DRUG PROFILE

Current Opinion in Molecular Therapeutics 2008 10(2):176-186
 ISSN 1464-8431

VB4-845, a conjugated recombinant antibody and immunotoxin for head and neck cancer and bladder cancer

Kristen Biggers[1] & Noah Scheinfeld[2]*

Addresses
[1]West Virginia University School of Medicine, Robert C Byrd Health Sciences Center, PO Box 9100, Morgantown, WV 26506-9100, USA

Columbia University School of Medicine, St Lukes Roosevelt Hospital Center
150 West 55th Street, Brooklyn, New York, NY 11220, USA
Email: NSS32@columbia.edu

*To whom correspondence should be addressed

Viventia Biotech Inc, under license from the University of Zurich, is developing VB4-845, comprising a Pseudomonas exotoxin fused to an anti-epithelial cell adhesion molecule single-chain antibody fragment, for the potential treatment of head and neck cancer (intratumoral) and bladder cancer (intravesical). VB4-845 is currently undergoing phase II and III clinical trials in patients with head and neck squamous cell carcinomas, as well as phase II clinical trials for the treatment of superficial transitional cell carcinoma of the bladder.

Introduction

Conventional surgical, chemical and radiological therapies are inadequately effective against cancer of the head and neck. Head and neck cancers include those that occur in all parts of the oral cavity, salivary glands, sinus regions, nasal cavities, pharynx and larynx. By far the most common histological subtype are head and neck squamous cell carcinomas (HNSCCs) [848835]; in addition lymphomas, sarcomas, adenocarcinomas and other cancer types can occur in diverse locations [881558]. Treatment is challenging in recurrent cancers, particularly when critical nerves and blood vessels are encased by cancer and extensive surgery can leave patients disfigured or requiring reconstructive surgery [881335]. Head and neck cancer is the sixth most common category of cancer worldwide [848999], with more than 500,000 cases diagnosed and 250,000 deaths per year [848835]. The overall five-year survival rate of all head and neck cancers in the US from 1988 to 2001 was estimated to be 57% [848835].

Originator University of Zurich

Licensee Viventia Biotech Inc

Status Phase III Clinical

Indications Bladder tumor, Head and neck tumor

Actions Anticancer, EpCAM modulator, Immunotoxin, Protein synthesis inhibitor

Technologies Antibody fragment, Intratumoral formulation, mAb (conjugated)

Synonyms and analogs Anti-EpCAM-Pseudomonas exotoxin conjugate (head and neck cancer), Proxinium, VB-845, Vicinium

The effectiveness of treatment depends greatly on the stage and type of cancer and decreases markedly in more advanced, invasive or metastatic disease [875209]. Treatments for early-stage disease, which comprises 30 to 40% of cases [880144], are individualized to the patient and often involve single modality therapy, usually surgery (open or endoscopic) or external beam radiotherapy [875209]. Locally advanced head and neck cancer accounts for approximately 50% of cases [880144]. Such patients are often treated with surgery, where viable, but the balance between sufficiently radical tumor resection (to minimize the likelihood of recurrence) and organ or tissue preservation is difficult to achieve [848835]. Radiation therapy (optimally with divided/fractionated doses) or chemotherapy (eg, docetaxel, cisplatin or 5-fluorouracil), or both are often administered after surgery; concurrent radiochemotherapy is also the standard nonsurgical treatment for unresectable head and neck cancers [875209], [881335], [881553]. The success of upfront chemoradiation treatment has become comparable with that of surgery followed by chemoradiation (with the option of salvage surgery); therefore, the treatment choice between the available approaches remains controversial and can depend on factors such as the organ preservation, operability, expertise of the institution and patient preference [880144]. The optimal regimens and the role of induction and adjuvant chemotherapy remain to be defined; however, in most cases adjunctive chemotherapy confers only a minimal improvement in the overall prognosis of patients with HNSCC [875209]. Various analyses estimate that adjuvant chemotherapy improves the five-year survival rate by 4 to 10% compared with radiotherapy alone [875232], [875234], [880152]. Despite this increase in survival, acute

adverse events (eg, muscular fibrosis, gastrointestinal, mucous membrane and hematological effects) can limit chemotherapy, particularly in patients with comorbidities [875232], [875234]. In addition, as up to 90% of HNSCCs overexpress the EGF receptor (EGFR) [880151], inhibitors of EGFR (notably cetuximab, which is approved for the treatment of colorectal cancer [523128]) are increasingly used as therapeutics in HNSCC. For example, cetuximab was tested as a front line treatment, in combination regimens, and in attempts to prevent tumor recurrence and progression [880144], [880151], [881553].

Despite treatment with surgery or radiochemotherapy, high proportions of head and neck cancers recur and may develop resistance to first-line chemotherapy [875209], [880144]. Up to 40% of patients are estimated to present with late-stage disease and metastasis [875209]. Metastatic HNSCCs are generally treated with platinum-based chemotherapy [875209]; for example, cisplatin alone or in combination with 5-fluorouracil and methotrexate [875209]. However, response rates are generally less than 35% and the improvement in median survival is low (6 to 9 months) [875209]. Therefore, improved treatment strategies are required.

Bladder cancer is the ninth most common cancer worldwide, with 145,000 deaths in 2002 [849007]. An estimated 90% of bladder cancers are transitional cell carcinomas and 10% are squamous cell carcinomas [848844]. Although the life expectancy for patients with superficial tumors (those restricted to the bladder mucosa or submucosal layer) is high, the five-year survival rate for patients with advanced, muscle-invasive carcinomas is less than 50% [848844]. Present treatment options include intravesical chemotherapy (eg, with cisplatin) or immunotherapy (eg, with BCG), or surgery or radiotherapy for more advanced cases. Surgery for advanced bladder cancers is often extensive; radical cystectomy to remove the bladder and lower ureters (and also the uterus and ovaries in women or prostate in men) is standard in several countries [881623]. However, the recurrence rate for bladder cancer remains high [848844], emphasizing the requirement for improved therapeutic options to be developed.

Recombinant immunotoxins have the potential to offer selective toxicity alongside a good safety profile and are thus promising anticancer modalities [583852]. Various toxins from diverse sources have the potential to be harnessed in an immunoconjugate. The best studied toxins include *Pseudomonas* exotoxin (eg, used in the IL-2 targeted LMB-2; National Cancer Institute), diphtheria toxin (eg, the IL-2 fusion protein denileukin diftitox for cutaneous T-cell lymphoma, and the transferrin conjugate TransMID; Celtic Pharmaceutical Holdings LP/Nycomed/PharmaEngine Inc) and plant toxins (such as bouganin, eg, VB6-845; Viventia Biotech Inc) [583852], [875449]. There have been two immunotoxin drugs that have received regulatory approval: gemtuzumab ozogamicin (calicheamicin conjugated to an anti-CD33 antibody) for the treatment of subtypes of leukemia [614736] and denileukin diftitox [313866].

VB4-845 is a humanized antibody fragment that specifically binds epithelial cell adhesion molecule (EpCAM), fused to a truncated *Pseudomonas* exotoxin [601540]. It is undergoing phase II and III development by Viventia Biotech Inc, under exclusive license from the University of Zurich [465618]. Two formulations are in development that are administered by different methods and for different indications, but are presumed to contain the same protein: Proxinium is administered by intratumoral injection for the potential treatment of head and neck cancer [627368], [848851] and the intravesical formulation Vicinium is administered via a catheter for the potential treatment of bladder cancer [627368], [853362]. After binding to EpCAM on the cell surface, the VB4-845 molecule is internalized; the toxin is then released and triggers cell death by blocking protein synthesis [849017]. EpCAM is a 40-kDa membrane-spanning protein that was first identified as a tumor marker because of its high expression on tumors of epithelial origin; it is also variably expressed, but to a lesser degree, on most healthy epithelial cells. In addition to its well-studied role in the regulation of cell matrix adhesion, EpCAM is involved in cellular signaling, migration, proliferation and differentiation [849010], [853364]. EpCAM is overexpressed on the surface of malignant squamous cells in several parts of the body, including those of the head and neck and bladder [849010], [849015], [849016], [853364], and is therefore a rational target for treatment of these diseases.

VB4-845 has completed two phase I clinical trials [601540], [662534] and is undergoing a phase II/III clinical trial (TARGET) for the treatment of recurrent HNSCC that is refractory to available treatment options [756933], [766105], [848851]. An additional phase II clinical trial has been completed [663136]; interim data demonstrated some potential in treating advanced cases of HNSCC [871828]. VB4-845 has also completed phase I clinical trials for non-invasive *in situ* bladder cancer [835237] and is now undergoing a phase II clinical trial [848849].

The FDA granted VB4-845 Orphan Drug designation in February 2005 for the treatment of advanced, recurrent head and neck cancer [583426]. In June 2005, the EMEA approved Orphan Drug status to VB4-845 for the treatment of head and neck cancer [604998] and in November 2005, the FDA awarded Fast Track status to the drug for the same indication [633960].

Synthesis and SAR

VB4-845 comprises a single-chain recombinant variable fragment (scFv) from the humanized anti-EpCAM antibody 4D5MOC-B fused to a truncated form of *Pseudomonas* exotoxin A (amino acids 252 to 608) [849021], [WO-00061635], [WO-2004096271]. Early studies aimed to improve the thermodynamic stability of the murine anti-EpCAM scFv MOC31 by modeling and systematically altering amino acid residues in the antigen-binding loop. Grafting of the antigen-binding loop of the scFv MOC31 onto the human consensus framework of scFv 4D5 resulted in the anti-EpCAM antibody 4D5MOC-A. A second chimeric scFv (4D5MOC-B) was designed in which eight additional amino acid residues

of the 4D5MOC31 antigen-binding loop were altered, to make the structural subclass and characteristics of the V_H domain and loop donor 4D5MOC31 more similar [849021].

Additional optimization studies tested the binding specificity of 4D5MOC31, 4D5MOC-A and 4D5MOC-B by radioassays with SW2 cells. The three scFv fragments exhibited similar dissociation constants in SW2 cells, with affinities of 3×10^{-9} M. However, only 4D5MOC31 inhibited the binding of the other graft variants to EpCAM; this scFv bound stably to EpCAM with a half-life of 38 min. No cross-reactivity was observed with HER2 or EGFRs, two other carcinoma-associated antigens, demonstrating a degree of specificity to EpCAM [849021].

Optimization of the sequence of 4D5MOC-B produced higher thermodynamic stability *in vivo* [849021]. At 24 h after intratumoral injection in SW2 tumor xenografts in mice, the proportion of the injected dose (ID)/kg of 4D5MOC31, 4D5MOC-A and 4D5MOC-B that remained in the tumor tissue was 1.24, 0.84 and 1.47%, respectively. The tumor:blood ratios were: 0.92, 1.95 and 5.25, respectively. These data suggest that 4D5MOC-B was the more stable scFv fragment, retaining its specific binding activity and localization to the tumor even with a lower blood ratio. In clearance studies, 4D5MOC-B was rapidly cleared from the blood, with an initial half-life of 6 min and an elimination half-life of 228 min [849021].

Based on its thermodynamic stability, binding activity to EpCAM and fast blood clearance, 4D5MOC-B was selected as the scFv to be conjugated to *Pseudomonas* exotoxin A [849017]. To construct the conjugate 4D5MOC-B-ETA (which was later named VB4-845), the DNA encoding the exotoxin was amplified by PCR and cloned into the pSW200 expression vector downstream of the EpCAM-binding 4D5MOC-B sequence. 4D5MOC-B-ETA was then transformed into the *Escherichia coli* strain SB536. The expressed protein was purified and analyzed; the molecular weight was approximately 70 kDa [849017]. In mice, VB4-845 had a half-life that was 1.5-fold longer than that of the parent scFv after 24 h, although no values were provided [WO-2004096271].

In PBS, the thermal stability of 4D5MOC-B-ETA decreased slowly with time (suggesting that the protein maintained its conformation) and retained its rapid clearance from the blood (suggesting that it did not aggregate into high-molecular weight fragments). The EpCAM dissociation constant was 4 nM [849017].

VB4-845 was formulated at a concentration of 1 mg/ml in sodium phosphate (20 mM) and NaCl (500 mM), at pH 7.2, and has a shelf-life of 6 months at -70°C [WO-2004096271].

Preclinical development

VB4-845 inhibited the growth of EpCAM-positive human tumor cell lines SW2 (small-cell lung carcinoma), CAL27 (tongue squamous cell carcinoma), MCF7 (breast adenocarcinoma) and HT29 (colon adenocarcinoma) with IC_{50} values of 0.005 to 0.2 pM. [^{3}H]Leucine incorporation assays in SW2 cells suggested that protein synthesis was inhibited with an IC_{50} value of 0.01 pM [849017].

In immunohistochemistry studies, VB4-845 did not cross react with non-tumorous mouse, rat, rhesus or cynomolgus monkey tissue; however, the drug did cross react with healthy chimpanzee colon, heart, kidney, liver, lung and pancreas tissue. VB4-845 displayed some cross reactivity with healthy human epithelial tissue, but did not cross react with the stromal components of epithelial tissue or non-epithelial tissue. In immunohistochemical analysis of arrays of human head and neck carcinomas samples of various disease stages and grades, VB4-845 bound to 52% of pharynx, 35% of larynx and 26% of oral cavity tumor tissue samples; staining positivity was associated with increasing grade and tumor severity [593666].

An *in vitro* study assessed cytotoxicity and cell growth inhibition in human squamous cell tongue carcinoma cell lines SCC15 and CAL27 before, during and after treatment with VB4-845 plus either radiotherapy or chemotherapy (cisplatin, carboplatin, paclitaxel, 5-fluorouracil, docetaxel, bleomycin or methotrexate). Concentrations of VB4-845 varied from 0 to 100% of the IC_{50} value (0 to 4 pM), whereas the chemotherapeutic concentration used was the IC_{50} for each cell line. Of the seven chemotherapeutic agents tested, five (cisplatin, carboplatin, paclitaxel, 5-fluorouracil and docetaxel) showed significant additive cytotoxic effects when combined with VB4-845 ($p \leq 0.05$). No difference in this additive cytotoxicity was observed when the sequence of treatments was changed (ie, whether VB4-845 was administered before, during or after the chemotherapeutic agents) [784577].

Additional studies were conducted in SCC15 cells incubated with VB4-845 (0 to 4 pM) before, during or after exposure to a 4-Gy dose of radiotherapy and in CAL27 cells exposed to VB4-845 (> 2 pM) plus RadioTheraCIM [784577], a ^{188}Re-labeled form of the anti-EGFR mAb nimotuzumab (YM Biosciences Inc/The Center of Molecular Immunology/ Biocon Biopharmaceuticals Pvt Ltd) [592034]. At 7 days after exposure, clonogenic assays demonstrated that the cytotoxicity of VB4-845 treatment was increased over that of radiotherapy alone (presumed to be for both cell lines as details were not provided). A synergistic cytotoxic effect was achieved when radiotherapy was followed by incubation with VB4-845, whereas an additive cytotoxic effect was observed when radiotherapy was administered during or after treatment with VB4-845 [784577].

In a separate *in vivo* study, intravenous VB4-845 was tested in mice bearing tumor xenographs of EpCAM-positive SW2, HT29 or CAL27 cells, or control mice bearing tumors of the EpCAM-negative COLO30 cell line. Mice received VB4-845 (5 μg) every second day for 3 weeks (total dose of 45 μg) or 10 μg for 1 week (total dose of 30 μg). Both treatment schedules produced responses in all xenograft types. Complete tumor regressions were observed in two out of seven mice when CAL27 tumors were administered the

5-μg dose. Overall, VB4-845 treatment led to major shrinkage (≥ 80%) of SW2 tumors [849017], [WO-2004096271]. Lower efficacy was observed for the 10-μg schedule than the 5-μg schedule, most notably against HT29 xenografts (tumor size decreased to 0.7-fold of initial volume at 3 weeks for the 5-μg schedule). On day 30, the approximate relative increases in tumor size from baseline were 26-, 2- and 15-fold for the control, 5-μg and 10-μg groups, respectively [849017].

In an *in vitro* study of VB4-845 in 14 bladder cancer cell lines (with varying EpCAM expression), strong cytotoxicity was observed against 11 of these cell lines. The IC_{50} values after 72 h of exposure varied from 0.001 to 320 pM; the four most sensitive cell lines were T24, SW870, UM-UC-10 and 1A6 (IC_{50} = 0.001 to 0.033 pM) [WO-2004096271]. When exposure to VB4-845 was reduced to 2 h, more than 93% cytotoxicity was observed against the SCaBER squamous bladder cancer cell line and 5637 transitional bladder cancer cell line. The cytotoxicity of a non-EpCAM-specific control *Pseudomonas* immunotoxin (4B5-PE) in these cell lines was less than 10% after up to 72 h incubation with up to 6 pM. The IC_{50} value for VB4-845 in SW780 cells was 0.002 pM after 3 h, whereas the 1A6 cell line was less sensitive, with an IC_{50} value of 0.033 pM after 37 h. After 2 h incubation with VB4-845 (6 pM), 96, 89 and 93% cell deaths were observed, respectively, in the UM-UC-10, 5637 and UM-UC-14 cell lines [WO-2004096271]. At 2 h, VB4-845 (500 pM) killed less than 93% of 5637 cells. Furthermore, 11 of these 14 bladder cell lines displayed > 30% EpCAM expression (determined by fluorescence assays) than the EpCAM-negative COLO320 cell line, suggesting a correlation between VB4-845 cytotoxicity and cellular EpCAM; for example, EpCAM expression in the most sensitive (T24, SW870, UM-UC-10 and 1A6) cell lines was 134.1 ± 35.9, 86.7 ± 3.1, 124.6 ± 5.3 and 154.7 ± 15.2 greater than the COLO320 control cell line, respectively [WO-2004096271]. Therefore, the required dose level of VB4-845 may be dependent on the EpCAM expression of the cell line or tumor.

Limited data are available on the characterization of the cytotoxic mechanism of VB4-845. When VB4-845 is administered to the site of a tumor, the antibody fragment specifically binds to EpCAM on the surface of the cancer cells. Once bound, the exotoxin enters the cell by receptor-mediated endocytosis. Under the low pH conditions of the endosome environment, an internal furin site in the protein is exposed, resulting in cleavage of the immunotoxin from the antibody [670969], [WO-2006087196]. In general, *Pseudomonas* exotoxin irreversibly prevents protein synthesis within the cell by ADP ribosylation of elongation factor-2 at a diphthamide residue, which leads to apoptosis [849017], [849019], [875449]. A single molecule of the toxin is capable of killing a cancer cell [875449].

Toxicity

In a study of the toxicity of VB4-845, Sprague-Dawley rats were administered a subcutaneous injection of the drug at 1.14, 4.29, 8.57, 17.10, 42.90 or 85.70 μg/kg. As all of the doses were tolerated, the MTD was assumed to be greater than the highest dose. Repeat-dose toxicity was tested by administering subcutaneous injections of VB4-845 at 1.0, 5.0, 35.0 or 77.8 μg/kg/day for 7 days. Reactions were mild (redness, edema and lesions were observed in a dose-dependent manner, a characteristic of local injection site reactions) and resolved after a 14-day rest period. There was no evidence of systemic toxicity [593666].

An additional study analyzing spleen and liver toxicity was conducted using C57BL/6 mice administered three intravenous injections of VB4-845 (250 or 500 μg/kg), every other day, or two intravenous doses of 1000 μg/kg every other day. There was no elevation in aspartate aminotransferase or alanine aminotransferase levels observed at 24 h after the final dose in either cohort, compared with saline-treated controls. Additionally, there were no histological changes to the liver, spleen or whole blood sample of mice in either treatment group. Elevated levels of aspartate aminotransferase or alanine aminotransferase were observed in mice receiving two doses of VB4-845 (1000 μg/kg), but minimal necrosis of hepatocytes was detected on histological analysis [WO-2004096271].

The researchers concluded from these preclinical studies that VB4-845 was safe to progress to clinical trials. The proposed single dose for humans was between 0.29 and 4.0 μg/kg [WO-2004096271].

Metabolism and pharmacokinetics

Studies to assess the biodistribution of VB4-845 after intratumoral injection (as for head and neck cancers) were conducted in mice bearing SW2 tumors. Mice bearing EpCAM-expressing SW2 xenografts or EpCAM-negative COLO30 tumors were administered radiolabeled VB4-845 (6 μg). Doses that remained in the tumor were: 2.93%ID/g after 4 h, 1.95 after 24 h and 1.13 after 48 h. In EpCAM-negative COLO320 control tumors, the maximum value was 1.65%ID/g after 30 min, 1.06 after 4 h and at background levels after 48 h. In addition, the tumor:blood ratio was 5.38 after 48 h, suggesting that VB4-845 is highly specific to EpCAM-expressing tissues and slow to dissociate from the tumor site [849017].

An *in vivo* study evaluated the pharmacokinetic parameters of VB4-845, administered with or without a chemotherapeutic agent, in Sprague-Dawley rats. Both cohorts received a subcutaneous injection of VB4-845 (77.8 μg/kg). One cohort additionally received an intravenous injection of paclitaxel (3 mg/kg), cisplatin (3.5 mg/kg) or 5-flurouracil (20 mg/kg) [784577]. Animals were monitored 24 h following treatment. VB4-845 did not alter the pharmacokinetic parameters of any of the chemotherapeutic agents tested, suggesting that it was safe to use in combination with these drugs [784577].

In a dose-escalation phase I trial, patients with HNSCC were treated with four weekly intratumoral injections of VB4-845 at dose levels of 20 to 280 μg [601540]. The majority of patients had a clearance time of < 4 h. A total of 15 of the 24 patients had detectable levels of the drug in the plasma. C_{max} values ranged from 18 to 2646 pg/ml, T_{max} from 10 to 130 min and the $AUC_{0\text{-last}}$ from 59 to 7847 pg.h/ml. The

half-life over the dose-range was 2.9 to 5.1 h, with a mean half-life of 4.0 ± 0.3 h across all doses [601540].

In a phase I/II clinical trial in 64 patients with EpCAM-positive superficial transitional cell carcinoma of the bladder, VB4-845 was not detected in the circulation of treated patients, signifying no leakage from the bladder [835237], [875472].

Clinical development

Phase I

Head and neck cancer

A phase I, open-label trial assessed the safety, tolerability, pharmacokinetics and preliminary efficacy of VB4-845 in 24 patients with advanced HNSCC; 17 had tumors that were EpCAM positive [567224], [601540]. Patients were administered intratumoral injections of VB4-845 (20, 40, 80, 130, 200 or 280 μg) once daily for 5 days, followed by 23 days of rest; this cycle was then repeated at an escalated dose (n ≥ 3 per dose level) [601540]. Of 14 evaluable patients treated with VB4-845, 43% had an objective response, either significant (n = 2) or minor (n = 4) tumor regression. Of the remaining eight patients, 28.5% had stable disease (n = 4) and 28.5% experienced tumor progression (n = 4). Furthermore, > 50% of the patients were still alive at the end of the trial, including several who had been enrolled in the trial for almost 12 months [567224]. The median survival time for treated patients with EpCAM-negative tumors was 125 days in contrast to 301 days for those patients with EpCAM-positive tumors [601540], [604998].

A second, phase I dose-escalation clinical trial of VB4-845 was conducted in 20 head and neck cancer patients to test a more intensive dosing regimen than the previous trial. Patients were administered VB4-845 (100, 200, 330, 500, 700, 930, 1240, 1650, 2200, 2950 and 4000 μg) as an intratumoral injection once weekly for 4 weeks [662534]. Viventia reported preliminary efficacy data from 18 evaluable patients. Of the 16 evaluable patients with EpCAM-positive tumors, 25% had a complete response to VB4-845, 37% had a partial response and 25% had stable disease (n = 4, 6 and 4, respectively) [592144]. Additionally, one of the four complete responses to VB4-845 was confirmed radiographically [662534].

Bladder cancer

In a phase I/II, dose-escalation trial, 64 patients with EpCAM-positive superficial transitional cell carcinoma of the bladder (stages Ta, Tis or T1; grades 2 or 3) who were unable to receive BCG therapy were treated with VB4-845 once weekly for 6 weeks. Administration was via a catheter into the bladder at doses of 0.1, 0.2, 0.33, 0.66, 1.32, 2.64, 5.28, 10.56, 13.73, 17.85, 23.2 or 30.16 mg [670969], [835237], [875472]. At the end of the trial period, 42% of patients had achieved a complete response, 13% had a partial response and 40% had stable disease (n = 27, 8 and 26, respectively). The overall response rate was 95% and responses were dose dependent [670969], [780234]. *Post hoc* analyses demonstrated that there was a sustained response after trial completion, lasting more than 6 months in most patients with many patients disease free at 12 months [798946].

Phase II

Head and neck cancer

A phase II, non-randomized, open-label, uncontrolled clinical trial (target enrolment n = 24) in patients with advanced, recurrent HNSCC considered refractory to conventional treatment was terminated [663136]. This clinical trial was designed to evaluate efficacy and safety as well as a recommended dosing schedule [663136], [878993]. Slow rates of accrual in North America [663136] were presumed to be the reason for ending the trial early and preliminary data from 15 patients suggested the prospect of efficacy and overall survival benefits [871828]. At the time of publication, no additional details were available.

Bladder cancer

A phase II, open-label, uncontrolled, single-group clinical trial was ongoing in patients with non-invasive, aggressive bladder cancer refractory to, or unsuitable for BCG treatment (expected n = 46) [848849], [871828]. Patients were to be administered VB4-845 (dose unstated) weekly for 6 to 13 weeks [780234], [871828]. Subsequently, patients responsive to therapy might receive up to three additional maintenance cycles over 51 weeks. The primary endpoint was to be overall response rate after 12 weeks as evaluated from histology and urinary cytology [780234]. Interim data released in January 2008 showed that, of 29 enrolled patients, 25 patients had completed the initial dosing and were evaluable for maintenance dosing, and 9 of the first 18 patients evaluated had complete tumor responses. No disease progression was observed [871828]. The trial was expected to be completed by August 2009 [848849].

Phase II/III

Head and neck cancer

A phase II/III, randomized, open-label trial (known as TARGET) of VB4-845 began in December 2005 and was expected to enroll approximately 300 patients with advanced HNSCC [756933], [766105]. This international trial was to compare once-weekly treatment with VB4-845 (700 μg) plus supportive care, administered until either complete resolution of tumors or tumor progression (determined by radiographic analysis) occurred, with supportive care alone [756933]. The primary endpoint for both phase arms of the trial was patient survival. Quality-of-life, progression-free survival and response rate were to be secondary endpoints [766105].

Based on interim data, an independent safety monitoring board for the TARGET trial recommended commencement of the phase III arm of the trial [766105]. At the time of publication, 150 patients had been enrolled [871828]. Interim efficacy data on patient survival demonstrated an advantage to VB4-845 treatment, with 40% improvement with the VB4-845-treated cohort and responses extending to over 12 months [871828]. The trial was expected to be completed by June 2009 [756933].

Side effects and contraindications

In phase I and II clinical trials with VB4-845, the drug was well tolerated, and side effects were considered mild to

moderate in severity and were mostly related to injection site reactions [601540], [670969], [766105]. During the phase I clinical trial in 24 patients, dose escalation reached 280 µg/day without an established MTD. The adverse effects were non-severe and mainly included local injection site pain [601540].

In the phase I/II clinical trial in 64 patients with EpCAM-positive superficial transitional cell bladder carcinoma, the majority of patients developed an immunological response to the antibody [835237]. In the phase I/II trial in HNSCC patients, the majority developed antibodies against VB4-845, most of which were directed against the toxin [601540]. No MTD was reached in this clinical trial [875472]. No major side effects or contraindications for VB4-845 were reported at the time of publication.

Patent summary

The first disclosure of 4D5-MOCB, the humanized anti-EpCAM scFv portion of VB4-845, was made by the University of Zurich in WO-00061635 (which has a priority date of April 1999). The use of VB4-845 for the treatment of HNSCC or bladder cancer was claimed in WO-2004096271 (priority date of April 2003), an application filed by researchers at the University of Zurich. Both these cases are licensed to Viventia for development and marketing. A novel variant of VB4-845 (IMMPA) was disclosed by the University of Zurich in WO-2006087196 (priority date of February 2005), without association to Viventia. This application claims a version of VB4-845 in which the furin cleavage site is replaced by a cleavage site for matrix metalloprotease subclasses 2 and 9. This immunotoxin targets EpCAM-expressing cells in the same way as VB4-845, but is stated to be slightly less toxic because of the lower activity of the activating enzymes.

An application published by Viventia alone, US-20060210572 (priority date of March 2005), claims methods for intratumoral administration of VB4-845 into a lesion (exemplified by HNSCC) that is connected via the lymphatic system to a second tumor. The method is designed to improve the effectiveness of treatment of the lymphatic nodes while minimizing systemic toxicity.

Current opinion

Preclinical studies have demonstrated the potential for VB4-845 to be used as an adjunct therapy for the traditional chemo- and radiation therapies for EpCAM-expressing cancers, in particular, HNSCC. *In vitro*, VB4-845 demonstrated additive efficacy in combination with chemotherapeutic drugs and synergistic cytotoxic activity in combination with radiation. Data from the phase I and II clinical trials have demonstrated VB4-845 to be safe and well tolerated in patients, which may be a consequence of its local administration. VB4-845 was efficacious against refractory and recurrent HNSCC (when administered as an intratumoral injection; Proxinium) and for superficial transitional cell bladder carcinoma (after intravesical administration via a catheter; Vicinium). The survival rate for the phase I clinical trial (4 and 10 months, respectively, for patients with EpCAM-negative and -positive tumors) [604998] exceeded the average 3- to 5-month survival rate for patients with advanced head and neck cancer [875209]. Furthermore, Viventia reported that interim analysis from the phase III arm of the TARGET trial suggested an improvement in median survival of 40% that was durable for over a year in some patients. However, all phase I and II trials, excluding the TARGET trial, have been non-randomized; therefore future trials should incorporate placebo arms to ensure proof-of-efficacy and should also provide more details with regards to definitions of disease measurements. As well as in HNSCC and bladder cancers, VB4-845 might also be effective in other carcinomas and tumors of epithelial origin, many of which express EpCAM [676822].

Vascular leak syndrome, a hallmark of immunotoxin or high-dose IL-2 treatment (presumed to be an immune response resulting in endothelial cell activation) [878994], does not appear to be a problematic toxicity for VB4-485, perhaps because VB4-845 is based on a humanized antibody. However, there are no data on the nature of the immunological response to VB4-845 in the majority of patients in the bladder cancer trial, other than most antibodies targeted the toxin portion of the drug. If this was a neutralizing antibody response, which would not be unexpected given the immunogenicity of the *Pseudomonas* exotoxin in other conjugates [881561], it might greatly limit the effectiveness of multiple doses of VB4-845. Potential contraindications to VB4-845 treatment are allergies to the components of the protein or cancers with mutations that confer resistance to the mechanism. It is possible that resistance to VB4-485 might evolve during treatment via mechanisms such as reduced expression of EpCAM [676822].

EpCAM is an attractive and rational target because of its function in sustaining and promoting cancers [849010]. However, its expression on healthy epithelial tissues (albeit at lower and variable levels) poses a significant risk of side effects. A study in transgenic mice that express human EpCAM suggested that a humanized antibody based on MOC31 exhibited limited bindings to non-cancerous tissues [875439]. However, the expression of EpCAM on various healthy human adult tissues (eg, at high levels in the colon) [676822], would suggest that at least some toxicity would be expected from VB4-845 or other EpCAM-targeting approaches. No details have been published on the cross reactivity of VB4-845 with non-cancerous human epithelial tissue, other than an abstract stating that 'some cross reactivity' was observed. These issues are critical and are expected to determine both the efficacy and safety of VB4-845 (and with other biological drugs or drugs targeting EpCAM). Without meaningful data on these issues, no speculation on the future of the drug can be valid.

The local delivery of VB4-845 would be expected to reduce the systemic toxicity of the drug to non-cancerous EpCAM-expressing tissues. However, the local delivery of VB4-845 would be expected to limit the efficacy of the drug against metastatic cancer, as systemic chemotherapy is standard

for metastatic cancers [875474]. This potential limitation might be overcome by the development of novel delivery methods or formulations that reduce toxicity, but that still permit effective concentrations to reach distal tumors. A nanodelivery system of EpCAM-directed immunoliposomes loaded with doxorubicin (that utilizes 4D5MOC-B to target EpCAM) was effective in treating mice with established human tumor xenografts [875428], suggesting a possible drug delivery method.

Of a number of immunoconjugates in clinical development, none target EpCAM and none are in development for the treatment of head and neck cancer or bladder cancer. Two other antibody-based therapeutics in clinical development target EpCAM: adecatumumab (Micromet Inc/Merck Serono SA) is undergoing phase II trials for prostate and breast cancers [748767] and the bispecific catumaxomab (Fresenius Biotech, TRION Pharma GmbH) for ovary and stomach cancers [649551]. Although these agents have not been tested against HNSCC or bladder cancers, they may be in future and thus represent the closest potentially competing agents for VB4-845. In its favor, VB4-845 was granted FDA Fast-Track status for the treatment of HNSCC [633960] and FDA and EMEA Orphan Drug designation for the same indication [583426], [604998]. Various novel chemotherapeutic agents are under development as potential treatments for HNSCC, either alone or as combinations, and refinements continue in regimens of approved agents (such as oxaliplatin with either gemcitabine or capecitabine) [849002]. With regards to bladder cancer, VB4-485 is also under development alongside the vinca alkaloid antitubulin agent vinflunine (Pierre Fabre SA; phase III for bladder cancer) [533749]. Furthermore, radioimmunotherapy is another strategy undergoing tests for head and neck cancer [518221], and also holds promise for the treatment of bladder cancer [875467]. Insufficient data are available to draw meaningful comparisons between VB4-485 and existing or novel drug treatments for HNSCC or bladder cancer. However, given the novel mechanism of VB4-845 and apparently low toxicity after local administration, it would likely be most effective in combination with cytotoxic chemotherapy or targeted agents such as growth factor inhibitors, or both, as well as surgery and radiotherapy.

As with various new therapies, defining endpoints other than an absolute increase in patient survival for the clinical trials with VB4-845 is challenging and the demonstration of efficacy in combination with chemotherapy agents should also be considered. It can be speculated that, just as the FDA has observed with bevacizumab for breast cancer [879351], reducing tumor size rather than extending survival can itself be the basis for approval of an oncology agent. Thus, VB4-485 might be approved on the basis of improvement of the parameters associated with cancer treatment. Data from additional clinical trials with VB4-845 are awaited with interest but, if the efficacy and safety that was observed in phase I and II trials continues, the drug may become a promising novel treatment for HNSCC, bladder cancer and other carcinomas.

Licensing

In October 2002, VB4-845 was licensed by Viventia from the University of Zurich for the treatment of head and neck cancer [465618].

Development status

Developer	Country	Status	Indication	Date	Reference
Viventia Biotech Inc	Brazil	Phase III	Head and neck tumor	14-DEC-05	756933
Viventia Biotech Inc	Russian Federation	Phase III	Head and neck tumor	14-DEC-05	756933
Viventia Biotech Inc	UK	Phase III	Head and neck tumor	14-DEC-05	756933
Viventia Biotech Inc	Canada	Phase II	Head and neck tumor	03-JAN-06	663136
Viventia Biotech Inc	Canada	Phase II	Bladder tumor	14-NOV-05	634542
Viventia Biotech Inc	US	Phase II	Head and neck tumor	03-JAN-06	663136
University of Zurich	US	Discontinued	Head and neck tumor	01-OCT-02	465618

Literature classifications

Chemistry

Study type	Result	Reference
Synthesis	To improve thermodynamic stability, the antigen-binding loops of the murine anti-EpCAM scFv MOC3 were grafted onto the human consensus framework of scFv 4D5 to produce 4D5MOC-A. Optimization of the structural subclass and characteristics of the V_H domain and loop donor region of MOC31 produced 4D5MOC-B, which was then developed on the basis of stable ($t_{½}$ = 38 min) and specific EpCAM binding activity.	849021
Synthesis	A cloned DNA sequence of truncated *Pseudomonas* exotoxin A (amino acids 252 to 608) was inserted into an expression vector downstream of the EpCAM-binding 4D5MOC-B sequence. The fusion protein MOC-B-ETA (later named VB4-845) was expressed in *Escherichia coli* SB536. The expressed protein was purified and analyzed, and observed to have a molecular weight of approximately 70 kDa.	849017

Biology

Study type	Effect studied	Model used	Result	Reference
In vitro	Activity	Growth inhibition assays in SW2, CAL27, MCF7 and HT29 cell lines incubated with VB4-845. [^{3}H]Leucine incorporation assays with SW2 cells incubated with VB4-845.	Cell growth was inhibited with IC_{50} values of 0.005 to 0.2 pM. Protein synthesis in SW2 cells was inhibited with an IC_{50} value of 0.01 pM.	849017
In vitro	Tissue binding	Immunohistochemistry studies to assess the binding of VB4-845 to healthy animal and human tissues, and to human head and neck carcinoma samples of various disease stages and grades.	VB4-845 did not cross react with non-tumorous mouse, rat, rhesus and cynomolgus monkey tissue, but bound to healthy chimpanzee colon, heart, kidney, liver, lung and pancreas tissue. VB4-845 displayed some cross reactivity with healthy human epithelial tissue, but not the stromal tissue components. VB4-845 bound to 52% of pharynx, 35% of larynx and 26% of oral cavity tumor tissue samples; staining positivity was associated with increasing grade and tumor severity.	593666
In vitro	Activity	SCC15 and CAL27 human squamous cell tongue carcinoma cell lines treated with VB4-845 (0 to 4 pM) before, during or after radiotherapy or chemotherapy (cisplatin, carboplatin, paclitaxel, 5-fluorouracil, docetaxel, bleomycin or methotrexate; at the IC_{50} for each cell line).	Cisplatin, carboplatin, paclitaxel, 5-fluorouracil and docetaxel had a significant additive cytotoxic effect when combined with VB4-845 ($p \leq 0.05$). No difference in this additive cytotoxicity was observed whether VB4-845 was administered before, during or after chemotherapy.	784577
In vivo	Efficacy	VB4-845 injected intravenously (5 µg every second day for 3 weeks, total dose of 45 µg; or 10 µg for 1 week, total dose of 30 µg) into mice bearing tumor xenographs of EpCAM-expressing SW2, HT29 or CAL27 cells (or non-EpCAM COLO30 cells).	The 5-µg dose caused complete tumor regressions in two out of seven mice with CAL27 tumors. Overall, VB4-845 treatment led to major shrinkage (≥ 80%) of SW2 tumors The 10-µg schedule had lower efficacy than the 5-µg schedule, most notably against HT29 xenografts. On day 30, the approximate relative increases in tumor size from baseline were 26-, 2- and 15-fold for the control, and the 5-µg and10-µg groups, respectively.	849017
In vivo	Activity	Cytotoxicity of VB4-845 (0.01, 0.6 or 6 pM) in 14 bladder cancer cell lines for 72 h exposure.	IC_{50} values for VB4-845 in bladder cancer cell lines varied from 0.001 to 320 pM; strong activity was observed against 11 cell lines, which correlated with the level of EpCAM expression. After 2 h incubation with 6 pM VB4-845, 96, 89 and 93% cell deaths were observed, respectively, in the UM-UC-10, 5637 and UM-UC-14 cell lines.	WO-2004096271

Metabolism

Study type	Effect studied	Model used	Result	Reference
In vivo	Pharmacokinetics	Radiolabeled VB4-845 (6 µg) administered to mice bearing EpCAM-expressing SW2 xenografts or EpCAM-negative COLO30 tumors.	The injected doses that remained in SW2 tumors were 2.93%ID/g after 4 h, 1.95 after 24 h and 1.13 after 48 h. In COLO320 control tumors, the maximum value was 1.65%ID/g after 30 min, 1.06 after 4 h and at background levels after 48 h. The tumor:blood ratio was 5.38 after 48 h, suggesting that VB4-845 is specific and slow to dissociate from the tumor site.	849017
In vivo	Pharmacokinetics	Rats administered VB4-845 (77.8 µg/kg), with or without intravenous paclitaxel (3 mg/kg), cisplatin (3.5 mg/kg) or 5-flurouracil (20 mg/kg).	VB4-845 did not alter the pharmacokinetic parameters of the chemotherapeutic agents tested and therefore appeared safe to use in combination with these drugs.	784577

Metabolism (continued)

Study type	Effect studied	Model used	Result	Reference
In vivo	Pharmacokinetics	Phase I trial in HNSCC patients administered once-weekly intratumoral injections of of VB4-845 20 to 280 µg for 4 weeks.	C_{max} values ranged from 18 to 2646 pg/ml, T_{max} from 10 to 130 min and the $AUC_{0\text{-last}}$ from 59 to 7847 pg.h/ml. The half-life over the dose-range was 2.9 to 5.1 h, with a mean half-life across all doses of 4.0 ± 0.3 h.	601540

Clinical

Effect studied	Model used	Result	Reference
Efficacy and safety	Phase I trial in 24 HNSCC patients administered cycles of VB4-845 as escalating intratumoral injections (20, 40, 80, 130, 200 or 280 µg/day) for 5 days, followed by 23 days of rest.	Objective responses were observed in 43% of patients, which included two significant and four minor tumor regressions. Of the remaining patients, four had stable disease. Median survival time for treated patients with EpCAM-negative tumors was 125 days in contrast to 301 days for those patients with EpCAM-positive tumors.	601540
Efficacy	Phase I trial of weekly intratumoral injections of VB4-845 (100, 200, 330, 500, 700, 930, 1240, 1650, 2200, 2950 and 4000 µg) for 4 weeks in 20 HNSCC patients.	Of 16 evaluable patients with EpCAM-positive tumors, 25% had a complete response to VB4-845, 37% had a partial response and 25% had stable disease (n = 4, 6 and 4, respectively).	592144
Efficacy	Phase I/II, dose-escalation trial in 64 patients with EpCAM-positive superficial transitional cell bladder carcinoma treated with VB4-845 (0.1, 0.2, 0.33, 0.66, 1.32, 2.64, 5.28, 10.56, 13.73, 17.85, 23.2 or 30.16 mg), via a catheter into the bladder, once weekly for 6 weeks.	At the end of the trial, 42% of patients had achieved a complete response, 13% had a partial response and 40% had stable disease (n = 27, 8 and 26, respectively). The overall response rate was 95% and responses were dose dependent.	670969
Efficacy	Phase II clinical trial in patients with bladder cancer unsuitable for BCG treatment (expected n = 46). VB4-845 (dose unstated) was administered over a period of 6 to 13 weeks by an intravesicular catheter.	Of 29 enrolled patients, at the time of publication 25 had completed the initial dosing and were evaluable for maintenance dosing; 9 of the first 18 patients evaluated had complete tumor responses. No disease progression had been observed.	871828
Efficacy	Phase II/III trial (TARGET) in patients with advanced HNSCC. Once-weekly intratumoral injections of VB4-845 (700 µg) plus supportive care was to be compared with supportive care alone.	Interim patient survival data demonstrated an advantage of VB4-845 treatment, with 40% improvement in the VB4-845-treated cohort and responses extending to over 12 months.	756933

Associated patent

Title Method for the stabilization of chimeric immunoglobulins or immunoglobulin fragments, and stabilized anti-EGP-2 scFv fragment.
Assignee Universitaet Zuerich
Publication WO-00061635 19-OCT-00
Priority EP-1999 99107030 09-APR-99
Inventors Plueckthun A, Honegger A, Willuda J.

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