal clearance and protein binding of melphalan in patients with cancer. *Cancer Chemother Pharmacol* 1988, **22**, 348–352.
10. McElwain TJ, Hedley DW, Burton G, *et al.* Marrow autotransplantation accelerates haematological recovery in patients with malignant melanoma treated with high-dose melphalan. *Br J Cancer* 1979, **40**, 72–80.
11. McElwain TJ, Powles RL. High-dose intravenous melphalan for plasma-cell leukaemia and myeloma. *Lancet* 1983, **2**, 822–824.
12. Maraninchi D, Pico JL, Hartmann O, *et al.* High-dose melphalan with or without marrow transplantation: a study of dose-effect in patients with refractory and/or relapsed acute leukemias. *Cancer Treat Rep* 1986, **70**, 445–448.
13. Mascret B, Maraninchi D, Gastaut JA, *et al.* Phase I–II study of

high-dose melphalan and autologous marrow transplantation in adult patients with poor-risk non-Hodgkin's lymphomas. *Cancer Chemother Pharmacol* 1985, **14**, 216–221.
14. Harousseau JL, Milpied N, Guilhot F, Garand R, Bourhis J. Traitement de première intention des myélomes grave du sujet jeune par melphalan a haute dose. *Presse Méd* 1988, **17**, 1471–1474.
15. Bosanquet AG, Gilby ED. Pharmacokinetics of oral and intravenous melphalan during routine treatment of multiple myeloma. *Eur J Cancer Clin Oncol* 1982, **18**, 355–362.
16. Brox L, Birkett L. Pharmacology of intravenous melphalan in patients with multiple myeloma. *Cancer Treat Rev* 1979, **6**, 27–32.
17. Chang SY, Alberts DS, Melnick LR, Walson PD, Salmon SE. High-pressure liquid chromatographic analysis of melphalan in plasma. *J Pharm Sci* 1978, **67**, 679–681.
18. Tattersall MHN, Jarman M, Newlands MJ, Holyhead L, Milstead RAV, Weinberg A. Pharmacokinetics of melphalan following oral or intravenous administration in patients with malignant disease. *Eur J Cancer* 1978, **14**, 507–513.
19. Woodhouse KW, Hamilton P, Lennard A, Rawlins MD. The pharmacokinetics of melphalan in patients with multiple myeloma: an intravenous/oral study using a conventional dose regimen. *Eur J Clin Pharmacol* 1983, **24**, 283–285.
20. EORTC Pharmacokinetic and Metabolism Group. Pharmacokinetically guided dose escalation in phase I clinical trials. Commentary and proposed guidelines. *Eur J Cancer Clin Oncol* 1987, **23**, 1083–1087.
21. Evans WE, Relling MV. Clinical pharmacokinetics—pharmacodynamics of anticancer drugs. *Clin Pharmacokinet* 1989, **16**, 327–336.
22. Collins JM, Grieshaber CK, Chabner BA. Pharmacologically guided phase I clinical trials based upon preclinical drug development. *J Natl Cancer Inst* 1990, **82**, 1321–1326.
23. Cano JP, Aubert C, Rigault JP, *et al.* Advantages and limitations of pharmacokinetic studies in the rationalization of anticancer therapy. Methotrexate and 5 FU. *Cancer Treat Rep* 1981, **65**, 33–42.
24. Favre R,Monjanel S, Alfonsi M, *et al.* High-dose methotrexate: a clinical and pharmacokinetic evaluation. Treatment of advanced squamous cell carcinoma of the head and neck using a prospective mathematical model and pharmacokinetic surveillance. *Cancer Chemother Pharmacol* 1982, **9**, 156–160.
25. Santini J, Milano G, Thyss A, *et al.* 5-FU therapeutic monitoring with the dose adjustment leads to an improved therapeutic index in head and neck cancer. *Br J Cancer* 1989, **59**, 287–290.
26. Zucchetti M, d'Incalci M, Willems Y, Cavalli F, Sessa C. Lack of effect of cisplatin on I.V. L-PAM plasma pharmacokinetics in ovarian cancer patients. *Cancer Chemother Pharmacol* 1988, **22**, 87–89.
27. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
28. Lott RS, Hayton WL. Estimation of creatinine clearance from serum creatinine concentration. A review. *Drug Intell Clin Pharm* 1978, **12**, 140–150.
29. Woodhouse KW, Henderson DB. High pressure liquid chromatographic method for the determination of melphalan in plasma. *Br J Clin Pharmacol* 1982, **13**, 605.

Acknowledgements—We thank Herbie Newell for help in reviewing the paper. This work was supported by a grant of the Ligue contre le Cancer du Rhône.

Eur J Cancer, Vol. 28A, No. 8/9, pp. 1315–1318, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Prognostic Relevance of pS2 Status in Association With Steroid Receptor Status and Proliferative Activity in Node-negative Breast Cancer

V. Cappelletti, D. Coradini, E. Scanziani, E. Benini, R. Silvestrini and G. Di Fronzo

Expression of the oestrogen-regulated pS2 protein was investigated on paraffin-embedded sections of primary breast tumours from 200 node-negative patients. Immunoreactivity was observed in 56% of the cases. pS2 expression was inversely correlated with tumour size and proliferative activity, whereas a direct correlation was observed with steroid receptor. 5-year relapse free survival was influenced by tumour size ($P = 0.02$), oestrogen receptor status ($P < 0.05$), and proliferative activity ($P < 0.01$). No difference in relapse-free survival was observed between patients subdivided according to pS2 expression alone. However, among patients with oestrogen-receptor-negative tumors, pS2 expression predicted a shorter relapse-free survival.
Eur J Cancer, Vol. 28A, No. 8/9, pp. 1315–1318, 1992.

INTRODUCTION

THE pS2 gene has been identified in the human hormone-responsive breast cancer cell line MCF-7 as a gene regulated by oestradiol at the transcriptional level [1]. Subsequent cloning and sequencing revealed that the pS2 gene codes for a secreted, low-molecular-weight protein similar to a porcine pancreatic protein known to inhibit gastrointestinal mobility and secretion [2]. Expression of the pS2 protein has been observed in human breast tumours, oestrogen-receptor-positive (ER+) breast cancer cell lines, and normal stomach mucosa [2]. Interestingly, pS2 could not be detected in normal breast tissue.

The pS2 gene appears to be potentially useful in basic studies to elucidate the molecular mechanism of oestrogen activity and in clinical studies for a better prognostic resolution within ER+ tumours [3]. In fact, identification of prognostic markers is particularly critical in breast cancer owing to the variable clinical outcome in patients with tumours with a similar histology or stage. Such clinical heterogeneity results from the multiple steps of malignant evolution, which are accompanied by the acquirement of genetic alterations resulting in abnormal expression of certain proteins [4, 5].

The biological prognostic indicators most widely used for