Cancer Chemother Pharmacol (2000) 46: 167–171 

CLINICAL TRIAL REPORT

**Nicholas Pavlidis · Steinar Aamdal · Ahmad Awada
Hilary Calvert · Pierre Fumoleau · Roberto Sorio
Cees Punt · Jaap Verweij · Allan van Oosterom
Rudolf Morant · Jantien Wanders · Axel-R. Hanauske**

# Carzelesin phase II study in advanced breast, ovarian, colorectal, gastric, head and neck cancer, non-Hodgkin's lymphoma and malignant melanoma: a study of the EORTC early clinical studies group (ECSG)

Received: 8 December 1999 / Accepted: 10 April 2000

**Abstract** *Purpose*: In a phase II trial, the activity of carzelesin, a cyclopropylpyrroloindole prodrug analog, was assessed. *Patients and methods*: Carzelesin was used as second- or third-line chemotherapy in patients with breast, ovarian, head and neck cancer and non-Hodgkin's lymphoma, and as first-line chemotherapy in patients with colorectal and gastric cancer and melanoma. The drug was given as a bolus infusion at a 4-weekly dose of 150 μg/m$^2$. A total of 140 patients were entered and a total of 285 courses were administered. *Results*: In general, the compound was well tolerated. Myelotoxicity was the most common toxicity. Grade 3 and 4 leukopenia was observed in 18.6% of the courses, neutropenia in 20.3%, thrombocytopenia in 16.2% and anemia in 8.7%. Double nadirs were seen in a total of 41 courses for neutrophils, in 40 for leukocytes and in 3 for platelets. Non-hematological toxicity was very mild. Only one partial response in a patient with melanoma was seen. *Conclusions*: At this dose and schedule carzelesin did not yield activity in the types of tumors studied.

**Key words** Carzelesin · Lymphoma · Solid tumors · Toxicity

N. Pavlidis (✉)
Department of Medical Oncology, Medical School,
University of Ioannina, Ioannina 45110, Greece
e-mail: npavlid@cc.uoi.gr
Tel.: +30-651-99394; Fax: +30-651-99394

S. Aamdal
The Norwegian Radium Hospital, 0310 Oslo 3, Norway

A. Awada
Department of Chemotherapy, Institut Jules Bordet,
Brussels, Belgium

H. Calvert
Division of Oncology, Newcastle General Hospital,
Newcastle-upon-Tyne, UK

P. Fumoleau
Centre René Gauducheau, Nantes-St Herblain, France

R. Sorio
Centro di Riferimento Oncologico, Aviano, Italy

C. Punt
University Hospital 6525 GA Nijmegen,
The Netherlands

J. Verweij
The Rotterdam Cancer Institute,
3008 AE Rotterdam, The Netherlands

A. van Oosterom · A.-R. Hanauske
Universitair Ziekenhuis Leuven, Belgium

R. Morant
Oncologie/Med. Klinic C, Kantonsspital St Gallen,
Switzerland

J. Wanders
NDDO Oncology, Free University Hospital,
1007 MB Amsterdam, The Netherlands

## Introduction

Carzelesin (U-80244) is a cyclopropylpyrroloindole (CPI) prodrug analog and is a semisynthetic compound based on cc-1065, a natural product isolated from the soil organism *Streptomyces zelenis*. CPI drugs are a class of compounds with unique DNA-interactive properties. These agents enter into the minor groove region of DNA and mediate the covalent bonding to the $N^3$-position of adenine in A-T rich regions, thus forming DNA adducts in a sequence-selective fashion [5, 9].

Three new CPIs have so far been developed: adozelesin, bizelesin and carzelesin. Adozelesin, which possesses the active CPI moiety, is the analog most closely related to CC-1065. Bizelesin is a CPI dimer, and thus has the potential to form DNA interstrand crosslinks, a characteristic that distinguishes this compound from the classical CPI drugs [6, 7].

Carzelesin was designed to be an inactive prodrug that is activated through hydrolysis of the phenylurethane substituent to yield U-76073, followed by ring closure to form the active prodrug U-76074 [9, 12]. All three compounds have been found to be extremely potent in tumor cell lines and in transplanted tumors in

mice. Carzelesin shows activity against Lewis lung carcinoma, B16 melanoma, colon 38 carcinoma, colon CX1 adenocarcinoma, lung LX-1 tumour, and ovarian 2780 and prostatic DU-145 carcinoma [4, 8].

Carzelesin has been selected for clinical development in Europe under the framework of the EORTC. Clinical multicenter phase I studies with carzelesin have been reported. Patients receive the drug either as a single dose intravenously every 4 weeks or in a daily ×5 schedule repeated every 4 weeks [1, 13]. The Early Clinical Studies Group (ECSG) of the EORTC initiated phase II studies with carzelesin administered as a single bolus infusion every 4 weeks at a dose of 150 $\mu g/m^2$ in patients with breast, ovarian, colon, gastric and head and neck cancer, non-Hodgkin's lymphoma (NHL) and melanoma.

## Materials and methods

These studies opened in May 1995 and closed in February 1997.

### Eligibility

All patients entered into these studies had to have histologically or cytologically verified advanced measurable malignant disease beyond resectability. Other eligibility criteria included: WHO performance status ≤2; age 18–75 years; adequate bone marrow, hepatic and renal function; neutrophils ≥2000/μl; platelets >100,000/μl; creatinine ≤140 μmol/l (1.6 mg/dl) (if borderline, a creatinine clearance had to be performed and to be ≥60 ml/min); serum bilirubin ≤26 μmol/l (1.5 mg/dl); and alkaline phosphatase, SGPT and SGOT not more than twice the upper limit of normal, unless related to liver involvement.

Patients with breast cancer were allowed to have received one and only one prior chemotherapy regimen for advanced disease. A minimum of 4 weeks (6 weeks in the case of prior mitomycin C or nitrosourea) was required between the last dose and the study treatment. Prior (neo)adjuvant chemotherapy was allowed. A prior high-dose chemotherapy regimen with hematopoietic rescue was excluded. Prior hormonal therapy both adjuvant and/or for metastatic disease was allowed, provided that an interval of 1 week between the last hormonal treatment and study entry was observed. Patients with ovarian cancer and NHL had to have had at least one, but no more than two, prior chemotherapeutic regimen before entry into the study. The treatment-free interval had to be at least 4 weeks. Patients with head and neck cancer were not allowed to have received more than one chemotherapy regimen either given for neoadjuvant or for advanced disease with a minimum of 4 weeks between the last treatment and study entry. For patients with colorectal cancer no prior chemotherapy for advanced disease was allowed. Adjuvant chemotherapy (5-FU, leucovorin, levamisole) was allowed provided that the therapy-free interval was at least 12 months. For patients with gastric cancer, no prior chemotherapy was allowed. In patients with melanoma, no prior chemotherapy was allowed with the exception of prior adjuvant or local chemotherapy (extracorporeal circulation). If the measurable lesions were outside the treated limb, the treatment-free interval had to be at least 4 weeks. Otherwise the treatment-free interval had to be at least 6 months. Prior immunotherapy was allowed.

Institutional human research committee approval was obtained and appropriate informed consent was obtained from all patients.

### Formulation, dosage and treatment procedures

Carzelesin was provided by The Pharmacia-Upjohn Company as a 0.25 mg (250 μg/ml) nonaqueous "PET-vehicle" in a 3-ml ampoule. The drug which was previously diluted to 50 ml with either 5% glucose or 0.9% sodium chloride, was given at a dose of 150 $\mu g/m^2$ as a single 10-min i.v. infusion every 4 weeks. After two full cycles only patients with tumour response or disease stabilization were allowed to continue treatment. Ancillary treatments were given as medically indicated.

Concerning dose modification, retreatment on day 28 was possible when the following criteria were met: (a) neutrophils $\geq 1.5 \times 10^9$/l, (b) platelets $100 \times 10^9$/l, (c) the values for the above-mentioned tests on day 28 were not lower than those measured on day 21 in view of the possibility of a double nadir as seen in phase I studies and (d) nonhematological toxicity had recovered to grade 2 or less. If the above criteria were not met on day 28 the treatment had to be delayed for 1 to 3 weeks. If there was no recovery on day 49 the patient had to be taken off study. Dose modifications were based upon the nadir values for white blood cells (WBC), granulocytes and platelets during the previous cycle. Whenever grade 4 toxicity lasting >7 days occurred in any of these tests, the dose for the next cycle had to be reduced by 25%.

### Response and toxicity criteria

Follow-up studies included weekly complete blood counts and every 4 weeks full biochemistry, tumor markers and urine analysis. Tumors were evaluated every two full cycles. Standard WHO response criteria were used. Toxicities were graded according to the NCI Common Toxicity Criteria for cancer clinical trials. A double nadir is defined as a decline in WBC, neutrophil or platelet count after initial recovery to CTC grade 0 within the same course.

### Statistics

Since for these tumor types (based upon selection criteria and prior chemotherapy) further development would only be of interest when activity in ≥20% of cases was shown, the Gehan design was chosen for this study. The trial for each tumor type consisted of two stages:

1. In the first stage 14 patients per tumor type would be entered. If there was no response (complete or partial), the trial would be terminated. This would ensure that if the drug was active in 20% or more of the patients the chance of erroneously rejecting the drug after the first 14 patients was 0.044.
2. If there was one response in the first 14 patients then one additional patient would be required. If two responses were observed then 6 additional patients would be required, if three responses, 9 patients, and if four or more responses, 11 patients. This would allow the response rate to be determined with a standard error of $<0.10$.

## Results

The characteristics of the patients and their previous treatment are shown in Table 1.

### Breast cancer

Of 19 patients with advanced breast cancer entered into the study, all were eligible. A total of 52 courses were administered. There were 12 delays, 10 due to hematological toxicity and 2 for non-drug-related reasons as well as 10 dose reductions due to myelotoxicity. No objective responses were observed in 16 evaluable patients. Six patients had stable disease, and the other ten progressed.

**Table 1** Patient characteristics (*NHL* non-Hodgkin's lymphoma)

| | Breast cancer | Ovarian cancer | Colorectal cancer | Gastric cancer | Head and neck cancer | NHL | Melanoma |
|---|---|---|---|---|---|---|---|
| Number of patients | | | | | | | |
| Entered | 19 | 21 | 26 | 19 | 19 | 17 | 20 |
| Men/women | 0/19 | 0/21 | 14/12 | 14/5 | 19/0 | 8/9 | 14/6 |
| Eligible | 19 | 19 | 26 | 19 | 19 | 17 | 20 |
| Age (years) | | | | | | | |
| Median | 63 | 61 | 60.5 | 62 | 54 | 65 | 53 |
| Range | 35–74 | 45–75 | 41–74 | 41–74 | 43–78 | 32–76 | 32–83 |
| Performance status | | | | | | | |
| 0 | 13 | 8 | 12[a] | 3 | 4 | 4 | 12 |
| 1 | 4 | 11 | 10 | 10 | 14 | 11 | 8 |
| 2 | 2 | 2 | 3 | 6 | 1 | 2 | 0 |
| Previous therapy | | | | | | | |
| Surgery | 19 | 21 | 26 | 12 | 14 | 7 | 20 |
| Radiotherapy | 15 | 0 | 4 | 0 | 18 | 4 | 5 |
| Immunotherapy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hormone therapy | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chemotherapy | 19 | 21 | 3 | 0 | 9 | 17 | 0 |

[a] Data on one patient missing

## Ovarian cancer

Of 21 patients with advanced ovarian cancer who failed first- or second-line treatment entered into the study, 2 were not eligible since they no longer met the eligibility criteria at the time their first treatment was scheduled. A total of 44 courses were given. Eight courses were delayed, five for hematological reasons, one because of mucositis and two upon patient request. No dose reductions were performed. No responses were seen in 13 patients evaluable for tumor response. Six patients had stable disease and seven progressive disease as best response.

## Colon cancer

Of 26 patients entered, all were considered eligible. A total of 50 courses were administered. Eight courses were delayed, five due to myelotoxicity. Only one course was reduced. Two patients were not evaluable for response and no partial or complete responses were observed. Three patients had stable disease.

## Gastric cancer

Of 19 patients entered, all were eligible. A total of 37 courses were given, 5 of which were delayed, 3 for hematological and 2 for non-hematological toxicity. Only one patient had a dose reduction. Four patients were not evaluable for response. There were no objective responses, while 3 patients showed no change, and 12 progressive disease.

## Head and neck cancer

Of 19 patients entered, all were eligible. A total of 36 courses were administered and 6 were delayed. Dose reduction due to myelosuppression was reported in one course. Of 11 patients evaluable, 1 had stable disease for 24 weeks and 10 had progressive disease.

## Non-Hodgkin's lymphoma

Of 17 patients entered, all were eligible. A total of 34 courses were administered and 11 were delayed. Ten courses were delayed due to hematological toxicity and only one patient had a dose reduction in one cycle due to neutropenic fever and thrombocytopenia. Another patient was hospitalized with grade 4 anemia, thrombocytopenia and grade 4 fever without neutropenia and died 1 week later. Of 13 patients evaluable for response, 1 had a partial response after two courses but went off study after three courses due to progressive disease, 4 had stable disease as best response and 8 had progressive disease.

## Melanoma

Of 20 patients entered, all were eligible. A total of 32 courses were given. Five courses were delayed because of hematological toxicity. No dose reductions were performed. Of 19 patients evaluable for response, 1 had a partial remission and 1 had stable disease, while the other 17 all progressed.

## Toxicity

In these seven phase II studies 285 courses were administered to 139 eligible patients, and 135 patients were evaluable for toxicity. Hematological toxicity is shown in Table 2. Of the 285 courses, 39 (13.7%) were delayed and 14 (5%) had dose reduction due to myelotoxicity. Four events of neutropenic fever were seen.

Only a few patients received more than two courses. In those who received more (up to seven courses) all courses were able to be given at the planned dose of 150 μg/m$^2$, indicating that hematological toxicity was not cumulative, at least not to such a degree as to cause severe myelosuppression in later courses. It seems, however, that some patients were more sensitive since they developed severe myelotoxicity as early as in the first cycle, whereas others did not develop this at all.

Double nadirs occurred in several courses. In breast cancer a double nadir for WBC and for neutrophils was seen in 11 and 10 courses, respectively. In ovarian cancer seven, five and one double nadirs for WBC, neutrophils and platelets were seen, respectively. In colorectal cancer four double nadirs for WBC and four for neutrophils were seen. In gastric cancer two double nadirs for WBC and four for neutrophils were seen. In head and neck cancer double nadirs were seen in four courses for WBC and in another four for neutrophils. In NHL double nadirs for neutrophils, WBC and platelets were seen in ten, eight and one courses, respectively. Finally, in melanoma double nadirs for WBC, neutrophils and thrombocytes were seen in four, four and one courses, respectively.

Non-hematological toxicity was generally infrequent and mild. Grade 3–4 asthenia was seen in 14 courses (4.9%), nausea and vomiting in six (2.1%), dyspnea in 3 (1.0%), stomatitis in two (0.7%), and skin, neurological and liver toxicities in two (0.7%) each. No alopecia was noted.

## Discussion

The DNA sequence-specific minor groove-binding alkylating agents have recently attracted much attention. One of these is carzelesin which inhibits DNA synthesis by stabilizing the native B-form DNA helix through reversible binding to the $N^3$ position of adenine in the minor groove [12].

The preclinical spectrum of activity of the DNA minor groove alkylators and their lack of cross-resistance in cancer cell lines, suggest that they may be effective against tumors with low sensitivity to currently used alkylating agents. From preclinical studies, carzelesin seems to be highly effective both in vitro and in vivo [4, 8]; however, no responses were seen in the phase I studies conducted [1, 13]. In the present series of phase II studies in seven different tumors no antitumor activity was observed. Only one patient with malignant melanoma had a partial clinical response. It should be pointed out that the doses of carzelesin and of other cc-1065 analogs required to produce their high efficacy in preclinical models were relatively high. The tolerable dose for these drugs in patients is much lower than the effective dose in preclinical models. Failure to fulfill the promise of animal studies in this series is probably related to unusual species-specific marrow sensitivity in humans.

Clinical investigation of carzelesin has shown that myelotoxicity is the dose-limiting toxicity [11, 13]. Among the DNA minor groove alkylating agents studied, carzelesin has shown a pattern of myelotoxicity comparable with that of adozelesin, but a less favorable pattern of myelotoxicity than that of tallimustine [3, 10]. The initial bone marrow toxicity caused by carzelesin shows a temporary improvement as a result of recruitment of committed myeloid progenitors and pluripotent stem cells around day 14. This event is followed by a "second" nadir, perhaps due to homing of the formal peripheral progenitors [2]. This phenomenon is consistent with the double nadir of neutrophils at approxi-

**Table 2** Hematological toxicity per cycle (*NHL* non-Hodgkin's lymphoma)

| | Breast cancer | Ovarian cancer | Colorectal cancer | Gastric cancer | Head and neck cancer | NHL | Melanoma | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number | % |
| No. of courses | 52 | 44 | 50 | 37 | 36 | 34 | 32 | 285 | 100.0 |
| Median no. of courses | 2 | 2 | 2 | 2 | 2 | 2 | 1.5 | | |
| Course delays[a] | 10 | 5 | 5 | 3 | 2 | 0 | 5 | 40 | 14.0 |
| Dose reductions[a] | 10 | 0 | 1 | 1 | 1 | 1 | 0 | 14 | 5.0 |
| Leukopenia | | | | | | | | | |
| Grade 3 | 10 | 8 | 4 | 7 | 6 | 6 | 1 | 42 | 14.7 |
| Grade 4 | 0 | 0 | 0 | 3 | 2 | 6 | 0 | 11 | 3.9 |
| Neutropenia | | | | | | | | | |
| Grade 3 | 7 | 3 | 8 | 7 | 4 | 7 | 2 | 38 | 13.3 |
| Grade 4 | 4 | 0 | 3 | 3 | 4 | 5 | 1 | 20 | 7.0 |
| Thrombocytopenia | | | | | | | | | |
| Grade 3 | 3 | 4 | 6 | 1 | 1 | 4 | 2 | 21 | 7.4 |
| Grade 4 | 4 | 1 | 3 | 3 | 4 | 9 | 1 | 25 | 8.8 |
| Anemia | | | | | | | | | |
| Grade 3 | 4 | 0 | 2 | 2 | 4 | 9 | 1 | 22 | 7.7 |
| Grade 4 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 3 | 1.0 |

[a] Due to hematological toxicity

mately 4 weeks occasionally observed in phase I studies [1]. In the present study double nadirs were observed in a total of 84 courses (41 for neutrophils, 40 for WBC and 3 for platelets).

Double nadirs can reach grade 2/3, but are usually not worse. Double nadirs for WBC and neutrophils would indeed be expected to coincide. However, the definition of double nadir requires in-between recovery to CTC grade 0. Therefore, WBC and neutrophil toxicity which recovers to grade 1 is not noted as a double nadir but still has the character of two valleys. Patient 6 from the colorectal study, for example, had a grade 2/grade 1 double nadir for WBC but no neutrophil double nadir because no neutrophil counts were available for this period. Patient 16 had a WBC grade 1/grade 2 double nadir and neutrophils grade 2/grade 2, in the same course. Patient 18 had two double nadirs for neutrophils in course two (grade 3/grade 4) and course six (grade 2/grade 3), but no double nadir for WBC. The reason for this was that the nadir for WBC was so low that no recovery to grade 0 could be reached. However, a decrease in the WBC nadir from grade 2 (on day 8) via grade 1 to again grade 2 (on day 36) was observed. In other patients recovery from grade 3 to grade 2 or 1, followed by a second decline to grade 3 was observed. If a double nadir were defined as any grade 3/4 hematological toxicity with an improvement of 1 or 2 grades within the same course rather than as recovery to grade 0, many more double nadirs would certainly be observed, providing enough blood evaluations were carried out and the follow-up was long enough after each course.

Double nadirs are observed more often in patients with initial deep nadirs since these patients often have their next course delayed. As a result, the second nadir is observed after the date when the next course should have started. The second nadir was noted mostly on day 29 and later.

In the phase I study by Awada et al., both neutropenia and to a lesser degree thrombocytopenia were dose limiting and a high interpatient variability was shown at all dose levels from 130 $\mu g/m^2$ upwards resulting in dose delay and/or reduction in several courses [1]. In our study hematological toxicity was also the most frequent toxicity with grade 3 and 4 neutropenia and thrombocytopenia in almost 20% and 16% of the courses, respectively. Only four events of neutropenic fever were noted.

In conclusion, this study demonstrated that carzelesin at this dose and in this schedule, despite its promising preclinical activity, had no activity in various solid tumors or in NHL, while myelotoxicity was found to be the most profound adverse event.

## References


1. Awada A, Punt CJA, Piccart MJ, Van Tellingen O, Van Manen L, Groot Y, Wanders J, Verweij J, Wagenet DJTh (1999) Phase I study of carzelesin (U-80,244) given (4-weekly) by intravenous bolus schedule. Br J Cancer 79: 1454
2. Filippini C, Bisiach M, Tagliabue G, D'Incalci M, Ubezio P (1997) Hematopoietic toxicity and cell cycle perturbations induced by new DNA minor groove-alkylating agents. Int J Cancer 72: 801
3. Fleming GF, Ratain MJ, O'Brien SM, Schilsky RL, Hoffman PC, Richards JM, Vogelzang NJ, Kasunic DA, Earhart RH (1994) Phase I study of adozelesin administered by 24-hour continuous intravenous infusion. J Natl Cancer Inst 86: 368
4. Houghton PJ, Cheshire PJ, Hallman JD, Houghton JA (1995) Therapeutic efficacy of the cyclopropylpyrroloindole, carzelesin, against xenografts derived from adult and childhood solid tumours. Cancer Chemother Pharmacol 36: 45
5. Hurley LH, Reynolds VL, Swenson DH, Petzold GL, Scahill TA (1984) Reaction of the antitumor antibiotic CC-1065 with DNA: structure of a DNA adduct with DNA sequence specificity. Science 226: 843
6. Lee CS, Gibson NW (1991) DNA damage and differential cytotoxicity produced in human carcinoma cells by CC-1065 analogues U-73,975 and U-77,779. Cancer Res 51: 6586
7. Li LH, Kelly RC, Warpehoski MA, McGovren JP, Gebhard I, DeKoning TF (1991) Adozelesin, a selected lead among cyclopropylpyrroloindole analogs of the DNA-binding antibiotic, CC-1065. Invest New Drugs 9: 137
8. Li LH, DeKoning TF, Kelly RC, Kruger WC, McGovren JP, Padbury GE, Petzold GL, Wallace TL, Ouding RJ, Prairie MD, Gebhard I (1992) Cytotoxicity and antitumor activity of Carzelesin, a prodrug cyclopropylpyrroloindole analogue. Cancer Res 52: 4904
9. Reynolds VL, Molineux IJ, Kaplan DJ, Swenson DH, Hurley LH (1985) Reaction of the antitumor antibiotic CC-1065 with DNA. Location of the site of the thermally induced strand breakage and analysis of DNA sequence specificity. Biochemistry 24: 6228
10. Shamdas GJ, Alberts DS, Modiano M, Wiggins C, Power J, Kasunic DA, Elfring GL, Earhart RH (1994) Phase I study of adozelesin (U-73,975) in patients with solid tumours. Anticancer Drugs 5: 10
11. Van Tellingen O, Punt CJA, Awada A, Wagener DJT, Piccart MJ, Groot Y, Schaaf LJ, Henrar REC, Nooijen WJ, Beijnen JH (1998) A clinical pharmacokinetics study of carzelesin given by short-term intravenous infusion in a phase I study. Cancer Chemother Pharmacol 41: 377
12. Warpehoski MA, Hurley LH (1988) Sequence selectivity of DNA covalent modification. Chem Res Toxicol 1: 315
13. Wolff I, Bench K, Beijnen JÇ, Bruntsch U, Cavalli F, De Jong J, Groot Y, Tellingen O van, Wanders J, Sessa C (1996) Phase I clinical and pharmacokinetic study of carzelesin (U-80244) given on a daily ×5 schedule. Clin Cancer Res 2: 1717