EUROPEAN JOURNAL OF CANCER 44 (2008) 1657–1662

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Phase I trial of r viscumin (INN: aviscumine) given subcutaneously in patients with advanced cancer: A study of the European Organisation for Research and Treatment of Cancer (EORTC protocol number 13001)

Lothar Bergmann[a,*], *Steiner Aamdal*[b], *Sandrine Marreaud*[c], *Denis Lacombe*[c], *Manfred Herold*[d], *Takuhiro Yamaguchi*[c], *Karin Wilhelm-Ogunbiyi*[e], *Hans Lentzen*[f], *Heinz Zwierzina*[d]

[a]*Klinikum der JW Goethe Universität, Medizinische Klinik III, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany*
[b]*Norwegian Radium Hospital, Clinical Research Montebello, N-0130 Oslo 3, Norway*
[c]*EORTC Headquarters, Ave E. Mounier 83/11, B-1200 Brussels, Belgium*
[d]*Universitätsklinik Innsbruck, Department of Internal Medicine, Anichstrasse 35, A-6020 Innsbruck, Austria*
[e]*PAION Deutschland GmbH, Martinstr. 10-12, 52062 Aachen, Germany*
[f]*CYTAVIS BioPharma GmbH, Schnackenburgallee 116a, 22525 Hamburg, Germany*

ARTICLE INFO

Article history:
Received 29 April 2008
Accepted 12 May 2008
Available online 2 July 2008

Keywords:
Aviscumine
Phase I trial
rViscumin
Ribosome-inactivating protein
Solid tumours

ABSTRACT

Safety of aviscumine by subcutaneous route was assessed in patients with advanced cancer refractory to chemotherapy. Patients with progressive disease received escalating doses twice weekly. Treatment of the accrued 26 patients (10 colorectal cancer (CRC), 6 soft tissue sarcoma (STS), 5 melanoma (MM), 5 others) was well tolerated without substance-related grade 3 or 4 toxicities. Grade 1/2 toxicities were predominantly injection site reactions. Aviscumine lacked dose-limiting toxicity (DLT) up to a maximal dose of 10 ng/kg. An increase of interleukin-1β and interferon-γ from baseline was seen in the patient's plasma between the 1st and 11th injection. Highest release of both cytokines was in the dose range of 4–5.9 ng/kg. Interferon-γ was not detected after doses higher than 6 ng/kg. Eight patients (5 CRC, 1 MM, 1 STS, 1 RCC) had disease stabilisation for 79–250 days (median122 days) associated with an increase of interleukin (IL)-1β and interferon (IFN)-γ. Aviscumine was well tolerated and appeared to possess clinical activity at a biologically active dose between 4 and 6 ng/kg.

1. Introduction

Aviscumine is a 57-kDa heterodimer consisting of a toxic A-chain, a site-specific type II ribosome-inactivating *N*-glycosidase[1] and a carbohydrate-binding subunit B responsible for cellular uptake.[2–4] It was achieved by recombinant cloning and separate expression of the A- and B-chain in *Escherichia coli*, yielding the active protein.[3,4]

Aviscumine exerts direct cytotoxic effects by the induction of apoptosis in tumour cells as well as indirect antineoplastic properties caused by immunomodulation *in vitro* and *in vivo*, and was well tolerated in experimental animal models.[5–9]

* *Corresponding author:* Tel.: +49 69 6301 5121; fax: +49 69 6301 3876.
E-mail address: L.Bergmann@em.uni-frankfurt.de (L. Bergmann).
0959-8049/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2008.05.005

The cellular receptors involved in the binding are terminal α2-6-sialylated structures on glycolipids and on glycoproteins.[10,11] Gangliosides with a Neu5Acα2-6Galβ1-4GlcNAc-terminus (CD75s) were defined as aviscumine receptors, leading to internalisation of the holoprotein.[10] The majority of tumours show an enhanced expression of CD75s compared with unaffected tissues. Furthermore, CD75s was detected in serum and is expressed on monocytes/macrophages, granulocytes, dendritic cells, endothelial cells and on murine CD8+ suppressor cells.[12] The expression of CD75s on immunocompetent cells such as CD8+ T-suppressor cells and the interaction between aviscumine and CD75s may be one explanation for the immunostimulation by aviscumine.

Based on the different modes of action, two development strategies were chosen in the phase I setting: intravenous (i.v.) application of relatively high doses (μg/kg) with the intention to define a maximum-tolerated dose (MTD)[16] and subcutaneous (s.c.) application of low doses in the nanogram range to define an optimal biologically active dose (OBAD).

In vitro studies showed that aviscumine induced the release of interleukin (IL)-1α and IL-6 from human keratinocytes and fibroblasts, of IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α from peripheral blood mononuclear cells (PBMCs) as well as the expression of the IL-2 receptor α chain and HLA-DR on peripheral blood T-lymphocytes.[13,5] Furthermore, aviscumine induced an increased expression of granulocyte macrophage colony stimulating factor (GM-CSF) RNA in $CD14^+$ positive cells (Dr. B. Schaffrath, Köln).

Aviscumine strongly bound to NK cells, granulocytes and monocytes, and induced apoptosis in these cells.[14] The generation of cytokines such as IL-1β and TNF-α in human monocytes correlated with the induction of apoptosis. The affinity of aviscumine to lymphocytes was relatively low.[14] However, activation of T-lymphocytes by aviscumine can be observed *in vivo*[7] and the cytotoxicity of human NK cells against cancer cells *ex vivo* is increased.[5]

Tumour growth inhibition by aviscumine was achieved in four different syngeneic mouse carcinoma models (C8 colon 38, Renca renal, Lewis lung, F9 teratocarcinoma) at a dose range from 3 to 3000 ng per kg after intraperitoneal (i.p.) application. A dose–response relationship was obtained.[15] Subcutaneous treatment of immunocompetent mice with aviscumine at doses from 0.3 to 150 ng/kg showed antimetastatic activity against two intravenously inoculated syngeneic sarcoma cell types (RAW 117-H10P lymphosarcoma, L-1 sarcoma) and revealed a significant overall prolongation of survival after lower doses than those administered in immunosuppressed animals. Immunophenotyping of the peripheral leukocytes of treated mice revealed the increased numbers of T-lymphocytes, NK cells and activated monocytes.[7] Administration of aviscumine (i.p.) at doses from 30 to 500 ng/kg into human ovarian cancer-bearing severe combined immunodeficient (SCID) mice led to a significant prolongation of survival.[6]

Toxicology studies showed that aviscumine can be safely applied in rodents and dogs and lacks genotoxic or mutagenic effects up to dosages of 1000 ng/kg i.v. Repeated i.v. or s.c. doses did not reveal any specific target organ toxicity in rats and dogs up to 1000 ng/kg. Reversible local injection site reactions were observed at concentrations above 50 ng/ml.

In conclusion, the preclinical data indicate that subcutaneous injection of aviscumine is a feasible application route for low doses of aviscumine. Therefore, in addition to intravenous application,[16,17] this tolerability and safety phase I trial of subcutaneous administration of aviscumine was performed in patients with refractory solid tumours.

2. Patients and methods

2.1. Inclusion criteria

Twenty six patients with progressive, measurable solid tumours were enrolled between October 2000 and October 2002. All the patients were aged at least 18 years and had a histologically or cytologically confirmed diagnosis of advanced solid tumours, refractory to standard therapies, but with a predicted life expectancy of 3 months or more. Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant 2$, no central nervous system involvement, adequate bone marrow function (white blood count $> 3 \times 10^9$/L, neutrophils $> 1.5 \times 10^9$/L, platelets $> 100 \times 10^9$/L), normal liver (serum bilirubin within 1.5 times the upper limit of normal (ULN), serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase < 2.5 times ULN) and renal function (serum creatinine < 120 μmol/l). Patients with previous exposure to mistletoe preparations were excluded. Prior to chemotherapy, hormone and/or radiation treatment had to be completed at least 4 weeks prior to trial enrolment. Patients were not allowed to receive any immunostimulating substances, biological response modifiers, colony stimulating factors, systemic steroids or monoclonal antibodies. Patients had to use effective contraception if of reproductive potential and were excluded if they were pregnant or lactating.

The trial protocol was approved by the European organisation for research and treatment of cancer (EORTC) Protocol Review Committee, the local Ethics Committees and the regulatory authorities, and the trial was conducted and monitored according to International Conference on Good Clinical Practice Harmonisation (ICH-GCP) guidelines. A written informed consent was obtained from all the patients according to Norwegian and German laws and regulations.

2.2. Trial drug formulation and administration

Aviscumine injection solution was supplied in 1 ml vials by the sponsor (VISCUM AG, Bergisch Gladbach, Germany) and kept in the hospital at 2–8 °C. The drug was formulated as a pyrogen free, phosphate-buffered saline at pH 8.0 with polysorbate 80 and glutamic acid. 0.1 ml of the injection solution was administered per 10 kg body weight by s.c. injection. Aviscumine was given twice weekly subcutaneously at varying injection sites. One cycle was defined as three consecutive weeks of treatment (six injections). Patients were continuously treated without interruption between the cycles. After each cycle the safety and tolerability of the treatment were assessed. Patients with progressive disease were removed from the trial, patients achieving stable disease (SD) or an objective response were allowed to remain on treatment until progression. Supportive care was at the discretion of the investigator.

2.3. Cohorts and treatment doses

Based on the preclinical data, a starting dose of 0.2 ng/kg body weight (BW) was considered to be safe and immunomodulatory active in men. Initially, dose escalation steps involved 1 patient per dose level as long as toxicity did not exceed common toxicity criteria (CTC), version 2.0, grade 1. Patient inclusion was blocked until potential toxicity of patients from the previous dose group within the first cycle was fully documented. When moderate (CTC grade 2) drug-related toxicity or a dose-limiting toxicity (DLT $\geqslant$ CTC grade 3) was observed, dose escalation was stopped until three more patients had been included at the same dose. If 2 patients out of 3 experienced DLT, the maximum-tolerated dose (MTD) was reached and further dose escalation stopped. If 1 out of 3 patients experienced a DLT, up to 6 patients had to be included. If 2 patients or more out of 6 experienced DLT, the MTD was reached. According to toxicological data, it was expected that the MTD for the s.c. injection would be determined by the local side reactions.

DLTs were defined as any non-haematological grade 3–4 drug-related toxicity according to common toxicity criteria (CTC), version 2.0, with the exclusion of nausea and vomiting, an absolute neutrophil count < 500/μl lasting for $\geqslant$7 days, febrile neutropenia (defined as an absolute neutrophil count < 500/μl lasting for $\geqslant$3 days associated with fever $\geqslant$38.5 °C for 24 h) or thrombocytopenia grade 4.

Dose steps initially planned were 0.2, 0.4, 0.8, 1.6, 2.4, 3.2 and so on (+0.8 ng/kg per further step). As the treatment was tolerated well and the relative increments between the levels were assumed to become too small the protocol was amended. From 7.2 ng/kg onwards the dose levels 8.5 and 10.0 ng/kg BW were chosen.

2.4. Pharmacokinetics, anti-aviscumine antibodies and cytokines

Plasma samples from 4 patients were collected for pharmacokinetic analysis prior to 1st and 11th injection (after 5 weeks) as well as 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after the injection of aviscumine on both treatment days. Pharmacokinetic analyses were performed using an immuno-polymerase chain reaction (PCR) method[18] by Chimera Biotech GmbH, Dortmund, Germany.

A possible induction of anti-aviscumine antibodies was measured in patient's plasma before the start of the treatment and 15 min prior to each treatment cycle (i.e. every 3 weeks) until the last application. Analysis was performed centralised at PHAST GmbH, Homburg/Saar, Germany. Anti-aviscumine antibodies were determined by using a Sandwich enzyme-linked immunosorbent assay (ELISA), with the captured anti-aviscumine B-chain antibody, aviscumine antigen, dilutions of plasma in phosphate-buffered saline (1:100, 1:1000, 1:10,000), a conjugated antihuman immunoglobulin M (IgM) POD from goat (Sigma A 0420) or a conjugated antihuman immunoglobulin G (IgG) POD from goat (Fc specific, Sigma A 0170).

Cytokines (IL-1β, IL-6, IL-10, F-γ, TNF-α) were measured by the commercially available ELISA kits (test kits Pharmingen, OPTEIA, Becton & Dickinson). Plasma samples were taken and immediately frozen at the 1st and at the11th application day immediately before injection as well as 1 h, 4 h, 8 h and 24 h after injection. The central analysis was performed at the laboratory facilities of the Department of Internal Medicine, Innsbruck Medical University, Innsbruck, Austria.

2.5. Response criteria

Tumour response was assessed according to response evaluation criteria in solid tumours (RECIST).[19] Initial assessment of disease (including the measurement of all target lesions) took place within two weeks prior to the administration of the first treatment. Follow-up assessments were performed after every six weeks (two cycles) until disease progression.

3. Results

3.1. Patient characteristics

Twenty six patients (21 male and 5 female) with solid tumours were recruited to the trial. The majority of patients were pretreated with surgery, chemotherapy and radiotherapy. All the patients who had documented progressive disease were eligible and assessable for safety and response. The patient characteristics are presented in Table 1. The ECOG performance status was 0–1 in all the patients. The number of doses given to each patient is shown in Table 2.

3.2. Toxicity

All the patients were evaluated for toxicity. In general, aviscumine was well tolerated at the doses studied and there were no grade 3/4 toxicities that were definitely attributed to the drug. The most frequent clinical toxicity was a local injection site reaction (erythema, pain and induration) which occurred in the majority of patients above doses $\geqslant$4.8 ng/kg and de-

Table 1 – Patient characteristics

Gender (no. of patients)	
Female	5
Male	21
Age (years)	
Median	56
Range	23–77
Eastern Cooperative Oncology Group, ECOG PS (no. of patients)	
0	13
1	13
Primary tumour (no. of patients)	
Colorectal cancer (CRC)	10
Soft tissue sarcoma (STS)	6
Melanoma (MM)	5
Renal cell cancer (RCC)	2
Lung cancer	2
Stomach cancer	1
Previous treatment (no. of patients)	
Surgery	23
Chemotherapy	17
Radiotherapy	9
Immunotherapy	2

Table 2 – Treatment administration

	No. of patients
No. of cycles per patient	
1	2
2	13
3	1
4	3
5	1
6	3
9	2
13	1
No. of patients per dose level (ng/kg)	
0.2	1
0.4	1
0.8	1
1.6	1
2.4	1
3.2	1
4.0	1
4.8	3
5.6	4
6.4	3
7.2	3
8.5	3
10.0	3
Reasons for discontinuation	
Disease progression	25
Impaired general health	1

creased in intensity with treatment duration. Other frequent side-effects were fatigue, fever and nausea. With one exception of hypertension grade 3 in cycle 2, the observed related toxicities were all grade 1/2 events (Table 3). Haematological toxicity was not observed. Serum chemistry (biochemistry) abnormalities in cycle 1 are shown in Table 4; an attribution to the drug was not assessable. There was no evidence of cumulative toxicity in the patients continuing treatment beyond the first treatment cycle. Dose-limiting events were not observed up to a dose of 10 ng/kg. The MTD was not reached. The majority of patients discontinued the treatment due to disease progression. One patient was taken off the trial due to impaired general health (see Table 2).

Table 3 – Non-haematological toxicity (maximum-related toxicity in cycle 1, no. of patients)

Grade common toxicity criteria (CTC)	1	2	3	4	1–4
Chest pain	1	1	0	0	2
Chills	6	0	0	0	6
Constipation	0	1	0	0	1
Diarrhoea	1	0	0	0	1
Dizziness	1	0	0	0	1
Fatigue	8	2	0	0	10
Fever	5	1	0	0	6
Flushing	1	0	0	0	1
Headache	5	1	0	0	6
Hypertension	1	0	0	0	1
Hypotension	1	0	0	0	1
Injection site reaction	9	10	0	0	19
Itching	1	0	0	0	1
Nausea	1	1	0	0	2
Vomiting	1	0	0	0	1

Table 4 – Laboratory (Biochemistry) abnormalities in cycle 1 (attribution to the drug not assessed; no. of patients)

Grade CTC	1	2	3	4	1–4
Aspartate aminotransferase (ALAT)	4	1	0	0	5
Alkaline phosphatase	5	5	0	0	10
Alanine aminotransferase (ASAT)	7	1	0	0	8
Bilirubinemia	1	0	0	0	1
Creatinine	2	0	0	0	2
Hypercalcemia	1	0	0	1	2
Hypoalbuminemia	10	0	0	0	10
Hypocalcemia	7	0	0	0	7
Hypokalemia	2	0	0	0	2
Hyponatremia	1	0	1	0	2

3.3. *Pharmacokinetics*

Analyses of aviscumine content were performed in plasma samples of patients numbers 9, 11, 24 and 25 (doses were 4.8, 5.6, 10.0 and 10.0 ng/kg, respectively). The data obtained indicated the presence of aviscumine and a decrease in its concentration during the monitored time as a qualitative result. However, the exact concentrations could be only interpreted as approximate values because the concentrations were below the lower limit of valid quantification (30 pg/ml). Therefore, further samples were not analysed and pharmacokinetic parameters could not be calculated.

3.4. *Antibodies to aviscumine*

Twice weekly s.c. injection of aviscumine induced production of antibodies of IgM and IgG classes. Six patients (patients nos. 6, 8, 18, 21, 22 and 24) developed antibodies of IgM class at a level of 100–600 (optical density value * dilution factor). Fourteen patients showed an induction of IgG antibodies. The low titres did not increase or decrease with treatment duration or with dose. The clinical relevance of these antibodies is unclear. With the exception of patients nos. 9 and 14, all the patients with SD showed this antibody induction (median titer: 1500; range: 370–10,200).

3.5. *Cytokine concentrations*

Relatively more patients showed increased IL-1β, IFN-γ and TNF-α plasma levels after the 11th injection as compared to the first injection. Because of the relatively low patient number in this phase I trial statistically significant differences were not defined though trends could be observed (Table 5). Additionally, the plasma levels of these cytokines were higher after 11th compared to the 1st injection (Fig. 1). A significant decrease was found for IL-6 after 11th injection compared to the first application ($p < 0.05$). Also for IL-10 a decrease could be seen. The increase in IL-1β and IFN-γ after 11th compared to the 1st injection was associated with SD. IL-1β and IFN-γ release demonstrated highest values (% increase from baseline)

Table 5 – Increases in cytokine variables (n = number of patients (%)) after 1st and 11th injection

	Day		McNemar test (*p*-value)
	1 (n = 26)	11 (n = 20)	
Interleukin (IL)-1β	10 (38.5%)	9 (45.0%)	1.000
IL-6	20 (76.9%)	8 (40.0%)	0.089
IL-10	11 (42.3%)	5 (25.0%)	0.545
Interferon (IFN)-γ	7 (26.9%)	7 (35.0%)	0.727
Tumour necrosis factor (TNF)-α	5 (19.2%)	6 (30.0%)	0.625

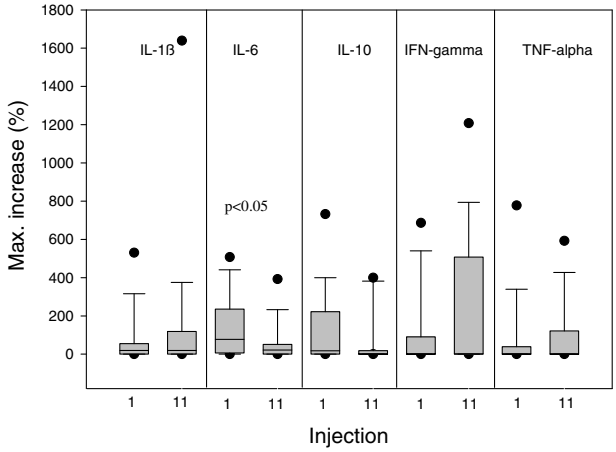

Fig. 1 – Relative maximal increase (% from baseline) in cytokine variables after 1st and 11th injection. Data are presented as interquartile ranges (boxes; $x_{0.75} - x_{0.25}$). The boundary of the box at zero indicates the 25th percentile, the line within the box marks the median and the boundary of the box farthest >from zero indicates the 75th percentile. Whiskers (bars) above the boxes indicate the 95th percentiles. Outliers are shown as dots.

in the dose range between 4 and 5.9 ng/kg. No stimulation was found after doses ⩾6 ng/kg. Due to the low patient numbers and high variability of the data, these findings were not statistically significant. For IL-1β, IFN-γ and IL-6 a bell-shaped curve was observed regarding the maximal stimulation. The OBAD assessed with regard to IL-1β and IFN-γ is in the mean dose range between 4 and 5.9 ng/kg.

3.6. *Efficacy*

Whilst tumour response was not a primary endpoint of the trial, stabilisation of disease was observed. Tumour evaluation (RECIST) was done every other cycle. The best overall response during the conduct of the trial was SD for 3–13 cycles (median: 122 days; range: 79–250): 5 out of 10 patients with colorectal cancer (median: 122 days, range: 87–250), 1 out of 2 patients with kidney cancer (80 days), 1 out of 6 patients with soft tissue sarcoma (79 days), 1 out of 5 patients with melanoma (128 days); the longest duration of SD (250 days) occurred in a 67-year-old man with liver, lung and lymph node metastases from colorectal carcinoma. There were no minor, partial or complete responses.

4. Discussion

In the preclinical models, aviscumine induces apoptosis of tumour cells at low (fM to pM) and necrosis in higher concentration ranges.[1,4,20] The mean aviscumine concentration required to inhibit tumour cell growth *in vitro* by 70% is 0.4 ng/ml and 2 ng/ml for 70% inhibition of tumour xenograft colony formation.[15] Aviscumine (i.p., s.c. and i.v.) exhibits growth inhibition in various heterotopic tumour and metastasis mouse models including the C8 colon 38-carcinoma-, Lewis lung sarcoma-, Renca renal carcinoma-, F9 testicular carcinoma-, RAW 117-H10P lymphosarcoma-, L-1 sarcoma- and the B16 melanoma model.[7,15] In the RAW 117-H10 P lymphosarcoma and the L-1 sarcoma model also a prolongation of survival is achieved by aviscumine.[7]

Based on the preclinical findings, we performed a phase I trial with subcutaneous application of aviscumine in patients with a histologically or cytologically confirmed diagnosis of progressive malignant solid tumours. Twice weekly s.c. injection of aviscumine was found to be safe up to a dose of 10 ng/kg BW and was well tolerated. After doses of ⩾4.8 ng/kg local reactions at injection sites occurred in almost every patient and decreased in intensity with treatment duration. DLT was not observed up to a dose of 10 ng/kg. MTD was not reached as the dose was not further escalated, because an OBAD had been seen at a dosage of 4–5.9 ng/kg.

After s.c. injection of maximally 10 ng/kg pharmacokinetic analysis revealed plasma concentrations below the lower limit of quantification. Therefore, pharmacokinetic parameters could not be calculated.

Injection of aviscumine induced the production of low titres of antibodies of IgM and IgG classes. At dosages from 6.4 ng/kg upwards the majority of patients showed an induction of anti-aviscumine antibodies of IgG class. The titres did not vary significantly with dose or with treatment duration and are of unknown clinical relevance.

Aviscumine injection induced an increase of IL-1β, IL-6 and IFN-γ plasma levels. Characteristic for the s.c. treatment was an increase of IFN-γ plasma concentrations between 1st and 11th injection. Furthermore, comparing the number of patients showing an IFN-γ response relatively more patients react after the 11th injection. The IFN-γ response indicated Th1-cell stimulation by aviscumine. The generation of cytokines in human monocytes correlated with the induction of apoptosis.[15,20] In this trial, aviscumine was found to induce the secretion of IL-1β and IFN-γ in plasma with the highest values in the dose range of 4–5.9 ng/kg. The most interesting clinical effect is the stimulation of T-cells that might mediate an *in vivo* antitumour T-cell response. Further studies need to investigate whether this effect is related to the binding of the drug to the CD75s epitope on CD8+ suppressor cells or to possible other mechanisms.

The best response (RECIST) was SD in 8 out of 26 patients (30.8%) lasting for in median 122 days (range 79–250 days; 3–13 cycles). The absence of progression had been seen in 23.1% of the patients after 3 months treatment. Stabilisations lasting longer than 4 months were seen in patients with melanoma (1 out of 5 patients lasting for 128 days) and with colorectal carcinoma (5 out of 10 patients lasting for 122 days, range 87–250 days). In the case of heavily pre-treated patients with

colorectal carcinoma, 5 patients out of 10 (50%) showed a progression-free rate at 3 months. It is interesting to note that the release of IL-1β and IFN-γ was associated with SD. With the exception of 2, all patients with SD showed IgG antibody induction.

The recommended dose for phase II is based both on the clinical benefit and on the OBAD estimated to be in the dose range of 4–5.9 ng/kg. SD was achieved in 9 out of 26 patients with 4.8 ng aviscumine/kg (median, range 0.2–10.0 ng/kg).

In conclusion, based on our phase I results, phase II trials with a recommended dose of 5 ng/kg and further investigations regarding the immunomodulatory effects of aviscumine are in preparation.

Conflict of interest statement

None declared.

Acknowledgments

The authors like to thank the study nurses Ingun Flatland Sve and Lone Hegg for their very valuable contribution to the trial.

REFERENCES

1. Langer M, Möckel B, Eck J, Zinke H, Lentzen H. Site-specific mutagenesis of mistletoe lectin: the role of RIP activity in apoptosis. *Biochem Biophys Res Commun* 1999; **264**(Suppl. 5):944–8.
2. Endo Y, Tsurugi K, Franz H. The site of action of the A-chain of mistletoe lectin I on eukaryotic ribosomes The RNA N-glycosidase activity of the protein. *FEBS Lett* 1988;**231**:378–80.
3. Eck J, Langer M, Möckel B, et al. Cloning of the mistletoe lectin gene and characterization of the recombinant A-chain. *Eur J Biochem* 1999;**264**:775–84.
4. Eck J, Langer M, Möckel B, Witthohn K, Zinke H, Lentzen H. Characterization of recombinant and plant-derived mistletoe lectin and their B-chains. *Eur J Biochem* 1999;**265**:788–97.
5. Hajto T, Hostanska K, Weber K, et al. Effect of recombinant lectin Viscum album agglutinin on the secretion of interleukin-12 in cultured human peripheral blood mononuclear cells and on NK-cell-mediated cytotoxicity of rat splenocytes in-vitro and in-vivo. *Nat Immun* 1998;**16**:34–46.
6. Schumacher U, Feldhaus S, Mengs U. Recombinant mistletoe lectin (rML) is successful in treating human ovarian cancer cells transplanted into severe combined immunodeficient (SCID) mice. *Cancer Lett* 2000;**150**:171–5.
7. Schaffrath B, Mengs U, Schwarz T, et al. Anticancer activity of rViscumin (recombinant mistletoe lectin) in tumour colonization models with immunocompetent mice. *Anticancer Res* 2001;**21**:3981–8.
8. Hostanska K, Vuong V, Rocha S, et al. Recombinant mistletoe lectin induces p53-independent apoptosis in tumour cells and cooperates with ionising radiation. *Br J Cancer* 2003;**88**:1785–92.
9. Elsässer-Beile U, Ruhnau T, Freudenberg N, Wetterauer U, Mengs U. Antitumoral effect of recombinant mistletoe lectin on chemically induced urinary bladder carcinogenesis in a rat model. *Cancer Lett* 2001;**91**:998–1004.
10. Müthing J, Burg M, Möckel B, et al. Preferential binding of the anticancer drug rViscumin (recombinant mistletoe lectin) to terminally α2-6-sialylated gangliosides. *Glycobiology* 2002;**12**:485–97.
11. Müthing J, Meisen I, Bulau P, et al. Mistletoe lecting I is a sialic acid-specific lectin with strict preference to gangliosides with terminal neu5 Acα2-6Galβ1-4GlcNAc residues. *Biochemistry* 2004;**43**:2996–3007.
12. Zimring JC, Levery SB, Kniep B, et al. CD75s is a marker of murine CD8+ suppressor cells. *Int Immunol* 2003;**15**:1389–99.
13. Schwarz T, Joller PW, Möckel B, Weyhenmeyer R, Lentzen H, et al. Recombinant lectin rML: a novel inducer of cytokines in vitro. *Eur J Cancer* 1997;**33**(Suppl. 5):S16.
14. Hostanska K, Hajto T, Fischer J, et al. Selective modulation of phosphatidylserine exposure on subpopulations of human peripheral blood lymphocytes by a plant lectin, Viscum album agglutinin (VAA)-I and its recombinant form (rVAA) in vitro. *Cancer Detect Prev* 1999;**23**:511–23.
15. Burger AM, Mengs U, Schüler JB, Zinke H, Lentzen H, Fiebig HH. Recombinant mistletoe lectin (rML) is a potent inhibitor of tumor cell growth in vitro and in vivo. *Proc Am Assoc Cancer Res* 1999;**40**:2639.
16. Schöffski P, Riggert S, Fumoleau P, et al. Phase I trial of aviscumine (rViscumin) in patients with solid tumours: a study of the European Organisation of Research and Treatment of Cancer New Drug Development Group. *Ann Oncol* 2004;**15**:1816–24.
17. Schöffski P, Breidenbach I, Krauter J, et al. Weekly 24 h infusion of aviscumine (rViscumin): a phase I study in patients with solid tumours. *Eur J Cancer* 2005;**41**:1431–8.
18. Adler M, Langer M, Witthohn K, Eck J, Blohm D, Niemeyer CM. Detection of rViscumin in plasma samples by immuno-PCR. *Biochem Biophys Res Commun* 2003;**300**:757–63.
19. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;**92**:205–6.
20. Möckel B, Schwarz T, Eck J, Langer M, Zinke H, Lentzen H. Apoptosis and cytokine release are biological responses mediated by recombinant mistletoe lectin in vitro. *Eur J.Cancer* 1997;**33**(Suppl. 5):S35.