Combined phase I/II study of imexon (AOP99.0001) for treatment of relapsed or refractory multiple myeloma

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Imexon [AOP99.0001 (4-imino-1,3-diazobicyclo[3. 1. 0]-hexan-2-one)] belongs to a novel class of promising anticancer agents that induce tumor apoptosis through oxidative stress. Clinical experience since the late 1960s has provided initial evidence for a clinical antitumor activity. Our open-label, multicenter phase I clinical trial was designed to further investigate the adverse event (AEs) profile and pharmacokinetics of AOP99.0001 in pretreated myeloma patients and collect initial data on the potential clinical efficacy in this indication. Thirty-six patients with relapsed or refractory myeloma, who had been pretreated with at least two lines of therapy earlier, were included. Imexon was applied intravenously on 5 consecutive days for 2 weeks (d1-5 and d8-12) for a 3-week cycle. The plasma half-life of AOP99.0001 and its active metabolite AOP99.0002 was found to be approximately 1.2 and 2.6 h, respectively. The mean duration of treatment with Imexon was 6.8 weeks in a dose range between 50 and 1000 mg/m^2 without reaching dose-limiting toxicity. Drug-related AEs occurring with a frequency of greater than 10% were fatigue, nausea, constipation, headache, asthenia, anemia, thrombocytopenia, leukopenia and creatinine increase. A total of nine severe adverse events occurred in three patients. No mortality was encountered when patients were on treatment with Imexon. Preliminary antimyeloma efficacy of AOP99.0001 was observed with 1 minimal response, 12 (36%) stable disease responses, and all other evaluable patients had progressive disease. Remarkably, the patient with minimal response also experienced a complete clinical resolution of myeloma-associated polyneuropathy. Overall, Imexon was safe and well tolerated in the dose range investigated. Imexon showed minor clinical activity as a single agent in heavily pretreated myeloma patients. On account of its unique mechanism of action, favorable toxicity profile, initial clinical evidence for antimyeloma activity, and its known synergistic activity in combination with approved agents for myeloma treatment, AOP99.0001 is recommended for future clinical studies in combination regimens in multiple myeloma. *Anti-Cancer Drugs* 21:708–715

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Introduction

There has been considerable progress in the treatment of multiple myeloma in recent years resulting in improved overall survival and disease-free survival particularly with the introduction of thalidomide, thalidomide derivatives, and bortezomib in combination therapy strategies [1–3]. Despite these advances, there is still an urgent need for the development of new compounds with a new mechanism of action, which will either improve the activity of the currently approved myeloma agents or provide significant activity as single agents.

AOP99.0001 (Heidelberg Pharma, Ladenburg, Germany) syn. Imexon as an isomer of 1-carboxamido-2-cyan-aziridine had initially been developed in the late 1960s as an immunostimulating agent for use in adjuvant cancer therapy [4].

Preclinical research since then has shown that AOP 99.0001 has activity against a wide range of tumor cells *in vitro* and in animal models with particularly high activity for cells of the B-cell lineage including myeloma cells [5,6]. The major antitumor mechanism of AOP 99.0001 is considered to be decreasing the cellular ability to scavenge reactive oxygen species (ROS). This leads to an accumulation of ROS, mitochondrial changes, and activation of a caspase-8-dependent apoptosis mechanism [7–9]. In addition, it was described that AOP99.0001 decreases protein expression and leads to a reduction in HIF-1 α expression [9]. The anticancer pathway of inducing apoptosis through ROS with compounds such as Imexon or arsenic trioxide was recently reviewed by Engels and Evens and considered to become a new paradigm in cancer treatment [10].

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The first clinical data with AOP99.0001 in cancer patients were obtained in the late 1970s [5,11], which described an acceptable and mild adverse event (AE) profile of mainly grade 1 or 2 (vomiting/nausea, fatigue, headache, anorexia). In all phase I and phase I/II studies published so far for patients with advanced malignancies, disease stabilizations were described in individual patients across a wide variety of entities. A few patients were described with objective responses: two complete response (CR) in a non-small-cell lung cancer and a melanoma patient, two partial response (PR) in a breast cancer, and one Hodgkin's lymphoma patient [5,12]. Recently, a phase I study of Imexon in combination with doxorubicin in patients with advanced malignancies came to a recommendation for further investigating the combination with established agents in phase II studies.

On the basis of the above-mentioned rationale, a phase I/II study with Imexon as the single agent was developed for patients with relapsed or refractory multiple myeloma.

Patients and methods

Study objectives

The primary objective of this study was to determine the maximum tolerated dose (MTD) of AOP99.0001, and to define the phase II recommended dose for patients with relapsed or refractory multiple myeloma.

The other objectives were to analyze the efficacy of AOP99.0001 as determined by the number and quality of responses (minimal response MR, PR, CR) and the pharmacokinetic characterization of AOP99.0001.

Study design and study drug

This study was conducted as an open-label phase I study in five centers in Germany, Austria, and Russia. The trial was approved by the local ethics committees of the participating centers and by respective regulatory agencies.

Phase I dose escalation was performed according to a modified Fibonacci/cohort scheme with three initial patients per cohort. On the basis of preclinical and toxicity studies, an initial dose of 50 mg AOP99.0001 per body surface area with planned dose escalations was decided.

A dose modification schedule was implemented that requested interruption of dosage in case of first occurrence of common terminology criteria for adverse events (CTCAE) grade 2 toxicity until resolution to grade 0 or 1. For grade 3 and re-occurrence of grade 2 toxicity, interruption of trial medication was intended until resolution to grade 1/0 with consecutive continuation of treatment at the next lower dose level. In the case of CTCAE grade 4 toxicity, study treatment was discontinued. Each cohort was entered after the safety and tolerability assessment of the earlier lower dose had been established after a minimum of 28 days of treatment. If one of the three patients developed a dose-limiting toxicity (DLT) during the first 28 days then additional six patients had to be enrolled on the same dose level.

For the recommended phase II dosage scheme, an additional cohort of a maximum of 19 patients should be enrolled to gain further experience on safety and toxicity. Blood and urine samples were collected for pharmacokinetic analysis on days 1, 2, and 12.

Study drug

Imexon (chemical name: 4-imino-1,3-diazabicyclo[3.1.0]-hexan-2-one) was supplied as lyophilisate and reconstituted using a solvent containing propyleneglycol/water (1 + 9, v/v) by a pharmacy on-site within 2 h of therapeutic application of Imexon. After reconstitution, Imexon (in the respective dose, see below) was applied as a 15-min short infusion on 5 consecutive days for 2 weeks (day 1–5 and 8–12) followed by a 1-week recovery period from day 15 onwards, which represented in total one cycle. A maximum of three cycles were planned per patient with the possibility to continue treatment in case of response and an acceptable AE profile at the discretion of the investigator.

Efficacy analysis

Response to treatment was determined according to the paraprotein response with the following categories: CR with complete disappearance of paraprotein in the urine and serum including negative immunofixation in both the compartments, PR as paraprotein reduction of $\geq 50\%$ in the serum with $\geq 90\%$ reduction in the 24-h-urine light chain excretion or reduction of the urine M-component below 200 mg/24 h. Minimal response (MR) was defined as reduction of serum M-protein between 25 and 49% and/or a reduction of urine M-protein of 50–89%. SD was defined as not falling under one of the other categories (paraprotein level between -25 and $+25\%$ according to baseline values and maintaining this status for at least 3 months).

Patients

Patients with symptomatic multiple myeloma with relapsed or refractory disease after at least two earlier chemotherapeutic regimens were eligible for this study. Patients were required to have a minimum paraprotein concentration (IgG: 10 g/l, IgA: 5 g/l, urine light chain 200 mg/24 h) and were not eligible if they suffered from severe untreated concurrent illnesses including infections. Treatments with other experimental drugs or agents that influence myeloma disease activity were not permitted. Treatment with acetylcysteine was not permitted either. Patient laboratory results had to be within the following limits: serum creatinine less than $2.0\times$ upper limit of normal (ULN), bilirubin less than $1.5\times$ ULN, transaminases less than $2.5\times$ ULN, neutrophil count ≥ 1/nl, thrombocytes ≥ 50/nl and Hb ≥ 8 g/dl. An earlier allogeneic transplantation was not allowed.

Pharmacokinetic analysis

Pharmacokinetic analysis of AOP99.0001 and its metabolite AOP99.0002 was performed from the plasma on day 1/day 2 (baseline/before infusion, 1, 5, 10, 30 min, 1, 2, 4, 8, and 24 h). After blood collection, plasma was derived by centrifugation and plasma samples were stored at −70°C. Two urine collections were performed on day 1 and day 2 from 0 to 12 h and 12–24 h after the first administration of the study drug (SD). During collection, urine was stored at +4°C. Later, 10-ml aliquots of urine were stored at −70°C. The SD and its metabolite were detected by high-performance liquid chromatography.

Adverse events, analysis of efficacy, and pharmacokinetics

Patients were monitored in weekly intervals throughout the treatment period for AEs. A study termination visit was planned for those patients completing all the three cycles on day 99, which was approximately 3 weeks after the completion of the third cycle according to protocol.

Assessments during the study included tumor assessments using laboratory and radiological assessments according to standard procedures for multiple myeloma patients. Blood sampling for pharmacokinetic measurements with an emphasis on day 1 was performed as well. The statistical and analytical plan used descriptive statistical tools.

Results

Thirty-six patients with multiple myeloma were included in this study between 2005 and 2007. One patient who had a MR to the SD was included in the study again upon relapse.

Patient characteristics are summarized in Table 1. According to the inclusion/exclusion requirements of the protocol, patients had a median of three earlier regimens before they entered the study with a range of 2–5 earlier pretreatments. Reflective of the extensively pretreated patient population, we recorded 10 patients who had been treated earlier with thalidomide as a single agent or within-combination therapies, 13 patients who had been treated earlier with bortezomib and 15 patients who had been subjected to high-dose therapy with melphalan before recruitment into the study.

The dose escalation steps were performed as follows: 50 mg/m^2 ($n = 3$), 100 mg/m^2 ($n = 3$), 200 mg/m^2 ($n = 3$), 300 mg/m^2 ($n = 3$), 400 mg/m^2 ($n = 12$), 500 mg/m^2 ($n = 5$), 600 mg/m^2 ($n = 4$), 1000 mg/m^2 ($n = 3$). On account of an AE with creatinine increase of grade 3 as described below, an additional exclusion criterion for the study was added that restricted eligibility of patients presenting with proteinuria below 0.5 g/24 h in 24-h urine. After this change to the inclusion/exclusion criteria, six additional patients were included at the 400 mg/m^2 dose level. No further related or possibly related severe adverse event (SAE)s occured and the dose increase was continued as planned.

Table 1 Patient characteristics

Characteristics	
Female [*n* (%)]	15 (42)
Age [median (range)]	60.8 (37–75)
Eastern Cooperative Oncology Group [*n* (%)]	
0	18 (50)
1	17 (47.2)
2	1 (2.8)
Durie salmon stage [*n* (%)]	
IIA	4 (8.3%)
IIIA	33 (81.7)
Type of M-protein [*n* (%)]	
IgG	24 (66.7)
IgA	8 (22.2)
Light-chain disease	4
Duration of disease in month [median (range)]	50 (7–209)
Prior lines of therapy [median (range)]	3 (2–5)
Previous high-dose therapy and autologous stem cell transplantation [*n* (%)]	15 (42)
Previous thalidomide [*n* (%)]	10 (28)
Previous bortezomib [*n* (%)]	13 (31.4)
Hemoglobin g/dl [median (range)]	12.2 (6.6–15.6)
Creatinine mg/dl [median (range)]	0.9 (0.48–2.1
β_2-microglobulin	2.81 (1.6–7.14)

Adverse events

Table 2 summarizes the AEs observed in this study and indicates those AEs that occurred with grade 3/4 and refers to the relationship of the AE with the SD.

Hematological adverse events

Eighteen (50%) patients developed an AE in the blood and lymphatic system category. Anemia was the most common reported AE, but only two patients (5.6%) developed grade 3 or 4 anemia. Grade 3/4 leukopenia and thrombocytopenia developed in three (8.3%) and four (11.1%) patients, respectively.

As the included patients were a heavily pretreated study population with active myeloma in 12 of the 18 patients, the hematological AEs were not considered to be related to AOP99.0001 by the investigators. There was neither a dose dependency for the number of hematological AEs, nor for the number of affected patients. However, we observed an increased risk for the development of hematological AEs in the higher-dose treatment groups (above 300 mg/m^2).

Nonhematological adverse events

The most common nonhematological AEs with an incidence of over 10% were nausea [27 (75%)], vomiting [20 (55.6%)], headache [11 (36.1%)], asthenia [10 (27.8%)], constipation [6 (16.7%], fatigue [4 (11.1%)], creatinine increase [4 (11.1%)], and back pain [4 (11.1%)]. For a detailed listing of the nonhematological AEs please refer to Table 2. From these more frequent

Table 2 Incidence of adverse events independent of relatedness with study drug (bold if over 10% for AE); $n = 36$ pts (safety population)

Adverse event	*n* (%) patients total	related	*n* (%) grade 3/4
Hematological AEs			
Blood and lymphatic system	18 (50.0)	12 (33)	7 (19.4)
Anemia	**10 (27.8)**	**5 (13.9)**	**2 (5.6)**
Leukopenia	**7 (19.4)**	**7 (19.4)**	**3 (8.3)**
Thrombocytopenia	**6 (16.7)**	**4 (11.1)**	**4 (11.1)**
Non-hematological AEs			
Cardiac	5 (13.9)	0 (0)	1 (2.8)
Arrythmia supraventricula	1 (2.8)		
Atrial fibrillation	2 (5.6)		
Cardiac failure	1 (2.8)	1 (2.8)	
Cardiomyopathy	1 (2.8)		
Tachycardia	1 (2.8)		
Gastrointestinal	30 (83.3)	28 (77.8)	0 (0)
Abd. distension	1 (2.8)	1 (2.8)	
Abd. pain	1 (2.8)	1 (2.8)	
Constipation	**6 (16.7)**	**3 (8.3)**	
Diarrhea	2 (5.6)		
Nausea	**29 (80.6)**	**27 (75)**	
Pancreatitis chronic	1 (2.8)	1 (2.8)	
Periodontitis	1 (2.8)		
Stomatitis	1 (2.8)		
Vomiting	**21 (58.3)**	**20 (55.6)**	
Epigastric discomfort	1 (2.8)	1 (2.8)	
General disorders and administration	17 (47.2)	13 (36.1)	0 (0)
Site conditions			
Asthenia	**12 (33.3)**	**10 (27.8)**	
Chest pain	1 (2.8)		
Fatigue	**6 (16.7)**	**4 (11.1)**	
Pyrexia	2 (5.6)		
Infections/infestations	9 (25)	1 (2.8)	1 (2.8)
Bronchitis acute	2 (2.8)		
Catheter-related infection	1 (2.8)		
Herpes simplex infection	2 (5.6)		
Herpes zoster infection	3 (8.3)	1 (2.8)	
Nasopharyngitis	1 (2.8)		
Pneumonia	1 (2.8)	1 (2.8)	
Rhinitis	1 (2.8)		
Sepsis	1 (2.8)	1(2.8)	
Urinary tract infections	2 (5.6)		
Investigations	11 (30.6)	4 (11)	3 (8.3)
Aspartate aminotransferase	1 (2.8)	1 (2.8)	
Serum creatinine	**4 (11.1)**	**4 (11.1)**	**2 (5.6)**
Serum LDH	1 (2.8)		
Serum triglycerides	1 (2.8)		
Serum urea	1 (2.8)	1 (2.8)	
Serum CRP	3 (8.3)	1 (2.8)	1 (2.8)
Creatinine-Cl. decreased	1 (2.8)		
Musculoskeletal	10 (27.8)	1 (2.8)	1 (2.8)
Arthralgia	2 (5.6)		
Back pain	**4 (11.1)**		
Bone pain	3 (8.3)	1 (2.8)	
Chest wall pain	1 (2.8)		
Osteonecrosis	1 (2.8)	1 (2.8)	
Pain in extremity	1 (2.8)		
Shoulder pain	1 (2.8)		
Nervous system disorders	16 (44.4)	13 (36.1)	1 (2.8)
Dizziness	1 (2.8)		
Headache	**13 (36.1)**	**11 (30.6)**	**1 (2.8)**
Paraesthesia	1 (2.8)		
Parsomnia	2 (5.6)	2 (5.6)	
Polyneuropathy	1 (2.8)		
Somnolence	1 (2.8)		
Tremor	1 (2.8)		
Psychiatric disorders	5 (13.9)	1 (2.8)	1 (2.8)
Insomnia	1 (2.8)	1 (2.8)	
Restlessness	2 (5.6)		
Stress	1 (2.8)		
Major depression	1 (2.8)		
Renal and urinary disorders	5 (13.9)	4 (11.1)	1 (2.8)
Oliguria	2 (5.6)	2 (5.6)	1 (2.8)
Pollakisuria	1 (2.8)		
Proteinuria	2 (5.6)	2 (5.6)	
Respiratory, thoracic, and mediast. disorders	5 (13.9)	3 (8.3)	
Cough	3 (8.3)		
Dyspnea	3 (8.3)	2 (5.6)	
Epistaxis	2 (5.6)	1 (2.8)	
Vascular disorders	6 (16.7)	4 (11.1)	0 (0)
Flushing	1 (2.8)	1 (2.8)	
Hypertension	1 (2.8)		
Phlebitis	1 (2.8)	1 (2.8)	
Thrombophlebitis	2 (5.6)	1 (2.8)	
Hemorrhage	1 (2.8)	1 (2.8)	

Abd, abdomen; AE, adverse event.

AEs, only headache and creatinine increase were recorded as grade 3/4 in one and two patients, respectively. Gastrointestinal AEs occurring as vomiting, nausea and headache, fatigue, asthenia, and creatinin increase were reported as related to study medication. As myeloma patients are particularly sensitive to renal complications, we performed a detailed analysis of the AEs reported for renal/urinary organ system and creatinine increase. For all the 36 patients, we did not detect a dose-dependent increase of creatinine, but AEs related to renal and urinary disorders were reported with an incidence of 13.9% affecting five patients. Oliguria occurred in two patients on the 100 mg/m^2 dose level and in one patient at 400 mg/m^2. Pollakisuria was reported for one patient on the 500 mg/m^2 dose and proteinuria for two patients treated with 1000 mg/m^2. In one patient proteinuria led to SD discontinuation. From a total of 11 patients in whom AEs related to the SD were reported, four were related to creatinine increase and one to urea increase. Therefore, creatinine increase was the most common AE in laboratory investigations (Table 2). The second most common was CRP increase in three patients. (8.3%). Remarkably, all the four AEs reported for creatinine increase were reported as 'related'. For one patient this increase was considered as 'severe'/grade 3. Creatinine increases occurred at the 100 ($n = 1$), 400 ($n = 2$), and 1000 mg/m^2 ($n = 1$) dose levels. The 'severe' increase in creatinine developed at 400 mg/m^2. In addition, the creatinine increase at the 100 mg/m^2 dose level was categorized as SAE. The creatinine increase of one patient treated with 400 mg/m^2 led to SD discontinuation. This patient had detectable light chain disease associated with kidney failure. As the investigator had categorized this creatinine increase as related, and as a contribution of AOP99.0001 to this AE could not be ruled out, the inclusion into this study was restricted to patients with a proteinuria level of 0.5 g/24 h and higher for the subsequent patients. All other AEs were reported as unrelated or unlikely related.

Laboratory investigations that were considered as AEs are listed in Table 2 as well. Regarding laboratory evaluations that were not reported as AE but were observed with a more than marginal change from baseline (< 30% increase from baseline), we identified six patients who experienced an increase in γ-glutamyl-transferase (GGT) that was more than three-fold above the upper limit of normal. None of these GGT elevations were considered as clinically relevant and returned to baseline or below either during the study or at study termination. For one patient, a concomitant increase in the ALT level was reported up to 220% of ULN.

Serious adverse events

There were no deaths of patients on study. The following SAEs were reported (nine SAEs in three patients): cardiac failure, pneumonia, septicemia, creatinine increase/renal insufficiency. Creatinine increase, which was recorded as a related SAE, was already described above. The other SAEs were recorded as not related.

Pharmacokinetic studies

AOP99.0001 had a linear pharmacokinetic profile in the dose range of 50–1000 mg (Table 3).

There was a strong dose relationship between the serum concentration of AOP99.0001 and its main metabolite AOP 99.0002, as measured by the maximum plasma concentration and the area under the plasma concentration–time curve after the intravenous infusion. Approximately 10–20% of the dose of AOP99.0001 was metabolized to AOP 99.0002 and appeared as such in plasma. The extent of this metabolic pathway was not dose dependent. Apparently, AOP99.0002 underwent further metabolism into at present unidentified metabolite(s).

Both the parent drug and its main metabolite were rapidly eliminated from plasma with a half-life of elimination of approximately 1.2 and 2.6 h, respectively. Twenty-four hours after the administration of AOP99.0001, no quantifiable concentration of either AOP99.0001 or

Table 3 Results of pharmacokinetic parameters

AOP 99.0001								
Dose (mg/m^2)	50		200		300		400	
N	3		2		3		6 (8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max} (μg/l)	3219.3	1455.5	20197.0	2897.7	40549.8	25367.5	29920.6	5293.8
C_{max}/dose (μg × m^2/mg × ml)	0.06	0.03	0.10	0.01	0.14	0.08	0.07	0.01
$AUC_{0-\infty}$ (μg × h/l)	4239.7	956.4	20571.5	4831.7	31434.6	8359.9	38002.6	9339.2
$AUC_{0-\infty}$/dose (μg × h × m^2/l × mg)	5.1	1.1	6.2	1.4	6.3	1.7	5.7	1.4
CL/BSA [l/(min × m^2)]	0.2	0.0	0.1	0.0	0.1	0.0	0.2	0.1
Vd/BSA [l/(m^2)]	10.6	2.8	10.9	0.7	6.0	1.4	13.8	3.7
$t^1/_2\beta$ (h)	1.1	0.1	1.3	0.2	1.2	0.2	1.2	0.2
Total urinary excretion in % dose	19.3%	2.4%	20.0%	1.0%	20.7%	4.8%	20.2%	5.5%
AOP 99.0002								
Dose (mg/m^2)	50		200		300		400	
N	3		2		3		6 (8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max} (μg/l)	167	56	985	538	1885	1736	1774	551
C_{max}/dose (ng × m^2/mg × ml)	3.3	1.1	4.9	2.7	6.3	5.8	4.4	1.4
$AUC_{0-\infty}$(μg × h/l)	535	19	2562	1217	3373	884	5754	1257
$AUC_{0-\infty}$/dose (ng × h × m^2/l × mg)	10.7	0.4	12.8	6.1	11.2	2.9	14.4	3.1
$t^1/_2\beta$ (h)	2.7	0.3	2.4	0.3	2.8	0.5	2.8	0.4
% AUC of AOP 99.0001	12.6		12.5		10.7		15.1	
$t^1/_2\beta$ (h)	1.1	0.1	1.3	0.2	1.2	0.2	1.2	0.2
$t^1/_2\beta$ (h)	1.1	0.1	1.3	0.2	1.2	0.2	1.2	0.2
AOP 99.0001								
Dose (mg/m^2)	500		600		1000			
N	2 (3)		1		3			
	Mean	SD	Mean	SD	Mean	SD		
C_{max} (μg/l)	45423.0	15568.9	56878.2	–	46209.9	24610.8		
C_{max}/dose (μg × m^2/mg × ml)	0.09	0.03	0.09	–	0.05	0.02		
$AUC_{0-\infty}$(μg × h/l)	50186.7	3060.3	66501.2	–	51871.8	24145.7		
$AUC_{0-\infty}$/dose (μg × h × m^2 × / l × mg)	6.0	0.4	6.7	–	3.1	1.4		
CL/BSA [l/(min × m^2)]	0.1	0.0	0.1	–	0.5	0.2		
Vd/BSA [l/(m^2)]	7.4	1.8	6.0	–	36.2	18.7		
$t^1/_2\beta$ (h)	1.1	0.1	1.1	–	1.3	0.2		
Total urinary excretion in % dose	22.0%	6.1%	25.0%		24.3%	9.4%		
AOP 99.0002								
Dose (mg/m^2)	500		600		1000			
N	2 (3)		1		3			
	Mean	SD	Mean	SD	Mean	SD		
C_{max} (μg/l)	3565	1257	3167	–	3607	1148		
C_{max}/dose (ng × m^2/mg × ml)	7.1	2.5	5.3	–	3.6	1.1		
$AUC_{0-\infty}$ (μg × h/l)	8953	885	8814	–	8302	2561		
$AUC_{0-\infty}$/dose (ng × h × m^2/l × mg)	17.9	1.8	14.7	–	8.3	2.6		
$t^1/_2\beta$ (h)	2.4	0.1	2.5	–	2.3	0.1		
% AUC of AOP 99.0001	17.8		13.3		16.0			

AUC: area under the curve; BSA: body surface area; C_{max}, maximum concentration; CL: clearance.

AOP99.0002 could be detected in any patient. The half-lives of elimination remained constant over the investigated dose range.

Approximately 10–20% of AOP99.0001 had been excreted as an unmodified drug by the kidneys. This indicates that 80–90% of the parent drug underwent a metabolism and/or a biliary/intestinal excretion. The body surface-corrected plasma clearance of AOP99.0001 ranged between 100 and 200 ml/(min × m^2) and remained constant over the entire investigated dose range. Accordingly, the plasma clearance of AOP99.0001 represented about 5% of the heart time volume, about 20% of the liver blood flow and about 30% of the renal blood flow. The extent of the renal excretion as AOP99.0002 remained constant over the investigated dose range.

The body surface-corrected apparent volume of distribution of AOP99.0001 ranged between 6 and 14 l/m^2 and remained constant over the investigated dose range. This corresponded to the apparent volume of distribution of approximately 0.2–0.4 l/kg of body weight, which was in line with the results of investigations on protein binding of AOP99.0001 (binding of proteins of approx. 40%). Considering pharmacokinetic parameters, the patients of the highest dose level (1000 mg/m^2) achieved lower AUC and Cmax levels than in the dose group below (600 mg/m^2). This indicates that the formulation of AOP99.0001 used in this study did not support this high dose (because of precipitations occurring during preparation amongst others).

Efficacy analysis

Efficacy was not the primary objective of this study; nevertheless, information on the antimyeloma activity of AOP99.0001 was collected.

Using a paraprotein-based response assessment, we identified three patients from the safety analysis that could not be evaluated for response for the following reasons: (i) SD discontinuation within the first cycle, (ii) SD duration less than 3 months for patients that discontinued the study after two cycles, (iii) one patient that developed an MR was re-entered into the study at a later time point. The treatment result of this second exposure to the SD was not integrated in the overall response assessment of this study. Therefore, efficacy evaluation was always related to 35 patients (compared with 36 for safety evaluations). Of the 33 patients, 20 patients (60%) developed progressive disease, 12 (36%) reached a SD and one patient achieved a MR. Patients were only categorized into SD if SD confirmed for at least 3 months. The patient with MR had received extensive treatment earlier with six cycles of M2 regimen (M2-regimen: BCNU, cyclophosphamide, melphalan, vincristine, prednisone; March 2003 until December 2004) with consecutive stable disease. In the first half of 2005, this patient received four courses of melphalan/prednisone therapy, but in August 2005 the condition of the patient deteriorated because of progressive polyneuropathy with numbness of both hands and feet and impairment of motor activity including spasms of the gastrocnemius muscle. In addition, the patient developed progressive asthenia, hyperhidrosis, and the Karnofsky index decreased to 60%. As a consequence, the patient was hospitalized and received an additional cycle of M2-combination chemotherapy without response and was included in our study. After the first cycle with Imexon 400 mg/m^2, the patient responded clinically with improvement of polyneuropathic symptoms, in particular, improvement of numbness in the hands and feet. Subsequently, clinical neurological symptoms further improved and resolved completely within 6 weeks. At the same time, the Karnofsky index reached 100%. After completion of three cycles according to study protocol, the patient was offered a continuation of treatment but refused because of personal reasons. The response remained for 3 months. The polyneuropathy re-occurred in August 2006 and the patient was offered to re-enter the study. The patient achieved an SD after this second course of AOP99.0001 treatment (three cycles), and the polyneuropathy improved as well but not to the extent as induced by the first course.

The planned maximum observation period for this study was 3 months (99 days) for the included patients. This time frame corresponded to three cycles of AOP as planned by the study protocol. Patients with progressive disease developed progression within this 3-month time frame. All patients with SD received three cycles of therapy and had stable disease for 3 months.

Discussion

Our report is the first publication of a clinical study on AOP99.0001 (syn. Imexon) in multiple myeloma patients. Overall, we identified a mild AEs profile for AOP99.0001. The main AEs for AOP99.0001 were hematological AEs with mainly grade1/2 anemia, leukopenia and thrombocytopenia. In addition, this study presents extensive pharmacokinetic data for AOP99.0001 in myeloma patients. The trial provides initial evidence for a possible clinical activity of single agent AOP99.0001 in multiple myeloma.

The AE profile of AOP99.0001 was favorable with mainly reversible grade 1/2 hematological toxicity in approximately 50% of the patients with anemia being the most common AE. All hematological AEs recovered until the next cycle. Most important nonhematological AEs were nausea and vomiting, which indicates that a prophylactic treatment with serotonin-antagonists before the application of AOP99.0001 should be an essential requirement. Other clinically significant AEs in our study were the deterioration of renal function and creatinine increase. Indeed, the only DLT that occurred was renal insufficiency. In this case, the relationship with the SD was

considered as potentially related. As some of these renal complications reached grade 3/4 or were even recorded as SAEs, we suggest for further investigations of Imexon in multiple myeloma to particularly monitor renal function and to exclude patients with clinically significant renal insufficiency (e.g. creatinine increase over 1.5-fold ULN or creatinine clearance below 50 ml/min). In addition, caution should be taken for patients with light chain excretion. As it is known that patients with significant light chain excretion in urine are prone to develop renal complication, we instituted a maximal light chain excretion level of 0.5 g/24 h as the exclusion criterion for this study as a protocol amendment. Regarding hepatobiliary function, there were only a limited number of patients that developed GGT increase, which was accompanied by an ALT increase in one case. None of these liver enzyme changes indicated clinically significant liver disease, and they all returned to baseline either within the ongoing study or shortly thereafter. Keeping this in mind and because of the biliary excretion of AOP99.0001, we suggest to exclude patients with severe liver disorders in future trials with this study agent.

Earlier studies with a similar but slightly less intense treatment schedule (day 1–5 and 15–19) established a DLT at 1000 mg/m^2 and a MTD at 875 mg/m^2 (with abdominal pain and neutropenia as DLTs) [11]. In a combination therapy study of Imexon with doxorubicin for patients with advanced malignancies (Imexon at d1-5 of a 21-day cycle, doxorubicin 75 mg/m^2), the DLT was at 1700 mg/m^2 and the MTD at 1400 mg/m^2 [13]. DLTs that were observed in this study were syncope (CTCAE grade 3), two patients with noncardiac chest pain (CTCAE grade 3) and diarrhea (CTCAE grade 3). Although our study formally did not reach the MTD/DLT level, we believe that the doses reached in this study were very close to this level. On account of the further safety and initial efficacy data of AOP as discussed below the authors would consider further clinical investigation of AOP99.0001 in multiple myeloma in a phase I/II study in combination with the standard Velcade/dexamethasone regimen. In the phase I part of this study, the DLT/MTD level of the combination could be determined.

The study was stopped after the inclusion of 36 patients and the reasons that led to termination are described in this paragraph. When the 36th patient was included, 30 patients were treated with a dose of 300 mg/m^2 and higher, which was considered to be the dose of biological activity based on the data available from preclinical and earlier clinical investigations. For these 30 patients, only one objective response (MR) was observed.

On the basis of these results, it was considered unlikely that AOP99.0001 application as a single agent would meet the goal, as set forward by the study protocol, for a phase II study to induce a response rate of more than 10% (four or more patients of the 30 additional patients in a phase II study). Therefore, the study was terminated without moving into a phase II setting.

Our statement of evidence for clinical activity of AOP99.0001 is based on three observations.

(1) As described above, one heavily pretreated patient being refractory to M2 regimen and melphalan/prednisone regimen developed an objective response that lasted for 3 months after the application of three cycles of AOP99.0001 according to the study protocol.
(2) Remarkably this patient developed an impressive complete resolution of a debilitating myeloma-associated peripheral sensory and motor polyneuropathy.
(3) In addition to this, approximately one-third of the patients developed a stabilization of the disease activity parameters over a period of at least 3 months.

Although the single-agent activity of AOP99.0001 is detectable but limited, several considerations argue for a further development of this compound in a combination setting. Preclinical data indicate that AOP99.0001, with its unique mechanism of action as an inducer of apoptosis by increasing cellular ROS, can synergistically interact with approved compounds for the treatment of multiple myeloma. Scott *et al.* described synergistic anti-myeloma activity *in vitro* for a combination of Imexon with DNA-binding agents (cisplatin, dacarbazine, melphalan) and pyrimidine-based antimetabolites (cytarabine, fluorouracil, gemcitabine) [14]. In addition, synergistic activity was observed for a combination with dexamethasone and bortezomib. Another argument for the combination therapy is that AOP99.0001 has a favorable toxicity profile that lacks skin and neurological AEs. In addition, future studies could integrate novel diagnostic techniques, such as gene expression profiling, to identify the subset of patients that have a higher likelihood of clinical benefit [15,16].

In summary, this first publication on a clinical trial of AOP99.0001 in pretreated multiple myeloma patients shows a mild toxicity profile of this agent and for this indication. Evidence for the clinical efficacy of this compound, which belongs to the novel class of oxidative stress inducers, is provided and a further development of AOP99.0001 in combination-therapy regimens is recommended based on the in-vitro data that describe a synergistic activity of AOP99.0001 with several approved antimyeloma agents.

References

1 Kastritis E, Zervas K, Symeonidis A, Terpos E, Delimbassi S, Anagnostopoulos N, *et al.* Improved survival of patients with multiple myeloma after the introduction of novel agents and the applicability of the International Staging System (ISS): an analysis of the Greek Myeloma Study Group (GMSG). *Leukemia* 2009; **23**:1152–1157.
2 Moehler TM, Neben K, Benner A, Egerer G, Krasniqi F, Ho AD, *et al.* Salvage therapy for multiple myeloma with thalidomide and CED chemotherapy. *Blood* 2001; **98**:3846–3848.

3 Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, *et al.* Improved survival in multiple myeloma and the impact of novel therapies. *Blood* 2008; **111**:2516–2520.
4 Bicker UF. BM 06.002: A new immunostimulating compound. In: Chirigos MA, editor. *Immune modulation and control of neoplasia by adjuvant therapy*. New York: Raven Press; 1978. pp. 389–401.
5 Sagaster P, Kokoschka EM, Kokron O, Micksche M. Antitumor activity of imexon. *J Natl Cancer Inst* 1995; **87**:935–936.
6 Salmon SE, Hersh EM. Sensitivity of multiple myeloma to imexon in the human tumor cloning assay. *J Natl Cancer Inst* 1994; **86**:228–230.
7 Dvorakova K, Waltmire CN, Payne CM, Tome ME, Briehl MM, Dorr RT. Induction of mitochondrial changes in myeloma cells by imexon. *Blood* 2001; **97**:3544–3551.
8 Evens AM, Prachand S, Shi B, Paniaqua M, Gordon LI, Gartenhaus RB. Imexon-induced apoptosis in multiple myeloma tumor cells is caspase-8 dependent. *Clin Cancer Res* 2004; **10**:1481–1491.
9 Samulitis BK, Landowski TH, Dorr RT. Inhibition of protein synthesis by imexon reduces HIF-1alpha expression in normoxic and hypoxic pancreatic cancer cells. *Invest New Drugs* 2009; **27**:89–98.
10 Engel RH, Evens AM. Oxidative stress and apoptosis: a new treatment paradigm in cancer. *Front BioSci* 2006; **11**:300–312.
11 Micksche M, Kokoschka EM, Sagaster P, Bicker UF. Phase I study of immunotherapy with Imexon. In: Chirigos MA, editor. *Immune modulation and control of neoplasia by adjuvant therapy*. New York: Raven Press; 1978. pp. 403–413.
12 Dragovich T, Gordon M, Mendelson D, Wong L, Modiano M, Chow HH, *et al.* Phase I trial of imexon in patients with advanced malignancy. *J Clin Oncol* 2007; **25**:1779–1784.
13 Moulder S, Dhillon N, Ng C, Hong D, Wheler J, Naing A, *et al.* A phase I trial of imexon, a pro-oxidant, in combination with docetaxel for the treatment of patients with advanced breast, non-small cell lung and prostate cancer. *Invest New Drugs* 2009 [E-pub ahead of print].
14 Scott J, Dorr RT, Samulitis B, Landowski TH. Imexon-based combination chemotherapy in A375 human melanoma and RPMI 8226 human myeloma cell lines. *Cancer Chemother Pharmacol* 2007; **59**:749–757.
15 Nair B, Shaughnessy JD Jr, Zhou Y, Astrid-Cartron M, Qu P, van Rhee F, *et al.* Gene expression profiling of plasma cells at myeloma relapse from tandem transplantation trial Total Therapy 2 predicts subsequent survival. *Blood* 2009; **113**:6572–6575.
16 Hose D, Reme T, Meissner T, Moreaux J, Seckinger A, Lewis J, *et al.* Inhibition of aurora kinases for tailored risk-adapted treatment of multiple myeloma. *Blood* 2009; **113**:4331–4340.