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Long-term Adjuvant Immunotherapy in Stage I High Risk Malignant Melanoma, Comparing Two BCG Preparations Versus Non-treatment in a Randomised Multicentre Study (EORTC Protocol 18781)

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The present study reports the results of a multicentre adjuvant trial with BCG (Bacillus Calmette-Guérin) in high risk patients (Breslow thickness ≥ 1.5 mm, Clark level ≥ III) with malignant melanoma, after surgical removal of their primary tumour. The trial was specifically designed in order to resolve the controversy and to provide some definite answers regarding the value of adjuvant BCG treatment in stage I malignant melanoma. Patients were randomised to either BCG RIV (108 patients) or BCG Pasteur (109 patients) for 3 years or to follow-up only (110 patients). The two vaccines used had greatly divergent properties regarding their mode of preparation, their composition and their immunomodulating activities. Of the 353 randomised patients, 23 were ineligible, 3 refused participation after randomisation and 327 were evaluable for final analysis. Median follow-up time was 6 years (range 0–10 years). The log-rank test comparison showed no statistical difference between the three arms regarding time to progression ($P = 0.55$) and duration of survival ($P = 0.82$). Treatment was generally well tolerated, with no major adverse events in either treatment arm. These findings confirm data with different BCG preparations and with stage II melanoma which also demonstrated no benefit regarding patient survival and time to relapse.
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INTRODUCTION

UP TO the present time, no therapeutic means are available that might influence the course of high risk stage I malignant melanoma apart from surgical removal of the primary tumour. Death results from the outgrowth of micrometastases, already present at the time of wide surgical excision. There is however good experimental and clinical evidence suggesting that the patient's immune defense plays an important role in controlling melanoma growth [1, 2]. This knowledge has been tested repeatedly in the past, using various immunomodulatory drugs including BCG, levamisol, transfer factor, *Corynebacterium parvum*, cimetidine and interferon [3–8]. BCG vaccination has been most widely practised among high risk stage I melanoma patients and has recently undergone a renaissance because of impressive effects in cancer of the bladder [9]. Initial encouraging reports on BCG therapy in malignant melanoma [10, 11] could not be supported by larger, more recently published studies [12, 13]. Nevertheless, detailed analyses of skin test reactions before and during immunotherapy [14, 15] suggest that the patient's immunological response to the BCG as well as his HLA-DR expression [16] might be of prognostic significance.

In 1978 when the present study was designed, all previous BCG studies had been criticised because they were either non-randomised, had too few patients, did not include a long enough follow-up, did not use the optimal BCG preparation, did not continue vaccination over a sufficiently extended period or did not analyse carefully the patient's immune status [17]. In the design of the present study, we have attempted to address these issues by doing a 10-year follow-up of a larger group of patients with high risk stage I malignant melanoma, after a 3-year vaccination. Two types of vaccines with very different properties, either BCG Pasteur or BCG RIV, were compared with no treatment. We here report on a first analysis of the overall data after the trial has been closed for 42 months.

PATIENTS AND METHODS

The study was open to all centres of the EORTC melanoma group willing to participate (for participating centres, see Table 1). Registered patients had to fulfil the following entrance criteria: age ≤75 or ≥15 years, with Clark grade III and a tumour

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*Writing committee, on behalf of the additional authors, including the responsible physicians of the participating centres (Table 1) and the histopathological review committee, consisting of F. Vakilzadeh, Hildesheim, F.R.G. and G. Kolde (Münster, F.R.G.) as coordinators and the following members: H. Berger, Göttingen, F.R.G., M. Goos, Essen, F.R.G., H.-G.W. Thies, Berlin, F.R.G., E. van der Esch, Amsterdam, The Netherlands.
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thickness of ≥1.5 mm or Clark grades IV or V irrespective of thickness or previous lymph node or other organ metastatic involvement or previous lymph node dissection. The tumour had to be surgically removed within the past month, with a safety margin of at least 3 cm and excision down to the level of the fascia. Previous radiation or chemotherapy were not allowed. The histological diagnosis of the tumour had to be confirmed at a later time point on a representative section by an independent panel of pathologists. Patients with previous or coexisting tumours, with a history of major allergic or autoimmune disease and with previous immunosuppressive or cytostatic therapies were excluded from the study.

Randomisation was performed centrally at the EORTC Data Center in Brussels. Patients were stratified according to institution and Clark grade (Clark III and Breslow thickness ≥1.5 mm, Clark IV or Clark V), using the random permuted block method.

Patients randomised into the BCG arms were treated with either of the two BCG vaccines which were kept and handled exactly according to the instructions of the manufacturers (Pasteur, Paris, France and RIV, Bilthoven, The Netherlands, respectively). The most important properties of the two vaccines are given in Table 2. Methods of preparation and immunopotentiating effects are detailed elsewhere [18, 19]. The main differences between the two vaccines are their effect on the immune system, with the BCG Pasteur stimulating primarily the humoral and the BCG RIV almost exclusively the cellular arm (information of the RIV).

Sterile suspensions (0.5 ml) of the vaccines were placed over cleansed skin and were pushed into it at four adjacent locations with a multipuncture device set at a depth of 2 mm, allowing the remaining suspension to dry subsequently on the skin. The first four vaccinations were given at the extremity closest to the lymph nodes draining the primary tumour. Subsequent vaccinations were given in a clockwise sequence on all four extremities (thighs or upper arms), for the first 10 times at weekly intervals and at monthly intervals thereafter. A reduction of dose (frequency or number of punctures) was allowed in case of very severe previous reactions to the vaccination. Physical examinations had to be performed monthly during the period of vaccination, chest X-ray and liver blood tests every 6 months, and liver, skeleton and brain scintigraphy every year. Non-vaccinated patients had to be seen every 2 months for the first half year, then every 3 months for up to 3 years. Thereafter, all patients had to be examined at 6 month intervals for up to 10 years.

Table 1. Contribution of patients from the different participating centres

Responsible physician	Centre	Patients entered
B.M. Czarnetzki E.B. Bröcker E. Macher	University of Münster	178
E. Christophers	University of Kiel	40
E. Jung	University of Heidelberg Klinikum Mannheim	33
H. Pehamberger	University of Vienna	33
C. Orfanos	Freie Universität, UKS	30
P. Fritsch	University of Innsbruck	17
P. Altmeyer	Ruhruniversität Bochum	10
E. Schöpf	University of Freiburg	7
M. Goos	University of Essen	3
G. Rassner	University of Tübingen	2
Total		353

Table 2. Basic properties of the two BCG vaccine preparations used in the trial

Properties	Pasteur lot 2 BCG lot 1173P2	RIV lot 057 BCG lot 1173P2
Dry weight/vial	19.5 mg	0.25 mg
Cult. part. $\times 10^6$/vial	760.0 mg	75.00 mg
Cult. part. $\times 10^6$/mg dry weight	39.0 mg	300.00 mg
O_2 uptake rate µl/h/vial	75.0 mg	9.60 mg
O_2 uptake rate µl/h/mg dry wt	3.85 mg	38.40 mg
Additives/vial	Dubos medium 50 mg glycerol 50 mg human albumin	166 mg Haemaccel 100 mg D-glucose 0.1 mg Tween 80
Mode of preparation	Surface culture homogenised by ball mill Fresh frozen, kept at −70°C	Homogeneous culture Freeze dried, kept at −20°C

Data were collected and analysed at the EORTC Data Center. Patients were reviewed regarding evaluability at regular intervals by the study coordinator (BMC).

For statistical analysis, time to progression and duration of survival were calculated from randomisation until latest follow-up (censored observation) or until "failure". For time of progression, "failure" meant the first relapse of any type (local, regional or distant), and for survival, "failure" meant death for any reason.

Actuarial curves were calculated according to the Kaplan–Meier technique [20]. Differences between curves were tested for statistical significance using the log-rank test, and for order variables (like Clark grade, Breslow thickness) the log-rank test for linear trend [21]. If the effect of one treatment arm (BCG Pasteur, for example) is constant over time in comparison to the control group (follow-up only), one may assume that:

$$S_T(t) = [S_c(t)]^h,$$

where $S_T(t)$ and $S_c(t)$ represent the probability to "survive" time t in the treatment group (T) and in the control group (C), respectively, and h is a constant, called the hazard ratio. These assumptions are the same as those of the Cox's Proportional Hazards Model. If the treatment group (BCG Pasteur, for example) has no or a small effect on "survival" in comparison to the control group, then h is close to 1; if treatment prolongs the duration of "survival", then h will be definitely lower than 1.

The calculations of hazard ratio and 95% confidence interval of the true h were performed using the confidence interval analysis (CIA) programme [22].

RESULTS

Of the 353 patients registered between June 1979 and February 1987, 23 were found to be ineligible either because they did not meet the requirements for disease stage or tumour histology

(20 patients), or because they fell under the exclusion criteria (3 patients). 3 additional patients were not evaluable because they refused to participate in the trial after randomisation.

The basic data on the 327 remaining patients are summarised in Table 3. As can be seen, randomisation is well balanced for most parameters except for the few cases with lentigo maligna melanoma (LMM) and for ulceration. Twice as many females than males were randomised, the predominant tumour type was the superficial spreading melanoma (SSM), followed by the nodular melanoma (NM), and most tumours were Clark grade IV, measuring 1.5–3.0 mm in thickness.

Additional data of potential interest are that 69 patients had an associated disease and 84 took chronic medications which were allowed according to the protocol. Most patients (315) had undergone wide excision and grafting of their primary tumour, 8 had been amputated, and in 7, the data were not given.

The mean numbers of vaccinations given were 30.00 for BCG Pasteur and 32.34 for BCG RIV, with the median and range being equal for both treatment groups (39, 0–47). The reasons for withdrawing from the study during the first 3 years of follow-up therapy are given in Table 4. Considering the fact that several of the losses to follow-up were unavoidable due to moving of patients to distant regions and countries, the conduction of the trial in the different centres and the compliance of the patients was generally good.

At the time of evaluation, the median time of follow-up was 6 years (range 0–10 years). The overall evaluation of all eligible patients showed that the following patient characteristics correlated with lower risk of recurrence: female sex, thinner tumour, lower Clark level and lack of ulceration (Fig. 1a–d).

The type of relapse did not differ among the three arms and included 23 local or in transit metastases, 76 regional lymph node metastases, 3 combined local and regional metastases, 19 distant metastases and 5 regional and distant metastases. The cause of death was given as melanoma in 78 patients, as cardiovascular in 4 and as "other" in 8 patients.

Table 3. Characteristics of evaluable patients by treatment group

		BCG Pasteur	BCG RIV	Control	Total	%
Sex	Female	70	78	73	221	67.6
	Male	39	30	37	107	32.6
Tumour type	SSM	58	47	58	163	49.8
	NM	40	33	35	108	33.0
	ALM	4	6	5	15	4.6
	LMM	1	8	3	12	3.9
	Other	6	14	9	29	8.9
Breslow thickness (mm)	<1.5	21	19	18	58	17.7
	<3.0	49	54	65	168	51.4
	<5.0	27	20	19	66	20.2
	≥5.0	11	14	8	33	10.1
	Not measurable	1	1	0	2	0.6
Clark grade	III	5	9	9	23	7.0
	IV	96	89	94	279	85.3
	V	8	10	7	25	7.6
Ulceration	No	88	94	91	273	83.5
	Yes	21	14	19	54	16.5
Primary site	Lower extr.	55	65	71	191	58.4
	Upper extr.	27	21	25	73	22.3
	Trunk	22	17	11	50	15.3
	Head and neck	4	4	0	11	3.4
	Other	1	1	3	2	0.6
Age (years)	<40	23	23	26	72	22.0
	<50	26	28	23	77	23.5
	<60	28	31	30	89	27.2
	≥60	32	26	31	89	27.2
Total		109	108	110	327	

Table 4. Reasons for eligible patients withdrawn from the study during or at the end of the first 3 years (treatment period) of the study

	BCG Pasteur	BCG RIV	Control	Total
Treatment completed	59	61	63	183
Progression	31	28	31	90
Death from melanoma	0	0	2	2
Death, non-melanoma	0	2	2	4
Excessive toxicity	4	0	0	4
Treatment refused	4	5	0	9
Lost to follow-up	8	9	9	26
No summary form	1	1	1	3
Protocol violation	1	0	1	2
Other*	1	3	3	7
Total	109	109	112	330

*Include 2 intervening pregnancies, 2 suspected but not confirmed melanoma metastases, 1 intervening benign and 1 non-melanoma malignancy, 1 with reason not given.

The treatment results of evaluable patients were comparable in terms of time to progression (Fig. 2a); the log-rank test yielded a $P = 0.55$. The estimated hazard ratio was 0.90 and the 95% confidence interval 0.58–1.40. Most recurrences were observed within 3 years after surgery; the risk of relapse remained, however, positive at 4, 5 and even at 8 years in this high risk group of patients. The duration of survival of the three treatment groups was very similar (Fig. 2b), with the log-rank yielding a $P = 0.82$. The treatment differences remained practically the same when all randomised cases were analysed: among the 23 ineligible patients there were only 3 progressions and 1 death. Exploratory analyses, comparing treatment results within the different subgroups (Table 3), did not yield statistically significant differences.

Undesirable side-effects (Table 5) were most expected, transient, of a mild to moderate nature and in no case, life threatening. The BCG RIV preparation was slightly better tolerated than the BCG Pasteur.

DISCUSSION

The present data show that adjuvant treatment with BCG has no significant effect on time to progression or survival in high risk stage I malignant melanoma patients. Although very detailed data on the patients had been collected in order to detect a possible clear benefit in favour of adjuvant BCG treatment or of one or the other BCG preparation on prognosis in the different subgroups, no evidence in this regard could be found. This might be due to a true "null effect" of the BCG preparations in the different patient categories or to a too low statistical power to detect a relatively small effect of one or both BCG preparations. The overall data on all patients confirmed, however, statistically significant differences regarding prognostic criteria for sex, tumour type, Breslow thickness, Clark grade and ulceration of the tumour (Fig. 1).

The study was designed to address a number of issues that were open to criticism in previous studies. Thus, the patients are evenly randomised regarding all major criteria (Table 3).

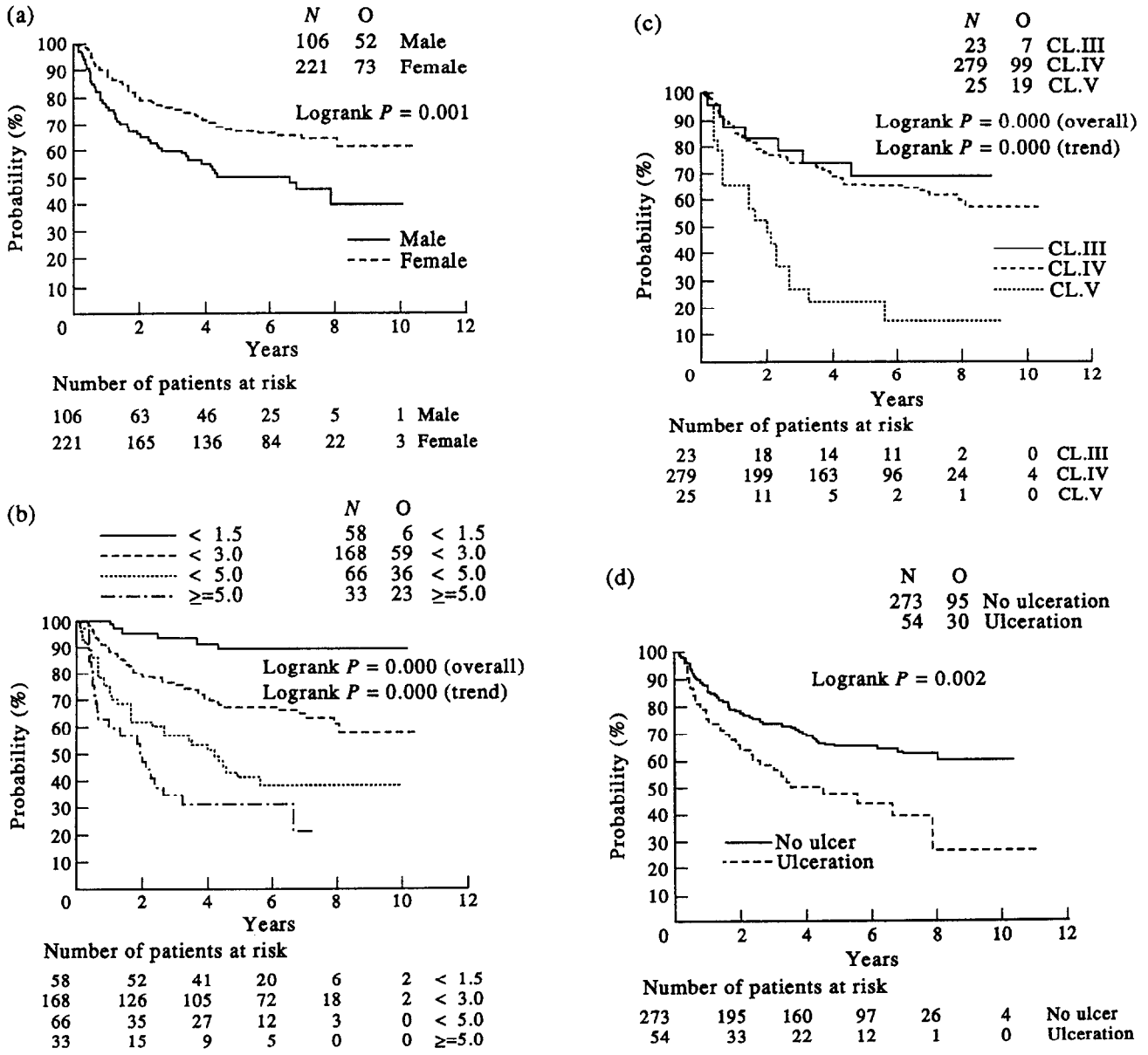

Fig. 1. Time to progression of all evaluable patients, based on sex. (a) Breslow thickness. (b) Clark grade. (c) Ulceration (d) of the primary melanoma. *N* = total number of patients, O = failures.

Histological criteria of the primary tumour were objectively assessed by a panel of independent experts. Treatment was extended over a period of 3 rather than the usual 2 years since there had been suggestions that relapse occurred more frequently at the end of the 2 year BCG vaccination and since an optimal effect of treatment was intended to be achieved. In addition, initial vaccinations were performed at the extremity closest to the tumour site in order to maximise the immune response at the location where it was most needed.

The number of patients and the length of follow-up in patients with stage I melanoma, as reported here, has not yet been achieved in any of the studies published so far. The large multicentre study of the WHO group included only 23 patients with stage I melanoma [12] who were treated with BCG Pasteur by multipuncture, in a similar protocol as in the present study, but only for 2 years. In a Canadian study [13], 99 patients were treated with an oral BCG (Connaught Laboratories Limited) for 2 years, after postsurgical intradermal BCG treatment around the site of excision. The median follow-up was 4 years. Neither of these two studies showed a benefit of BCG treatment.

So far, a number of different types of vaccines and modes of vaccination have been used in different studies, with claims that the one might be better than the other. The study presented here is the first where two types of vaccines are compared directly. The two vaccines are prepared differently and differ markedly regarding cultured particles and additives (Table 2). They also evoke very distinct immunological responses, with the BCG Pasteur but not the BCG RIV causing a marked rise in serum antibodies against BCG (internal information, RIV). In spite of this, the two preparations demonstrated no benefit regarding survival or disease free interval of the patients studied here (Fig. 2).

Thus, although adjuvant BCG vaccination had no significant deleterious effects for our patients with high risk stage I malignant melanoma, in agreement with the literature [23], it has no significant beneficial effect either. Even subgroups which have

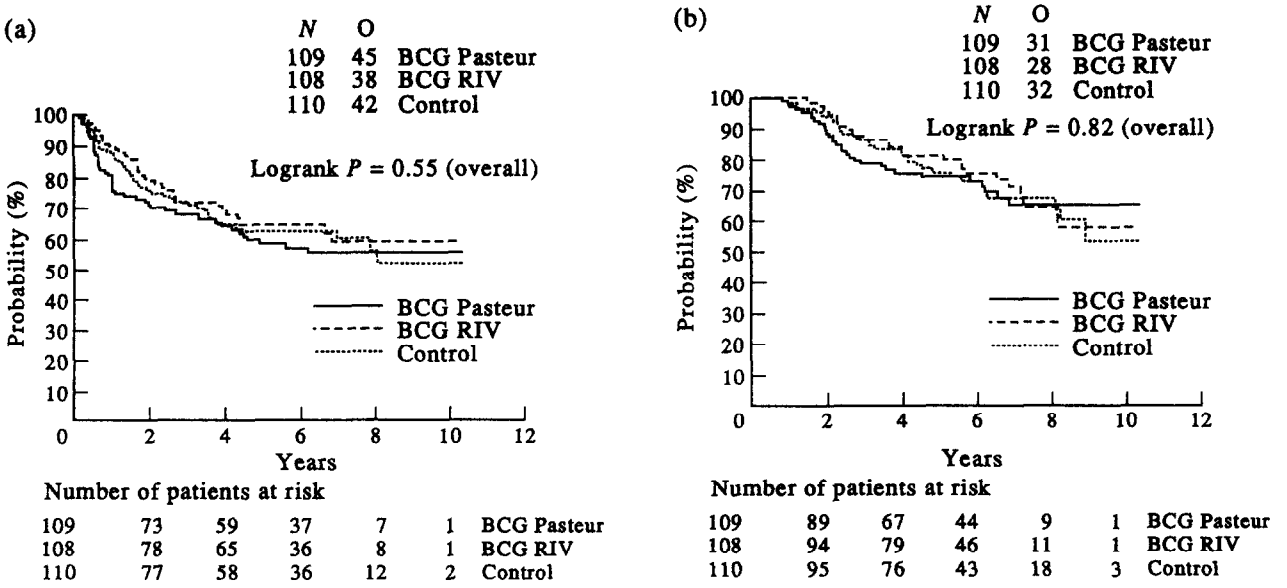

Fig. 2. Time to progression (a) and survival (b) of all evaluable patients, analysed separately according to type of treatment.

Table 5. Undesirable side-effects recorded in association with the BCG vaccinations

	BCG Pasteur	BCG RIV
Maximal toxicity total	73	71
Mild	32	41
Moderate	30	22
Severe	11	8
Malaise	45	43
Nausea/vomiting	16	10
Fever	24	20
Chills	24	16
Anorexia	12	10
Rash	21	13
Lymph node swelling	54	40
Renal dysfunction	1	3
Anaphylactic reaction	1	0

been found to benefit from BCG in previous studies may do so because of an intrinsically different status of the patient's immune function or immunogenicity of the tumour [14–16]. These disappointing results of BCG in an adjuvant setting could mean either that systemic treatment is not sufficient to influence favourably immune surveillance or that immune surveillance is not the decisive mechanism to contain or eliminate micrometastases in high risk melanoma. Future therapeutic strategies will have to address themselves therefore either to more specific aspects of the immune response of the tumour patients or to other mechanisms of tumour control, based on a hopefully better understanding of the biology and immunology of melanoma.

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Phase I–II Intraperitoneal Mitoxantrone in Advanced Pretreated Ovarian Cancer

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36 previously treated patients (25 with anthracyclines) with advanced epithelial ovarian cancer have been treated with intraperitoneal (i.p.) mitoxantrone (M) at increasing doses. The response was evaluated through repeated laparoscopy with multiple biopsies and serial measurement of Ovarian Cancer Antigen 125 (CA 125); 11/36 patients had a complete (6 patients) or partial (5 patients) response. Toxicity (both local and general) was observed starting from 25 mg/m² of M per cycle. The amount of drug reaching systemic circulation was monitored by measuring M plasma value after i.p. treatment. This study showed wide variations in serum levels obtained after i.p. doses ranging from 23 to 36 mg/m². The area under the curve (AUC) of mitoxantrone plasma samples, did not correlate with the i.p. administered dose. Conversely, a correlation seems to exist between the plasma AUC and the responder status. Patients who showed clinical responses to i.p. treatment with mitoxantrone had AUCs and plasma peak levels of the drug that were significantly higher than those in non-responders ($P = 0.03$, Fisher's exact test).
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INTRODUCTION

INTRAPERITONEAL (i.p.) treatment of ovarian cancer with drugs delivered in large fluid volumes represents a valid alternative to the more traditional intravenous (i.v.) route [1–4].

Mitoxantrone (M) is a new anthraquinone derivative of anthracene with established activity in breast cancer, acute leukaemia and malignant lymphoma [5, 6].

In ovarian cancer the clinical efficacy of this new drug given by conventional i.v. treatment has been only moderate in various experiences [5–10] (Table 1). In contrast, M proved to be the most effective cytotoxic agent against human ovarian cancer cells in the human clonogenic assay [11]. The concentrations tested *in vitro* (0.1–10 μg/ml) however, are not commonly attained after i.v. administration [12].

We started treating patients with intraperitoneal M to ascertain whether local high drug concentrations could improve the benefit/toxicity ratio.

PATIENTS AND METHODS

Patients' characteristics

36 patients entered this trial from June 1987 to October 1990. Patients' characteristics are shown in Table 2. All patients had recurrent or advanced ovarian cancer and all were previously treated with multiple chemotherapy regimens; 25 of them had received anthracyclines at the total mean dose of 258 mg/m² (range 128–500). A total of 116 M cycles were administered (Table 2). Patients entered into this trial if they met the following criteria: (a) life expectancy of at least 2 months and a WHO performance status less than 3; (b) laboratory evidence of normal renal and hepatic functions, with white blood cells (WBC) more than 4000 and platelet count more than 100 000 per μl; (c) no clinical evidence of cardiac disease (assessed by electrocardio-

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