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Review

The preclinical and clinical activity of aviscumine: A potential anticancer drug

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ABSTRACT

Extracts from the European mistletoe plant *Viscum album* have been studied for decades for their direct and indirect anticancer activity. Therefore, scientists were interested in identifying the active compound (mistletoe lectin) in these extracts and making it available as a highly purified molecule for drug development. Recombinant mistletoe lectin (INN: aviscumine) was produced in *Escherichia coli*. It has been shown to have immunomodulatory and cytotoxic activity in *in vitro* and in animal models and can target tumour cells. Clinical phase I studies also demonstrated immunomodulatory activity, which appears to have a positive effect on disease stabilisation. This review explores the current knowledge base for aviscumine's mechanism of action, efficacy and side-effects in both preclinical studies and clinical trials, and it considers aviscumine's potential as a cancer therapy.

1. Introduction

Extracts from European mistletoe *Viscum album* have been used as complementary or alternative cancer therapies for decades based on their presumed immunostimulatory and antineoplastic effects.[1–6] There has been some interest in identifying the active compound (mistletoe lectin) in these extracts and making it available as a highly purified molecule for drug development. Recombinant mistletoe lectin (INN: aviscumine) was produced in *Escherichia coli*. Aviscumine is now in large-scale production, allowing investigations of its structure, mechanism of action, toxicity profile and efficacy. This review focuses on studies related specifically to the recombinant protein aviscumine and its potential utility in cancer therapies.

Aviscumine is a 57-kDa heterodimer composed of a toxic A-chain, which is a site-specific type II ribosome-inactivating *N*-glycosidase[7], and a carbohydrate-binding subunit B that is responsible for cellular uptake[8–10] (Fig. 1). Aviscumine selectively cleaves the *N*-glycosidic bond of the adenine-4324

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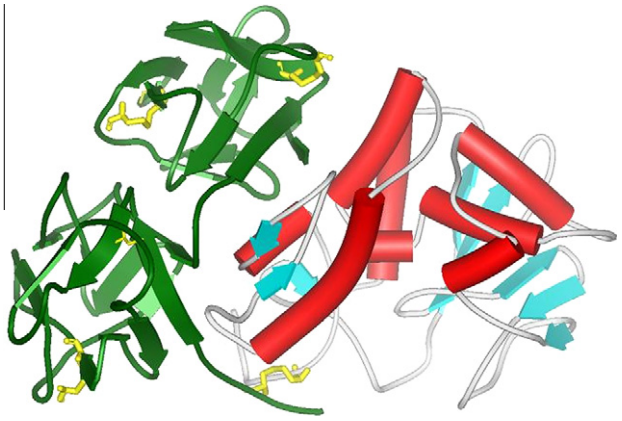

Fig. 1 – Structure of aviscumine. The B-chain is represented by green β-sheets, the A-chain by red α-helices and the β-sheets by pale green.

residue in eukaryotic 28S ribosomal RNA, leading to a catalytic inactivation of ribosomes that causes the inhibition of translation and protein synthesis. Aviscumine's ability to debilitate 28S ribosomal RNA does not depend on a specific point in the cell cycle or the proliferative state of the target cell.[11]

The cell-surface structure involved in aviscumine preferential binding, CD75s, is an alpha-2,6-sialyllactosamine.[12,13] CD75s structures are expressed due to increased alpha-2,6-sialyltransferase activity. CD75s is expressed predominantly on activated B-, T-, and immature dendritic cells (DCs), macrophages, monocytes and granulocytes[14–16], but it can be found upregulated in haematological cancers[17,18] and has been shown to be present or to be upregulated in cells in solid tumours compared with normal tissue.[19–25] Studies of aviscumine binding in human adenocarcinoma of the lung found that most of the adenocarcinomas (86 out of 93, or 92.5%) bound aviscumine, while only 7.5% ($n = 7$) did not express aviscumine-binding sites.[26] Thus, aviscumine is likely to bind and be taken up by cells via CD75s receptor.

2. Aviscumine's mechanism of action: cytotoxic and immunomodulatory activity

Preclinical studies have shown aviscumine to have antineoplastic and immunomodulatory properties both *in vitro* and *in vivo*.

2.1. *Cytotoxic activity*

Aviscumine exerts significant cytotoxic activity similar to that of plant lectin, and it induces apoptosis in a low concentration range (fM to pM) and necrosis in higher concentration ranges.[10,27–34] The mechanism of aviscumine-mediated cell death has been studied in multiple cell types; the rate of apoptosis was directly correlated to the A-chain activity, revealing that apoptosis is induced solely by the toxic A-chain.[7,31] The mechanism appears to be independent from the death receptor *Fas* because apoptosis could be induced in *Fas*-deficient cells.[35]

Aviscumine's cytotoxic activity appears to be independent of mechanisms of irradiation resistance. In radiation-sensitive human colon adenocarcinoma cells, combined treatment with irradiation and aviscumine resulted in an additive inhibition of proliferation.[29] In drug-resistant pancreatic carcinoma cells (CAPAN), aviscumine induced apoptosis in a manner independent of the activity of the antiapoptotic transcription factor NFκB (data not shown). This evidence indicates that certain tumour cells with deficiencies in some of the common pro- or anti-apoptotic regulators (*Fas*, p53, NFκB) are susceptible to aviscumine, which suggests a possibility for using aviscumine to treat tumour cells with resistance to other apoptotic anticancer drugs.

On a molar basis, aviscumine is approximately 5000 times more potent than the standard therapeutic agent adriamycin against human tumour cell lines and 1500 times more potent than paclitaxel. Independent of multidrug resistance mechanisms, it is more active in doxorubicin-resistant breast tumour cells and vindesine-resistant pleural mesothelioma cells than in the respective drug-sensitive parental cell lines. Prolonged incubation with aviscumine reduces the amount of drug needed for the same cytotoxic effect.[36–40] Treatment with aviscumine (intraperitoneal, subcutaneous and intravenous) inhibits growth in various syngeneic and heterotopic tumour and metastasis mouse models, including C8 colon 38-carcinoma, Lewis lung sarcoma, Renca renal carcinoma, F9 teratocarcinoma, RAW 117-H10 P lymphosarcoma, L-1 sarcoma and the B16 melanoma model.[36,41,42] In the RAW 117-H10 P lymphosarcoma and the L-1 sarcoma models, prolonged survival is achieved by administering aviscumine in a very low dose range (ng/kg). In rats, a significant inhibitory effect on experimental urothelial carcinogenesis is seen after intravesical instillation.[43]

Aviscumine binds to natural killer (NK) cells, granulocytes, and monocytes.[28] NK-mediated cytotoxicity is also stimulated *in vitro*, implicating aviscumine's immunomodulatory activity in its anticancer properties.[44]

2.2. *Immunologic activity*

Numerous immunological factors are influenced by the administration of aviscumine *in vitro* in a variety of cell types (Table 1). The secretion of interleukin-12 (IL-12) and its active heterodimeric form, p70, were significantly induced by aviscumine in human peripheral blood mononuclear cells (PBMC) in culture.[44] Interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) are also released from PBMC.[44,45] Interleukin-1α (IL-1α) and IL-6 are induced in human keratinocytes and fibroblasts in the presence of aviscumine, and the expression of the IL-2 receptor alpha chain (CD25) and HLA-DR on peripheral blood T lymphocytes (both activated T-cell markers) are upregulated.[44,45]

Enhanced NK-mediated cytotoxicity is apparent in PBMC and rat splenocytes in the presence of aviscumine; this cytokine-induced NK activity and the presence of activated monocytes are also apparent *in vivo*.[44] Aviscumine increases the activity of human NK cells against lymphoma cells *ex vivo*,[43,44] and the cytotoxicity of human NK cells against human cancer cells *in vitro* is also increased.[44] In tumour-bearing mice, it enhances the number or activates

Table 1 – Cellular responses to aviscumine.

Cell type	Parameter	Effect	References
Mouse L-1 sarcoma cells (*in vitro*)	Effect of aviscumine	Cytotoxic	41
Mouse RAW-117-P sarcoma cells (*in vitro*)	Effect of aviscumine	Cytotoxic	41
RAW-117-P bearing mice (*in vivo*)	Peripheral blood leucocyte counts	Enhanced	41
	T-cells ($CD3^+45^+$/μl)	Enhanced	41
	NK^+/MAC-1^+/$CD45^+$/μl	Enhanced	41
	MAC-3^+/$CD45^+$/μl	Enhanced	41
Rat splenocytes (*in vitro*)	NK cytotoxicity against YAC-1 target cells	Enhanced	44
Rat large granular lymphocytes (LGL) (*in vivo*)	Number of LGL	Enhanced	44
Rat MAC-1^+ (*in vivo*)	Percentage of MAC-1^+	Enhanced	44
Human keratinocytes (HaCaT cell line) (*in vitro*)	Secretion of interleukin-1α (IL-1α)	Enhanced	45
	Secretion of IL-15	Enhanced	45
	Expression of IL-6 mRNA	Enhanced	45
Human peripheral blood mononuclear cells (PBMC) (*in vitro*)	Secretion of IL-6	Enhanced	44
	Secretion of TNF-α	Enhanced	44
	Secretion of IL-12, IL-12p70	Enhanced	44
	Secretion of IFN-γ	Enhanced	45
	Secretion of IL-1β	Enhanced	45
	Expression of CD25	Enhanced	45
Human monocytic tumour cell line (THP-1 cells) (*in vitro*)	Secretion of IL-1β	Enhanced	45
	Expression of IL-1β mRNA	Enhanced	45
	Expression of IL-6 mRNA	Enhanced	45
	SAPK/JNKinase	Activation	Not published
	p38 MAP kinase	Activation	Not published
Human ovarian carcinoma cells (SKOV-3) (*in vitro*)	SAPK/JNKinase	Activation	11
	p38 MAP kinase	Activation	11
	p42/44 MAP kinase	Activation	11
Human monocytic tumour cell line (HL-60 cells) (*in vitro*)	Expression of IL-1β mRNA	Enhanced	45
	Expression of IL-6 mRNA	Enhanced	45

leucocyte subpopulations (T-lymphocytes and activated monocytes).[41]

The intracellular pathway involved in immunomodulation by aviscumine has also been examined. Aviscumine activates the cascade of MAP kinase pathway p38, Erk1/2 and SAP/JNK in SKOV-3 cells in a manner that depends on the HER-2 level,[11] and it activates caspases and two MAP-kinase pathways (p38 and SAPK/JNK) in keratinocytes *in vitro*. These pathways appear to affect both cytokine release and direct cytotoxic activity induced by aviscumine. Aviscumine-triggered apoptosis depends on both the caspase and the MAPK pathway. IL-1β is released from monocytes as a result of aviscumine treatment through the activation of caspase-1. The transcript for IL-1β is downregulated by both caspase-3 inhibitor Z-VAD-fmk and p38 inhibitor SB203580 (data not shown). Although the affinity of aviscumine to lymphocytes was found to be relatively low, the activation of T-lymphocytes by aviscumine can be observed *in vivo*.[41]

These immunomodulatory effects of aviscumine, demonstrated both *in vitro* and *in vivo*, may contribute to its indirect antineoplastic properties through activation of the host immune response to cancerous cells.

3. Preclinical development: *in vitro* and *in vivo* studies

Aviscumine is particularly active in breast, lung, renal, colon, prostate, melanocytic and ovary tumour panels of the cell line screening model and in breast, lung, gastric, pancreas and testicular tumour panels in the xenograft screening model. The mean IC_{70} against the growth of tumour xenograft *in vitro* models was 2 ng/mL. Growth inhibition (GI_{50}) at 1 ng drug/mL (20 pM) in the antiproliferative activity screen was seen against 20 human tumour cell lines (Freiburg screen).[37–40,46] Interestingly, tumour cells with resistance to other apoptosis-inducing anticancer drugs that are based on deficiencies in some of the common pro- or anti-apoptotic regulators (*Fas*, p53, NFκB) are susceptible to aviscumine (data not shown). The sensitivity profile of the drug determined in the panel's cell lines derived from nine different tumour entities was unique; no similarity was observed in comparison with the set of standard anticancer agents in a National Cancer Institute (NCI) database. A comparison of the drug profile with the molecular targets database revealed that the mode of action of aviscumine does not depend on the expression of the common targets for anticancer drugs and is not related to any common molecular mechanism (e.g. oncogenes, mutations) (data not shown).

Intraperitoneal (i.p.), subcutaneous (s.c.) or intravenous (i.v.) administration of aviscumine inhibits growth in various heterotopic tumours and mouse models of metastasis.[36,41,42] Aviscumine increases the likelihood of survival for mice with syngeneic orthotopic urinary bladder tumours after intravesical instillation, and it significantly decreases the number of the animals with tumours or metastases.[43] In immunodeficient mice, intratumoural administration into human ectopic

Table 2 – Growth inhibitory effects of aviscumine in animal models after intraperitoneal (i.p.) injection[36,41,42,46].

Syngeneic murine model	Dose (ng aviscumine/kg/d)	%T/C[a]
C8 colon 38-carcinoma	1000	32
	300	34
	30	52
Renca renal carcinoma	3000	18
	300	35
	30	41
Lewis lung carcinoma	3000	12
	300	32
	30	59
F9 teratocarcinoma	3000	1
	300	7
	30	14
Human tumour xenograft model		
LXFS 538 small cell lung cancer	3000	27
	300	74
	30	88
	3	0
A 549 non-small-cell lung cancer (NSCLC)	1500	29
	1000	48
	1500 + 75 mg carbo-Pt	24
	1000 + 75 mg carbo-Pt	25
	75 mg carbo-Pt	37
CXF 280 colon carcinoma[b]	7500	28
	2250	27
	750	54
	250	59
5776 Human colon carcinoma	1500	48
	750	47
	375	70
6044 Human colon carcinoma	1500	28
	750	67
	375	66

[a] $\%T/C = \frac{\text{Median tumour volume in test group} \times 100\%}{\text{Median tumour volume in control group}}$.
[b] After intratumoural injection.

CXF-280 colon xenograft carcinoma and i.p. therapy of LXFS 538 small cell lung cancer led to strong, dose-dependent inhibition of tumour progression.[46] In immunodeficient mice with SoTu3 human ovarian cancer, i.p. administration of aviscumine also led to a significant increase in survival[47] (Table 2).

In an orthotopic murine model, more than 90% of animals survived MB 49 urinary bladder carcinoma. Aviscumine has also exhibited antimetastatic activity against three syngeneic sarcoma cell types in immunocompetent mice.[36,41,42]

In preclinical studies, aviscumine has been found to be safe, with no relevant genotoxic or mutagenic potential up to 1 μg/kg i.v. Repeated i.v. or s.c. dosing did not reveal any specific toxicity in target organs of rats and dogs up to 1000 ng/kg.[48] Toxicity as indicated by bleeding is observed at much higher doses, i.e. at 4000 ng/kg in rats and at 10,000 ng/kg in dogs after 1 week of daily i.v. administration. Local lesions at the injection sites are to be expected at concentrations equal to or greater than 50 ng/mL. The genotoxicity/mutagenicity potential is comparable to that of other vital cytotoxic treatments. Injection-site reactions are observed at concentrations greater than 50 ng/mL but are reversible.

Aviscumine appears to be more active against doxorubicin-resistant breast tumour cells and vindesine-resistant pleural mesothelioma cells than against the respective drug-sensitive parental cell lines, indicating that the drug is not affected by common resistance mechanisms such as MDR mechanisms. In addition, it enhances the cytotoxic effects of vincristine, mafosfamide, idarubicin and cisplatin in the human leukaemia cell lines K562 and KG1a.[49] Moreover, aviscumine exhibited an additive antiproliferative effect with the cytostatic agents adriamycin and cisplatin in the human lung carcinoma cell line A549.[50]

4. Phase I clinical studies of aviscumine

Because solid tumours overexpress CD75s, they have been the focus to test the efficacy of aviscumine. Based on the indirect and direct functions of this agent, two developmental strategies were employed in a phase I trial: i.v. injection of a relatively high dose (μg/kg) to define a maximum-tolerated dose,[51,52] and s.c. application at a lower dose (ng/kg) to define the optimal biologically active dose.[53]

In 2004, a phase I trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) resulted in a recommendation of 5.6 μg/kg i.v. aviscumine for doses in future trials.[51] The 2004 trial, designed to determine dose-limiting toxicity, enrolled patients with refractory solid tumours, predominantly colorectal, ovarian, renal cell and breast cancer. Because aviscumine has a relatively short half-life (13 min), the investigators concluded that the dosing regimen needed to be improved, and as a result the 2004 trial was followed shortly thereafter by a study that analysed prolonged i.v. infusion of aviscumine (24 h once weekly).[52] This study included subjects with advanced, refractory malignant solid tumours, including colorectal cancer (CRC), soft tissue sarcoma and pancreatic cancer. Pharmacokinetic data indicated that potentially active plasma levels of aviscumine were maintained during the entire infusion (5 μg/kg).

In 2008, Bergmann et al. published the results of a phase I clinical trial that considered s.c. administration of aviscumine in patients with a histologically or cytologically confirmed diagnosis of progressive malignant solid tumours.[53] Twenty-six patients received escalating doses of aviscumine as s.c. injection twice weekly, starting with 0.2 ng/kg body weight. Aviscumine has also been tested in patients with superficial bladder cancer in a phase I/II study designed to determine maximum tolerated dose (MTD) via intravesical instillation.[54] The details of these initial human trials of aviscumine to treat cancer are discussed here in terms of toxicity, pharmacokinetics, immunomodulatory activities and efficacy.

4.1. *Toxicity*

Common clinical toxicities with short-term i.v. application have included fever, fatigue, nausea, vomiting, allergic reactions, increased tumour pain, and elevated liver enzymes.[51] The increases in liver enzymes have often been transient. Dose-limiting reversible grade 3 toxicities have included fatigue and liver toxicity.[51] With prolonged infusion of aviscumine, fatigue, fever, nocturia, urticaria, erythema, and

pruritus were found to be the most common side-effects.[52] Dose-limiting toxicities after 6 μg/kg of aviscumine included elevated transaminases and gamma-GT, fatigue, and hypokalaemia.

When aviscumine was given s.c., no grade 3/4 toxicities were definitely attributed to the drug (Common Toxicity Criteria [CTC] version 2.0).[53] The most frequent clinical toxicity was a reaction at the local injection site that occurred in the majority of patients at doses exceeding 4.8 ng/kg and decreased in intensity with duration of treatment. Other frequent side-effects were fatigue, fever and nausea. The s.c. injection of aviscumine was not associated with neutropenia, granulocytopenia or thrombocytopenia and there was no evidence of cumulative toxicity in patients who continued treatment beyond the first treatment cycle. Dose-limiting events were not observed up to a dose of 10 ng/kg and the maximum tolerated dose was not reached. The majority of patients discontinued treatment because of progression of their disease.

4.2. *Pharmacokinetics*

Pharmacokinetic data after 1-h i.v. infusion confirmed previous animal studies.[51] Aviscumine exhibited biphasic features, with the first part of the curve indicating that 85–90% of the drug is most probably rapidly distributed and presumably also degraded due to proteolytic processes. The initial half-life ($t_{1/2\alpha}$) after 1-h i.v. infusion was 13 min (range 3.2–35.5). Plasma levels reached about 27 ng/mL after a dose of 5600 ng/kg,[51] and aviscumine did not seem to accumulate in the plasma or any other site. Preliminary data after the 24-h i.v. infusion showed that plasma levels about 4 ng/mL could be maintained during and up to 1 h after the infusion. Prolonged i.v. infusion of aviscumine resulted in plasma levels greater than 2 ng/mL throughout the infusion in the majority of cases; the initial half-life was comparable to previous results.[52] After s.c. injection of up to 10 ng/kg aviscumine, pharmacokinetic analysis revealed plasma concentrations below the lower limit of quantification (30 pg/mL).[53] Based on these results and those from clinical benefit, the researchers recommended a dose range of 4–5.9 ng/kg for future s.c. phase II trials.

4.3. *Immunologic activity*

With i.v. administration of aviscumine, IL-1β, IL-6 and interferon gamma (IFN-γ) were stimulated independent of dose.[51] When aviscumine was injected subcutaneously, plasma levels of IL-1β, IFN-γ and TNF-α increased, and concentrations of IL-6 and IL-10 decreased after the 11th injection (versus the first dose).[53] IFN-γ response indicates that aviscumine stimulates Th1 cells that may mediate an *in vivo* antitumour T-cell response. Whether this effect is related to aviscumine's ability to bind CD75s on $CD8^+$ suppressor cells or whether other mechanisms are at play is not known. IL-1β, IFN-γ and IL-6 were found to have a bell-shaped response curve to the dose of aviscumine.[53]

Increases in IL-1β and IFN-γ appeared to be associated with stable disease, with the highest expression of these cytokines at doses between 4 and 5.9 ng/kg. No stimulation was found after doses >6 ng/kg. Because of the small number of patients, however, statistically significant differences could not be defined. Furthermore, high intra- and inter-individual variabilities can be assumed. This immune response was observed in a concentration range (calculated from the applied dosage) where apoptotic cell death also occurs *in vitro*. The generation of cytokines in human monocytes was correlated with the induction of apoptosis, implicating this action of aviscumine in both immunomodulatory and cytotoxic activities.[28,31]

4.4. *Antibodies to aviscumine*

IgG or IgM antibodies to aviscumine have been apparent in all clinical trials to date. Of the 26 subjects that received s.c. injection, 20 exhibited expression of either antibody form.[53] The titres were low and did not vary widely with dose or with the duration of treatment. Although the clinical relevance of these antibodies is unknown, with the exception of 2 of the 26 patients, patients with stable disease showed induction of IgG antibody (median titre: 1500; range: 370–10,200).

4.5. *Efficacy*

Although the trials performed to date have not been designed to test efficacy, the antitumour activity was monitored. In the initial i.v.-administered aviscumine trial by EORTC, 11 of 41 participants (26.8%) exhibited stable disease for multiple cycles (median 10 weeks, range 6–27 weeks) in previously progressing patients.[51] When aviscumine was given by prolonged infusion, 4 of 14 participants (28.6%) showed indications of stable disease lasting multiple cycles (4–8) during the course of the trial, ranging from 11 to 21 (median: 12) weeks.[52]

When s.c. injection was tested, stable disease was seen in 8 of 26 patients (30.8%) (5 with CRC, 1 with melanoma, 1 with soft tissue sarcoma, and 1 with kidney cancer) for a median of 122 days (range: 79–250 days), which was associated with increases in plasma IL-1β and IFN-γ.[53] After 3 months of treatment, 23.1% of patients had absence of progression. Disease that was stable for longer than 4 months was seen in patients with melanoma (1 of 5 patients lasting 128 days) and colorectal carcinoma (5 of 10 patients lasting 122 days, range 87–250 days). In the case of heavily pretreated patients with colorectal carcinoma, 5 of 10 patients (50%) remained progression free at 3 months. The longest duration of stable disease (250 days) was seen in a 67-year-old man with liver, lung and lymph node metastases from colorectal carcinoma. The best response (RECIST criteria) was stable disease (SD). Because of the progressive status of the patients before inclusion into the trials, these stabilisations suggest a potential efficacy of the drug and thus may be indicative of aviscumine antitumour activity.

5. Phase I/II clinical trials of aviscumine

Aviscumine has been tested in patients with superficial bladder cancer in a non-randomised trial.[54] This phase I/II study was designed to determine the MTD via intravesical instillation. This dose level was not reached, but 3 of 17 patients experienced complete remissions (18%) of marker lesions, with one remission lasting >36 months. Aviscumine has

Table 3 – Outcome of the phase I trials regarding stable disease by route of aviscumine administration.

Trial	Rate/duration of stable disease (SD) ⩾ 6 weeks	Rate/duration of SD ⩾ 12 weeks	Rate/duration of SD ⩾ 16 weeks	Primary tumour (*n*)
Intravenous (i.v.) (*n* = 41)[52] 1 h Infusion	26.8% Median: 10.0 weeks	12.2% Median: 18.0 weeks	9.8% Median: 19.0 weeks	Colorectal carcinoma (14) Ovarian cancer (6) RCC (6) Breast cancer (5) STS (2) Pancreatic cancer (2) Other (6)
i.v. (*n* = 14)[53] 24 h Infusion	28.6% Median: 12.0 weeks	14.3% Median: 17.1 weeks	7.1% Median: 21.3 weeks	Colorectal carcinoma (7) STS (3) Pancreatic (2) Prostate cancer (1) Urothelial bladder cancer (1)
Subcutaneous (s.c.) (*n* = 26)[54]	30.8% Median: 17.4 weeks	23.1% Median: 18.2 weeks	19.2% Median: 21.1 weeks	Colorectal carcinoma (10) RCC (2) STS (6) Melanoma (5) Lung cancer (2) Stomach cancer (1)

Abbreviations: RCC = renal cell cancer; STS = soft tissue sarcoma.

fewer side-effects compared to the better-studied and most commonly prescribed immunotherapeutic for bladder cancer, Bacillus Calmette-Guerin (BCG).

Intrapleural therapy with aviscumine was assessed in four patients with malignant pleural effusion in a phase I/II trial that tested efficacy and tolerability (data not shown). Doses starting from 6 μg/50 mL up to 48 μg/50 mL (two patients) and up to 60 μg/50 mL (two patients) were well tolerated. Thirty days after completion of therapy, one patient receiving up to 60 μg/50 mL was relapse-free (no recurrent effusion) and the other responded partially (did not require pleural puncture).

6. Clinical dose and administration

In phase I trials, patients experienced prolonged stable disease with aviscumine treatment of solid tumours that were refractory to other treatments (Table 3).[52–54] Studies to date indicate that s.c. injection may be superior to i.v. administration. Therefore, based on the present clinical data, the clinical development of the s.c. route should continue. The adverse events associated with treatment were similar in the two routes, although no grade 3 or 4 toxicities were associated with s.c. administration, and there was no evidence of cumulative toxicity in patients who were treated beyond the first cycle.

7. Future use of aviscumine

Based on preclinical and early-stage clinical research, there is a need for further investigations of aviscumine in a broad range of solid tumours. Phase II trials and further investigations regarding the immunomodulatory effects of aviscumine are in preparation, including studies of progressive patients with unresectable stage IV metastatic melanoma (running phase) and with metastatic CRC after failure of standard therapies (running phase). The primary objective of these studies is to determine the length of progression-free survival, which should help to further understand the role that aviscumine may play in cancer therapeutics.

Clinical trials will have to demonstrate whether aviscumine offers potential benefit for patients with solid tumours that are refractory to other treatments, and whether it may offer patients an option in cases where standard therapies failed. Preclinical and initial clinical data suggest that the cytotoxic and immunomodulatory activities of aviscumine offer a treatment with a unique mode of action that does not appear to succumb to multi-drug-resistance mechanisms.

Further study of aviscumine treatment through s.c. administration is warranted to evaluate its potency as a possible maintenance therapy to extend a stable disease state achieved by the first- or second-line chemotherapy. In principle, aviscumine may hold potential as a combination partner with chemotherapy or other immunotherapeutics such as vaccines. It appears that this protein, originally identified decades ago in mistletoe extracts, could become in its recombinant form a new drug for cancer treatment. It might be speculated whether aviscumine offers an additional option as an immunotherapeutic in cancer patients.

A workshop convened by the NCI in 2007 brought together representatives from academia, industry and the NCI to rank immunotherapeutic agents that are not broadly available for testing in patients with cancer despite substantial evidence of immunologic activity and great potential for use in treating cancer. The result was a well-vetted ranking of 20 agents based on the cumulative knowledge of the broad immunotherapy and cancer research communities to encourage further development through (1) Rapid Access to Intervention Development (RAID) applications for manufacture, (2) NCI distribution of company-manufactured agents, or (3) reinvigoration of pharma/biotech efforts to develop them.[55] We suggest that aviscumine deserves to be considered for inclusion in such efforts to fast-track its clinical development.

Conflict of interest statement

Heinz Zwierzina and Lothar Bergmann: involved in phase II trial as national principal investigators. Heiner Fiebig, Steinar Aamdal, and Patrick Schöffski have no conflict of interest. Klaus Witthohn and Hans Lentzen: employees of CYTAVIS BioPharma GmbH.

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