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Papers

High Failure Rate of Carboplatin–Etoposide Combination in Good Risk Non-seminomatous Germ Cell Tumours

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24 patients with good risk non-seminomatous germ cell tumours (GR-NSGCT) were enrolled in a phase II trial combining carboplatin (C) and etoposide (E). Carboplatin was given at a fixed dose of 450 mg/m² at d2, and E 120 mg/m², d1–3, every 4 weeks × 4 cycles (cy). Myelosuppression was the major toxicity with neutropenia grade 4 in 18 cy (19%) and grade 3 in 26 cy (27%). Thrombocytopenia grade 3 and 4 occurred in 7 and 1 cy, respectively. Responses included: 20 complete responses (CR) (83%) with 16 clinical CR and 4 pathological CR; 3 additional patients had complete surgical removal of residual disease (SRRD) with viable tumour (surgical CR); 1 patient progressed during C + E therapy. 5 of the 16 clinical CR relapsed, and all the 3 surgical CR progressed despite post-operative salvage chemotherapy. Adverse events occurred in 9 patients (37.5%; 95% C.I., 19–59%). After a median follow-up of 24 m (range 14 to 38) 4 patients had died [3 progressive disease (PD), 1 suicide while in CR], 3 were alive with PD, and 17 had no evidence of disease. No significant correlation between area under the curve values of carboplatin, overall treatment failure and the platelet nadirs was observed. We conclude that the efficacy of the C + E regimen as given in our protocol is inferior to the standard cisplatin-containing regimens. The low dose-density (D/I) of carboplatin could be responsible for the high failure rate.
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INTRODUCTION

CISPLATIN BASED chemotherapy has induced dramatic improvements in treatment results for patients with non-seminomatous germ cell tumours (NSGCT), with cure rate of approximately 70–90% [1].

Prognostic factors in NSGCT include serum tumour marker levels: alphafetoprotein (AFP), human chorionic gonadotropin (HCG) and lactate dehydrogenase (LDH), stage of the disease, and tumour volume. These prognostic factors allow patients to be assigned into good risk and poor risk groups [2–5].

Patients with good risk characteristics achieve a high complete remission rate with cisplatin-based chemotherapy, and experience few treatment failures [6]. In the past decade, most investigators have turned their efforts to reducing the treatment toxicity in good risk patients [6–10].

As a first step, the reduction of vinblastine dosage and the omission of maintenance therapy was allowed [1]. Later on, etoposide replaced vinblastine in the PVB regimen (cisplatin + vinblastine + bleomycin) [11]. Moreover, the deletion of the fourth cycle of BEP (bleomycin, etoposide, cisplatin) reduced the toxicity and maintained the same efficacy in good risk-NSGCT patients [7].

In an effort to evaluate the role of bleomycin, an EORTC trial comparing four cycles of BEP versus EP (etoposide, cisplatin) demonstrated similar activity and lower toxicity of the EP regimen [12]. The EP two drug regimen was also tested at the Memorial Sloan Kettering Cancer Center (MSKCC) in a randomised trial comparing four cycles of EP versus three cycles of VAB-6 (vinblastine, bleomycin, cisplatinum, cyclophosphamide, dactinomycin) in good risk-NSGCT. EP was proved to be equally active and less toxic than VAB-6 [13]. However, an ECOG trial showed that omitting bleomycin, when three cycles of EP were administered, resulted in a higher failure rate than three cycles of BEP [8]. Thus, the standard treatment of good risk-NSGCT is either four cycles of the EP two drug combination or three cycles of the BEP three drug combination.

Cisplatin induces a wide variety of toxicities such as emesis, renal damage, acoustic and peripheral nerve toxicity. In addition, it is often necessary to accompany the administration of cisplatin with large volumes of intravenous saline in the in-patient setting. Frequently, this drug is administered over several days. Carboplatin, a cisplatin analogue, causes less toxicities and may be delivered in the out-patient setting without hydration [17, 18]. In an attempt to decrease cisplatin related side-effects, carboplatin was tested as a replacement for cisplatin in good risk-NSGCT patients [10, 14].

In our institution good risk-NSGCT patients were defined according to the Institut Gustave-Roussy (IGR) prognostic

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model which is based exclusively on serum tumour marker levels [4]. The standard treatment of good risk patients in our institution was four cycles of EP at 4-week intervals.

In an attempt to substitute cisplatin with carboplatin, a prospective phase II trial of the etoposide and carboplatin combination in good risk-NSGCT patients was undertaken.

PATIENTS AND METHODS

Patients with good risk-NSGCT entered this prospective trial, according to a combination of serum HCG concentration and serum AFP levels as determined by our model [4]. Patients with either HCG > 2 300 mIU/ml or AFP > 1 000 ng/ml are considered in the poor risk group. The probability of complete response (CR) is calculated for the other patients according to $P = \exp\ h/(1 + \exp\ h)$, where $h = 1.90–0.003 \sqrt{\text{AFP}}$-$0.021 \sqrt{\text{HGC}} + 0.033\ \text{HCG}/1.000$, with HCG in mIU/ml and AFP in ng/ml. Patients with more than a 70% predicted CR rate with standard chemotherapy are considered good risk [4, 5]. The same prognostic model was established for NSGCT of both testicular and extragonadal origin. However, ovarian NSGCT and pure seminoma were not included in the study.

Pathological orchiectomy specimens were reviewed in our institution. No prior chemotherapy or radiotherapy was given.

Pretherapeutic investigations included physical examination, serum HCG, beta-HCG subunit, AFP assays, LDH concentration, liver function tests, usual blood chemistry, chest X-ray, and computed tomographic scan of the thorax and of the abdomen. Complete clinical examination, chest X-ray, tumour marker assays, full blood count and usual blood chemistry were repeated prior to each cycle. Complete blood count with platelet count and differential was obtained each week during treatment.

Renal function was assessed prior to each course by serum creatinine level measurement and creatinine clearance.

Creatinine clearance was measured by urine collection or calculated by the Cockroft and Gault formula [15].

$$\text{Creatinine clearance (ml/min)} = \frac{(140\text{-age}) \times \text{weight (kg)}}{0.814 \times \text{serum creatinine (mmol/l)}}.$$

Chemotherapy consisted of etoposide 120 mg/m^2 as a 1 h infusion on days 1, 2 and 3, and carboplatin at a fixed dose of 450 mg/m^2, administered as a 30 min infusion in 500 ml of 5% dextrose on day 2. Cycles were repeated every 28 days for a total of four cycles.

The serum concentration × time or area under the curve (AUC) of carboplatin was retrospectively calculated for each course according to the Calvert formula [16]: AUC (mg × min/ml) = dose (mg)/(GFR + 25), where GFR = glomerular filtration rate. The GFR value was estimated by the creatinine clearance calculated by the Cockroft and Gault formula.

The evaluation of response was performed prior to the third cycle and 4 weeks after the fourth cycle of chemotherapy. Patients with complete disappearance of the disease and tumour marker normalisation were considered in clinical CR (cCR). Patients with residual disease and normal serum tumour markers after chemotherapy completion, were submitted to the surgical removal of residual disease (SRRD). A pathological CR (pCR) was defined as the complete resection of either necrotic debris, fibrosis, or mature teratoma without any viable disease. A surgical CR (sCR) was defined by the complete surgical excision of viable tumour. In these patients, four post-surgery additional cycles of vinblastine, ifosfamide, cisplatin (VeIP) combination chemotherapy regimen were administered. Adverse effects were defined as either primary refractory disease, viable tumour at SRRD, or relapse. Follow-up was calculated from the start of chemotherapy.

RESULTS

Patients' characteristics

Between June 1989 and May 1991, 24 patients entered this prospective phase II trial.

All patients had a testicular primary. However, during the same period no extragonadal NSGCT fulfilled the good risk criteria. Median age of patients was 30 years (range : 17–43 years). The testicular tumour was left and right-sided in, respectively, 11 and 13 patients. Histological review revealed that embryonal carcinoma was the most frequent subtype, which was found alone in 6 patients and in association with other components in 17. Mature or immature teratoma was found in 12 specimens. Yolk sac tumour, choriocarcinoma and seminoma were present in five specimens each.

The work-up revealed that the disease was limited to elevated marker levels in 4 patients (elevated AFP in 2, HGC in 1, and both in 1), 19 patients presented retroperitoneal lymph node enlargement, lung metastases in 6 patients, and enlarged mediastinum in 2 patients.

Assignment to prognostic groups of the Indiana University (IU) [2] and MSKCC [3] prognostic models was studied. According to the IU classification, 20 patients had minimal disease, 3 patients had moderate disease (3 palpable abdominal mass as the only metastatic site), and 1 patient had advanced disease (multiple pulmonary metastases of more than 3 cm in diameter with a non-palpable abdominal disease). In the MSKCC model, all 24 patients were assigned to the good risk group. Apart from the 3 patients with palpable abdominal mass (lymph node enlargement > 10 cm) the remaining 21 patients fulfilled the characteristics for good prognosis according to the MRC/EORTC eligibility criteria described in the PEB versus CEB randomised study.

A total of 96 cycles of chemotherapy were administered to 24 patients. All patients were evaluable for response and toxicity.

Response

16 patients experienced a cCR after the completion of the four cycles of chemotherapy. 4 other patients had a pCR; mature teratoma was found in 2 patients at SRRD, and fibrosis and necrosis in the 2 others. 5 of the 16 cCR patients relapsed after 1, 5, 6, 9 and 13 months, respectively (patients 4, 6, 7, 8 and 9 in Table 1). Patient 4 died of progressive disease (PD) despite four different salvage regimens. Complete remission was obtained in 2 patients: patient 7 who had the VeIP regimen followed by one cycle of high-dose chemotherapy (HDCT) and autologous bone-marrow transplantation (ABMT), and patient 8 who had complete retroperitoneal lymph node dissection followed by four cycles of VeIP regimen. The other 2 patients failed to respond to VeIP therapy, but were rendered disease-free by a second-line salvage chemotherapy and will receive consolidation by HDCT and ABMT (patients 6 and 9).

3 patients underwent a complete SRRD, since viable germ cell residual disease was present. These 3 sCR patients received VeIP regimen. 2 patients progressed during VeIP treatment (patients 2 and 3) one of whom (patient 2) died due to PD. The other patient has NED after two subsequent relapses three lines of chemotherapy, and two cycles of HDCT and ABMT. The third patient relapsed after 11 months and is alive with disease despite further salvage regimens (patient 5).

Table 1. Treatment failures

Patient (inclusion number)	Age	Histology	Stage (IU)	AUC 1 of Carboplatin	Type of failure	Salvage therapy	Current status
1 (1)	42	MT + EC + YST	Moderate	6.72	Refractory	VIP, NIF,	Dead
2 (18)	19	S + EC	Minimal	4.4	Viable tumour	VIP, refused ABMT	Dead
3 (7)	31	S + EC + MT	Advanced	5.67	Viable tumour	VIP, ABMT, Surgery, ABMT, Surgery, Cisca II VB IV	NED
4 (5)	33	MT + EC + YST	Minimal	6.55	Relapse	VIP, surgery, NIF Epirubicin, oral VP16	Dead
5 (19)	29	EC	Minimal	5.7	Viable tumour	VIP, surgery, Cisca II VB IV, refused ABMT	Alive with PD
6 (15)	27	MT + IT + EC	Moderate	6.28	Relapse	VIP, Navelbine, Cisca II VB IV	Under treatment
7 (14)	26	MT + EC + YST + S	Minimal	4.6	Relapse	VIP, ABMT	NED
8 (12)	30	EC + S	Moderate	6.52	Relapse	Surgery + VIP	NED
9 (21)	20	EC	Minimal	6.35	Relapse	VIP, surgery, Cisca II VB IV	Under treatment

MT = Mature teratoma; EC = embryonal carcinoma; YST = yolk sac tumour; S = seminoma; IU = Indiana University; AUC = area under the curve; VIP = vinblastine + ifosfamide + platinum; NIF = navelbine + ifosfamide + farmorubicin; ABMT = autologous bone marrow transplantation; Cisca II, VB IV = alternation of cisplatinum + cyclophosphamide + doxorubicin and vinblastine + bleomycin; PD = progressive disease; NED = no evidence of disease.

Finally, 1 patient progressed during C + E regimen (patient 1 in Table 1). He continued to progress despite VeIP regimen and further salvage therapy. He died 22 months after the start of C + E.

After a median follow-up of 24 months (range 14–38), 4 patients had died (3 PD and 1 suicide while in CR), 1 is still alive with PD, 2 are in salvage treatment, and 17 are NED (71%; 95% C.I., 49–87%).

In summary, 23 patients attained CR (16 cCR, 4 pCR and 3 sCR) and 1 patient had progressive disease. Overall treatment failure was observed in 9 patients (37.5%; 95% C.I., 19–59%): 1 PD, 3 viable active residual disease and 5 relapses. In addition, 6 patients were refractory to subsequent salvage chemotherapy (1 PD, 2 sCR and 3 relapses).

Factors of treatment failure

All 4 patients with advanced or moderate disease according to the IU classification failed. The other five adverse events had minimal IU disease.

The AUC of carboplatin actually received was calculated at each cycle according to the Calvert formula. The GFR was estimated from the creatinine clearance. The mean AUC of carboplatin was 6.1 mg × min/ml. All the AUC values at the first cycle were greater than 5 except in 2 patients in whom it was 4.6 and 4.4, respectively. One relapsed 5 months after cCR, and still has NED (patient 7 in Table 1). The other patient had viable tumour at SRRD. He continued to progress despite salvage treatment and died of PD after 14 months (patient 2). No significant difference in the mean AUC of the four cycles was observed between patients categorised as treatment success and those who failed treatment (Students t-test). Although, 13 out of 24 patients had a carboplatin AUC greater than 6 mg × min/ml at the first cycle, 5 of these 13 patients presented adverse events (1 PD and 4 relapses).

The dose intensity (D/I) was uniformly 112 mg/m²/w for carboplatin, and 90 mg/m²/w for etoposide.

Toxicity

The toxicity was mostly mild and included predominantly myelosuppression (Table 2). Grade 3 neutropenia was encountered in 26 cycles (27%) and grade 4 neutropenia in 18 cycles (19%). There were only three febrile neutropenic episodes without microbiological documentation. Hospitalisation was required in one episode. Grade 3 and 4 thrombocytopenia occurred in less than 10% of the cycles. Only one cycle with a 6000/mm³ platelet nadir was observed in a 43-year-old patient, who received platelet transfusion for a grade 1 haemorrhage. Haemoglobin level fell below 8 g/dl in four cycles and required red blood cell transfusion. Grade 3 nausea and vomiting occurred in 20% of the cycles and was well managed by metoclopramide. All patients presented grade 3 alopecia. There was no renal toxicity as assessed by repeated serum creatinine dosage and

Table 2. Toxicity during 96 cycles (expressed in number of cycles)

Toxicity	WHO grade 1	2	3	4
PMN	14	24	26	18
Platelets	20	14	7	1
Haemoglobin	—	—	4	—
Nausea/vomiting	43	32	17	1
Mucositis	2	—	—	—
Infection	—	—	1	—
Fever	—	2	—	—

WHO = World Health Organization; PMN = polymorphonuclear leucocytes.

creatinine clearances. Neither treatment delay, nor dose reduction was required. The correlation between carboplatin AUC at cycle 1 and the nadir of thrombocytopenia was non significant within the range of values observed in this study (Spearman correlation coefficient, $R = -.29$, $P < .20$). However, the platelet levels tended to decrease with increased AUC.

DISCUSSION

Cisplatinum containing chemotherapy regimens are active in good risk-NSGCT patients, and the problem of drug toxicity is currently the most important issue in the selection of chemotherapy regimens.

Carboplatin, a clinically active cisplatin analogue, has proven to exhibit useful clinical advantages when compared to its parent compound: it is less emetogenic and has no significant nephrotoxicity, neurotoxicity nor ototoxicity [17, 18]. However, because its major toxicity is myelosuppression, the use of carboplatin in association with other myelotoxic drugs is compromised.

The introduction of carboplatin in the armamentarium of good risk-NSGCT chemotherapy was successfully realised at the Royal Marsden Hospital. Among 76 patients with good risk-NSGCT treated by the association of carboplatin, etoposide and bleomycin (CEB), only 5 patients [6.5%] experienced adverse events. VP16 was given at a dose of 120 mg/m^2/d days 1, 2, 3, bleomycin 30 mg/d days 2, 9, 16 and carboplatin was given at day 2 at a variable dosage (300–550 mg/m^2) adjusted according to the Calvert formula. Cycles were repeated every 21 days. The failure in these 5 patients was considered a consequence of an insufficient dosage of carboplatin (carboplatin < 360 mg/m^2). All these 5 patients were rendered disease-free after salvage chemotherapy [10].

Conversely, our experience with carboplatin containing regimens are disappointing. The failure rate was as high as 37%, and the salvage treatment was mostly unsuccessful. So our study was discontinued, since it is clear that this regimen was less effective than standard regimens such as BEP or EP.

Our protocol schema and dosage were established according to known phase I and II trials of VP16 and carboplatin. A fixed dose of 450 mg/m^2 of carboplatin was chosen according to previous phase I experiences [17, 18]. The dose of VP16 was 360 mg/m^2 according to the Royal Marsden and Charing Cross Hospital trials [10, 19].

In an attempt to explain our unfavourable results compared to those of the CEB combination, three points can be raised. The first issue concerns the optimal equivalent dosage of using carboplatin instead of cisplatin. One must be aware that the antitumour activity of carboplatin against heterotransplanted human testicular cancer cell lines in the nude mouse was significantly weaker than that of cisplatin [20]. So, carboplatin as a replacement for or an alternative to cisplatin in NSGCT remains an unanswered question. If this is true, then the adequate dosage of carboplatin remains a problem. As shown by Childs *et al.* [21], the dose of carboplatin must be optimised according to the Calvert formula [16]. These authors found from the carboplatin dose response analysis in 121 patients with good risk-NSGCT treated by CEB regimen, that the mean carboplatin AUC in patients who had successful treatment was 4.85 mg × min/ml, and 4.03 mg × min/ml in those patients who failed the CEB regimen [21]. The relationship between tumour response and carboplatin AUC was also confirmed in ovarian cancer patients treated by carboplatin as a single agent [22]. This study was performed retrospectively in 1 028 patients and illustrated the lack of significant increase in the likelihood of response above an AUC of 5. Since the co-administration of etoposide and carboplatin did not alter either the pharmacokinetic behaviour or the carboplatin AUC accuracy [23], one can set an AUC threshold of 4.5–5.0 mg/ml to ensure a favourable outcome.

In our study, the retrospective measurement of AUC demonstrated the lack of a significant difference of the mean AUC value between patients who did or did not experience adverse events. Moreover, 7 out of 9 patients with adverse events had an AUC higher than 5, and therefore, they did not receive underestimated doses of carboplatin. Bajorin *et al.* found an inferior activity of EC (etoposide + carboplatin) compared with EP in good risk-NSGCT randomised trial since 29 unfavourable responses occurred in the carboplatin arm versus 14 in the cisplatin arm among 131 and 134 patients, respectively [14]. The retrospective calculation of the carboplatin AUC in the EC arm failed to predict treatment failure. Regarding the AUC values in our trial as well as those in the MSKCC [14], we can exclude the carboplatin underdosage according to the AUC simplified formula as an explanation of failures. However, the carboplatin dose-intensity must be considered. In the MSKCC trial and in our trial, cycles were administered at a 4 week interval. Conversely, patients in the Royal Marsden trial were treated every 3 weeks and therefore received a 25% higher carboplatin D/I. This observation may be of major importance and will be discussed below.

The second issue concerns the dosage of VP16. One can suppose that 90 mg/m^2/w as D/I is a reduced dose. The dose efficacy relationship of VP16 was validated by Cavalli *et al.* [24] in small-cell lung cancer chemotherapy, and by Crawford *et al.* [25] in germ cell tumours. In the latter study, authors compared relapses after POMB/ACE treatment to matched control patients who did not relapse. They found that the cause of reduced treatment dose-intensity in most patients who relapsed, was a reduced dose of etoposide. Moreover, Husband and Green reported a highly significant effect of the relative D/I of etoposide on survival in assessing the outcome of POMB/ACE chemotherapy in 53 males [19]. In this study, the D/I of etoposide in the standard regimen was 90 mg/m^2/w (the same as in our protocol), and patients who received a relative D/I of VP16 > 0.75 had a significant improvement of survival at 5 years [19]. However, the D/I of VP16 in the CEB regimen, which was given at 3-week intervals, was higher than in our protocol (120 mg/m^2/w versus 90 mg/m^2/w). Thus, an underdosage of etoposide could not be excluded as explanation of failure in our trial.

The third issue concerns the usefulness of bleomycin. Many non-randomised trials confirmed its activity in combination chemotherapy without cisplatin [26, 27], or in cisplatin containing regimens [9]. However, well conducted randomised trials [12, 13] failed to prove the impact of bleomycin when four cycles of standard dose etoposide–cisplatin regimens were administered. However, when only three cycles were given, the long term NED rates seem lower [8]. This observation supports the view that bleomycin is important in suboptimal dosages of etoposide and cisplatin [28]. It is thus suspected that bleomycin may make the difference between Royal Marsden trial results and our own results. An ongoing randomised EORTC trial comparing CEB and BEP may add information to the role of bleomycin in combination with etoposide and carboplatin.

Table 3 shows the results of three trials: the first is the CEB regimen of the Royal Marsden Hospital in which five treatment

Table 3. Comparison of carboplatin-containing regimens

Protocol	Nb	Adverse events	TD VP16	D/I VP16	TD CBCDA	D/I CBCDA	Mean AUC	Bleomycin
CEB RMH [10]	76	5 (6.5%)	360	120	400–550	133–136	4.8	+
EC arm [14] MSKCC	131	29 (22%)	500	125	500	125	No prediction	–
E + C IGR	24	9 (37.5%)	360	90	450	112	6.1	–

RMH = Royal Marsden Hospital; CEB = carboplatin, etoposide, bleomycin, Q3W; MSKCC = Memorial Sloan-Ketterin Cancer Center; IGR = Institut Gustave-Roussy; EC = E + C : etoposide, carboplatin, Q4W; TD = total dose (mg/m²/cycle); D/I = dose intensity (mg/m²/w); AUC = area under the curve of carboplatin (mg × min/ml); CBCDA = carboplatin.

failures were observed. The mean carboplatin AUC was equal to 4.8 [10]. The second is the carboplatin–etoposide (CE) arm of the MSKCC trial [14], and the third is our trial. In Table 3, our protocol represents the lowest D/I of etoposide and carboplatin. Since the MSKCC CE arm -which was less active than the platinum arm- represents the highest VP16 D/I, the impact of the dose intensity of VP16 in the prediction of response may be unlikely. The only difference that could be retained as significant is a lower D/I of carboplatin in the MSKCC and IGR trials compared to CEB regimen. This difference in dose efficacy of carboplatin was not apparent in the AUC calculation according to the Calvert formula. Thus, we can suggest that the D/I of carboplatin could be the determinant factor in predicting response.

As demonstrated in the studies of Childs *et al.* and Bajorin *et al.* [21, 14], the carboplatin AUC in our trial was not significantly correlated with platelet nadir. However, a relationship between AUC, pretreatment platelet count and desired platelet nadir was established by Van Echo *et al.* [29].

In conclusion, the combination of carboplatin and etoposide with this schedule and at this dosage is clearly less active than standard BEP or EP chemotherapy regimens in the treatment of good risk-NSGCT patients. Several factors may play a role in this lower activity: carboplatin AUC less than 5 mg × min/ml, deletion of bleomycin, decreased etoposide dosage and obviously the extent of the disease. Carboplatin dose intensity seems to be the most important factor. The administration of a dose of carboplatin which allows the attainment of an AUC > 5 is recommended every 3 weeks. When this condition is observed, two questions remain unanswered: the role of bleomycin and the optimal dosage of etoposide. Ongoing randomised trials may partially answer these questions. However, the standard treatment of good risk-NSGCT patients remains either four cycles of EP or three cycles of BEP. Thus, carboplatin cannot be considered as standard treatment of a good risk-NSGCT but may be used only in prospective clinical trials specifically designed to evaluate its activity and its optimal dosage.

1. Einhorn LH. Testicular cancer as a model for a curable neoplasm : the Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1981, **41**, 3275–3280.
2. Birch R, Williams S, Cone A, *et al.* Prognostic factors for favourable outcome in disseminated germ cell tumors. *J Clin Oncol* 1986, **4**, 400–407.
3. Bosl G, Geller N, Cirrincione C, *et al.* Multivariate analysis of prognostic variables in patients with metastatic testicular cancer. *Cancer Res* 1983, **43**, 3403–3407.
4. Droz JP, Kramar A, Ghosn M, *et al.* Prognostic factors in advanced nonseminomatous testicular cancer. A multivariate logistic regression analysis. *Cancer* 1988, **62**, 564–568.
5. Droz JP, Kramar A, Rey A. Prognostic factors in metastatic disease. *Semin Oncol* 1992, **19**, 181–189.
6. Garrow G, Johnson D. Treatment of 'good risk' metastatic testicular cancer. *Semin Oncol* 1992, **19**, 159–165.
7. Einhorn LH, Williams D, Loehrer PJ, *et al.* Evaluation of optimal duration of chemotherapy in favourable-prognosis disseminated germ cell tumors : a Southeastern Cancer Study Group Protocol. *J Clin Oncol* 1989, **7**, 387–391.
8. Loehrer PJ, Elson P, Johnson DH, Williams SD, Trump DL, Einhorn LH. A randomized trial of cisplatin (P) plus etoposide (E) with or without bleomycin (B) in favorable prognosis disseminated germ cell tumors (GCT). *Proc Am Soc Clin Oncol* 1991, **10**, 169.
9. Brader M, Horwich A, Peckham MJ. Treatment of favorable-prognosis non seminomatous testicular germ cell tumors with etoposide, cisplatin and reduced dose bleomycin. *Cancer Treat Rep* 1987, **71**, 655–656.
10. Horwich A, Dearnaley DP, Nicholls J, *et al.* Effectiveness of carboplatin, etoposide and bleomycin combination chemotherapy in good-prognosis metastatic testicular nonseminomatous germ cell tumors. *J Clin Oncol* 1991, **9**, 62–69.
11. Williams S, Birch R, Einhorn L, Irwin L, Greco A, Loehrer P. Treatment of disseminated germ-cell tumors with cisplatin, bleomycin and either vinblastine or etoposide. *N Engl J Med* 1987, **316**, 1435–1440.
12. Stoter G, Kaye S, Jones W, *et al.* Cisplatin and VP16 +/– bleomycin (BEP vs EP) in good risk patients with desseminated nonseminomatous testicular cancer : a randomized EORTC, GU Group Study. *Proc Am Soc Clin Oncol* 1987, **6**, 110.
13. Bosl G, Geller N, Bajorin D, *et al.* A randomized trial of etoposide - + cisplatin versus vinblastine + bleomycine + cisplatin + cyclophosphamide + dactinomycin in patients with good-prognosis germ cell tumors. *J Clin Oncol* 1988, **6**, 1231–1238.
14. Bajorin D, Sorosdy M, Bosl J, *et al.* Good risk germ cell tumor (GCT) : a randomized trial of etoposide +carboplatin (EC) vs etoposide + cisplatin (EP). *Proc Am Soc Clin Oncol* 1992, **11**, 203.
15. Cockroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976, **16**, 31.
16. Calvert AH, Newell DR, Gumbrell LA, *et al.* Carboplatin dosage : prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989, **7**, 1748–1756.
17. Van der Vijgh W. Clinical pharmacokinetics of carboplatin. *Clin Pharmacokinet* 1991, **21**, 242–261.
18. Canetta R, Bragman K, Smaldone L, Rozincweig M. Carboplatin : current status and future prospects. *Cancer Treat Rev* 1988, **15** (suppl B), 17–32.
19. Husband D, Green J. POMB/ACE chemotherapy in non-seminomatous germ cell tumours : outcome and importance of dose intensity. *Eur J Cancer* 1992, **28A**, 86–91.
20. Harstrick A, Casper J, Guba R, Wilke H, Poliwoda H, Schmoll HJ. Comparison of the antitumor activity of cisplatin, carboplatin and iproplatin against established human testicular cancer cell lines *in vivo* and *in vitro*. *Cancer* 1989, **63**, 1079–1083.
21. Childs W, Nicholls E, Horwich A. The optimisation of carboplatin dose in carboplatin, etoposide and bleomycin combination chemotherapy for good prognosis metastatic nonseminomatous germ cell tumours of the testis. *Ann Oncol* 1992, **3**, 291–296.

22. Jodrell D, Egorin M, Canetta R, *et al.* Relationship between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. *J Clin Oncol* 1992, **10**, 520–528.
23. Newell D, Eeles R, Gumbrell L, Boxall F, Horwich A, Calvert H. Carboplatin and etoposide pharmacokinetics in patients with testicular teratoma. *Cancer Chemother Pharmacol* 1989, **23**, 367–372.
24. Cavalli F, Sonntag RW, Jungi F, *et al.* VP16-213 monotherapy for remission induction of small cell lung cancer : a randomized trial using three dosage schedules. *Cancer Treat Rep* 1978, **62**, 473–475.
25. Crawford S, Newlands S, Begent R, Rustin G, Bagshawe K. The effect of intensity of administered treatment on the outcome of germ cell tumours treated with POMB/ACE chemotherapy. *Br J Cancer* 1989, **59**, 243–246.
26. Samuels M, Holoye P, Johnson D. Bleomycin combination chemotherapy in the management of testicular neoplasia. *Cancer* 1975, **36**, 318–326.
27. Samuels M, Johnson D, Holoye P. Continuous intravenous bleomycin (NSC-125066) therapy with vinblastine (NSC-49842) in stage III testicular neoplasia. *Cancer Chemother Rep* 1975, **59**, 563–570.
28. Culine S, Mahjoubi M, Philippot I, Droz JP. Treatment of good-risk disseminated non-seminomatous germ cell tumours : the less bleomycin, the more cisplatin. *Eur J Cancer* 1991, **27**, 1715.
29. Van Echo D, Egorin M, Aisner J. The pharmacology of carboplatin. *Semin Oncol* 1989, **16** (suppl 5), 1–6.

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Cell Proliferation of Breast Cancer Evaluated by Anti-BrdU and Anti-Ki-67 Antibodies : Its Prognostic Value on Short-term Recurrences

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The prognostic value of breast cancer proliferative activity was evaluated in 385 women operated for primary, non-metastasised mammary carcinoma. Cell kinetics was measured using two immunohistochemical techniques. Cells in S-phase of cell cycle were labelled *in vitro* by incubation of fresh tissue fragments with 5-bromo 2-deoxyuridine (BrdU), a thymidine analogue. Nuclei of cells in active DNA synthesis were stained by an anti-BrdU monoclonal antibody (Mab). Cells in interphase and mitosis were detected with Ki-67, a Mab that is known to react with a nuclear antigen present in G1/S/G2/M phases of cell cycle, but not in resting cells. This reagent provides a means of evaluating the growth fraction of neoplastic cells. BrdU was incorporated in a proportion of tumour cells ranging from 0.1 to 65.5% (median 6.8%). In the panel of tumours presented in this report the median percentage of Ki-67 positive cells (Ki-67 score) was 9.0% (range 0.1–77%). The relationship between disease-free survival (DFS), BrdU labelling index, Ki-67 score and 13 different clinico-pathological variables was investigated by multivariate analysis, using the Cox proportional hazards model. Axillary node status ($P = 0.009$) and Ki-67 score ($P = 0.038$) emerged as independent prognostic factors. Nodal status and tumour growth fraction allowed division of patients into groups at different risk of relapse : tumours with a proliferative index below the median value showed a lower recurrence rate than tumours with a high proliferative activity ($P < 0.001$). In particular, no relapse occurred in pN0 patients bearing carcinomas with a Ki-67 labelling < 9.0% 4 years after surgery. These findings suggest that the evaluation of proliferative activity in breast cancer enhances the probability of correctly predicting outcome after surgery and could be of assistance in the planning of adjuvant therapies.
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INTRODUCTION

IN BREAST cancer the histological status of axillary lymph nodes is widely accepted as the most reliable prognostic marker for the risk of relapse after surgery [1, 2]. The correct planning of adjuvant therapies in node negative patients demands, however, the use of additional prognostic factors [3].

A number of studies have shown a correlation between the proliferative activity of human primary breast carcinomas and prognosis [4–6]. Thymidine labelling index (TLI), an autoradiographic method that measures the proportion of tumour cells in S-phase of cell cycle, has been the most commonly used indicator of proliferative activity in breast cancer [7]. This procedure, however, is time consuming and the use of a radioactive isotope with a long physical half life, such as tritium, is not accessible to all clinical laboratories, making it unsuitable for large studies involving different institutions.

More recently, several immunohistochemical methods for the assessment of tumour proliferative activity have become available. A non-radioactive alternative to tritiated thymidine,

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