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CLINICAL TRIAL REPORT

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Phase I trial with weekly EO9, a novel bioreductive alkylating indoloquinone, by the EORTC Early Clinical Study Group (ECSG)

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Abstract *Purpose*: EO9 is a new synthetic bioreductive alkylating indoloquinone, with preferential activity against solid tumors and higher antitumor activity under anaereobic conditions compared with aerobic conditions. In preclinical models EO9 demonstrated no major organ toxicity. The aim of the present phase I study was to determine the toxicities and the maximal tolerated dose (MTD) of EO9 administered as a 5-min i.v. infusion weekly to patients with solid cancers. *Methods*: Twenty-eight patients entered the study. The dose was escalated from 2.7 mg/m^2 according to a Fibonacci-like schedule. *Results and conclusion*: The dose-limiting toxicity was proteinuria. No other major toxicities were detected and in particular there was no significant increase in serum creatinine. This was in contrast to findings in a previous phase I trial using EO9 in a 3-weekly schedule, where a number of patients experienced severely decreased kidney function. The MTD in the present study was 15.0 mg/m^2 weekly and the recommended dose for phase II studies was 12.0 mg/m^2 weekly. Compared with 3-weekly EO9, the dose intensity could be increased from 22 mg/m^2 to 36 mg/m^2 with the weekly administration. Phase II studies have been performed by the EORTC Early Clinical Study Group in advanced breast, gastric, colorectal, pancreatic, and non-small-cell lung cancer.

Key words Phase I · EO9 weekly

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Introduction

The indoloquinone EO9 [3-hydroxy-5-aziridinyl-1-methyl-2-(1*H*-indole-4,7-dione)-prop-βb-en-αa-ol] was developed under the auspices of the EORTC [6, 10]. EO9 is a new fully synthetic bioreductive alkylating agent, structurally related to mitomycin C [6] but with a different antitumor profile [10]. The preclinical evaluation in the National Cancer Institute disease-oriented cell line panel EO9 demonstrated preferential cytotoxicity in cell lines derived from solid tumors and lacked activity in lympho-leukemic cell lines [6]. The panel included cell lines from colon cancer, central nervous system, melanoma, renal cancer and non-small cell lung cancer.

EO9 undergoes bioreductive activation by the two-electron-reducing flavoenzyme DT-diaphorase [14, 16]. The reduction of EO9 by DT-diaphorase generates monofunctional and bifunctional alkylating agents capable of forming adducts with DNA [21]. Experiments in vitro and in vivo have demonstrated that DT-diaphorase is often over-expressed in tumors compared with normal tissues [1, 15, 16] and that sensitivity to EO9 cytotoxicity at least under aerobic conditions is correlated to the DT-diaphorase activity in the tumor cells [2, 5, 18, 20, 21]. Under anaerobic conditions, however, a negative correlation with DT-diaphorase activity has been reported. [12a, 12b, 16]. Model experiments have also demonstrated that EO9, unlike most other cytotoxic drugs, is much more active under anaerobic conditions than under aerobic conditions [12a, 12b], however only at reduced pH [8]. This may indicate that enzymes other than DT-diaphorase must be involved in the bioactivation of EO9 under anaerobic conditions [3, 12b].

The LD_{10} (lethal dose to 10% of the animals) of a single administration of EO9 to mice was 9 mg/kg (equivalent to 27.0 mg/m^2). In mice EO9 induced gastrointestinal obstruction but no notable bone marrow or renal toxicity [6]. A dose of one tenth of the mouse equivalent was recommended as a starting dose for phase I studies in humans. This dose was also found to

be safe in rats and dogs. Because of its interesting antitumor activity against solid tumors, its unusual mechanism of bioactivation, and lack of bone marrow toxicity, EO9 was chosen for further clinical studies.

EO9 had initially been tested in a 3-weekly schedule [17, 19]. The dose-limiting toxicity in that particular study was proteinuria. Since EO9 had shown no apparent schedule dependency in preclinical models [6] and had a short half-life [9, 17], it was decided to test a weekly EO9 schedule to see if the treatment could be intensified. The purpose of the present study was to determine the maximum tolerated dose (MTD) and the toxicity of EO9 when administered as a bolus injection every week.

Patients and methods

Eligibility criteria included informed consent and microscopically confirmed diagnosis of a solid tumor not amenable to any established forms of treatment. Patients with known brain or leptomeningeal involvement, or other serious illnesses were excluded. Other eligibility criteria were age between 18 and 70 years, life expectancy of >3 months, and performance status ECOG <2. Prior chemotherapy was allowed provided that there was a 4-week treatment-free interval prior to entry in the study (6 weeks for nitrosourea, mitomycin C, and extensive radiotherapy).

Prior to the start of treatment, each patient had a complete medical history and physical examination, complete blood count and platelet count, and determination of serum chemistries, including albumin, total protein, electrolytes, uric acid, urea, creatinine, and liver function tests.

All patients had to have adequate renal and liver function, i.e., serum creatinine <120 μmol/l, serum bilirubin <25 μmmol/l, and SGOT and SGPT within 2× the normal upper limit (unless related to liver metastases), white blood cells $>4.0 \times 10^9$/l, and platelet count $>100 \times 10^9$/l. Complete blood and platelet counts, serum chemistries, and urinanalysis were repeated weekly. Standard common toxicity criteria (CTC) were used for assessment of toxicity. The tumor parameters were assessed by CT scan and/or ultrasound every 6 weeks. Response was defined according to standard World Health Organization (WHO) criteria.

Drug administration

EO9 was administered as an i.v. bolus injection over 5 min every week, or after full recovery from the previous dose. A full treatment cycle was defined as three EO9 injections. The drug was supplied by the EORTC New Drug Development Office as a freeze-dried pink-red colored cake consisting of 5 mg EO9 and 50 mg lactose and NaOH to adjust pH to about 9. Each vial was reconstituted with 10 ml saline yielding a final concentration of 2.5 mg/ml.

Dose schedule and dose escalation

Doses were escalated in decreasing rates (Fibonacci-like schedule) and depending on the clinical judgement of the investigators. If no or minimal (grade I) toxicity was observed, doses were escalated with 100% to 50% steps. If significant grade II toxicity was observed, doses were escalated with 33% to 20% steps. If late toxicity was observed, the time interval to re-treatment was appropriately lengthened in the subsequent cycles.

Maximum tolerated dose

The MTD was the highest dose which could be safely administered to a patient producing tolerable, manageable, and reversible toxicity of CTC grade III or grade IV, in the case of hematological toxicity or nausea/vomiting, or CTC grade II for organ toxicities in at least two to six patients. The recommended dose for phase II studies would be one dose level below the MTD.

The recommended starting dose ($MELD_{10}$), for the study was 2.7 mg/m^2. Subsequent dose levels were 5.4, 7.0, 10.0, 12.0 mg/m^2, and 15 mg/m^2. A minimum of three patients with a total of four evaluable courses were to be entered in non-toxic dose levels. It was decided that at least 1 week or 2 weeks at higher dose levels should pass between the entry of the first and the next two patients at a particular dose level.

Results

Characteristics of the patients

The demographic characteristics of the patients are shown in Table 1. A total of 28 patients (14 females and 14 males), ranging from 19 to 64 years of age entered the study. Twenty-four patients had received prior chemotherapy with or without radiation therapy.

The EO9 dose was escalated (Table 2) from dose level 2.7 mg/m^2 to dose level 15.0 g/m^2. When the MTD had been reached, three additional patients were entered at the dose level below (12.0 mg/m^2), which is the recommended treatment dose for later phase II trials. One of the patients, at dose level 10.0 mg/m^2, had a dose reduction to 8.5 mg/m^2 during the second and third course due to proteinuria. At dose level 12.0 mg/m^2 one patient was not evaluable due to progressive brain metastases 6 days after the first drug administration. Another patient received by miscalculation 12.5 mg/m^2 instead of the planned 12.0 mg/m^2, which resulted in a 4% overdosage. In the toxicity evaluations, this patient is included in the 12.0-mg/m^2 dose level. A total number of

Table 1 Characteristics of the patients. *WHO* World Health Organization, *NSCLC* non-small-cell lung cancer

Total number of patients		28
Number of eligible patients		24
Sex (M/F)		14/14
Age (mean, range)		50; 19–64
Performance status	WHO	Number of patients
	0	11
	1	16
	2	1
Prior treatment		
Surgery/chemotherapy/ radiotherapy		7
Surgery/chemotherapy		16
Surgery only		3
Chemotherapy only		1
Radiotherapy only		1
Tumor types		
Colorectal		10
Melanoma		8
Lung (NSCLC)		3
Sarcoma		3
Hepatocellular carcinoma		1
Gastric carcinoma		1
Uterus carcinoma		1
Unknown		1

Table 2 Number of patients at each dose level

Dose level (mg/m^2)	Number of patients	Evaluable patients
2.7	3	3
5.4	3	3
7.0	4	4
8.5	1	1
10.0	6	5
12.0	7	6
15.0	5	5

77 courses were administered in the study. Several patients received only one or two injections in their last treatment cycle due to toxicity or tumor progression.

Renal toxicity

The dose-limiting toxicity of EO9 in this study was proteinuria (Table 3). At the 12.0-mg/m^2 dose level three of six patients developed grade I proteinuria, while at the 15.0-mg/m^2 level all five patients developed proteinuria, two patients grade I and three patients grade II. In one patient albumin in the urine increased from 8 μmol/ml after the first EO9 administration to 15 μmol/ml after the second. Another patient with pre-existing grade I proteinuria developed grade II proteinuria after the first EO9 administration. In five of the patients the EO9 therapy was either stopped or delayed due to the proteinuria. Mild hematuria (grade I) was observed in a total of seven patients, one at the 5.4-mg/m^2 dose level, two at the 7.0-mg/m^2 dose level, one at the 10-mg/m^2 dose level, one at the 12.0-mg/m^2 dose level, and three at the 15.0-mg/m^2 dose level. In one patient (15.0 mg/m^2) the pre-existing hematuria deteriorated from grade I to grade II grade during the EO9 treatment. Only two patients, at the 5.4-mg/m^2 and 12-mg/m^2 dose levels, developed a grade I increase of serum creatinine.

Bone marrow toxicity/Other toxicities

Nine of the patients developed grade I anemia and one patient grade II. Only one patient developed grade I thrombocytopenia. None of the patients developed leukopenia. Two patients had grade I fever and seven mild headache. Nausea grade I was seen in eight patients and vomiting grade I in four, occurring on the treatment day or on the following day. No other side-effects were observed.

Antitumor activity

Two patients were not evaluable for response due to early progression. One progressed after the first EO9 administration and the other after the second. Of the remaining 26 patients, four had stable disease and 22 disease progression.

Discussion

The present phase I study shows that the dose-limiting toxicity with weekly EO9 is proteinuria. No other major toxicities were observed. The moderate nausea, vomiting, and low-grade fever were easily managed with standard medication. In a previous phase I study with EO9 using an every 3 week schedule the dose-limiting toxicity was also proteinuria [19]. Two of the patients in that particular study also developed significant increases in serum creatinine and one of the patients had to be treated with hemodialysis. Renal biopsies taken demonstrated minimal change nephropathy and no other changes [19]. In the present study with weekly EO9 administration none of the patients developed a significant increase in serum creatinine. The renal toxicity observed in the two phase I studies was unexpected since in the preclinical toxicity evaluation EO9 had only induced vascular congestion in the gastrointestinal tract and no major organ toxicities [6]. This illustrates that extrapolation of preclinical toxicity data to the clinic may not always be valid and should be done with caution.

The pharmacokinetics of EO9 [9] showed rapid elimination with a median half-life of 10.1 min and wide inter-patient variability. An eightfold range in systemic EO9 clearance was observed. In the previous 3-weekly EO9 study a linear correlation between the degree of proteinuria and the area under the plasma curve was detected, suggesting that patients with inherent low

Table 3 EO9 renal toxicity. *CTC* common toxicity criteria

Dose level (mg/m^2)	Number of evaluable courses	Proteinuria/albuminuria					Creatinine↑					Hematuria				
Toxicity grade	(CTC)	1	2	3	4	9[a]	1	2	3	4	9[a]	1	2	3	4	9[a]
2.7	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.4	6	–	1	–	–	–	1	–	–	–	–	2	–	–	–	–
7.0	13	2	–	–	–	–	–	–	–	–	–	4	–	–	–	–
8.5	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10.0	22	2	1	–	–	–	–	–	–	–	–	2	–	–	–	–
12.0	16	4	–	–	–	1	1	–	–	–	–	1	–	–	–	–
15.0		10	3	6	–	–	–	–	–	–	–	–	2	–	–	–

[a] 9 = Pre-existing abnormality, aggravating at least one CTC

clearance will be at risk of toxicity. In the present study no such correlation could be detected.

When EO9 was administered weekly instead of 3 weekly the dose-intensity could be increased from 22.0 mg/m^2 to 36.0 mg/m^2 (12.0 mg/m^2 × 3), respectively, in each 3-weekly cycle. Weekly administration was therefore selected for the subsequent phase II trials and the recommended dose for these studies was 12.0 mg/m^2. In a recent randomized phase II study with weekly versus 3-weekly scheduled EO9 the major toxicity was proteinuria [4]. The frequency of proteinuria was, however, different in the weekly and 3-weekly schedule (34.5% and 62.5%, respectively), indicating that the weekly schedule was less nephrotoxic than the 3-weekly one.

In the previous phase I trial with 3-weekly EO9 [19] two of 32 patients with heavily pretreated adenocarcinoma of unknown primary achieved partial responses in liver and lung metastases. In the current phase I study, however, with higher EO9 dose intensity, none of the 24 patients demonstrated clinical responses. In fact in five different recently published EO9 weekly phase II trials involving a total of 113 patients suffering from non-small-cell lung cancer, gastric, colorectal, pancreatic or breast cancer no clinical responses were observed [11].

It can be speculated that the disappointing clinical results may have been related to low levels of the EO9 activating enzyme DT-diaphorase in the tumors. From preclinical studies it is also known that EO9 may be more active under anaerobic conditions than under aerobic conditions. Therefore measuring the reductase expression in tumors, and at the same time the pO2, may be important for the further development of EO9 in cancer therapy. It is also likely that such bioreductive agents mainly will be used in conjunction with radiotherapy or other chemotherapeutic agents [13].

In summary the dose-limiting toxicity of weekly EO9 is proteinuria. The 12.0-mg/m^2 dose produces tolerable and predictable toxicity and is the recommended dose for single drug treatment in clinical trials.

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