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A clinical phase I trial of gemcitabine and treosulfan in uveal melanoma and other solid tumours

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Abstract

This trial was performed to define the maximum tolerated dose (MTD) of treosulfan administered in combination with a fixed dose of gemcitabine in uveal melanoma patients. Preclinical studies suggested synergistic activity against uveal melanoma. Gemcitabine (1 g/m^2) and treosulfan (2.5–4 g/m^2) were administered on days 1 and 8, and cycles were repeated on day 29 for a maximum of six cycles. For treosulfan, dose escalation cohorts of 2–4 patients were enrolled. An additional 25 patients were entered at treosulfan dose levels II (3 g/m^2) and III (3.5 g/m^2). Thirty three patients with uveal melanoma and six patients with other histologies were accrued. Side-effects were predominantly haematological. The MTD was 3.5 g/m^2 of treosulfan together with 1 g/m^2 of gemcitabine. In the uveal melanoma patients, one partial response (PR) and 15 stablisations of disease (SD) were recorded and whether this translates into a survival gain should be explored further.

Keywords: Uveal melanoma; Gemcitabine and treosulfan

1. Introduction

Uveal melanoma is the most common primary intraocular malignant tumour. Despite the ability to make an accurate diagnosis and availability of various primary treatments, mortality from this disease has remained unchanged [1–3]. The main problem is the development of haematogenous metastases (mainly to the liver) which often are highly resistent to chemotherapy. A variety of systemic chemotherapeutic drugs have been tested, among them dacarbazine, temozolomide, 9-nitrocamptothecin, and combination regimens (BOLD: bleomycin, vincristine, lomustine, dacarbazine). Response rates were rather low and results sometimes conflicting [4–7]. Median survival time ranged between 5 and 7 months [8]. Longer durations of survival were observed in phase II trials with the novel nitrosourea fotemustine (with or without immunotherapy) and in locoregional approaches with either fotemustine or platin-containing therapies [8–13].

Recently, several highly chemoresistant tumour cells, including human uveal melanoma cells, have been shown to have considerable sensitivity to the alkylating agent treosulfan. This agent is licensed for the treatment of ovarian cancer in Europe [14]. The capability of treosulfan to alkylate or crosslink DNA is preserved in multidrug-resistant cell lines [15]. Using an adenosine triphosphate (ATP)-based chemosensitivity assays (ATP-TCA), a considerable synergistic *in vitro* interaction between treosulfan and the nucleoside analogues, cytarabine and gemcitabine, was demonstrated in uveal melanoma cells [16]. The mechanism of synergy may be explained by the inhibition of DNA repair mechanisms by both nucleoside analogues [17].

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Based on these *in vitro* findings, we initiated a clinical phase I trial to evaluate the combination therapy gemcitabine/treosulfan (termed GeT) for the treatment of solid tumours.

2. Patients and methods

Patients meeting the following criteria were selected for inclusion: patients with uveal melanoma were eligible when they had received no prior chemotherapy or only fotemustine or the BOLD combination. There is no standard chemotherapy regimen for renal cell cancer, therefore, first-line treatment within this protocol was allowed for these patients. Patients with other histologies were only eligible if they had failed at least one standard chemotherapy regimens. Further inclusion criteria were a Karnofsky performance status of at least 60% and adequate hepatic, renal, cardiac and bone marrow function.

Gemcitabine (Gemzar^R, Lilly, Bad Homburg, Germany) was administered at 1 g/m^2 in 250 ml of 0.9% NaCl on days 1 and 8 as a 30 min intravenous (i.v.) infusion. This was immediately followed by the i.v. administration of treosulfan (Ovastat^R, Medac, Hamburg, Germany) on days 1 and 8 at the allocated dosages. For dose finding studies, cohorts of 2–4 patients were treated with four different dose regimens, with the treosulfan dose ranging from 2.5 to 4 g/m^2 (Table 1). Subsequent patient cohorts received either 3 or 3.5 g/m^2 of treosulfan per dose. The treatment was repeated on day 29 in the cases who had adequately recovered from toxicity and in the absence of tumour progression upto a maximum of six cycles. In cases of persisting $\geqslant$ grade 3 leucopenia or $\geqslant$ grade 2 thrombopenia, the next treatment cycle was postponed for 1 week, and/or the dose of treosulfan was reduced by one level. All patients received supportive treatment with 5-HT3 antagonists (in most cases 5 mg tropisetron).

The study protocol was approved by the institutional ethics committee. Patients were enrolled after informed consent was obtained. Tumour response, as well as the toxicity evaluations, were performed according to the Common Toxicity Criteria v2.0 (CTC, http://ctep.cancer.gov/reporting/ctc.html). Reevaluation of the patient's status was scheduled after two, four and six cycles, and three monthly thereafter.

Table 1
Treatment regimen and dose escalation

Dose level	Treosulfan (Ovastat^R)	Gemcitabine (Gemzar^R)
I	2.5 g/m^2 days 1 and 8	1 g/m^2 days 1 and 8
II	3.0 g/m^2 days 1 and 8	1 g/m^2 days 1 and 8
III	3.5 g/m^2 days 1 and 8	1 g/m^2 days 1 and 8
IV	4.0 g/m^2 days 1 and 8	1 g/m^2 days 1 and 8

3. Results

3.1. Patient characteristics

Thirty nine patients with advanced malignancies were enrolled in this study. Patient characteristics are given in Table 2.

3.2. Toxicity analysis

Chemotherapy was generally well-tolerated. Acute toxicity consisted of mild nausea in nine patients, all without vomiting. The predominant delayed toxicity was myelotoxicity (Table 3). Grade 3 or 4 thrombopenia was observed in three and one patient, respectively. The patient with grade 3 thrombopenia on dose level I had a uveal melanoma and had previously received fotemustine, with the last dose being given 6 weeks prior to inclusion in this study. One patient with grade 3 anaemia on dose level II already had grade 3 anaemia prior to therapy. On dose levels III and IV, one and two patients, respectively, developed thrombopenia requiring a dose reduction in two cases. Grade 3 leucopenia was observed in one patient on dose level III. Further cases, where dose reductions were necessary, were due to persisting toxicities of grades 1–2. Non-haematological side-effects exceeding grade 2 toxicity were alopecia and neutropenic fever of grade 3 in two patients. Mild side-effects (toxicity grade I or II) included fatigue, transient fever, pain and headache.

The dose escalation part of this study demonstrated grade 3 and 4 thrombopenia to be the dose-limiting toxicity. The maximum tolerated dose (MTD) of treosulfan was between 3 and 3.5 g/m^2. To gain more experience with this regimen in uveal melanoma patients, we included additional patients with uveal melanoma ($n = 25$) at these two dose levels, 3 and 3.5 g/m^2 treosulfan. Two patients were excluded from analysis due to incomplete observations. Grade 3 or 4 haematotoxicity occured in five of 15 patients (dose level II) and five of 17 patients (dose level III). Considering all patients, a total of 131 treatment cycles were administered, with a median of three cycles per patient (range 1–6). In Figs. 1–3, the kinetics of the haematological toxicities are represented. Leucopenia had a nadir around day 14. Grade 3 leucopenia was observed in two of 15 and four of 17 patients for dose levels II and III, respectively. Treatment associated infections were not observed. The frequency of grade 3/4 thrombopenia was similar for dose levels II and III, with the nadir occurring around day 14. There was a trend of cumulative toxicity for thrombopenia and leucopenia for patients on dose level III.

Table 2
Patient characteristics

	Uveal melanoma			Non-melanoma
	All	Dose level		
		I/II	III/IV	
Number of patients	33	14	19	6
Age (in years)				
Median (range)	62 (33–78)	57 (36–74)	67 (33–78)	59 (40–68)
Gender				
Female/male	18/15	7/7	11/8	2/4
Histology				
Uveal melanoma	33			
Renal cell cancer				2
Non-small cell lung cancer				2
Ovarian cancer				1
Colorectal cancer				1
Prior cytotoxic therapy				
No systemic pretreatment	28	12	16	1
1 Chemotherapy regimen	5	2	3	2
2 Chemotherapy regimens				3
Number of metastatic sites				
1 Organ site	15	8	7	2
2 Organ sites	10	2	8	2
>2 Organ sites	8	4	4	2
Pretreatment serum LDH				
Below 2× UNL	22	8	14	n.a.
Above 2× UNL	11	6	5	n.a.

UNL, upper normal limit; n.a., not applicable; LDH, lactate dehydrogenase.

Table 3
Common Toxicity Criteria: grade 3 and 4 toxicities

	Cohort 1				Cohort 2	
Dose level	I	II	III	IV	II	III
Patients per dose level[a]	2	3	4	4	12	13
Patients with Haematotoxicity Grade 3/4	1	1	1	2	4	4
Anaemia Grade 3/4		1/0				
Thrombopenia Grade 3/4	1/0		0/1	2/0	4/0	2/0
Leucopenia Grade 3/4			1/0		1/1	3/0
Neutropenic fever Grade 3/4			1/0			
Toxicity caused						
Dose reduction	1		3	1	1	1
Cycle delay			1			1
Treatment discontinuation			1	1		

[a] One patient (dose level III) was excluded from analysis.

3.3. Clinical response in patients with uveal melanoma patients and other tumour histologies

For cases treated at dose levels I/II, no objective responses were observed, whereas two patients (uveal melanoma, renal cancer) on dose level III and one patient on dose level IV (ovarian cancer) had a partial response. Furthermore, we observed a stablisation of disease for more than three months in 15 patients with uveal melanoma. Despite the small patient numbers, a significant trend for improved overall survival with higher treosulfan doses was recorded (data not shown).

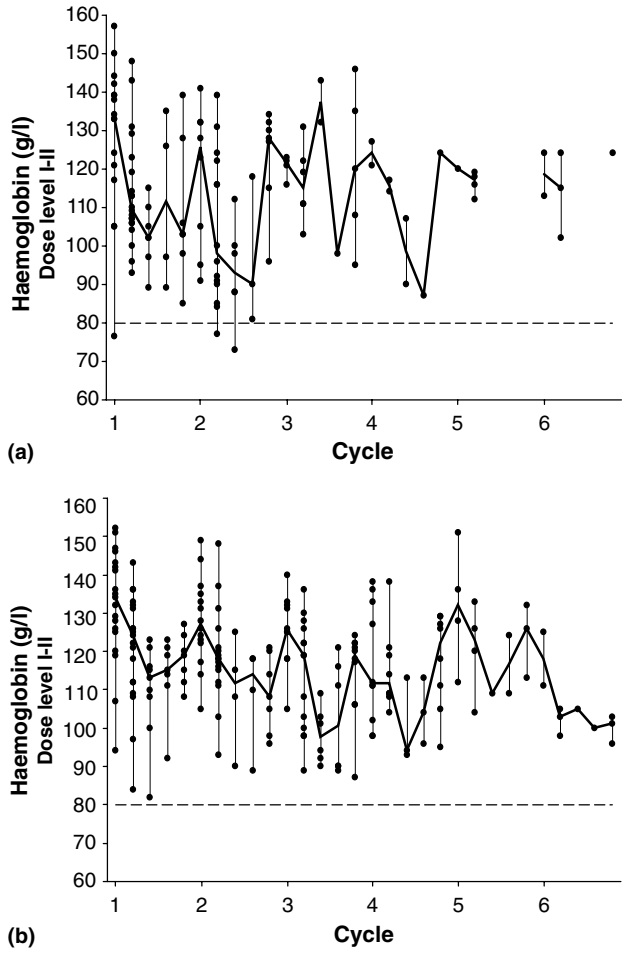

Fig. 1. Haemoglobin concentrations from peripheral blood of patients treated at dose levels I–II (a) and III–IV (b). Solid line, median values; dotted line, threshold to grade 3 toxicity; points, single values.

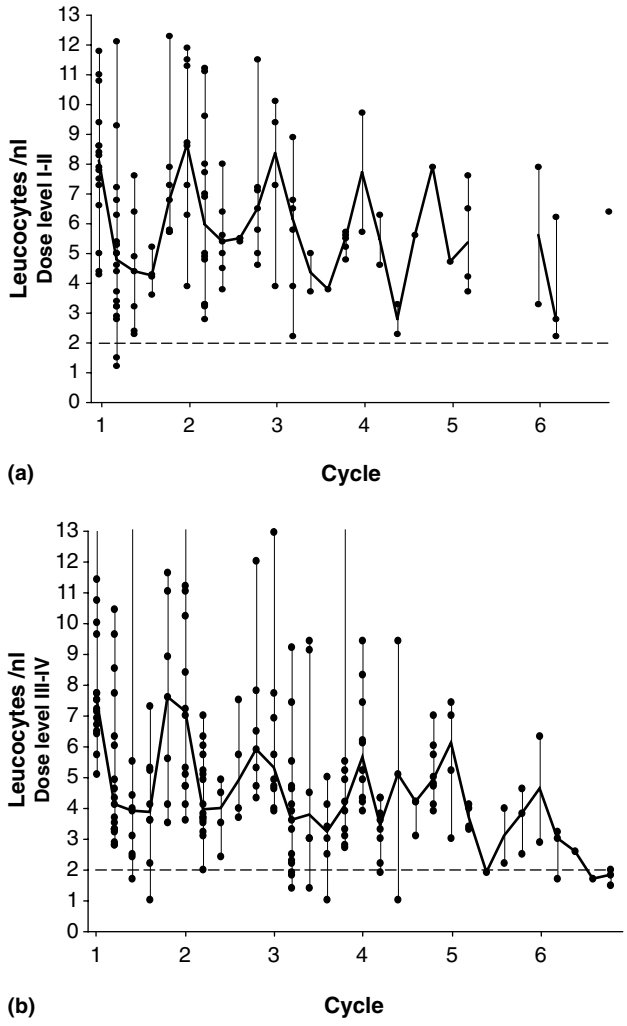

Fig. 2. Leucocyte count from peripheral blood of patients treated at dose levels I–II (a) and III–IV (b). Solid line, median values; dotted line, threshold to grade 3 toxicity; points, single values.

4. Discussion

This phase I evaluation was performed to assess the dose-limiting toxicity of treosulfan administered in combination with a fixed dose of gemcitabine. As expected from the toxicity profile of the single agents, the dose-limiting toxicity was myelotoxicity. Haematotoxicity in excess of grade 2 was observed in 50% of patients at dose level IV, in 29% at dose level III and 33% at dose level II, and most patients had thrombopenia and/or leucopenia. Grade 3 anaemia (two cases) was a rare event and in one of these cases was observed in a patient with preexisting anaemia. Two uveal melanoma patients (on dose levels I and III) had been pretreated with fotemustine. Despite of an interval of 6 weeks between the last administration of fotemustine and entry into this study, it is likely that the fotemustine treatment contributed to the elevated toxicity observed in these two patients, since both of them already had marked haematotoxicity following the administration of fotemustine. Non-haematological side-effects were predominantly mild, reversible and lasted a maximum of 10 days after treatment. The toxicity spectrum was similar to that observed in other studies, but the exact frequencies of myelotoxicity (grade 3/4: 8–50%) are difficult to compare, since the patient cohorts in the other studies were always small and the treatment regimens heterogeneous [18–20].

Up to now, there is no known effective chemotherapy for metastatic uveal melanoma. The median survival time for this patient group is approximately 5–7 months [8,21]. One problem in performing clinical trials in metastatic uveal melanoma patients is the rareness of the tumour with an overall incidence of approximately six cases per million per year and 15–35% of patients with metastatic spread. We included 33 uveal melanoma patients in this trial and observed a partial response (PR) in one patient which lasted for two years. Due to recent disease progression, this patient is now undergoing more treatment with GeT. For the remaining 32

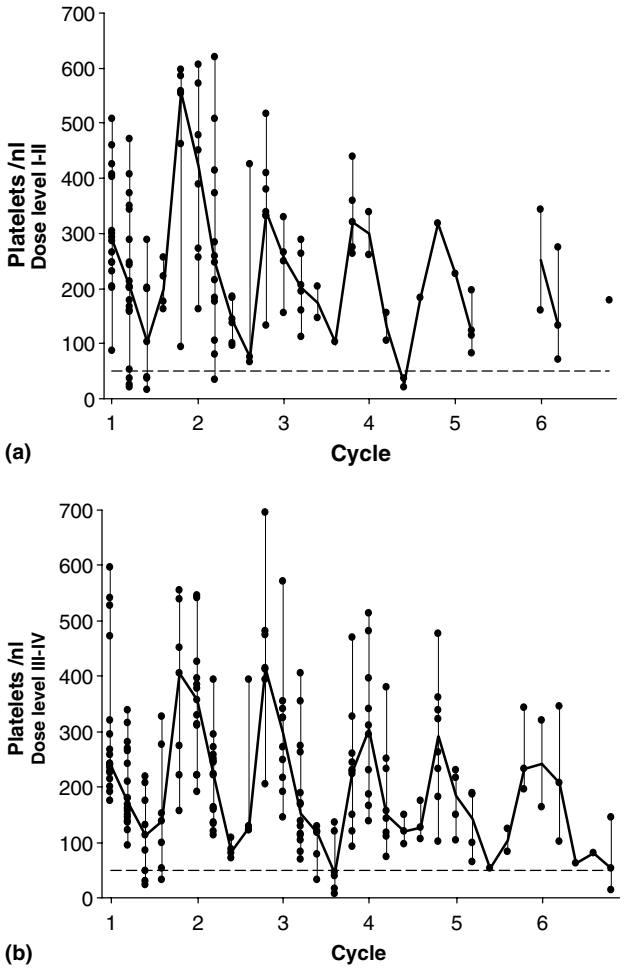

Fig. 3. Platelet count from peripheral blood of patients treated at dose levels I–II (a) and III–IV (b). Solid line, median values; dotted line, threshold to grade 3 toxicity; points, single values.

uveal melanoma patients, a stabilisation of disease exceeding 3 months in previously progressing tumours was noted for 44%.

In patients with other histologies, we observed two objective antitumour responses. One partial response occured in a patient with ovarian cancer refractory to first-line and second-line standard agent chemotherapy. Both drugs used in this study, gemcitabine and treosulfan, are known to be active in ovarian cancer [20,22–25]. Other promising results in platinium-refractory ovarian cancer were obtained when individually planned therapy according to an ATP-TCA was applied [26]. It is worth noting that we were able to harvest sufficient numbers of peripheral blood stem cells for autografts during the recovery period subsequent to the fourth cycle of treatment, encouraging us to investigate the GeT-Protocol as a possible stem cell mobilising chemotherapy regimen in patients with ovarian cancer. The second objective response occured in a patient with renal cell cancer refractory to pretreatment with 5-fluorouracil (5-FU) and interferon-α. Renal cell cancer is one of the most chemo-resistant tumours known and there is no standard treatment. The patient with highly advanced disease (pulmonary and pleural metastases, pleural effusion, performance status reduced to 60%) initially responded to four courses of GeT protocol treatment. During a subsequent 3 month treatment-free interval, there was a 25% regrowth of tumour manifestations, which responded again to a second series of GeT treatments. Thus, the combination of gemcitabine and treosulfan should be further investigated in renal cell cancer, although gemcitabine or treosulfan administered as single agents have been found not to be effective [27–31]. Promising results have also been obtained by combinations of gemcitabine with other agents, e.g., 5-FU, oxaliplatin or interferon and interleukin-2 [32–34].

In summary, the results of this study are encouraging, and evaluation of the GeT protocol in a phase II study in uveal melanoma, ovarian and renal cell cancer is recommended. The MTD and recommended dose for further phase II/III testing is 1 g/m^2 gemcitabine and 3.5 g/m^2 treosulfan on days 1 and 8 of each treatment cycle.

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