Med Oncol
DOI 10.1007/s12032-008-9111-x

ORIGINAL PAPER

A phase I clinical study of intratumorally administered VB4-845, an anti-epithelial cell adhesion molecule recombinant fusion protein, in patients with squamous cell carcinoma of the head and neck

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Received: 19 March 2008 / Accepted: 13 October 2008

Abstract VB4-845 is a novel recombinant fusion protein that targets the epithelial cellular adhesion molecule (EpCAM). This initial clinical trial was conducted to determine the maximum tolerated dose of intratumoral injections in patients with advanced squamous cell carcinoma of the head and neck and to assess pharmacokinetics and immunogenicity. Twenty-four patients with advanced, recurrent squamous cell carcinoma of the head and neck received two cycles of five daily intratumoral VB4-845 injections of 20, 40, 80, 130, 200, or 280 μg. The maximum tolerated dose was established to be 280 μg administered daily for 5 days. Common adverse events were pain due to intratumoral injection and reversibly elevated liver enzymes. Of the 24 patients, 15 had detectable blood levels with a mean drug half-life of 4.0 ± 0.3 h. VB4-845 reduced or stabilized tumors in 71.4% of epithelial cell adhesion molecule-positive patients. VB4-845 intratumoral injection therapy was well tolerated and feasible.

Keywords Clinical trials · Immunotoxin · EpCAM · Squamous cell carcinoma of the head and neck · VB4-845

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Introduction

Treatment options for many cancers are increasing as novel therapies are introduced. However, therapies for head and neck cancer, that improve long-term survival in recurrent, metastatic disease without serious toxic side effects are still absent from the treatment regime. Here we present an initial clinical trial for a novel recombinant fusion protein therapy for squamous cell carcinoma of the head and neck (SCCHN), which targets epithelial cell adhesion molecule (EpCAM) and is administered through intratumoral (IT) injection.

SCCHN comprises several distinct soft tissue carcinomas of the oral cavity, pharynx, and larynx that locally metastasize to regional lymph nodes. The estimated new case incidence in 2007 of SCCHN in the US is 45,660 with an estimated 11,210 deaths [1]. With early diagnosis (stage I/II) prognosis is good with a five-year survival rate of 60–80%. However, up to 70% of patients present with advanced disease (stage III or IV), which has only a 10–50% five-year survival rate [2]. Treatment options are limited for patients with recurrent or locally metastatic disease who have failed with first-line treatments of surgery, platinum-based chemotherapy, radiation, or chemoradiotherapy. Second-line chemotherapy and re-irradiation are options; however, the prognosis is usually poor, with serious side effects, and the mean overall survival rates for second-line treatments are very low (103 days) [3]. Newer antibody therapies are in clinical trials; however, their benefit over standard treatment regimes in recurrent SCCHN has yet to be proven [4].

EpCAM, a 40 kD transmembrane glycoprotein, mediates epithelium-specific, Ca^{2+}-independent, homotypic cell–cell adhesions and is expressed by the majority of normal adult epithelial tissues with the exception of

squamous stratified epithelium [5, 6]. In normal epithelial cells, EpCAM expression is localized to the basolateral surface [7]; however, in cancer, cell surface expression becomes more heterogenous [8]. EpCAM's overexpression on a wide variety of adenocarcinomas and squamous cell carcinomas relative to normal tissue expression [8–10] has made it an attractive target for immunotherapy [11]. Overexpression of EpCAM in SCCHN increases invasive potential [12] and has a negative impact on overall survival [13]. Several monoclonal antibody therapies to EpCAM have been developed with many in clinical trials; however, none have been specifically directed to SCCHN [11].

Immunotoxins are an emerging cancer therapy, which combine the specificity of antibody targeting with a toxic molecule such as *Pseudomonas* exotoxin, ricin, or diphtheria toxin [14–17]. Continued efforts to increase specificity and efficacy and reduce systemic toxicity in the treatment of hematologic cancers and some solid tumors have identified immunotoxins as an effective alternative cancer treatment [14]. However, a common side effect with systemic (IV) immunotoxin therapy is vascular leak syndrome (VLS), a non-specific effect on endothelial cells, which culminates in the release of fluids into tissue [18].

The immunotoxin VB4-845 (Proxinium^{TM}) is a recombinant fusion protein constructed through the fusion of a humanized anti-EpCAM single chain variable fragment (scFv) to a truncated *Pseudomonas* exotoxin A ($ETA_{252–608}$) lacking the cell surface binding domain [19]. Specific binding of VB4-845 to the EpCAM antigen on carcinoma cells allows internalization through an endocytic pathway. The activated form of the ETA is released by furin cleavage of a proteolytic site within the recombinant fusion protein (ETA region), resulting in cell death by the inhibition of protein synthesis via ADP ribosylation of elongation factor 2 [20, 21]. The anti-tumoral activity of VB4-845 has been demonstrated in EpCAM-positive human lung, colon, and squamous cell carinoma xenografts in nude mice with strong inhibition of tumor growth and regression of tumors following systemic administration of VB4-845. No effect was seen in EpCAM-negative human tumor xenografts identifying this as EpCAM-specific targeting [19]. Preclinical studies in rats were used to establish human dose regimes with repeated subcutaneous doses up to 77.8 μg/kg having no systemic toxicity [22]. IT-administered therapy is feasible for SCCHN because primary tumors and local metastases often present with accessible tumors for injection. IT administration has proven to be an effective mode of immunotoxin drug delivery in other cancer indications, including metastatic breast and colorectal cancers and malignant melanomas [23]. An added benefit to IT administration over systemic administration is that it avoids potential toxicity due to VLS and reduces the effect of circulating antibodies.

This is a phase 1 trial to assess the safety, tolerability, and pharmacokinetics profile of VB4-845 in a dose escalation study in patients with advanced, recurrent SCCHN using IT injection.

Materials and methods

Patient selection

Patients 18 years of age or older, with histological confirmation of recurrent SCCHN following radiotherapy and/or chemotherapy, were eligible for enrollment. At least one bidimensional, measurable target lesion that was amenable to direct injection was required to be present. Other inclusion criteria were Karnofsky performance status of $\geq$70, estimated life expectancy of more than 3 months, adequate hepatic function, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq$2.5 times the upper limit of normal (ULN) and bilirubin levels $\leq$1.5 times ULN, and serum creatinine $\leq$1.5 times ULN, or creatinine clearance $\geq$60 ml/min. Hematologic values required for inclusion were granulocytes $\geq$1,500/μl, platelets $\geq$100,000/μl, and hemoglobin >8 g/dl. Patients should have received stable doses of analgesic medication for at least one week prior to treatment. Females of reproductive age either had to be medically or surgically incapable of bearing children or had to agree to use a highly effective contraceptive method from the time of screening until 4 weeks after the study ended. Written informed consent was obtained from each eligible patient before any study-related activity was performed. Patients with impending airway obstruction or carotid artery involvement by the targeted tumor were excluded from the study.

Study design and dose escalation

The phase 1 study was an open-label, multicenter, single-arm trial assessing the safety, tolerability, and pharmacokinetics of VB4-845. The drug was administered via intratumoral injection in two treatment cycles; each treatment cycle consisted of daily injections of a single drug concentration for five consecutive days followed by a rest period of 23 days. The ascending dose levels used were initially doubling doses of 20, 40, and 80 μg, followed by a modified Fibonacci escalation of 130, 200, or 280 μg doses. In subjects with more than one lesion, the largest and most accessible lesion was identified as the target tumor. A standard method for injection was established to equally distribute the drug throughout the tumor by dividing the tumor into five equal areas for the five-day cycle. The daily dose was administered through one needle puncture centered within the prescribed day's area through

six to eight radiating needle tracks. Dose limiting toxicity (DLT) was defined as the occurrence of adverse events related to the treatment including grade four toxicity for local reaction at the injection site, grade four flu-like symptoms, grade four hematological toxicity, or any other non-hematological grade three toxicities with the exception of alopecia. Dose escalation at the 20, 40, and 80 μg levels was permitted once one patient at that dose level completed one cycle of treatment without DLT, and dose escalation at the 130, 200, and 280 μg levels was permitted once a minimum of three patients at the lower dose had completed one cycle of treatment without DLT. If two of the patients experienced DLT at any dose, that dose was determined to be the maximum tolerated dose (MTD).

Safety was evaluated by monitoring adverse events, hematology, blood chemistry/serology, and urinary laboratory parameters, and through the regular performance of physical examinations. The toxicity grade of adverse events was classified according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC), version 2.0. To fully characterize the local effects of IT VB4-845 administration, a local injection toxicity scale was included based on the injection site reaction event in NCI CTC Version 2.0: pain, erythema, and swelling of the skin or mucosa limited to the injection site and an area $\leq$3 cm adjacent to the tumor borders was classified as grade one, with a similar reaction in an area >3 cm and $\leq$6 cm adjacent to the tumor borders classified as grade two. Extension of the area to >6 cm, or the presence of necrosis or ulceration that was severe or prolonged and required surgery, or arterial bleeding projecting from the wound, which was self-limited with pressure, defined a grade three toxicity. A grade four injection toxicity was a life-threatening or chronically disabling local reaction or a hemorrhage. Adverse events were judged to be treatment-related by the investigators if they were considered possibly, probably, or definitely related to VB4-845 administration.

All procedures were in accordance with the Helsinki Declaration of 1975 (Consolidated Guideline adopted in 1996 and revised in Edinburgh, Scotland 2000); International Conference on Harmonization guideline E6 (Good Clinical Practice); Title 21, Parts 50 and 56 of the United States Code of Federal Regulations.

Patient evaluation

Pretreatment screening evaluations were done within two weeks of the baseline visit and included history and physical examination, a 12-lead electrocardiogram, identification, measurement, and digital photography of target and non-target tumors, chest radiograph, urinalysis, blood collection for serum chemistry and hematology, and a biopsy to confirm recurrent disease (not done if a biopsy had been done in the preceding eight weeks). Biopsy tissue samples were taken for immunohistochemistry analysis to determine if the tumors were positive or negative for EpCAM expression. Concomitant medications were recorded at this evaluation, as well as at every subsequent evaluation. Baseline evaluations were done on day 0 or 1 prior to drug administration, and included CT scans and caliper measurements of target and non-target tumors, as well as assessment of Karnofsky performance status. Physical examination, urinalysis, and blood collection for biochemistry and hematology were performed at this visit if they were not done previously at the pretreatment evaluation.

Prior to dosing on days 1 through 5 of each treatment cycle, vital signs were recorded, and urinalysis, blood biochemistry, and hematological analyses performed. After dosing on days 1 through 5 of each treatment cycle, vital signs were taken every 30 min for 4 h, then once an hour for the next 12 h, and at 24 h after dosing. On day 1 of cycles 1 and 2, pain was assessed from a standardized visual analog scale. On days 7, 14, and 21 of both treatment cycles, physical examination, urinalysis, blood biochemistry, and hematological analyses were performed. Patient evaluation at the final visit (day 28 of the last treatment cycle, or earlier if treatment was discontinued) included physical examination, assessment of pain from a standardized analog scale, assessment of Karnofsky performance status, CT scan, measurements and digital photography of target and non-target tumors, 12-lead electrocardiogram, and urinalysis, blood biochemistry, and hematological analyses.

Pharmacokinetics

Blood samples were taken prior to dosing on days 1 and 5 of treatment cycle 1, and on day 1 of cycle 2. After dosing on day 1, cycle 1, samples were taken at 10 min, 30 min, 1, 2, 4, 6, 12, and 24 h. For each sample, 3 ml of venous blood was collected into a lithium heparinate tube, centrifuged at 1000 RCF for 10 min and the resulting plasma was kept frozen at −80°C until analysis. The amount of VB4-845 in the plasma was evaluated by a MTS-based potency assay (tetrazolium compound; Promega, Madison, WI), using VB4-845-mediated cytotoxicity on the EpCAM-positive CAL-27 cell line (ATCC, Manassas, VA). CAL-27 cells were plated into 96-well plates (50 μl of 10^5 cells/ml suspension) and grown for 2 h at 37°C. Known amounts of VB4-845 (0–112 pg/ml) were spiked in normal human plasma and were used to establish a calibration curve. Plasma samples (50 μl) were added to CAL-27 cell cultures and incubated at 37°C under 5% CO_2 for 72 h and the supernatant was removed. The cells were washed once with culture medium and 100 μl of fresh medium was added followed by 20 μl of MTS reagent. The cells were

incubated for a further 2 h at 37°C under 5% CO_2 and the O.D. read at 490 nm using a plate reader spectrophotometer (VMax, Molecular Devices, Sunnyvale, CA). VB4-845 in the patient plasma sample was determined from a calibration curve of standard VB4-845 (correlation coefficient of $r^2 = 0.98$; accuracy 92–112%). The lower and upper limits of quantification were 7 pg/ml (0.1 pM) and 112 pg/ml (1.6 pM), respectively, with intra- and inter-day variability of <25%. Plasma pharmacokinetic variables, including area under the curve (AUC), maximum concentration (C_{max}), and elimination half-life ($t_{1/2}$), were calculated using WinNonlin Pro (version 4.1, Pharsight Corporation, Mountain View, CA).

Immunogenicity

Blood samples for assessing humoral immune reactivity to VB4-845 were the same as collected for the pharmacokinetic analysis and taken on days 1, 7, 14, 21, prior to dosing on day 1 of cycle 2, and at the final visit. Antibody titers to the antibody portion (4D5 scFv), exotoxin ($ETA_{252–608}$), and VB4-845 were measured using an ELISA. Briefly, equimolar $ETA_{1–608}$ (Sigma-Aldrich Chem. Co., St. Louis, MO), 4D5 scFv (Viventia Biotech), or VB4-845 were coated onto ELISA plates and blocked with PBS containing 1% BSA. Serially diluted patient samples in PBS containing 1% BSA were added to the coated plates; the presence of captured antibodies to $ETA_{1–608}$, 4D5 scFv, or VB4-845 were detected by adding HRP (horseradish peroxidase)-labeled goat anti-human-IgG, IgM, IgA (H + L) (KPL Inc., Gaithersburg, MD) followed by the substrate, TMB (3, 3′, 5, 5′ tetramethyl benzidine; KPL Inc., Gaithersburg, MD). The plate was read (VMax, Molecular Devices, Sunnyvale, CA) at 450 nm to determine the optical density (O.D.). The patient immune response is expressed as the titer of the anti-drug antibodies, which is the reciprocal dilution required to obtain 0.1 O.D. after subtracting pretreatment OD values. The cutoff value was defined as 4 × SD of the O.D. of the blank buffer samples because normal plasma could not be used as a control.

Results

Patient characteristics

Twenty-four patients with advanced, recurrent SCCHN, with a median age of 65 years were enrolled in this study (Table 1). Of these patients 67% had received at least one cycle of chemotherapy, and all had received radiotherapy. Immunohistochemistry (IHC) evaluation of biopsy samples determined that 17 patients had EpCAM-positive tumors, and seven had EpCAM-negative tumors.

Table 1 Patient characteristics

Total number of patients	24
Median age (range)	65 years (48–77)
Gender	
Male	19
Female	5
Ethnicity	Caucasian
Karnofsky performance status	
70	1
70–80	21
90	2
Prior therapy	
Chemotherapy	16
1 Cycle	2
2 Cycles	2
3 Cycles	12
Radiotherapy	24
EpCAM status	
Positive	17
Negative	7

Dose escalation and maximum tolerated dose

This study was a progression through increasing doses of 20, 40, 80, 130, 200, and 280 μg (Table 2) delivered intratumorally. Dosing of one drug concentration was given daily for 5 days, followed by a rest period of 23 days, and a second cycle of treatment. Elevated liver enzymes (grade three) were determined to be the DLT in this study and occurred in three patients. All 24 patients completed the first cycle of treatment, and 20 patients completed the second cycle. Of the four patients that did not complete the second cycle of treatment, one patient at

Table 2 Dose escalation scheme and number of observed DLTs

Dose (μg/day)	Patients enrolled	Patients completing Cycle 1	Patients completing Cycle 2	DLT
20	2	2	2	
40	3	3	3	
80	2	2	1[a]	1
130	8	8	7[b]	
200	3	3	2[c]	
280	6	6	5[a]	2[d]
Total	24	24	20	3

[a] Patient discontinued due to DLT

[b] Patient discontinued due to death not related to VB4-845

[c] Patient discontinued due to patient request

[d] A second patient completed cycle 2, but DLT was determined following cycle completion

the 80 μg dose experienced grade three elevated liver enyzme test results (DLT) after the first dose of the second cycle, and discontinued treatment; one patient at the 130 μg dose level died during the first rest period from a hemorrhage, which was deemed to be due to the natural history of the disease and other complications and not related to VB4-845; one patient at the 200 μg dose requested to discontinue with the study; and one patient at the 280 μg dose experienced elevated liver enzymes and treatment was discontinued at the time of the elevated test results in the second cycle of treatment. Another patient at the 280 μg dose experienced elevated alanine aminotransferase (ALT) levels (grade three) in the first cycle alone, which was not assessed as a DLT at the time, so treatment was continued. This event was subsequently reclassified as a DLT since it met the criteria in the study protocol. The MTD was determined to be 280 μg daily for five days, as this was the dose at which two or more patients of the dosage group experienced DLTs.

Safety

VB4-845 was generally well tolerated when administered by intratumoral injection in five daily doses of up to 280 μg/day over two cycles. Eighteen patients (75%) reported at least one adverse event during the course of the study (Table 3); however, only 11 patients (46%) reported adverse events related to VB4-845 treatment. The most common drug-related adverse event was pain at the injection site (seven patients or 29.2%), which was mitigated by prophylactic and concurrent pain management. Elevated liver enzymes were present in four patients (16.7%), three of which were at the 280 μg dose. The elevated liver enzymes were not associated with any signs of liver damage or toxicity and were asymptomatic, transient, and reversible in all patients. Three patients had tumor bleeding, two of which were possibly due to the drug treatment. The cause of grade four tumor bleeding in the third patient at the 130 μg dose was deemed to be not

Table 3 Patients with adverse events during dose escalation

Adverse events	Dose level (μg)					
	20	40	80	130	200	280
Injection site pain	1/2[a]	1/2[a]	2/2[a]	1/2[a]		2/2[a]
Tumor pain	1/1[a], 1/2	1/1, 1/2	1/2, 1/3	1/2, 1/3		1/3[a]
Tumor swelling	1/1[a]					
Neoplasm progression				1/1		
Tumor hemorrhage			1/3[a,b]	1/4		1/3[a]
Headache	1/1					
Blood/Lymphatic system disorder		1/1, 1/2[a]		1/2		
Weight decrease		1/1	1/1			1/1
Nausea	1/1[a]					
Anemia			1/4[a,b]			1/2
Constipation			1/3			
Dysphagia			1/3[b]			1/2
Elevated liver enzymes (AST, ALT)			1/3[a,b]			1/2[a], 2/3[a]
Metabolism/Nutrition			1/2, 1/3	1/2		1/2[a]
Hypotension			1/2[b]			
Hypertension						1/1
Neutrophil increase				1/2		
Alkaline phosphatase increase				1/2, 1/3		1/3[a]
Bilirubin increase						1/2[a]
Urea increase				1/2		
Lymphocyte decrease				1/2		
Tongue hemorrhage					1/1	
Hematocrit decrease					1/1	
Hemoglobin decrease					1/1	
Cardiac disorder						1/1
Fatigue						1/2
Hyperthermia						1/1

Values indicate the number of adverse events per grade at each dose level. If a patient experienced an adverse event more than once, the event with the highest grade was tabulated

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Represent adverse events which are possibly, probably, or definitely related to drug administration

[b] Events are all related to one patient

due to the drug treatment. Tumor bleeding is not unexpected in this patient population, given the advanced nature of their disease and the propensity for tumor hemorrhage to occur in advanced head and neck cancers. The types and distribution of adverse events were comparable between treatment cycles. Treatment-related adverse events were reported by patients in all of the dose cohorts. One patient at the 80 μg cohort experienced five adverse events, three of which were deemed to be due to treatment. One event was a grade three tumor hemorrhage, which occurred two days after treatment was discontinued and resulted in grade four anemia. Although the hemorrhage was assigned as possibly due to treatment, the patient had received anticoagulants, which were likely a contributing factor. The patient completely recovered from this event.

Immunogenicity

The immunogenicity of VB4-845 was examined by analyzing the titers of anti-scFv and anti-ETA in blood samples of patients taken at various time points (Table 4). Patient titers to VB4-845 were also analyzed prior to treatment and 14 (58.3%) patients were positive, with levels of patients' antibodies to VB4-845 and ETA in similar titer ranges (data not shown). The presence of these antibodies is possibly due to prior exposure to ETA from *Pseudomonas* bacteria. Over time, an increasing proportion of patients developed anti-ETA responses, and by the end of the study, all patients analyzed had detectable anti-ETA titers. After the first week of treatment, four patients had detectable anti-scFv responses, and by the end of treatment, 18 out of 22 patients had detectable anti-scFv responses. At the end of the study, the ETA portion of the molecule (titer 2,000 to 1,600,000) had generally elicited a much higher response than the scFv (titers 1,000 to 74,000).

Table 4 Antibody responses to the antibody (scFv) and exotoxin (ETA) portions of VB4-845

Day	Anti-ETA response		Anti-scFv response	
	# of patients[a]	Titer range ($\times 10^{-3}$)	# of patients[a]	Titer range ($\times 10^{-3}$)
0	14/24	1–8[b]	NA	NA
7	12/24	1.6–1,600	4/24	1–12
28	19/22	1.5–1,200	4/22	2–31
56	21/21	2–1,600	18/21	1–74

[a] # of patients with detectable response/# of patients evaluated

[b] Titer ranges for antibody response to VB4-845 at day 0 were similar to ranges to the anti-ETA response. *NA* not analyzed

Table 5 Pharmacokinetics of VB4-845 dosing

Dose level (μg)	# of patients	C_{max} (pg/ml)	AUC (pg/ml h)	$T_{1/2}$ (h)[a]
20	2	18 ± 25	59 ± 83	3.76 (1)
40	3	202 ± 349	1407 ± 2436	4.6 (1)
80	2	102 ± 127	1039 ± 1385	4.73 (1)
130	8	1051 ± 1639	3132 ± 4172	2.6 ± 1.66 (3)
200	3	258 ± 261	1203 ± 1063	4.66 (1)
280	6	2646 ± 4081	7847 ± 12034	3.46 ± 1.35 (5)

Area under the curve (AUC), maximum concentration (C_{max}), and elimination half-life ($T_{1/2}$) were calculated using WinNonlin Pro (version 4.1, Pharsight Corporation, Mountain View, CA)

[a] Values in parentheses indicate the number of patients analyzed for $T_{1/2}$

Pharmacokinetics

Pharmacokinetic studies were performed to evaluate the clearance of VB4-845 from the solid tumor into the blood following intratumoral injection. Detectable plasma levels of VB4-845 were observed in 15 out of 24 patients using a quantitative bioassay with a lower limit of detection of 7 pg/ml (0.1 pM). Of the nine patients that had no detectable VB4-845 in their blood, seven had antibodies to ETA prior to drug administration, which would allow clearance of the VB4-845 from circulation. The presence of anti-VB4-845 antibodies may in part account for the large variation of pharmacokinetic parameters from patient to patient. Standard pharmacokinetic variables were determined for all dose levels and included patients with no detectable levels for C_{max} and AUC determinations (Table 5). The mean maximum concentration (C_{max}) ranged from 18 to 2,646 pg/ml; however, the kinetics were non-linear. The elimination half-life ($t_{1/2}$) ranged from 2.6 to 4.73 h, with the mean $t_{1/2}$ across all doses of 4.0 ± 0.3 h.

Discussion

The recombinant fusion protein VB4-845 was well tolerated in patients with SCCHN when administered via intratumoral injection over the range of 20–280 μg. The preliminary MTD was established to be 280 μg administered daily for 5 days, based on the occurrence of DLTs of elevated liver enzymes in two patients at this dose level. Intratumoral injection was determined to be a well-tolerated form of drug administration for this recombinant fusion protein. Adverse events were manageable and compatible with those expected following intratumoral injections. The most common adverse event related to VB4-845 therapy was pain at the injection site. Vascular

leak syndrome, a common adverse effect associated with systemic immunotoxin therapy [18], was not observed. To establish a recommended dose using this dosing schedule would require further expansion of the 200 μg cohort. However, a second phase 1 trial explored a weekly dosing regime and found greater tolerance for higher doses of drug (manuscript in preparation).

A common problem in immunotherapy is the induction of a humoral response against the drug [24]. The development of fully humanized antibodies through the use of recombinant technology has reduced the immune response against many therapeutic monoclonal drugs. Although the scFv region of VB4-845 is a humanized antibody fragment with little to no expected antigenicity, the ETA component is known to be antigenic, and thus the development of patient antibodies to the drug was anticipated. Both components of VB4-845 elicited antibody titers in patients, although the exotoxin was much more immunogenic than the scFv portion. The finding that the majority of patients had pre-existing antibodies to the exotoxin portion may be a result of the patient's previous exposure to *Pseudomonas* bacteria [25]. By the end of the study, all patients developed antibodies to the exotoxin portion of VB4-845, and most patients had lower titers of antibodies to the scFv portion. The anti-scFv response may have been enhanced by a carrier effect, via the immunogenic ETA region. Although an antibody response to VB4-845 is potentially neutralizing and would promote clearance from circulation, it is unlikely that these antibodies can enter the tumor microenvironment to any great degree due to vascular barriers [26, 27], and therefore these responses would not likely inhibit the anti-tumor activity of VB4-845. The presence of VB4-845 anti-tumor activity in a large number of patients, concomitant with the development of anti-recombinant fusion protein antibodies during the course of the study, supports this hypothesis. Clinical trials with an increased number of drug cycles are currently in progress to evaluate the effect of drug antibodies in the long term.

Pharmacokinetic analyses of VB4-845 in the blood following intratumoral injection established that the drug may be released into circulation. However, only 15 of the 24 patients had detectable levels of VB4-845 in their blood after doses of up to 280 μg. For most of the patients the failure to detect VB4-845 could be explained by a pre-existing anti-ETA response. For these patients, VB4-845 would be rapidly cleared from the systemic circulation to below the level of detection of the assay. Alternatively, since VB4-845 is quantified indirectly based upon potency, it is possible that neutralizing antibodies may be present in the circulation, which may block killing in the assay thereby preventing the detection of the VB4-845. The elimination half-life of VB4-845 denotes a rapid clearance from circulation. The non-linearity of C_{max} over the dose range may be a reflection of a variety of factors that could influence the clearance of the drug from the tumor into circulation, such as tumor size, vascularization of the tumor, and clearance by antibodies.

Preliminary, exploratory efficacy data using radiographic and investigator assessments of tumors suggest that VB4-845 can reduce or stabilize tumor growth in patients with EpCAM-positive tumors (data not shown). Although dosing in this study was not optimized for therapeutic benefit and efficacy was not the primary objective, anti-tumor activity was seen at a number of dose levels. Most patients (71%, 10/14) with clinically evaluable EpCAM-positive tumors had a response, whereas all of the patients with clinically evaluable EpCAM-negative tumors exhibited disease progression over the course of the study. In addition, the anti-tumor activity of VB4-845 was seen in a patient population with recurrent, advanced tumors that had already been unresponsive to traditional front-line therapies. The median overall survival in patients with advanced, non-responsive SCCHN tumors is 103 days [3]. Although not statistically significant because of the sample size, patients with EpCAM-positive tumors that responded to VB4-845 therapy had a median survival time of 278 days, compared with the overall study group of 125 days. This may improve under optimized dosing conditions, and clearly warrants further controlled studies.

Chemoradiotherapy is a standard treatment regime for patients with recurrent SCCHN, and even though more intense radiation and chemotherapies have increased survival rates in recent years, resistance to chemotherapy and severe toxic effects accompany these treatments [28]. Clearly, alternative forms of treatment are required. Non-conjugated monoclonal antibody therapy to epidermal growth factor receptor (Cetuximab) has undergone clinical trials in combination with radiotherapy with some success [29–32]. However, efficacy and toxicity studies comparing Cetuximab with standard chemoradiotherapy treatments in larger trials are currently under way [32, 33]. Two other antibodies are in early stage clinical trials: Bevacizumab (anti-Her-2) therapy has resulted in some severe vascular complications and Tratuzumab (anti-vascular endothelial growth factor) has not improved response [4].

Immunotoxins produced as recombinant fusion proteins have shown the most promise in the treatment of hematological malignancies where the target cells are more easily accessible and the immune system is impaired, thus reducing neutralizing antibodies. Clinical responses to systemically delivered recombinant fusion proteins in patients with solid tumors have been more difficult to demonstrate [24]. Thus, IT injection was chosen for drug administration to concentrate the drug at the target as well as to reduce potential vascular side effects and immunogenic response. The preliminary anti-tumor data observed

in this study, combined with the encouraging safety profile, strongly suggest that recombinant fusion protein therapy using intratumoral administration may have potential as an effective treatment in SCCHN. These promising results in patients with recurrent, refractory SCCHN will be extended in a larger clinical trial with optimized dosing to evaluate the efficacy of the drug in the treatment of head and neck cancer.

Acknowledgments We would like to thank Dr. Jennifer Brown, Dimitri Fitsialos, Dr. Barry Wenig, Joseé Quenneville, Jacinthe Guindon, Caroline Woods, and Heather Graham for their contributions to the study and/or the review and preparation of the manuscript, and Dr. Philippa Bridge-Cook for drafting assistance. We gratefully acknowledge Professor Eugene Grigoryevich Matyakin, Professor Georgey Moiseyevich Manikhas, and Doctor Gennadij Abramovich Ginzburg for their participation in the study.

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