PERGAMON

European Journal of Cancer 38 (2002) 70–74

European Journal of Cancer

www.ejconline.com

Phase II study of XR 5000, an inhibitor of topoisomerases I and II, in advanced colorectal cancer

F. Caponigro*, C. Dittrich, J.B. Sorensen, J.H.M. Schellens, F. Duffaud, L. Paz Ares, D. Lacombe, C. de Balincourt, P. Fumoleau

EORTC Early Clinical Studies Group, New Drug Development Program, Brussels, Belgium

Received 6 July 2001; accepted 24 July 2001

Abstract

XR 5000 is one of a series of tricyclic carboxamide-based cytotoxic agents. It binds to DNA by intercalation and stimulates DNA cleavage by inhibition of both topoisomerase I and II, thus possibly overcoming the resistance resulting from downregulation of either enzyme. Twenty patients with advanced or metastatic colorectal cancer, unpretreated for metastatic disease, received XR 5000 at the dose of 3010 mg/m^2 in a 120-h central intravenous (i.v.) infusion every 3 weeks. Response was evaluated every two cycles. No complete (CR) or partial responses (PR) were observed in eligible patients (response rate, 0 of 19, 0%; 95% confidence interval (CI): 0–18%). 5 patients had stable disease, which lasted from 79 to 157 days. Haematological toxicity was low, since only one grade 4 neutropenia and two grade 3 anaemia were observed. Other treatment-related grade 3-4 toxicities were: deep venous thrombosis (2 cases), liver toxicity, diarrhoea, anorexia, dyspnoea, chest pain, infection (1 case each). Despite the good toxicity profile, these results do not support further trials with XR 5000 in metastatic colorectal cancer. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Colorectal cancer; Phase II study; Topoisomerase; XR 5000

1. Introduction

Colorectal cancer is one of the most chemoresistant tumours. While nearly 15% of patients with colorectal cancer have metastases at presentation, nearly two-thirds of patients with resected tumour involving locoregional lymph nodes will recur within 5 years of initial treatment [1]. Over the past 40 years, 5-fluorouracil (5-FU), with or without modulation by levofolinic acid (LFA), has been the mainstay of treatment for metastatic colorectal cancer. However, over the last few years, new drugs have enriched the therapeutic armamentarium against this disease. In particular, irinotecan, a topoisomerase I inhibitor, has been approved for the treatment of patients who have failed 5-FU [2,3] and, more recently, has qualified as the new standard for first-line treatment when used in combination with 5-FU and LFA [4,5]. None the less, even considering these recent breakthroughs, the prognosis of patients with metastatic colorectal cancer remains poor, since response rates to the new gold standard do not exceed 50%, and, more importantly, median survival is not higher than 17 months. Therefore, the search for new active drugs in metastatic colorectal cancer is a major demand in medical oncology.

XR 5000 (N-[2-(dimethylamino)ethyl]acridine-4-carboxamide) is an acridine derivative, which binds to DNA by intercalation and stimulates the formation of cleavable complexes between DNA and topoisomerases I and II. Preclinical studies have shown that this drug is a potent cytotoxic and it is able to overcome several types of drug resistance, including the multidrug resistance exhibited towards a number of other topoisomerase I or topoisomerase II inhibitors. XR 5000 has been administered to 72 patients within two phase I clinical trials, which evaluated a 3-h treatment regimen [6,7]. In the first study, a single 3-h infusion was used in doses up to 750 mg/m^2 free base equivalent. In the second study, a 3-h infusion was administered on 3 consecutive days in doses up to 800 mg/m^2. The toxicological profile of the drug was similar in the two studies. In fact, pain in

* Corresponding author at: Istituto Nazionale Tumori "Fondazione G. Pascale", Via M. Semmola, 80131 Napoli, Italy. Tel.: +39-081-590-3225; fax; +39-081-590-3821.

E-mail address: fracap@sirio-oncology.it (F. Caponigro).

0959-8049/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0959-8049(01)00336-7

the infusion arm was the dose-limiting toxicity (DLT) in both studies. At the highest dose levels, several patients experienced also flushing, paraesthesias around the mouth, eyes and nose, lacrimation, sedation and a feeling of agitation. Furthermore, in the 3-day schedule study, typical chest pain occurred in a patient who had drug infusion via a Hickman line; in the same study, 1 patient, in whom a peripheral line was used, developed thrombosis of the right innominate vein. A different phase I study has evaluated the safety and pharmacokinetics of a 120-h infusion of XR 5000 administered through a central venous catheter to patients with advanced solid tumours [8]. A total of 18 patients were treated in this study with doses ranging between 700 and 4060 mg/m^2. At the last dose level, DLT (consisting of chest and abdominal pain, without cardiac enzyme or electrocardiogram (ECG) changes) was observed. However, these symptoms disappeared once the infusion was stopped. The dose level immediately below (3010 mg/m^2) was judged as feasible, since the most relevant toxicities were mild to moderate deep venous thrombosis, constipation, diarrhoea, fatigue, anorexia, nausea, vomiting and lethargy, while only 1 patient had grade 4 neutropenia. Therefore, the dose for phase II was set at 3010 mg/m^2; at this dose level, the 120-h infusion of XR 5000 achieved a 4-fold higher area under the concentration versus time curve (AUC) than was achieved at the maximum tolerated dose (MTD) of the 3-h infusion regimen, and this represented one of the main reasons (along with better tolerability) for the choice of the prolonged infusion regimen to be taken forward for phase II studies.

Following these findings, the European Organization for Research and Treatment of Cancer (EORTC) decided to undertake a broad phase II programme for XR 5000. In particular, a phase II study in colorectal cancer was planned, aimed at determining the response rate and duration, at further characterising toxicities and at collecting pharmacokinetic variables.

2. Patients and methods

2.1. Patient selection

Eligibility criteria for study entry included histologically-proven colorectal carcinoma not amenable to curative surgery or radiotherapy; presence of at least one target lesion bidimensionally measurable by computed tomography (CT) scan; age >18 years, Eastern Cooperative Oncology Group (ECOG) performance status 0–2; adequate bone marrow, liver, renal, cardiac function; life expectancy of at least 3 months. Patients were ineligible if they had received prior chemotherapy for metastatic disease (adjuvant chemotherapy was allowed if completed at least 6 months before study entry). Pregnancy, lactation, uncontrolled infections, unstable systemic diseases, prior radiotherapy in the last 4 weeks, major surgery in the last 14 days, symptomatic brain metastases, previous or concurrent malignancies at other sites were also exclusion criteria. The study was approved by the Protocol Review Committee of the EORTC and by the Ethics Commitee of each participating centre. Written informed consent was obtained from each patient before registration.

2.2. Treatment plan

Patients received XR 5000 at the dose of 3010 mg/m^2 free base equivalent. The drug was diluted in physiological saline to a total volume of 250 ml to be delivered at a constant infusion rate over 120 h. Antiemetic coverage was used at discretion of the investigator. In the first treatment cycle, blood samples were taken for therapeutic plasma level monitoring immediately before infusion, 24 hours after the start of infusion, and at the end of the infusion itself. Blood samples were taken into lithium heparin tubes and centrifuged within 10 min of collection. Plasma was separated into plain plastic tubes and immediately deep-frozen and retained at −20 °C until transfer to the analytical laboratory. The treatment was repeated every 3 weeks and withheld for a week in presence of drug-related toxicity (grade 4 haematological and grade 3-4 non-haematological toxicity) on the planned day for re-treatment. If recovery of toxicity did not occur within two weeks, treatment was discontinued, and the patient was taken off study. No dose modification was allowed. Supportive care was left at the physician's discretion.

2.3. Patient evaluation

At enrolment, patients were evaluated by a complete history and physical examination, performance status recording, complete blood cell (CBC) count, serum chemistries, urinanalysis, ECG, chest X-ray, carcinoembryonic antigen (CEA) assay, thoracic and abdomino-pelvic CT scan. Other exams were performed only in the presence of a clinical indication. Patients were monitored weekly throughout treatment by clinical examination, toxicity assessment and CBC count. Evaluation for tumour response was performed every two courses of chemotherapy with repetition of all tests which were abnormal at baseline, plus additional tests, when clinically indicated. Response was assessed according to standard World Health Organization (WHO) criteria. A follow-up scan obtained at least 4 weeks later was required to confirm complete response (CR) or partial response (PR). All objective responses documented by CT scans were required to be reviewed and confirmed by an external expert panel. After two courses of chemotherapy, patients with an objective

response and patients with stable disease received additional treatment, up to a maximum of six courses (unless further treatment continuation was felt to be in the patient's best interest), or unacceptable toxicity. Patients with progressive disease were taken off study. Toxicity was graded according to National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 2.0.

2.4. Statistical methods

The sample size calculation was based on the standard two stage Gehan's rule. In the first stage, 14 evaluable patients were required. If no response was observed in this cohort of patients, the study was to be closed. In case of one or more responses in the first 14 patients, additional patients would be added up to a maximum of 25. This design ensures that, if the drug is active in 20% or more of the patients, the chance of erroneously rejecting the drug after the first 14 patients is 0.044. However, the number of patients included in this trial went up to 20 due to simultaneous consent and registration of 7 patients in the participating institutions. The duration of CR or PR was measured from the date of first documentation of response to the date of objective progression. The duration of stable disease was measured from the time of study entry to the first evidence of disease progression.

3. Results

3.1. Patient characteristics

Between August 1999 and February 2000, 20 patients were entered onto the study. One patient was declared ineligible, both because he did not have any suitable target lesions for evaluation of response, and because he was treated before registration. The majority of patients had PS = 0, moderately differentiated tumour, and had not received any kind of prior chemotherapy; in fact, only 3 patients had previously received adjuvant (2 cases) or neoadjuvant (1 case) chemotherapy. The characteristics of eligible patients are detailed in Table 1.

3.2. Response

A total of 68 cycles of XR 5000 were administered to all registered patients, for a median number of two courses per patient (range 2–16). All nineteen eligible patients received at least two courses of chemotherapy and were assessable for treatment response. However, 1 patient refused treatment re-evaluation after two cycles because of a grade 3 thrombosis, which induced him to withdraw his consent to continue treatment. No complete or partial responses were observed in the eligible patients, after an external review panel examined all the CT scans (response rate, 0 of 19, 0%; 95% confidence interval (CI): 0–18%). 5 patients had stable disease, which lasted from 79 to 157 days. 5 eligible patients died of progressive disease during follow-up.

3.3. Toxicity

Median dose intensity was 99% of the planned dose (range 59–102%). Doses were reduced (lowered to 90% or less with respect to the theoretical dose) in 3 patients because of deep venous thrombosis, pain and infection (1 case), common practice which allows rounding down to 2 m^2 of a greater body surface area (2 cases). Treatment was delayed in 3 patients mostly because of problems in port-a-cath or central venous catheter, which required settlement or replacement. Treatment was interrupted (infusion not completed) in seven courses because of deep venous thrombosis (2 cases), overt progressive disease (1 case), or pump-related problems (4 cases). One patient died immediately after the fourth course of therapy. This patient had a very high tumour burden, with metastatic disease involving both lungs, abdominal nodes and probably bone. During the fourth course of treatment, she showed clinical signs of overt disease progression, such as worsening of respiratory function, increase of left leg oedema and back pain. She died a few days later because of respiratory insufficiency;

Table 1
Characteristics of eligible patients (total no. = 19)

Age (years) Median (range)	60 (40–73)
Sex	
Male	9
Female	10
Performance status	
0	14
1	4
2	1
Primary site	
Colon	11
Rectum	8
Tumour grading	
Well differentiated	1
Moderately differentiated	12
Poorly differentiated	2
Unknown	4
Previous surgery	
No	3
Yes, biopsy	6
Yes, resection	8
Yes, other	2
Prior radiotherapy	
No	16
Yes	3
Prior chemotherapy	
No	16
Yes, (neo) adjuvant	3

this death was not judged by the investigator as drug-related, but it was considered due to disease progression. Haematological toxicity was low in our study, since only 1 patient had an uncomplicated grade 3 leucopenia and grade 4 neutropenia, while 2 patients had grade 3 anaemia. Gastrointestinal side-effects were in general mild to moderate; in fact, no grade 3 nausea/vomiting was recorded, and only one patient had promptly reversed grade 3 diarrhoea. One patient had grade 4 liver toxicity (hyperbilirubinaemia); 1 patient had grade 3 left flank pain. Fatigue was observed in 15 patients, but it never exceeded grade 2. Anorexia was observed in 6 patients, and it reached grade 3 in 1 patient. One patient had grade 3 chest pain during infusion; however, the ECG was unchanged, cardiac enzymes were normal, and the pain decreased following treatment with paracetamol. Grade 3 dyspnoea occurred in 3 cases; in 1 case, it occurred during treatment infusion, and was clearly drug-related; in the other 2 cases, it was felt due to the underlying disease. 2 patients had grade 3 deep venous thrombosis during treatment. In both cases, patients had arm oedema, redness and pain at the port-a-cath. Ultrasound scan showed thrombosis in the left jugular, subclavian and axillary veins in 1 case, and in the left subclavian vein in the other. In both cases, the event resolved following appropriate medical treatment. Toxic effects observed in all treated patients are summarised in Table 2.

Table 2
Toxicity (total no. = 20)

	CTC grade				
	0	1	2	3	4
Leucopenia	17	2	0	1	0
Neutropenia	19	0	0	0	1
Anaemia	8	8	2	2	0
Thrombocytopenia	20	0	0	0	0
Nausea/vomiting	12	7	1	0	0
Liver	19	0	0	0	1
Stomatitis	18	2	0	0	0
Anorexia	14	4	1	1	0
Diarrhoea	13	5	1	1	0
Constipation	12	6	2	0	0
Dyspnoea	14	1	2	3	0
Deep venous thrombosis	18	0	0	2	0
Chest pain	19	0	0	1	0
Atrial fibrillation	19	0	1	0	0
Neurotoxicity	11	8	1	0	0
Fever	19	0	1	0	0
Infection	17	1	1	1	0
Fatigue	5	6	9	0	0
Alopecia	19	1	0	0	0

CTC, common toxicity criteria.

3.4. Pharmacokinetics

Pharmacokinetic data will be pooled together with those of concomitant other phase II studies and will be reported in a separate paper.

4. Discussion

DNA topoisomerases are important targets for anticancer drugs. Compounds like doxorubicin, etoposide, which target topoisomerase II, and camptothecin derivatives, which target topoisomerase I, have an established role in the treatment of a number of tumours. In particular, irinotecan is now acknowledged as the most active single agent against metastatic colorectal cancer, and it represents the standard therapy for patients with this disease, when used in combination with 5-FU and LFA [4,5].

Acridine derivatives, long known for their antimicrobial properties, have formed the basis for the development of agents which target topoisomerase II [9], such as amsacrine, which is active in the treatment of acute leukaemia [10]. A series of acridine derivatives lacking the anilino side-chain of amsacrine, but containing a charged side-chain at the 4-position have shown high activity against Lewis lung carcinoma [11]. In particular, one of these compounds (XR 5000, DACA) has shown to be very active against a number of murine tumours [12]. The unusual and unexpected cytotoxic properties of this new compound were attributed to its ability to stimulate DNA cleavage with either topoisomerase I or II. This is theoretically an extremely interesting mechanism of action, since single enzyme-targeting drugs often suffer from the development of drug resistance due to downregulation of the targeted enzyme. Furthermore, it has been suggested that resistance to inhibitors of one topoisomerase may be associated with increased sensitivity to inhibitors of the other [13], thus raising the possibility of synergy between topoisomerase I and II inhibitors. XR 5000 can be considered the leader of this emerging new class of compounds and it is the first to enter clinical trials.

After the phase I programme for this compound was completed, the 120-h continous infusion schedule via a central line every 3 weeks was chosen to be taken forward for phase II studies, since it was better tolerated and permitted the achievement of a 4-fold higher AUC at the MTD with respect to the 3-h infusion schedule. A broad phase II programme was undertaken by the EORTC Early Clinical Studies Group (ECSG), which included the present study. The acknowledged activity of topoisomerase I targeting drugs, such as irinotecan, in colorectal cancer, further increased the appeal of the study.

However, the study results are quite disappointing. No objective responses were observed in 19 eligible patients, while only 5 eligible patients had a stable disease. The toxicity profile confirmed that which emerged from phase I trials. In particular, myelosuppression was minimal; gastrointestinal tract toxicities were mild to

moderate and manageable. Most of the toxic effects were linked to the toxicity of the drug at the infusion site; in fact, 2 patients had grade 3 deep venous thrombosis, one patient had grade 3 chest pain and 1 patient had treatment-related grade 3 dyspnoea. Furthermore, problems at the port-a-cath or central venous catheter, requiring settlement or substitution, were the cause of nearly all treatment delays and consequent reduction of the drug dose intensity. No toxic deaths occurred, since the only patient who died during treatment had a clear and rapid disease progression, which was considered the reason for her death.

In conclusion, despite the good toxicity profile, these results do not support further trials with XR 5000 in unpretreated patients with metastatic colorectal cancer. However, it has to be pointed out that the occurence of chest pain as the DLT in phase I studies probably prevented adequate dose intensification. This is borne out by the low number of haematological toxicities, and it might be the main reason why XR 5000 failed, after having been so promising in the preclinical studies. Nonetheless, dual inhibition of topoisomerases represents a very appealing mechanism of action for an anticancer drug; the search for new compounds which are more potent and are suitable for a more convenient route of administration, such as the oral route, is strongly being pursued [14].

Acknowledgements

This work was supported by Xenova Limited, Slough, UK.

References

1. Cohen AM, Minsky BD, Schilsky RL. Cancer of the colon. In De Vita VT, Hellman S, Rosenberg SA, *et al*, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia, Lippincott-Raven, 1997, 1144–1197.
2. Cunningham D, Pyrhonen S, James RD, *et al*. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 1998, **352**, 1413–1418.
3. Rougier P, Van Cutsem E, Bajetta E, *et al*. Randomised trial of irinotecan versus fluorouracil by continous infusion after fluorouracil failure in patients with metastatic colorectal cancer. *Lancet* 1998, **352**, 1407–1412.
4. Douillard JY, Cunningham D, Roth AD, *et al*. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000, **355**, 1041–1046.
5. Saltz LB, Cox JV, Blanke C, *et al*. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *New Engl J Med* 2000, **343**, 905–914.
6. McCrystal M, Evans B, Baguley B, *et al*. A phase I study of DACA (N-[2-(dimethylamino)ethil]acridine-4-carboxamide) (SN22995, CRC88/05, XR 5000). Sponsored by the Cancer Research Campaign (CRC), UK. *Proc ASCO* 1999, **18**, 692 (180a).
7. Twelves CJ, Gardner C, Flavin A, *et al*. Phase I and pharmacokinetic study of DACA (XR 5000): a novel inhibitor of topoisomerase I and II. *Br J Cancer* 1999, **80**, 1786–1791.
8. De Bono JS, Propper D, Ellard S, *et al*. A phase I study of XR 5000 (DACA) by 120 hour intravenous infusion. *Ann Oncol* 1998, **9**(Suppl. 2), 449 (117a).
9. Baguley BC. DNA-intercalating anti-tumour agents. *Anticancer Drug Des* 1991, **6**, 1–35.
10. Arlin ZA. A special role for amsacrine in the treatment of acute leukemia. *Cancer Invest* 1989, **7**, 607–609.
11. Atwell GJ, Rewcastle JW, Baguley BC, Denny WA. Potential antitumor agents.50. In vivo solid tumor activity of derivatives of N-(2-(dimethylamino)ethyl)acridine-4-carboxamide. *J Med Chem* 1987, **30**, 664–669.
12. Baguley BC, Zhuang L, Marshall E. Experimental solid tumor activity of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *Cancer Chemother Pharmacol* 1995, **36**, 244–248.
13. Gupta RS, Gupta R, Eng B, *et al*. Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. *Cancer Res* 1988, **48**, 6404–6410.
14. Dangerfield W, Mistry P, Stewart AJ, *et al*. In vitro and in vivo characterization of XR11576, a novel dual inhibitor of topoisomerase I and II. *Proc AACR* 2001, **42**, 548 (101a).