Chapter 19
The Preclinical and Clinical Evaluation of VB6-845: An Immunotoxin with a De-Immunized Payload for the Systemic Treatment of Solid Tumors

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Introduction

One of the challenges in cancer therapy is to eradicate tumor cells while minimizing the toxic side effects to normal tissue that can rapidly become dose-limiting. In this regard, the unique specificity of antibodies enables the targeting of antigens that are differentially or aberrantly expressed on tumor cells while ignoring their normal counterparts [1]. To date, six IgG antibodies have received FDA approval for the treatment of cancer, Herceptin™ (Trastuzumab), Rituxan™ (Rituximab), Avastin™ (Bevacizumab), Campath™ (Alemtuzumab), Erbitux™ (Cetuximab), and Vectibix™ (Panitumumab), and all have shown varying degrees of clinical and commercial success [2]. While designed to target tumor cells with nanomolar affinity, clinical evidence would suggest that the anticancer mechanisms mediated by these antibodies are not on their own sufficient to provide a prolonged clinical benefit [3].To that end, other strategies have been explored to enhance antibody potency while still exploiting their targeting function. One such approach has been to attach a cytotoxic payload to an antibody that when delivered to a cancer cell induces a highly potent cell death signal [4]. The most common payloads attached to antibodies or antibody fragments are small molecule drugs, radionucleotides, and toxins [1, 5–8]. Two radionucleotide-conjugated antibodies Zevalin (Ibritumomab tiuxetan) and Bexxar (Tositumomab-/I131) and one antibiotic-conjugated antibody Mylotarg (Gemtuzumab ozogamicin) have been approved, although Mylotarg was subsequently withdrawn [9]. In addition, Ontak a diptheria toxin (DT) conjugated to an IL2 cytokine received approval for the treatment of cutaneous T cell lymphoma [10]. A variety of antibody–drug conjugates (ADCs) such as the anti-HER2 trastuzumab-DM1 are currently being evaluated in the clinic as antibody conjugates have proven themselves superior to the naked antibody in xenograft tumor models [11].

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Similarly, a variety of immunotoxins have been evaluated in the clinic, but as yet none have received FDA approval; however, those targeting leukemic cancers such as BL22, an anti-CD22 dsFv linked to truncated *Pseudomonas* exotoxin A (ETA), have been particularly successful [12, 13].

Despite the potency of immunotoxins, their inherent immunogenicity has limited their clinical use [14, 15]. In most cases the targeting antibodies are human or humanized scFv or Fab fragments which minimize the likelihood of a patient immune response; however, toxins are entirely foreign proteins and therefore highly immunogenic [16–18]. As a consequence, the humoral response in patients elicits the formation of antidrug antibodies (ADAs), resulting in rapid drug clearance, and hence, limited therapeutic effectiveness. Historically, cancers of hematological origin have responded better to immunotoxin-based therapies due to both the accessibility of the malignant cells as well as the immunocompromised state of these patients, thereby permitting multiple cycles of treatment [8]. For solid tumors, ADAs rapidly become dose-limiting after only a few weeks of treatment [19]. One strategy for circumventing immunogenicity is to use loco-regional administration, an approach that is only applicable in a limited number of indications. The immunotoxin, VB4-845, an anti-EpCAM scFv (4D5MOCB) linked to a truncated form of *Pseudomonas* ETA, has been evaluated in early clinical trials for squamous cell carcinomas of the head and neck (SCCHN) as well as transitional cell carcinomas of the bladder; drug administration was intratumoral for SCCHN and intravesical for bladder cancer [20–22]. In both indications, the drug was efficacious, well tolerated, and unaffected by a humoral response. However, most solid cancers cannot be treated effectively using loco-regional delivery strategies and thus require a systemically administered drug given in multiple cycles. Several approaches have been used to reduce the immunogenicity of the toxin moiety including the co-administration of immunosuppressant drugs, PEGylation, as well as the identification and removal of putative B and T cell epitopes from the protein [23–28]. Each approach has demonstrated some success in preclinical studies, but a final assessment of a de-immunization strategy can only be obtained by analyzing patient samples following repeat administration. The approach we have taken is to create a toxin, de-bouganin, in which the T cell epitopes were removed to create a cytotoxic payload with negligible immunogenic potential. In this chapter, we describe the 'bench to clinic' development of an anti-EpCAM immunotoxin carrying the de-immunized payload, de-bouganin, designed for the systemic treatment of solid tumors.

De-Bouganin for Use in Immunotoxins

Bouganin is a plant-derived toxin isolated as a 29 kDa single polypeptide chain (Type 1) ribosome inactivating protein with RNA N-glycosidase activity that directly inhibits translation [29–31]. The selection of bouganin was based upon its proven cytotoxic potential when conjugated to a targeting antibody as well as its favorable toxicity profile in animal models when compared to other type I RIPs [32, 33].

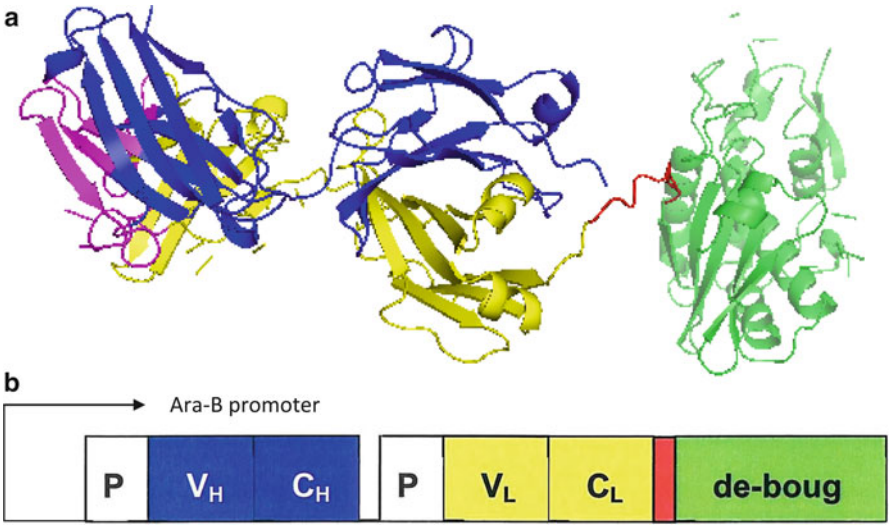

Fig. 19.1 (**a**) Ribbon representation of VB6-845. The de-bouganin moiety (*green*) is fused to the light chain (*yellow*) of the Fab fragment via a peptidic linker containing the furin proteolytic site (*red*). The heavy chain is shown in *blue* and CDRs loop in *magenta*. (**b**) Schematic representation of the VB6-845-C_L dicistronic unit under the control of the Arabinose (Ara-B) promoter of pING3302 expression vector. V_H, V_L, C_H, and C_L abbreviations correspond to the variable heavy and light chain and heavy and kappa chain conserved domain, respectively, P to the *Pel*B leader sequence and de-boug to de-bouganin. The furin linker is indicated in *red*

The absence of a cell binding domain, often associated with other toxins, contributes to its favorable toxicity profile. In order to address the potential immunogenicity of bouganin, overlapping peptides covering the entire sequence were tested in a T cell proliferation assay. Subsequently, reactive peptides corresponding to potential T cell epitopes were identified and removed [34]. This T cell epitope-depleted form of Bouganin (de-bouganin) was shown to have minimal immunogenic potential in vitro while preserving the potency of the parent molecule [34].

In order to clinically evaluate de-bouganin, the 77 kDa VB6-845 immunotoxin was constructed as a recombinant fusion protein comprised of an anti-EpCAM humanized Fab fragment, derived from 4D5MOCB scFv [34, 35] (Fig. 19.1a). There are several compelling reasons that make EpCAM a clinically relevant target for immunotherapy. First, although expressed on normal epithelia, EpCAM is generally overexpressed on carcinomas and increased expression is often associated with disease progression and poor patient prognosis [36–38]. Second, the cell surface distribution differs between normal and tumor epithelia such that EpCAM is more readily accessible on tumor cells [39–41]. Third, the pivotal role of EpCAM in proliferation, mitogenic signal transduction, and transformation underscores its importance as a therapeutic target [42]. To create VB6-845, de-bouganin was attached to a humanized EpCAM targeting Fab fragment using a furin-cleavable linker (Fig. 19.1b). The choice of a Fab-toxin format for VB6-845 was based upon

Table 19.1 VB6-845 reactivity and potency

Cell line	Indication	Reactivity[a]	IC_{50} (nM)
NIH:OVCAR-3	Ovarian	59	0.4
Caov-3	Ovarian	107	0.4
MCF 7	Breast	113	0.4
NCI-H69	Lung	31	1.5
HT-29	Colon	58	1.7
CAL 27	Head and neck	87	1.8
LNCaP	Prostate	43	11
HT-3	Cervical	29	23
HEC-1-A	Endometrial	42	43
RL95-2	Endometrial	4.9	100
SK-OV-3	Ovarian	4.3	>100
A-375	Melanoma	1.1	>100

[a]Values are representative of three independent experiments. Reactivity is defined as the mean fluorescence fold increase over the PBS control

the consideration of an optimal molecular size that would permit extravasation from the vasculature and subsequent penetration into the tumor bed while remaining stable in serum. The Fab-toxin format was manufactured as a fusion protein using a scalable, cost-effective microbial expression system.

Preclinical Evaluation of VB6-845

A preclinical evaluation of VB6-845 was performed to support clinical development in accordance with the ICH S6 (ICH S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals) guidelines. The preliminary investigation of VB6-845 examined its specificity, cytotoxicity, and reactivity with normal human tissue and identified appropriate clinical indications for the drug. This was followed by a comprehensive series of pharmacology and toxicology studies to determine the safety profile of VB6-845 for human use as well as establish a safe starting clinical dose.

In Vitro Specificity and Cytotoxicity of VB6-845

To illustrate the specificity and potency of VB6-845, a panel of epithelial tumor cell lines was tested for binding reactivity and cytotoxicity (Table 19.1). The tumor cell line selection was based, in part, upon the availability of established mouse xenograft models for follow-up efficacy studies as well as areas of perceived clinical need. As expected, VB6-845 potency varied according to EpCAM expression with no measurable cytotoxicity in EpCAM-negative cell lines. High EpCAM expression was associated with a sub-nanomolar IC_{50}. To further demonstrate the targeted

Table 19.2 Cytotoxic activity of common chemotherapeutic drugs

	IC_{50} (nM)			
Drug	NIH:OVCAR-3	A-375	Daudi	HMEC
Paclitaxel	$<10^{-6}$	4.9×10^{-6}	$<10^{-6}$	$<10^{-6}$
Docetaxel	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Vincristine	4.4×10^{-6}	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Topotecan	0.071	1.5	0.009	4.1
VB6-845	**1.0**	**>1,000**	**>1,000**	**220**
Doxorubicin	3.0	2.8	16×10^{-6}	16
Mitomycin C	28	14	2.8	50
Bleomycin Sulfate	30	170	22	600
Bleomycin A5	150	290	130	1,000
Irinotecan	180	900	190	1,000
Etoposide	210	280	1.7	600
Methotrexate	>1,000	6.0	3.6	41
Fluorouracil	>1,000	>1,000	>1,000	>1,000
Cyclophosphamide	>1,000	>1,000	>1,000	>1,000
Cisplatin	>1,000	>1,000	>1,000	>1,000

Representative IC_{50} of two independent experiments. Adapted with permission from Cizeau et al. [34]

specificity of VB6-845, the potency of VB6-845 was compared against a panel of chemotherapeutics on representative EpCAM-positive and EpCAM-negative tumor cell lines as well as a normal cell line (Table 19.2). In direct contrast to chemotherapeutics that showed no specificity, VB6-845 exhibited potent killing only against the EpCAM-positive tumor cell line (NIH:OVCAR-3) with minimal to no potency against the EpCAM-negative melanoma cell line (A-375), the lymphoid-derived tumor B cell line (Daudi), or the normal human mammary epithelial cell line (HMEC).

Immunohistochemical Staining with VB6-845

In accordance with FDA regulations, the reactivity of VB6-845 with normal human tissues was investigated using immunohistochemical analysis [43]. The GLP study tested a panel of 35 normal frozen human tissues for immune reactivity using an optimized concentration for staining. Of the 35 tissues examined, some degree of binding was noted in 20 tissue types with no binding being detected in the adrenal, bone marrow, brain, cerebellum, cervix, esophagus, eye, heart, liver, lymph node, muscle, placenta, skin, spinal cord, spleen, tonsil tissues, or white blood cells. The binding of VB6-845 in epithelia was membrane associated and consistent with the expression of EpCAM in normal tissues reported for other antibodies [44–46]. VB6-845 bound strongly to carcinomas of various origins including colon, rectum, head and neck, breast, prostate, esophagus, lung, endometrial, and ovarian, all of which are known to express higher levels of EpCAM relative to their normal counterparts [46].

In Vivo Efficacy

Since VB6-845 was highly potent against ovarian cell lines, SCID mice bearing established subcutaneous NIH:OVCAR-3 human tumor xenografts were used to evaluate the in vivo efficacy and tolerability of VB6-845 using 10 and 20 mg/kg doses. No significant weight loss was observed over the course of the treatment, indicating that both dose levels were well tolerated. The maximum tumor volume in the 10 mg/kg treated group was on average 40 mm^3 at the end of the study with 3/15 mice being tumor free. However, tumor growth was negligible in the 20 mg/kg treated group with a significantly higher number of tumor free mice (12/15) than observed in the control group [34] (Fig. 19.2a). Of the 15 mice in the untreated group, 11 reached the 750 mm^3 endpoint tumor volume. In contrast, treatment with 10 and 20 mg/kg of VB6-845 resulted in 100% survival by the end of the study with none of the treated mice reaching the 750 mm^3 endpoint tumor volume (Fig. 19.2b).

Given the promising in vitro and in vivo efficacy data, a comprehensive pharmacokinetic and toxicokinetic program was undertaken to determine the safety profile of this drug and to establish a safe starting dose in humans.

Selection of an Animal Model

In order to establish a pharmacologically relevant model for use in toxicology studies, several animal species (mouse, rat, and dog) including non-human primates (cynomolgus monkey, Rhesus monkey, and chimpanzee) were screened for binding cross-reactivity with VB6-845 through immunohistochemical analysis. With the exception of chimpanzees, no tissue cross-reactivity was observed. Given the ethical concerns surrounding the use of chimpanzees, this species was not considered suitable for toxicological testing. Therefore, it was determined that VB6-845 would not be pharmacologically active in terms of EpCAM binding in species typically used to conduct toxicology studies. This outcome was expected and observed in a previous preclinical evaluation of an EpCAM targeting scFv linked to *Pseudomonas* ETA [47, 48]. Consequently, the Sprague–Dawley rat was chosen for single-dose toxicological testing as it represented a well-characterized model for immunotoxin testing and in the case of some ETA-based immunotoxins has shown symptoms closely resembling VLS in humans [49, 50]. Given the lack of a cross-reactive species, it was decided that a second repeat-dose toxicity study would be performed in a non-human primate model (cynomolgus monkey) to provide another level of safety that would mimic the treatment regimen and route of administration to be used in the clinic. This same approach was used in the repeated-dose toxicology testing of the ADC Mylotarg, where its target antigen was only expressed in humans and larger primates [51]. It is important to note that subsequent to this study, IHC S6 regulations were amended to stipulate that tissue cross-reactivity is not an appropriate criterion for selecting a relevant species for the safety evaluation of immunotherapeutics.

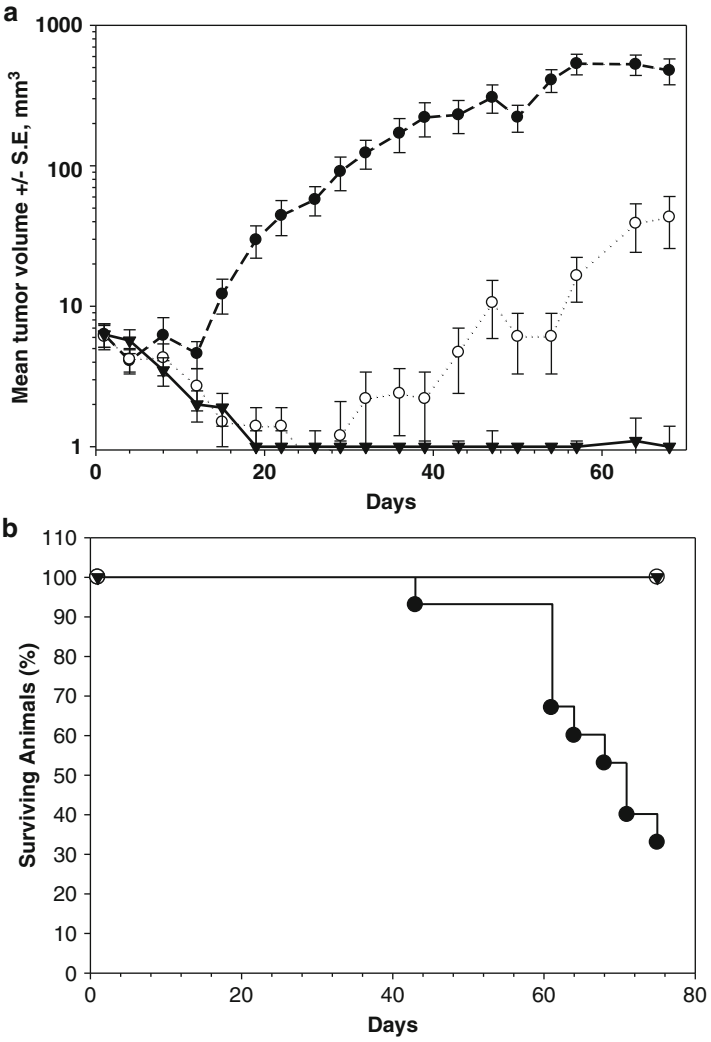

Fig. 19.2 Median tumor growth curves and survival plots. (**a**) NIH:OVCAR-3 human ovarian xenografts were generated from tumor fragments implanted subcutaneously into the flank of each mouse. On Day 1 of the study (8 days post-implantation), mice were randomly sorted into three groups ($n=15$). Group 1, untreated mice (*filled circles*), served as tumor growth controls. Mice in Groups 2 (*open circles*) and 3 (*inverted filled triangle*) received 10 and 20 mg/kg doses, respectively, administered on a 5-days-on, 2-days-off cycle for 3 weeks, followed by twice weekly for 4 weeks. The route of administration was a bolus intravenous (*IV*) injection into the tail vein until Day 26, followed by intraperitoneal (*IP*) injection for the remaining doses due to tail swelling. Animals were monitored for tumor size and were euthanized when their tumors reached the endpoint volume (750 mm^3) or on the last day of the study, Day 75. Animals dosed at 20 mg/kg showed no increase in median tumor volume, $p<0.001$. Reprinted with permission from Cizeau et al. [34]. (**B**) Kaplan–Meier survival plots. Animals from both treatment groups survived beyond the end of the study

Transgenic animals were also considered; however, their pattern of EpCAM expression was different when compared to humans and they were therefore unsuitable as pharmacologically relevant models [52–54].

Animal Toxicology Studies

Single-Dose Toxicology in Sprague–Dawley Rats

Sprague–Dawley rats (three/sex/dose) were administered a single IV bolus of VB6-845 via the lateral tail vein at doses of 6.25, 25, 50, 100, or 200 mg/kg, followed by a 2-week observation period. No overt adverse clinical signs were observed in animals administered up to and including 100 mg/kg of VB6-845. However, rats administered 200 mg/kg demonstrated clinical signs that included excessive licking of forepaws, reddened skin on fore and hind paws, edema of the forepaws, and a slight decrease in activity level. Rats in both the 100 and 200 mg/kg dose groups showed less body weight gain when compared to the control group.

Dose-dependent increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were detected following the administration of VB6-845 at 100 and 200 mg/kg. In both treatment groups, the changes in hepatic function were transient as AST returned to control levels by Day 8, and ALT returned to control levels by Day 15. On the basis of the elevated AST and ALT levels, as well as the paw oedema, the MTD was determined to be 200 mg/kg. The no-observable-adverse-effect level (NOAEL) for VB6-845 was determined to be 100 mg/kg. From these results, a repeated-dose toxicology study was undertaken in a non-human primate model mimicking the proposed clinical strategy for drug delivery.

Repeated-Dose Toxicology in Cynomolgus Monkeys

A GLP study was conducted to evaluate the systemic effects of repeated doses of VB6-845 administered via IV infusion in cynomolgus monkeys. In all, four treatment groups of Two/sex/group were administered VB6-845 at 10, 30, 60, or 90 mg/kg on Days 1 and 8 with additional recovery groups of one/sex/group (administered 60 or 90 mg/kg) maintained for a 20-day recovery period. The test article was administered to all groups via a 3 h infusion on Days 1 and 8, at a dose volume of 10 mL/kg/h, to mimic the route and infusion time intended for the clinic. Given the foreign nature of VB6-845 in this species and the expected immune response, repeat dosing was limited to two treatments. Parameters monitored included mortality, clinical signs, and body weight and food consumption. Blood samples were collected for hematology and clinical chemistry evaluations as well as for the determination of the pharmacokinetic profile and immunogenicity of VB6-845. At the end of the study, complete necropsy examinations were performed and a standard panel of tissues was microscopically examined.

There were no mortalities or changes in body weight. Treatment-related clinical signs were limited to decreased activity and hunched posture following the second treatment at all dose levels, with no indication of a dose relationship. These clinical signs generally resolved by the next day. Red cell parameters (erythrocytes, hemoglobin, and hematocrit) were comparably decreased in all treatment groups on Day 7, and continued to decrease through Day 14 in the Recovery Group, suggestive of a test article-related effect. Increased reticulocytes on Day 14 were compatible with a delayed bone marrow regenerative response. Transient and reversible increases in liver enzymes (AST, ALT, and SDH) were observed throughout the treatment; however, the changes did not appear to be dose-dependent and resolved by Day 20 in the Recovery Group.

Histopathological changes related to the administration of VB6-845 were dose-dependent and were limited to the kidneys, liver, spleen (female only), and lymph nodes (female only). All treatment-related effects, with the exception of tubular degeneration at 60 and 90 mg/kg that was still ongoing, were resolved by the end of the observation period. Due to the lack of tissue cross-reactivity in this model, these findings would not be unexpected as these organs represent the primary routes of metabolic elimination for a protein of this size.

Based on the results, the NOAEL was determined to be 30 mg/kg and therefore used as the basis for calculating a safe starting dose in the clinic [55, 56]. A dose level of 30 mg/kg in cynomolgus monkeys approximates a 10 mg/kg dose in humans [55] and applying the generally accepted 1 log lower safety margin resulted in the starting dose for humans being set at 1 mg/kg.

Pharmacokinetics in Cynomolgus Monkeys

The toxicokinetic profile of VB6-845 was evaluated as part of the repeated-dose toxicology study in cynomolgus monkeys, using an ELISA. Plasma samples were taken from two males and two females in the main study group at 0, 0.5, 1, 2, 4, and 24 h following a 3 h infusion of 10, 30, 60, and 90 mg/kg of VB6-845. Analysis indicated a proportional relationship between dose level and pharmacokinetic parameters in samples collected on both Day 1 and Day 8 (Table 19.3). Dose escalation was directly proportional to the mean peak (C_{max}) as well as the extent of the exposure ($AUC_{(last)}$). Although C_{max} values increased in a dose proportional manner on both Day 1 and Day 8, peak exposure levels were lower on Day 8 at all dose levels. The lower C_{max} on Day 8 at all dose levels had an effect on clearance (CL) and volume distribution (V_d) that was most likely due to an immune response in the animals. The mean half-life ($t_{1/2}$) values of VB6-845 on Days 1 and 8 were 2.5 ± 0.1 and 2.4 ± 0.5 h, respectively. The half-life was in the expected range for a 77 kDa drug and was similar to that obtained in a previous study with VB4-845 an anti-EpCAM scFv-ETA immunotoxin [48]. Some variability was observed between males and females; however, a statistical difference could not be calculated due to the limited sample size in each dosing group. The approximate 2.5 h half-life of VB6-845 indicated that full clearance of the drug would be expected between doses when patients are administered a once-weekly dose.

Table 19.3 Pharmacokinetic parameters in Cynomolgus monkeys

Dose level (mg/kg)	Day	C_{max} (μg/mL)	$AUC_{(last)}$ (h μg/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_d (mL/kg)
10	1	139 ± 30.8	801 ± 348	2.4 ± 0.1	14.5 ± 6.4	50.9 ± 24.4
	8	74.6 ± 22.5	291 ± 124	2.7 ± 0.4	39.6 ± 17.1	158 ± 84.0
30	1	335 ± 47.3	1,784 ± 107	2.5 ± 0.1	16.9 ± 1.0	59.5 ± 4.4
	8	245 ± 49.6	792 ± 160	2.7 ± 0.6	39.1 ± 8.3	147 ± 14.9
60	1	716 ± 134	3,682 ± 415	2.6 ± 0.1	16.4 ± 1.9	62.0 ± 6.5
	8	433.5 ± 179	1,354 ± 496	2.3 ± 0.3	48.7 ± 16.4	165 ± 70.9
90	1	1,255 ± 270	6,770 ± 2214	2.5 ± 0.1	14.2 ± 3.7	51.1 ± 15.3
	8	648 ± 230	2,585 ± 902	1.9 ± 0.2	38.2 ± 13.3	108 ± 45.3

Data for males and females are combined. Values are means ± SD, $n = 4$. C_{max} maximum observed drug concentration in plasma; $AUC_{(last)}$ area under the drug concentration–time curve from time 0 to time t, where t is the time of the last measurable plasma concentration; $t_{1/2}$ elimination; *CL* apparent plasma clearance; V_d apparent volume of distribution

Immunogenicity in Cynomolgus Monkeys

As both the antibody and toxin moieties of VB6-845 were foreign proteins to the cynomolgus monkeys, an immune response was expected and not considered predictive for humans. However, determining the immunogenic potential of protein therapeutics in animal models, particularly non-human primates, is important for identifying potential safety concerns that may arise in patients [57].

The immunogenicity of VB6-845 was evaluated in cynomolgus monkeys as part of the repeated-dose toxicology study with plasma samples taken from Main Study Group animals on Days 0 and 7 (Two/sex/dose) and from Recovery Group animals on Days 0, 7, 14, 21, and 28 (one male and one female per dose cohort) following IV administration of VB6-845. Samples were analyzed by ELISA to determine the presence of antibodies against the Fab fragment (4D5MOCB) and de-bouganin. Antibodies were detected 14 days after the first infusion with the majority of the response being directed towards the Fab portion of the molecule and to a lesser extent against the de-immunized bouganin moiety (Fig. 19.3). While this result demonstrated that de-bouganin was minimally immunogenic, a final assessment of the effectiveness of the de-immunization strategy can only be determined following repeat dosing in patients.

Summary of Preclinical Evaluation

The in vitro and in vivo preclinical data demonstrated the specificity and nanomolar potency of VB6-845 against EpCAM-positive cell lines and human tumor xenografts, respectively. A comprehensive toxicological program showed the safety and tolerability profile of VB6-845 and determined a safe starting dose of 1 mg/kg in the clinic.

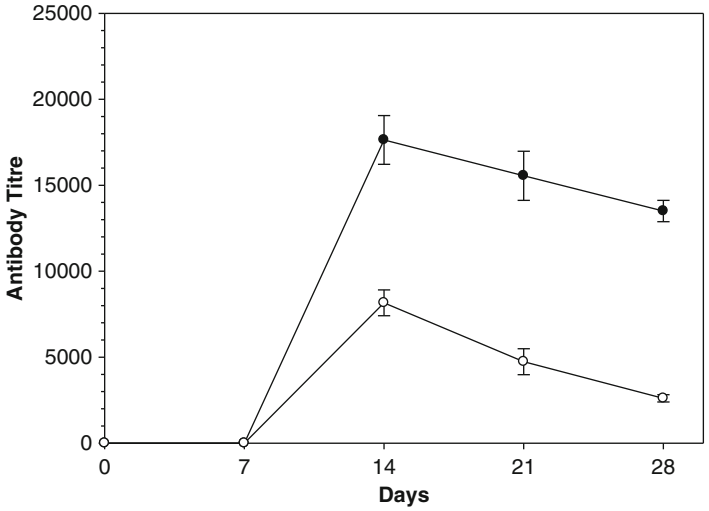

Fig. 19.3 Immunogenicity in Cynomolgous monkeys dosed at 90 mg/kg against the Fab fragment (*filled circles*) and de-bouganin (*open circles*). Mean antibody titers ± SE, $n = 2$.

Clinical Experience with VB6-845

Study Design and Dose Escalation

The primary study objective of the Phase I trial was to determine the maximum tolerated dose (MTD) and evaluate the safety and tolerability of VB6-845 when administered as an IV monotherapy infusion, once weekly, in 4-week cycles. Secondary objectives included evaluating the pharmacokinetic profile, assessing exploratory efficacy, and in particular examining the immunogenicity of VB6-845.

Dose cohorts of a minimum of 3 subjects with EpCAM-positive, advanced refractory solid tumors of epithelial origin as detected by immunohistochemistry were entered into the study which was carried out at a total of six investigative sites. The occurrence of a dose-limiting toxicity (DLT) in a cohort required the expansion of that dose cohort to six subjects and subjects who discontinued from the study prior to having received a minimum of four doses of VB6-845 were replaced. The starting dose was defined as 1 mg/kg and doses were escalated, according to a modified Fibonacci design, until an MTD was reached. The MTD was defined as the highest dose at which <2 out of six patients experienced a DLT. Subjects continued to receive treatment until an unacceptable toxicity occurred, all lesions completely disappeared, disease progression was determined, or the study was terminated. Patients were assessed for safety by monitoring of adverse events (AEs), clinical laboratory tests, standard 12-lead ECGs, vital signs, and physical examinations. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (NCI CTC AE v3.0).

A total of 15 subjects were enrolled into the study with solid tumors that included renal, ovary, breast, gastric, pancreas, non-small cell lung, and colorectal cancers. Three subjects were enrolled at the first cohort dose level of 1 mg/kg, ten subjects at the second cohort dose level of 2 mg/kg, and two subjects at the third cohort dose level of 3.34 mg/kg. The study was terminated when sufficient data had been collected to assess the immunogenicity of VB6-845 (see below). The maximum treatment duration was 16 weeks.

Safety Evaluation

Only one DLT was reported. This event was a grade 4 acute infusion reaction which occurred in a subject (cohort 2 at 2.0 mg/kg) with metastatic renal cell carcinoma. The subject developed hypotension and weakness during the third infusion, which was assessed as probably related to VB6-845. The event responded well to medicated therapy and was considered to be resolved 1 day after onset, without any sequelae. The MTD of VB6-845 was not reached at the time of study termination.

Of the 15 subjects who received treatment, 5 subjects reported a total of 5 serious adverse events (SAEs). Two of the reported SAEs were reported as related to study treatment. Both treatment-related SAEs were infusion reactions and consisted of a symptom complex characterized in both cases by hypotension, fever, and nausea, and on an individual basis included dizziness, weakness, drowsiness, chills, and hyperemia of the face and neck. The first infusion reaction event was grade 3 and resolved with standard therapy. The subject continued subsequent VB6-845 infusions with corticosteroid and H1 and H2-receptor antagonist pre-medication; the second event was grade 4 and assessed as the single reported DLT, as described above. The subject was discontinued from the study in accordance with the protocol treatment stopping criteria.

Due to the early closure of the trial, adverse event results are based on data available in the clinical database at the time of study termination. At least one treatment-related AE (defined as possibly, probably, or definitely related) was experienced by ten subjects. The majority of the treatment-related AEs reported were assessed as mild or moderate in severity and resolved within 1–2 days. The most frequently reported treatment-related AEs were associated with general disorders and administration site conditions. Within this group, pyrexia was reported most frequently.

Exploratory Efficacy Evaluation

A secondary endpoint of the study was to make an exploratory assessment of efficacy. Patients had full imaging performed (including, but not limited to, the chest, abdomen, pelvis, and bone architecture) at baseline in order to establish all existing lesions using standard imaging techniques (CT/MRI for chest, abdomen,

and pelvis; and a bone scan for bone architecture with following CT/MRI if bone scan is positive for any lesions). Post-baseline assessments of all sites of disease were made every 4 weeks using the same techniques as used at baseline. Up to ten sites of measurable disease were identified as "target" lesions for the assessment of tumor response. Non-radiographically measurable tumors were assessed for a clinical response by the Investigator.

Exploratory efficacy data reported on seven subjects enrolled in cohorts 1 and 2 who completed one full cycle (4 weeks) of treatment revealed encouraging preliminary results. Five of the seven subjects showed stable disease on CT scans 1 week after the completion of the fourth dose. Of the three subjects who continued to receive study treatment past the first cycle, one subject continued to have stable disease at the completion of their second (8 weeks) and third (12 weeks) cycles. In addition, objective tumor responses, based on data reported by the investigative sites at the time of study termination, demonstrated a decrease in measurable target tumor size in two patients in the second dose cohort; one with renal cell carcinoma and another with breast carcinoma. At baseline, the subject with renal cell carcinoma had six measurable target lesions in the lungs, as well as a measurable target lesion in a pulmonary lymph node and the pelvic mesentery. At the final visit (following the week 3 infusion), reported CT scan results showed decreases in all measurable target lesions, with decreases in individual lesions ranging from 11 to 29%. Other nontarget, nonmeasurable lesions appeared unchanged and the appearance of a potentially new brain lesion (inaccessible to VB6-845 therapy) was noted. At baseline, the subject with breast carcinoma had five measurable target lesions in the liver as well as three additional measurable target lymph nodes in the mediastinal, pre-tracheal, and bifurcational areas. CT scan results reported following the completion of 4 weekly infusions of VB6-845 revealed decreases in four of the five measurable target lesions in the liver, with decreases in individual lesions ranging from 4 to 15%. Nontarget, nonmeasurable lesions in lungs, liver, and bones were reported as stable.

Pharmacokinetics

Patient blood samples were taken for pharmacokinetic analysis on weeks 1 and 3 before, during, and after the 3 h infusion; analysis was completed only for the 1 and 2 mg/kg cohorts. The slow infusion rate was chosen to avoid a cytokine response and to maintain an elevated drug plasma concentration for a longer period of time than would have been achieved with a bolus injection. Sampling was performed according to the following schedule: pre-infusion, midpoint infusion (1.5 h), end of infusion, post-infusion 10 and 30 min; and 1, 2, 4, 6, 8, 12, and 24 h post-dose. The level of VB6-845 was measured using a GLP-validated, MTS-based potency assay. The assay detected VB6-845-mediated killing of CAL-27 cells, an EpCAM-positive cell line, and the IC_{50} values obtained are directly proportional to the concentration of intact drug; the lower detection limit of the assay was 14 pg/mL.

Table 19.4a Pharmacokinetic parameters in patients

Dose level (mg/kg)	Week	C_{max} (μg/mL)	$t_{1/2}$ (h)	$AUC_{(last)}$ (h μg/mL)	Cl (mL/h/kg)	V_d (mL/kg)
1	1	9.6 ± 2.3	3.8 ± 1.3	35.2 ± 13.3	30.8 ± 9.5	183 ± 99.6
	3	1.4 ± 1.8	2.2 ± 1.6	4.6 ± 6.1	859 ± 936	4,020 ± 5970
2	1	16.3 ± 5.8	4.9 ± 1.5	60.3 ± 24.8	40.5 ± 22.8	309 ± 245
	3	10.9 ± 9.6	2.6 ± 2.2	41.7 ± 46.5	299 ± 399	552 ± 503

Data for males and females are combined. Values are means ± SD. C_{max}, maximum observed drug concentration in plasma; $t_{1/2}$ elimination half-life; $AUC_{(last)}$ area under the drug concentration–time curve from time 0 to time t, where t is the time of the last measurable plasma concentration; CL apparent plasma clearance; V_d apparent volume of distribution

Table 19.4b Kinetics of VB6-845 plasma clearance in patients

Dose level (mg/kg)	Mean VB6-845 plasma concentration (nM)							
	Infusion time interval		Post-infusion time intervals (h)					
	Pre-dose	Infusion-3 h	1	2	4	8	12	24
1	N/A	123.95 (206.6)	63.53 (105.9)	39.07 (65.1)	15.50 (25.8)	2.68 (4.5)	1.02 (1.7)	0.2 (0.3)
2	N/A	196.93 (328.2)	108.98 (181.6)	52.15 (86.9)	21.98 (36.6)	3.80 (6.3)	1.46 (2.43)	0.41 (0.68)

Values in parentheses represent the fold increase in nM concentration of VB6-845 expressed as a function of the VB6-845 IC_{50} concentration versus ovarian carcinoma cell line NIH:OVCAR-3

Standard pharmacokinetic parameters were determined for both dose levels (Table 19.4a). The maximum concentration (C_{max}) measured for patients dosed at 1 mg/kg ranged from 8.08–12.26 μg/mL in week 1 to 0.07–3.50 μg/mL at week 3. For patients dosed at the 2 mg/kg level, the C_{max} values ranged from 4.76–23.2 μg/mL in week 1 to 1.15–21.6 μg/mL at week 3. The mean maximal plasma concentration for both dose cohorts was at the end of the 3 h infusion. A comparison of the two dose cohorts indicated a dose proportionality that was concentration dependent similar to that observed in the non-human primate study. The mean elimination time ($t_{1/2}$) for patients dosed at 1 mg/kg was 3.8 h on week 1 and 2.2 h on week 3 and for the 2 mg/kg group was 4.9 h on week 1 and subsequently 2.6 h on week 3. The peak exposure levels and half-life of VB6-845 were lower in all patients by week 3; however, the reduction was considerably less in the 2 mg/kg group. VB6-845 was cleared fairly rapidly within the first 24 h following infusion consistent with its molecular size. The highest concentration of drug detected for each dose cohort was >2 logs over the IC_{50} value of VB6-845 (0.6 nM) versus the ovarian carcinoma cell line NIH:OVCAR-3, whereas the lowest drug concentration at the 24 h time point approximated the IC_{50} concentration (Table 19.4b).

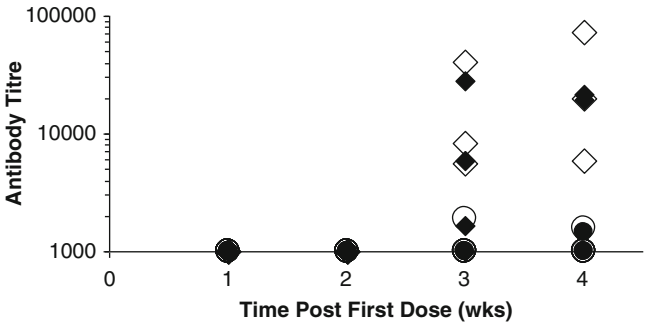

Fig. 19.4 Antibody titers measured against de-bouganin (1 mg cohort (*filled circles*); 2 mg cohort (*open circles*) and the Fab moiety (1 mg cohort (*open diamonds*); 2 mg cohort (*filled diamonds*)) of VB6-845. For 1 mg cohort n = 3 for all weeks. For 2 mg cohort, n = 9 for weeks 1 and 2, n = 4 for week 3, and n = 2 for week 4

Immunogenicity

To assess the effectiveness of the bouganin de-immunization strategy, patient plasma samples were tested for immune responsiveness to the humanized Fab and de-bouganin portions of VB6-845 (Fig. 19.4). No measurable antibody titers were directed against either molecule in any of the patients after 2 weeks. A relatively weak anti-de-bouganin titer was measured in only one of the patients after the first 3 weeks of treatment, as compared to six of seven patients who showed anti-Fab titers at the same time point. By week 4, all patients from both dose cohorts had measurable anti-Fab titers (mean titer = 27,858 ± 25,744, n = 5). In contrast, only two of these patients had berely detectable anti-de-bouganin titers (mean titer 1,513 ± 65.1). The lack of immune responsiveness towards de-bouganin, a totally foreign protein, in these patients illustrates the validity of the T cell epitope-depletion approach to dampen the immune response and strongly supports the utility of de-bouganin as a cytotoxic payload for systemic delivery. Even though the CDR loops of the mouse anti-EpCAM antibody were grafted onto a humanized framework, immune reactivity is not necessarily unexpected as humanized and even fully human antibodies can exhibit some degree of immunogenicity in the clinic [58]. The strength and degree of this antibody response on efficacy and/or safety will often depend upon the antibody itself and the indication being treated. In order to reintroduce VB6-845 to the clinic, the T cell epitopes of the Fab fragment have been identified and removed while preserving specificity and potency.

Summary

The potency and efficacy of immunotoxins as cancer therapeutics, particularly for treating cancers of hematologic origin, have been well demonstrated over the last two decades. However, the clinical effectiveness of immunotoxins for solid cancer

therapy has been limited by their immunogenicity directed primarily at the toxin moiety. VB6-845 was well tolerated and showed preliminary efficacy in the exploratory Phase I trial. The clinical data supported the continued development of VB6-845 as a promising new therapy for advanced solid tumors of epithelial origin. An important endpoint of this study was to assess the immunogenicity of VB6-845 as the appearance of ADAs would reduce the number of treatment cycles and limit clinical benefit. The study showed the de-bouganin payload to be of low immunogenic potential with a minimal de-bouganin response and therefore represents a first-in-man demonstration of a successfully de-immunized protein toxin. On the strength of the clinical experience with VB6-845, Viventia is currently evaluating several Fab-de-bouganin molecules specific to solid tumors. These antibodies were characterized using an immune driven antibody platform which comprises a novel screening method to generate fully human antibody fragments, thus circumventing the need to de-immunize the targeting moiety [59]. Reducing or completely ablating the appearance of anti-toxin antibodies with de-immunization strategies will permit a true assessment of the clinical benefit of immunotoxins in targeted cancer therapy.

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