PERGAMON

European Journal of Cancer 36 (2000) 341–347

European Journal of Cancer

www.elsevier.com/locate/ejconline

Chronotherapy with 5-fluorouracil, folinic acid and carboplatin for metastatic colorectal cancer; an interesting therapeutic index in a phase II trial☆

C. Focan *, F. Kreutz, D. Focan-Henrard, N. Moeneclaey

Department of Medical Oncology, Les Cliniques Saint-Joseph, Rue de Hesbaye 75-4000, Liege, Belgium

Received 21 April 1999; accepted 29 October 1999

Abstract

The aim of this study was to develop a phase II study gauging the contribution of a daily low-dose of carboplatin combined with 5-fluorouracil (5-FU) and folinic acid (FOL) in a chronotherapy schedule for advanced colorectal cancer patients. 60 patients with metastatic colorectal cancer were included in a phase II trial in which 50% of patients had received prior chemotherapy of up to three regimens. Treatment consisted of a combination of 5-FU (700 mg/m^2/day) and FOL (300 mg/m^2/day) infused from 10.00 pm to 10.00 am peaking at 4.00 am with carboplatin infused from 10.00 am to 10.00 pm at 40 mg/m^2/day with a peak at 4.00 pm. 4-day courses were repeated every 2 weeks in an ambulatory setting with a programmable pump. Patients experienced excellent tolerance (grades III–IV%): diarrhoea, 8.1; nausea/vomiting, 4.8; mucositis, 3.2; skin or neurological 1.7; granulocytes 29.0; platelets and haemoglobin (Hb) 9.7. Major tumour responses were observed in 47% of cases, 4 complete response (CR), 24 partial response (PR); 3 CR and 6 PR (69%) were recorded in 13 previously untreated patients; 11 (18%) underwent subsequent surgical resection of residual metastases. Median survival was 14.6 months with 22% patients surviving over 2 years (35% survival for responders versus 0 for non-responders). In conclusion, this chronotherapy determined administration of 5-FU/FOL and carboplatin yielded an excellent therapeutic index for the combination and warrants further evaluation in the first-line treatment of metastatic colorectal cancer.

Keywords: Colorectal cancer; Ambulatory medicine; Chronotherapy; Carboplatin; 5-Fluorouracil

1. Introduction

5-Fluorouracil (5-FU), the reference drug in the treatment of colorectal cancer, has only achieved 10% of objective responses when given as a single agent for first-line treatment of metastatic disease [1]. This objective response rate may be doubled either by the combination of 5-FU with biochemical modulators [mainly folinic acid (FOL) or methotrexate] [1,2] or by a protracted continuous venous administration [3,4]. High-doses of 5-FU and FOL given as a 48-h continuous infusion were recently proved superior to a bolus intravenous (i.v.) administration of the same drugs [5].

Various platinum derivatives have been combined with 5-FU based chemotherapies in an attempt to further improve the results. Cisplatin and carboplatin, given in monotherapy, are considered inactive against metastatic colorectal cancer, even at their maximum tolerated doses; their response rates are respectively 0–9% and 0–5% [6–10]. In randomised trials, no clinical benefit could be obtained from the addition of cisplatin to 5-FU, despite the ability of the former to modulate 5-FU cytotoxicity in experimental systems [11–16]. Generally, despite some results suggesting enhanced response rates, most authors consider their experience as negative because of the lack of survival improvement and the increased toxicity and complexity of the treatment [11–16]. Only a few reports have dealt with carboplatin combined with 5-FU (±FOL) [17,18]. Some are encouraging and one study even showed the feasibility of a chronotherapy with low 5-FU doses and carboplatin doses gauged according to scheduled area under the curve (AUC) (Calvert's formula) [18].

☆Presented at the Sixth International Conference of Anticancer Research, Kallithea, Halkidiki, Greece (October 1998) and awarded at the yearly meeting of the Belgian Society of Medical Oncology (January 1999).

* Corresponding author. Tel.: +32-4-224-89-90. Fax: 32-4-224-89-91.
E-mail address: christian.focan@lescliniquesstjoseph.be (C. Focan).

0959-8049/00/$ - see front matter
PII: S0959-8049(99)00282-8

Chronotherapy is a new strategy of drugs administration based on circadian rhythms [19,20]. The expression of specific genes and their co-ordination by at least one hypothalamic biological clock modulates the rhythms of cellular metabolism and proliferation in normal tissues [20–23].

Furthermore, in laboratory rodents, tolerability and/or antitumour efficacy of up to 30 anticancer drugs, including 5-FU and platinum derivatives, vary largely according to their dosing time during the 24-h timescale of the regimen [24]. In human bone marrow, skin and digestive mucosae, DNA synthesis, a stage of the cell-division cycle associated with increased susceptibility to S-phase-specific agents, decreases by 50% or more between midnight and 4.00 am from its daytime level [20,22–24]. The activity of dihydropyrimidine dehydrogenase in human mononuclear cells increases by 40% around midnight [20]. This enzyme is involved in the intracellular catabolism of 5-FU and contributes to the improved tolerability of this drug observed between midnight and 4.00 am [25]. The application of these concepts to clinical practice is primarily aimed at increasing the dose intensity through an adjustment of drug delivery using 24-h rhythms. The selection of peak times of drug delivery is based on the results obtained from chronopharmacological studies on rodents. The demonstration of relevant rhythms in normal host tissues, which may be damaged by chemotherapy, and the drug pharmacokinetics dependency according to the dosing time also contribute to this selection.

In metastatic colorectal cancer (CRC), phase I, II and III trials performed by the European Chronotherapy Group (International Organisation for Cancer Chronotherapy acting now as the EORTC Chronotherapy Study Group) have validated the chronotolerance of a complex combined chronotherapy with oxaliplatin (LOHP), 5-FU and FOL [26–28]: chronotherapy reduced 5-fold the rate of severe mucosal toxicity and halved functional impairment from peripheral sensitive neuropathy [27,29] whilst antitumour efficacy was nearly doubled with an objective response rate (ORR) of 51% with the 3-drug chronotherapy compared with an ORR of 30% using a flat infusion strategy [27,28]. The role of oxaliplatin which seems to be active for first- or second-line treatments of metastatic CRC [29–32], has been further established in a multicentre randomised trial [33].

A phase II study to gauge the contribution of a daily low-dose of carboplatin combined with 5-FU and FOL in a chronotherapy schedule for advanced colorectal cancer patients was developed in our centre for the following reasons: (1) a large fraction of colorectal cancer patients referred to our centre failed to fulfil inclusion criteria of the European Chronotherapy Group; (2) from the end of 1997 to the end of 1998 no active European study was undertaken; and (3) oxaliplatin was (at the time of this study) not refunded by the National Health Insurance in Belgium.

Pharmacokinetics studies have shown a short terminal half-life for carboplatin of around 120 min [34,35]. The rate of platinum excreted in patients' urine during the first 24 h after having received carboplatin ranged from 58 to 77% of the whole administered dose [34,35]. As a result of the low proliferative activity of most colorectal cancers and the rather rapid clearance of carboplatin, continuous infusion of the latter might be an attractive way to optimally exploit the characteristics of both the tumour and the drug. Phase I studies were conducted and they have confirmed the safety of carboplatin use in continuous infusions for 5, 14 or 21 days [36,37]. Furthermore, chronotolerance to carboplatin has been established in humans: Kerr and colleagues demonstrated that myelotoxicity of carboplatin was influenced by the time of its administration; an injection at 6.00 am induced greater thrombocytopenia than an injection at 6.00 pm [38]. Finally, interesting therapeutic indexes were recently reported in non-small cell lung and in upper-aerodigestive tract tumours with an infusional chronotherapy of 5-FU, FOL and carboplatin [39–41]. A prior pilot chronotherapy study performed on metastatic colorectal cancer with the same drugs has reported similar findings [18]. Based on the promising results obtained by the European group with a bi-weekly intensified chronotherapy with 5-FU, FOL and LOHP performed 4 days every 2 weeks (4 days on, 10 days off) [42,43], we designed a similar programme in which carboplatin replaced LOHP.

2. Patients and methods

This phase II trial was conducted in accordance with the Helsinki declaration on patients' rights and after approval by the local ethical medical committee of our hospital centre.

Patients with histologically proven CRC and measurable metastases at computed tomography (CT) scan and with a Karnofsky index >80, (WHO $\leqslant 2$), who fell out of inclusion criteria for IOCC or EORTC studies because of their progression during or within 6 months after an adjuvant treatment, were proven resistant to previous chemotherapy or were too old (>75 years of age) and/or had been previously treated with 5-FU based chronotherapy, were eligible for the study as well as patients previously treated with chemotherapy for advanced disease. After informed consent and a general medical evaluation (including at least general biology with tumour markers CEA and CA 19.9, CT scans of thorax, abdomen, pelvis), implantation (if necessary) of a double lumen venous access port (Port-a-Cath system: Pharmacia & Upjohn, Uppsala, Sweden or Bard, Salt Lake City, UT, USA) was carried out. Treatment was

administered using time programmable pumps with four channels (Intelliject or Melodie; Aguettant Laboratories, Lyon, France) and consisted of 4-day courses repeated every 2 weeks (4 days on, 10 days off) with 5-FU (700 mg/m^2/day) and FOL (300 mg/m^2/day for racemic form or 150 mg/m^2/day for levogyre form) infused sinusoidally from 10.00 pm to 10.00 am (peak at 04.00 am) and carboplatin (40 mg/m^2/day) infused from 10.00 am to 10.00 pm (peak at 4.00 pm) (Fig. 1).

Toxicity was recorded on each treatment day and graded according to WHO criteria [44]. The assessment of tumour targets was repeated after 4 and 8 courses and thereafter every 3 to 4 courses; WHO criteria [44] were also used to define tumour evolution as complete (CR), partial (PR) or minor responses (MR), no change (NC) or progressive disease (PD). Tumour evaluations were carefully assessed independently by a team of 2 oncologists and 1 radiologist, none of them involved in the management of patients' care. Tumour responses considered for this report were those reviewed by the panel; the evaluation of the treating physician, if any, was not recorded in the patients' files. Time to progression and survival were determined by Kaplan–Meier estimates.

3. Results

3.1. Patients' characteristics

Between August 1996 and July 1998 60 patients fulfilling the inclusion criteria were incorporated in the study. Tables 1 and 2 summarise their main characteristics. The reasons for ineligibility in the studies of the European group are analytically presented in Table 2. 50% of patients had previously received chemotherapy for advanced disease. Amongst the 30 previously untreated cases, 8 (13%) were progressing within 12 months of initiation of adjuvant chemotherapy (in one case after resection of a second primary tumour), 5 (8%) had undergone surgery for relapse, 3 (5%) were $\geqslant$75 years old, 2 (3%) had measurable lesions not fulfilling requirements of the EORTC and finally 11 (18%) patients were treated during a break in active European studies (end of 1997 and 1998). Only 13 (22%) patients had not received prior chemotherapy (Table 1). A median number of 8 chemotherapy courses (range: 1–24; mean 5.7 ± 5.2) was delivered to the patients.

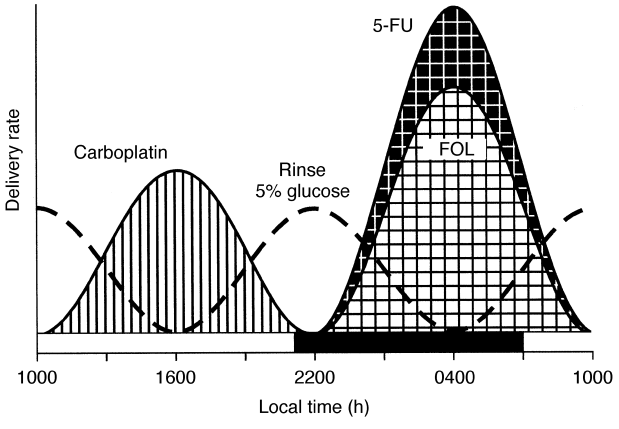

Fig. 1. Schedule of chronomodulated intravenous infusion of 5-fluorouracil (5-FU), folinic acid (FOL) and carboplatin. The local time in hours is plotted against the drug delivery rate. This cycle was repeated automatically for 4 consecutive days.

3.2. Toxicity

Toxicity was assessed on 549 courses. An excellent overall tolerance was observed. Table 3 presents the observed grades III–IV toxicities.

Table 1
Patients characteristics (n = 60)

Sex: male/female	27/33
Age: median (range)	65 (37–83) years
Primary tumour	*n* (%)
Colon/rectum	45 (75)/15 (25)
Grade 1/2/3/x	26 (43)/21 (35)/4 (7)/9 (15)
Dukes A/B/C/D/x	2 (3)/8 (13)/27 (45)/18 (30)/5 (8)
T 1/2/3/4/x	1 (2)/3 (5)/29 (48)/20 (33)/7 (12)
N 0/1/2/3/x	12 (20)/14 (23)/19 (32)/8 (31)/7 (12)
Previous treatment	
Surgery — for primary tumour	58 (97)
For metastases	28 (47)
Once	26[a]
Twice	1
> Twice	1
Radiotherapy	20 (33)
Chemotherapy	
Adjuvant	35 (58)
Palliative	30[b] (50)
Both	18 (30)
None	13[c] (22)
Chronotherapy	26 (43)
Metastases	
Sites; Isolated	27 (45)
Liver	18
Lung	4
Peritoneum	3
Abdominal wall	1
Locoregional	1
Multiple	33 (55)
Synchronous/metachronous	21 (35)/39 (65)
Number of sites 1/2/3	27 (45)/25 (42)/8 (13)
Progression within 1 year after initiation of adjuvant treatment	11[d] (18)

[a] Including 1 case of hypernephroma; 4 exploratory laparotomies.
[b] Two lines in 5 cases; three lines in 4 cases.
[c] 9 no active EORTC protocol; 2 measurable lesions not fulfilling requirements of EORTC; 1 too old; 1 prior resection and radiotherapy for relapse.
[d] Of whom 3 also received one line of palliative chemotherapy.
x, unknown/undetermined.

Table 2
Analytical reasons for ineligibility in studies of the European Chronotherapy Group

	n (%)
No previous chemotherapy for advanced disease	
No active protocol ongoing (end 1997–end 1998): isolated reason	11 (18)
Progression within 12 months of initiation of adjuvant treatment	8 (13)
Previous resection surgery (± radiotherapy) for relapse	5 (8)
⩾75 years (isolated reason)	3 (5)
No measurable lesion according to EORTC requirements	2 (3)
Second primary tumour resected and progression at the end of adjuvant chronotherapy	1 (2)
Previous chemotherapy for advanced disease	
One line	21 (35)
Two lines	5 (8)
Three lines	4 (7)
Total (*n*)	60 (100)

Dose delays were required in 18.2% of courses and 59.7% of patients. Dose reductions were required in 2.9% of courses and 24.2% of patients for 5-FU and in 6.6% and 43.5% for carboplatin respectively. The adaptations of the treatment were basically proposed to minimise grades I–III toxic events (i.e. for platelets between $50–99\times10^9$/l).

3.3. Tumour evolution

For the whole group, 4 CR, 24 PR (47% responses), 7 MR and 10 NC were recorded. 4 patients were not appraisable (1 too early, 1 early withdrawal after one course, 1 early death and 1 refusal before course four). In patients previously treated with chemotherapy for advanced disease, 12/30 PR (40%) were observed whilst 12/26 (46%) previously treated with chronotherapy responded; 6/18 responses (33%) were assessed in cases previously given both adjuvant and palliative chemotherapy. 9 PR, 3 MR and 3 NC were observed in the group of 21 cases previously treated with one line of chemotherapy. Of interest, 3 PR, 1 MR and 1 NC were also obtained amongst the 5 patients previously treated with two lines of chemotherapy whilst only 1 NC was obtained within the 4 cases previously exposed to three lines of therapy. Amongst the 26 patients having previously received 1 or 2 5-FU based treatment, 14 had progressed during or within 6 months after withdrawal of therapy; 4 PR (29%), 2 MR and 4 NC could be assessed in this last group. Similarly 5 PR (63%) and 1 NC were observed amongst the 8 subjects progressing during or within 6 months of adjuvant therapy. Finally, 3 CR and 6 PR (69%) were recorded amongst the 13 chemotherapy naïve subjects. Median progression free survival was 8.3 (95% confidence interval (CI): 6.1–11.6) months; median survival of the whole group was assessed at 14.6 (95% CI: 10.7–17.5) months (18.0 m for responders versus 10.7 m for non-responders; $P<0.01$). 11 patients (18%) could benefit from further hepatic (7 cases), pulmonary (2 cases), both (1 case) and liver and colon (1 case) resections with a complete surgical response being obtained in 10 cases. For the whole group, 22% of patients survived 2 years (35% for the responders versus 0 for the non-responders) (Fig. 2).

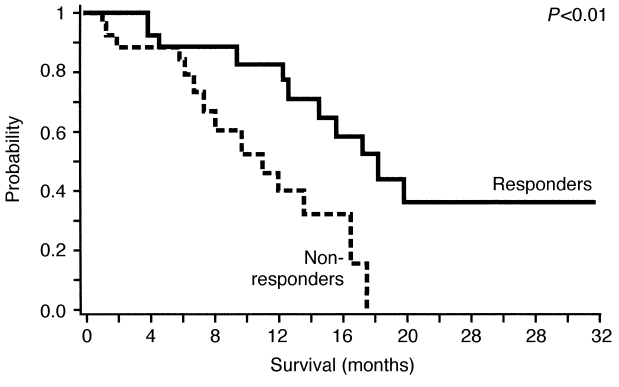

Fig. 2. Probability of survival according to response to chronotherapy.

Table 3
Toxicity — WHO grades III–IV (%)

	% Courses (549)	% Patients[a] (59)
Haematological		
Granulocytes	6.6	29.0
Leucocytes	3.6	21.0
Platelets	2.0	9.7
Haemoglobin	1.5	9.7
Clinical		
Nausea-vomiting	1.1	4.8
Mucositis	0.9	3.2
Diarrhoea	1.5	8.1
Skin	0.5	1.7
Neurological	0.02	1.7
Alopecia	0.0	0.0

[a] One case was in-evaluable due to early withdrawal after course 1. % courses and % patients as maximum toxicities per patient.

4. Discussion

The chronotherapy programme proposed in this phase II study developed an interesting therapeutic index in a group of colorectal cancer patients with, in most cases (78%), worst prognostic characteristics. Clearly, the use of a low daily dose of carboplatin has allowed the administration of the schedule without any significative clinical or haematological toxicity, at least until the eighth course. All patients were treated in ambulatory convenience with a maintained excellent quality of life; sometimes, they suffered from mild nausea-vomiting (easily controlled with anti-5HT3) and developed no alopecia.

Table 4
Comparative toxicities between densified chronotherapy (4 days on, 10 days off) with 5-fluorouracil (5-FU), folinic acid (FOL) and either oxaliplatin (FFL4-10) [43] or carboplatin (FFC4-10) (this trial) (% WHO grades III–IV)

	FFL4-10		FFC4-10	
	% Courses (823)	% Patients (90)	% Courses (549)	% Patients (59)
Granulocytes	1.8	13.4	6.6	29.0
Platelets	0.4	3.4	2.0	9.7
Haemoglobin	0.3	2.2	1.5	9.7
Diarrhoea	8.3	41.1	1.5	8.1
Nausea-vomiting	7.6	37.8	1.1	4.8
Mucositis	5.1	30.0	0.9	3.2
Skin	1.7	7.9	0.5	1.7
Peripheral neuropathy (any grade)	72.1	92.2	0.02	1.7

A high level of major tumour responses (47%) was recorded. These were independently assessed by a team of oncologists and a radiologist not involved in the patients' care. Moreover, 18% of patients could benefit from further surgical resection with curative intent of their residual metastases. With this strategy, long-term survival (or even cures) of half of those patients has been recently reported [45]. Our results also suggest that carboplatin (like oxaliplatin) is partly able to reverse acquired tumoral resistance to 5-FU based chemotherapy [31,32]. Indeed, a response rate of approximately 30% was obtained in previously treated patients either progressing under a 5-FU treatment or with a short free interval. In addition, 5/8 patients (63%) progressing during or within one year of adjuvant treatment also enjoyed a clinical response. These results were obtained with a low-dose of carboplatin and it was clear that the maximum tolerated dose (MTD) of the combination was not reached. These observations must be considered along with the fact that carboplatin alone, even at its MTD, has no significant antitumour effect against CRC [8–10]. In monotherapy, the optimal carboplatin doses administered in CVI were larger than those used in our study. They were established at 75 mg/m^2/day in 5-day schedules [35,36] or 25 to 30 mg/m^2/day in 14- or 21-day schedules [36,37]. Probably, like oxaliplatin, carboplatin may act as a potentiator and a modulator of the cytolytic effect of 5-FU and FOL, allowing tumour cells to retrieve some sensitivity to 5-FU [31,32]. The role of platinum derivatives in the combined treatment of metastatic CRC has also been emphasised recently in 2 large multicentre phase III studies with the use of oxaliplatin [33,46].

The high level of major tumour responses (69%) obtained in previously untreated patients suggests that, again like oxaliplatin, carboplatin may play a definitive role in the enhancement of 5-FU and FOL tumoral activity. Indeed, this response rate of 69% fits well with the recurrent response rate of 66% observed in the trials with oxaliplatin performed in a parallel study by the European Chronotherapy Study Group [42,43]. In phase II studies, including a large multicentre one, more than 100 chemotherapy naïve patients with measurable metastatic CRC were treated with a bi-weekly intensified chronotherapy schedule (4 days on, 10 days off) with 5-FU, FOL and oxaliplatin [42,43]. In those trials, haematological toxicity was negligible; however, clinical toxicity (diarrhoea, nausea-vomiting, mucositis, skin and neurology) was significantly higher than in the present report (Table 4). In particular, patients previously untreated receiving the oxaliplatin-based chronotherapy complained of grades 3–4 mucositis (30% cases) or gastrointestinal tract (approximately 40% cases) toxicities and almost universally of peripheral sensitive neuropathy which was frequently consuming [43]. Low subjective toxicities were observed in our study despite the fact that 78% of our patients ($n=47$) had previously been exposed to 5-FU-based chemotherapies. Finally, a densified chronotherapy (4 days on, 10 days off) with a progressive increase of the 5-FU dose (in combination with FOL) up to individual MTDs could only achieve a response rate of 41% in a more favourable group of patients, since they were previously untreated, and at the cost of grade III mucositis and skin toxicities in 45 and 65% of patients respectively [47].

To conclude, the excellent therapeutic index observed in our phase II study warrants further evaluation of the role of carboplatin as a potentiator of the effects of fluorinated pyrimidines in first line combined treatment of metastatic CRC, and the results should be compared to those obtained with oxaliplatin.

Acknowledgements

We gratefully thank our colleagues from the surgical and gastro-enterological teams of our hospital and of the CHU Sart Tilman (Liège-B) for referral patients for this trial. We thank also Drs L. Longrée, M.-P. Graas, A.C. Davin (oncologists) and J.F. Biquet (radiologist) for their participation in the independent review of medical imaging. The excellent editorial assistance of R. Franssen and the assistance of M. Focan for reviewing the English formulation have also been fully appreciated.

References

1. Advanced Colorectal Cancer Meta-analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. *J Clin Oncol* 1992, **10**, 896–903.
2. Advanced Colorectal Cancer Meta-Analysis Project. Meta-analysis of randomized trials testing the biochemical modulation of fluorouracil by methotrexate in metastatic colorectal cancer. *J Clin Oncol* 1994, **12**, 960–969.

3. Lokich J, Ahlgren J, Gullo J, *et al*. Prospective randomized comparison of continuous infusion of fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program study. *J Clin Oncol* 1989, **7**, 425–432.
4. Piedbois P. The Meta-Analysis Group in Cancer: Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. *J Clin Oncol* 1998, **16**(1), 301–308.
5. De Gramont A, Bosset JF, Milan C, *et al*. Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French Intergroup study. *J Clin Oncol* 1997, **15**(2), 808–815.
6. Lokich J, Zipoli T, Greene R, *et al*. Protracted low-dose cisplatin infusion in advanced colorectal cancer. *Cancer Treat Rep* 1986, **70**(4), 523–524.
7. DeSimone PA, Davila E, Jochimsen PR, Bartolucci AA. High-dose cisplatin in the treatment of advanced adenocarcinoma of the colon and rectum: a southeastern cancer study group trial. *Cancer Treat Rep* 1986, **70**(10), 1229–1230.
8. Nolè F, Biganzoli L, Buzzoni R, Bajetta E. Carboplatin in patients with advanced colorectal cancer pretreated with fluoropyrimidines. *Eur J Cancer* 1993, **29A**, 1330–1331.
9. Perry DJ, Weiss RB, Creekmore SP, Micetich KC, Curt GA. Carboplatin for advanced colorectal carcinoma: a phase II study. *Cancer Treat Rep* 1986, **70**(2), 301–302.
10. Pazdur R, Samson MK, Baker LH. CBDCA: phase II evaluation in advanced colorectal carcinoma. *Am J Clin Oncol* 1987, **10**(2), 136–138.
11. Loehrer PJ, Einhorn LH, Williams SD, *et al*. Cisplatin plus 5-FU for the treatment of adenocarcinoma of the colon. *Cancer Treat Rep* 1985, **69**(12), 1359–1363.
12. Dy C, Gil A, Algarra SM, *et al*. Combination chemotherapy of cisplatin and 5-FU in advanced colorectal carcinoma. *Cancer Treat Rep* 1986, **70**(4), 465–468.
13. Loehrer PJ, Turner S, Kubilis P, *et al*. A prospective randomized trial of fluorouracil versus fluorouracil plus cisplatin in the treatment of metastatic colorectal cancer: A Hoosier Oncology Group trial. *J Clin Oncol* 1988, **6**(4), 642–648.
14. Kemeny N, Israel K, Niedzwiecki D, *et al*. Randomized study of continuous infusion of fluorouracil versus fluorouracil plus cisplatin in patients with metastatic colorectal cancer. *J Clin Oncol* 1990, **8**(2), 313–318.
15. Diaz Rubio E, Jimeno J, Anton A, *et al*. A prospective randomized trial of continuous infusion of 5-fluorouracil (5-FU) versus 5-FU plus cisplatin in patients with advanced colorectal cancer. *Am J Clin Oncol* 1992, **15**, 56–60.
16. Lokich JJ, Ahlgren JD, Cantrell J, *et al*. A prospective randomized comparison of protracted infusional 5-fluorouracil with or without weekly bolus cisplatin in metastatic colorectal carcinoma. *Cancer* 1991, **67**, 14–19.
17. Gil A, Dy C, Hidalgo OF, et al. Carboplatin (CBDCA) and 120 hours 5-fluorouracil (5FU) in patients (PTS) with colorectal cancer. *Proc ECCO 4*, 1987, 42, abstract 161.
18. Natoli C, Martino MY, Irtelli L, *et al*. Chronomodulated infusion of 5-fluorouracil, folinic acid and carboplatin in colorectal cancer: a pilot study. *Anticancer Res* 1995, **15**, 2841–2842.
19. Focan C. Marker rhythms for cancer chronotherapy. From laboratory animals to human beings. *In Vivo* 1995, **9**, 283–298.
20. Focan C. Circadian rhythms and cancer chemotherapy. *Pharmacology and Therapeutics* 1995, **67**, 1–52.
21. Lévi F, Giacchetti S, Adam R, *et al*. for the International Organization for Cancer Chronotherapy. Chronomodulation of chemotherapy against metastatic colorectal cancer. *Eur J Cancer* 1995, **31A**, 1264–1271.
22. Smaaland R, Laerum OD, Lote K, *et al*. DNA synthesis in human bone marrow is circadian stage dependent. *Blood* 1991, **77**, 2603–2611.
23. Antoch MP, Song EJ, Chang AM, *et al*. Functional identification of the mouse circadian clock gene by transgenic BAC rescue. *Cell* 1997, **89**, 655–667.
24. Mormont MC, Boughattas N, Lévi F. Mechanisms of circadian rhythms in the toxicity and the efficacy of anticancer drugs: relevance for the development of new analogs. In Lemmer B, ed. *Chronopharmacology: cellular and biochemical interactions*. New York, Marcel Dekker, 1989, 395–437.
25. Harris B, Song R, Soong S, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels: evidence for circadian variation of plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990, **50**, 197–201.
26. Lévi F, Misset JL, Brienza S, *et al*. A chronopharmacologic phase II clinical trial with 5-fluorouracil, folinic acid and oxaliplatin using an ambulatory multichannel programmable pump. High antitumor effectiveness against metastatic colorectal cancer. *Cancer* 1992, **69**, 893–900.
27. Lévi F, Zidani R, Vannetzel JM, *et al*. Chronomodulated versus fixed infusion rate delivery of ambulatory chemotherapy with oxaliplatin, 5-fluorouracil and folinic acid in patients with colorectal cancer metastases. A randomized multi-institutional trial. *J Natl Cancer Inst* 1994, **86**, 1608–1617.
28. Lévi F, Zidani R, Misset JL. Randomized multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. *Lancet* 1997, **350**, 681–686.
29. Becouarn Y, Ychou M, Ducreux M, *et al*. Phase II trial of oxaliplatin as first-line chemotherapy in metastatic colorectal cancer patients. *J Clin Oncol* 1998, **16**, 2739–2744.
30. Lévi F, Perpoint B, Garufi C, *et al*. Oxaliplatin activity against metastatic colorectal cancer. A phase II study of 5-day continuous venous infusion at circadian rhythm modulated rate. *Eur J Cancer* 1993, **29A**, 1280–1284.
31. Machover D, Diaz-Rubio E, De Gramont A, *et al*. Two consecutive phase II studies of oxaliplatin (L-OHP) for treatment of patients with advanced colorectal carcinoma who were resistant to previous treatment with fluoropyrimidines. *Ann Oncol* 1996, **7**, 95–98.
32. Garufi C, Brienza S, Bensmaïne MA, *et al*. Addition of oxaliplatin (L-OHP) to chronomodulated (CM) 5-fluorouracil (5-FU) and folinic acid (FA) for reversal of acquired chemoresistance in patients with advanced colorectal cancer (ACC). *Proc Am Soc Clin Oncol* 1995, **14**, 192.
33. Giacchetti S, Zidani R, Perpoint B, *et al*. Phase III trial of 5-fluorouracil (5-FU), folinic acid (FA) with or without oxaliplatin (OXA) in previously untreated patients (pts) with metastatic colorectal cancer (MCC). *Proc Am Soc Clin Oncol* 1997, **16**, 229a.
34. Van Echo DA, Egorin MJ, Aisner J. The pharmacology of carboplatin. *Semin Oncol* 1989, **2**(Suppl. 5), 1–6.
35. Van Echo DA, Egorin MJ, Whitacre MY, Olman EA, Aisner J. Phase I clinical and pharmacologic trial of carboplatin daily for 5 days. *Cancer Treat Rep* 1984, **68**, 1103–1114.
36. Lokich J, Anderson N, Bern M, *et al*. Infusional carboplatin. Phase I studies of 5-day and 14-day infusions. *Cancer* 1991, **68**, 68–71.
37. Smit EF, Willemse PHB, Sleifer DTh, *et al*. Continuous infusion carboplatin on a 21-day schedule: a phase I and pharmacokinetic study. *J Clin Oncol* 1991, **9**, 100–110.
38. Kerr DJ, Lewis C, O'Neil B, *et al*. The myelotoxicity of carboplatin is influenced by the time of its administration. *Hematol Oncol* 1990, **8**, 59–63.
39. Focan C, Denis B, Kreutz F, Focan-Henrard D, Lévi F. Ambulatory chronotherapy with 5-fluorouracil, folinic acid and carboplatin for advanced non-small cell lung cancer. A phase II feasibility trial. *J Infus Chemother* 1995, **5**(Suppl. 1), 148–152.
40. Focan C, Kreutz F, Denis B, Focan-Henrard D, Moeneclaey N. Ambulatory chronotherapy with 5-fluorouracil, folinic acid and

carboplatin for advanced non-small cell lung and esophagus cancer. An interesting therapeutic index. *Proc Am Soc Clin Oncol* 1997, **16**, 468a.

41. Focan C. Chronotherapy of human solid tumors other than colorectal. *In Vivo* 1995, **9**, 549–554.
42. Bertheault-Cvitkovic F, Jami A, Itzhaki M, *et al*. Biweekly intensified ambulatory chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil and folinic acid in patients with metastatic colorectal cancer. *J Clin Oncol* 1996, **14**, 2950–2958.
43. Lévi F, Zidani R, Brienza S, *et al*. A multicenter evaluation of intensified ambulatory chronomodulated chemotherapy with oxaliplatin, fluorouracil and leucovorin as initial treatment of patients with metastatic colorectal cancer. *Cancer* 1999, **85**, 2532–2540.
44. *WHO Handbook for Reporting Results of Cancer Treatment*. Geneva, World Health Organisation, 1979.
45. Giacchetti S, Itzhaki M, Gruia G, *et al*. Long-term survival of patients with unresectable colorectal cancer liver metastases following infusional chemotherapy with 5-fluorouracil, leucovorin, oxaliplatin and surgery. *Ann Oncol* 1999, **10**, 663–669.
46. De Gramont A, Seymour M, *et al*. A randomized trial of leucovorin (LV) and 5-fluorouracil (5 FU) with or without oxaliplatin in advanced colorectal cancer (CRC). *Proc Am Soc Clin Oncol* 1998, **17**, 257a.
47. Curé H, Adenis A, Tubiana-Mathieu N, *et al*. Phase II trial of chronomodulated (CM) high dose 5-fluorouracil (5-FU) and l-folinic acid (l-FA) in patients with metastatic colorectal cancer (MCC). *Proc Am Soc Clin Oncol* 1998, **17**, 273a.