A preclinical profile of VB6-845: a recombinant immunotoxin for targeting ovarian cancer

J. Brown[a], J. Cizeau[a], D. Bosc[a], M. Rasamoelisolo[a], J. Entwistle[a], N. Glover[b], and G. C. MacDonald[a].

[a]Viventia Biotech Inc. 147 Hamelin St., Winnipeg, MB, Canada, R3T 3Z1; [b]5060 Spectrum Way, Suite 405, Mississauga, ON, L4W 5N5

ABSTRACT

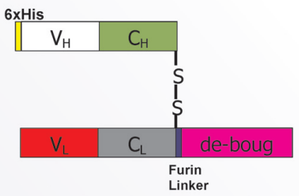
Ovarian cancer is the fourth most common cause of cancer mortality in women. Most patients present with advanced disease due to non-distinctive symptoms. Advances in surgery and treatment modalities have improved survival, but prognosis remains poor with a five-year survival rate of about 30% signifying the lack of effective treatment. VB6-845 is a recombinant fusion protein consisting of a tumor-targeting Fab linked to de-immunized Bouganin. VB6-845 specifically targets the epithelial cell adhesion molecule (Ep-CAM) that is highly expressed on many epithelial carcinomas, including gynecological cancers. Examination of VB6-845 cross-reactivity in gynecological carcinomas was conducted using Immunohistochemistry (IHC) analysis that included various disease stages and grades. VB6-845 immunoreactivity against ovarian primary and metastatic carcinomas was >90%. For endometrial and cervical carcinomas the reactivity was 95% and 64% respectively. IHC also was used to assess the potential for VB6-845 cross-reactivity with normal human tissue. Positive binding was seen in some epithelial tissues whereas binding was not observed in non-epithelial tissues or stromal components. To evaluate safety of VB6-845, toxicology studies were conducted. An acute dose toxicology study was conducted in the Sprague-Dawley rat. The MTD was determined to be 200 mg/kg at which animals presented with odema of the paws and liver enzymes levels were elevated more than 10 fold above normal. A non-human primate toxicology study was conducted in Cynomolgus monkeys. Animals were I.V. dosed twice, one week apart, at 10, 30, 60, 90 mg/kg in a dose escalation study. There were no mortalities, changes in body weight, and few clinical signs attributed to VB6-845 dosing. Elevated liver enzyme levels correlated to microscopic changes in the liver of animals dosed at 60 and 90 mg/kg. Microscopic changes also were noted in the kidneys, spleen and lymph nodes. All VB6-845 related changes had resolved by the end of the recovery period and the NOAEL was 30 mg/kg. The pharmacokinetic profile indicated that there was dose proportionality of VB6-845 (a 9-fold dose increase resulted in a 9-fold C_{max} increase) to the mean peak exposure. The mean half-life ($T_{1/2}$) values of VB6-845 were similar between Days 1 and 8 (2.49 $\pm$ 0.13 and 2.40 $\pm$ 0.48 hours, respectively). The preclinical profile concerning specificity and safety indicate that VB6-845 was well tolerated and will be effective in directing its potent cytolytic effect against ovarian cancer.

INTRODUCTION

Epithelial ovarian cancer is the fourth most common cause of cancer mortality in women and the leading cause of death from gynecological cancers. Of all newly diagnosed cases of ovarian cancer, approximately 75% present with advanced stage III or IV disease, for which, recurrence rates are high with a median survival rate of 2 years. Options for late stage or recurrent disease are limited and patients that have previously been treated with chemotherapy regimens typically do not benefit from further chemotherapy treatment indicating a lack of effective treatment.

Immunogenicity and toxicity represent two challenges that limit the effectiveness of immunotoxins as cancer therapeutics. The cancer treatment strategy for Viventia's immunotoxin development has been to overcome these impediments. VB6-845 is a recombinant fusion protein consisting of a humanized FAb version of a single chain variable fragment (scFv) specific for the epithelial cell adhesion molecule (Ep-CAM) antigen linked to a de-immunized form of Bouganin, a type I ribosome inactivating protein (RIP) (Figure 1). The de-immunized Bouganin has been engineered to retain the active domains necessary prevent protein synthesis and induce cell death, but the antigenic domains have been eliminated thereby preventing de-bouganin from launching an immune response following systemic exposure to VB6-845. Ep-CAM is a cell surface marker that is highly expressed on a variety of different carcinoma cells of various epithelial origins, but has limited expression on normal cells making it a suitable target for immunotherapy against these cancers.

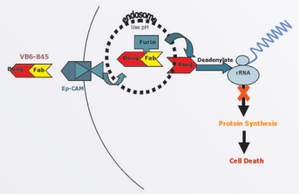
Figure 1 Schematic Representation of VB6-845



Mechanism of Action

VB6-845, bound to the Ep-CAM antigen found on the surface of carcinoma cells, is internalized through an endocytic pathway. Furin contained within the endosomal compartment cleaves a proteolytic site in the furin linker, releasing an activated form of de-bouganin. The de-immunized version of bouganin has been engineered to retain the active domains necessary to induce cell death, but the antigenic domains have been eliminated thereby preventing bouganin from launching an immune response following systemic exposure. Cell death is induced via a mechanism similar to other type I RIPs, by removing a single adenine base from the highly conserved rRNA (28S rRNA) in eukaryotic cells. This results in a conformational change in the rRNA, inhibiting the binding of elongation factors, halts protein synthesis and ultimately results in cell death. Cells that do not express the Ep-CAM antigen do not bind VB6-845 and are not subject to bouganin mediated effects

Figure 2 Mechanism of Action of VB6-845



RESULTS

VB6-845 has a high level of reactivity against ovarian cancers

Table 1 A high degree of VB6-845 immunoreactivity against various types of ovarian carcinoma was demonstrated by immunohistochemistry analysis. Tissue micro arrays of ovarian carcinomas were examined for cross-reactivity with VB6-845. Normal colon mucosa was used as the positive tissue control; lymphatic tissue (tonsil) was used as the negative tissue control.

			Cell Surface Staining (% cells)			
		n	positive	weak	moderate	strong
Histology	samples	200	93.2			
	serous-papillary	72	93.1	9.7	15.3	68.1
	endometroid	48	98.0	4.2	12.5	81.3
	mucinous	24	100	8.3	8.3	83.3
	clear cell	20	100	15.0	0.0	85.0
	rare types	36	75.0	16.7	8.3	50.0
Stage	T1	39	94.9	12.8	7.7	74.4
	T2	23	87.0	0.0	4.3	82.6
	T3	69	91.3	11.6	8.7	71.0
	T4	0	-	-	-	-
Nodal stage	N0	7	85.7	14.3	0.0	71.4
	N1	6	100	16.7	0.0	83.3
Silverberg grade	G1	57	96.5	8.8	17.5	70.2
	G2	62	98.4	11.3	9.7	77.4
	G3	63	93.6	6.3	6.3	81.0

VB6-845 is reactive only against human epithelial tissues

Table 2 IHC analysis examining VB6-845 immunoreactivity with normal human tissues indicated cross-reactivity limited to epithelial tissues. The number of positive staining tissues and the percent of the cells that stained positive at the optimal staining concentration (1.25) µg/mL are indicated.

Normal Tissue	Pathology Score: Positive Tissue Samples N = 3	% cells in Positive Range: weak	moderate	strong
Gall Bladder	3	0-25	0-25	50-100
Breast	3	10-20	30-80	50-60
Colon	3	0-10	50-80	20-50
Fallopian Tube	3	0-10	10-40	50-90
Thyroid	3	0-10	40-50	50-60
Trachea	3	0-30	40-50	20-50
Pancreas	3	0-15	10-50	20-90
Parathyroid	3	0	10-30	70-90
Parotid	3	5-30	5-30	10-90
Small Intestine	3	0-20	20-50	30-80
Prostate	3	10-30	50-60	0-40
Kidney	3	30-50	20-30	5-20
Ureter	3	10-40	10-70	0-20
Uterus	3	0-50	10-50	0-90
Bladder	3	50-80	30	0
Lung	3	20-60	0-10	0
Stomach	3	20-50	0-30	0
Pituitary	2	0-20	0-45	0-30
Testis	2	0-60	0-10	0
Thymus	2	0-7	0-5	0
Cervix	0	0	0	0
Esophagus	0	0	0	0
Skin	0	0	0	0
Adrenal	0	0	0	0
Bone Marrow	0	0	0	0
Brain	0	0	0	0
Cerebellum	0	0	0	0
Eye	0	0	0	0
Heart	0	0	0	0
Liver	0	0	0	0
Lymph Node	0	0	0	0
Muscle,skeletal	0	0	0	0
Ovary[1]	1	50-80	20-50	0
Placenta	0	0	0	0
Spinal Cord	0	0	0	0
Spleen	0	0	0	0
Tonsil	0	0	0	0
WBC	0	0	0	0

1 epithelium cell of small benign cyst is stained and scored

In vitro cytotoxicity demonstrated with VB6-845

VB6-845 exhibited potent in vitro activity against the ovarian Ep-CAM positive cancer cell line, OVCAR-3 as determined using an MTS assay, with an IC_{50} of 0.6 nM, (0.26 µg/mL). In vivo efficacy studies demonstrated the potential cytotoxic effect of VB6-845 in OVCAR-3 and MCF-7 xenograft models (Poster #5555, Wednesday April 5, 8:00 AM).

Toxicological Analysis of VB6-845

VB6-845 was not cross-reactive with mice, rats, dogs, or Cynomolgus monkeys. To evaluate the safety of the de-bouganin, toxicology studies were conducted in Sprague-Dawley rats and Cynomolgus monkeys.

Single dose rodent toxicology study

VB6-845 was administered as an IV bolus in a dose escalation study (6.25, 25, 50, 100, and 200 mg/kg) in Sprague-Dawley rats. An MTD was established.

- **No unscheduled deaths during the study**
- **Clinical signs at 200 mg/kg: edema of the paws, slight cyanosis**
- **Elevated liver enzyme levels (AST, 15-fold and ALT, 37-fold)**
- **Recovery AST, Day 8 and ALT, Day 15**
- **MTD = 200 mg/kg**

Repeated dose rodent toxicology study

Table 3 Sprague-Dawley rats were administered five intravenous bolus VB6-845 injections. The doses were administered every 3rd day for 2 weeks. A dose-dependent anaphylactic reaction occurred with the 4th dose. The 40 mg/kg group did not receive the 5th dose.

Group	Dose level mg/kg	Treatment group	Recovery group	PK group
1	Vehicle control	20	10	0
2	10	20	0	18
3	20	20	10	18
4	40	20	10	18

- **Anaphylaxis after 4th dose**
- **Unscheduled deaths during the study**
- **Clinical signs: decreased activity, labored breathing, cold to touch,and cyanosis**
- **Elevated liver enzyme levels (AST and ALT)**
- **Microscopic changes: heart, stomach, small intestines, large intestines, liver, lung, spleen, kidney, epididymis, skeletal muscle, and tongue**
- **Complete recovery by day 14 after the completion of dosing at 20 & 40 mg/kg/day except for heart and lungs**

Immunogenicity analysis

Plasma samples to determine the antibody response induced in Sprague-Dawley rats were taken from all animals on Days 0, 7, and 13 with additional samples from the recovery group animals on Day 27. No antibody titres were found on Day 0. Antibody titres against the whole molecule, VB6-845, were detected by an ELISA on Day 13. An antibody response directed to the 4D5 MOCB FAB portion was only detected on Day 27 and no antibody response was detected to the de-immunized Bouganin.

Table 4 The majority of the animals examined for an immune response were found to have antibody titres against the full molecule VB6-845 with no antibody titre detected against the de-immunized Bouganin. Results are based on the number of available samples.

Dose level mg/kg	Anti-VB6-845 Titers			Anti-4D5-Fab Titers			Anti-de-Bouganin Titers		
	D 7	D 13	D 27	D 7	D 13	D 27	D 7	D 13	D 27
10	0	5 / 20	-	0	0	0	0	0	0
20	0	20 / 24	3 / 4	0	0	1 / 3	0	0	0
40	0	11 / 13	5 / 5	0	0	1 / 4	0	0	0

Pharmacokinetic analysis

VB6-845 was evaluated at each dose level on Days 1 and 13 at 8 sampling times (0, 0.25, 0.5, 1, 2, 4, 6, 24 hours). VB6-845 was not quantifiable in any samples collected predose. The maximum plasma concentration of VB6-845 was estimated to be 433,107 ng/mL in a female rat 15 minutes after IV administration of 40 mg/kg. At 20 and 40 mg/kg, an initial peak in VB6-845 concentration was observed 15 minutes following administration, followed by a second peak at approximately 4-6 hours after dosing on Day 1. This elimination pattern may result from circulatory proteins in the rat interacting with VB6-845, making it initially unavailable for quantification, and thus a second peak is observed.

Escalating Dose Toxicology Study in Cynomolgus monkeys

Table 5 Cynomologous monkeys received two weekly VB6-845 intravenous infusions. Each treatment group, consisting of four monkeys (two females and two males) with recovery groups at the two upper doses, received two doses of VB6-845 as 3 hour intravenous infusions given one week apart. The highest dose of 90 mg/kg in monkeys is equivalent to 210 mg/kg in rats or 30 mg/kg in humans.

Group	Dose level mg/kg	Treatment group	Recovery group	PK group
1	10	4	0	4
2	30	4	0	4
3	60	4	2	4
4	90	4	2	4

- **No unscheduled deaths during the study**
- **Clinical signs after 2nd dose limited to decreased activity and hunched posture (not dose related)**
- **Reduction in food consumption related to treatment**
- **Elevated liver enzyme levels (AST and ALT)**
- **Microscopic changes were seen in animals dosed at 30 mg/kg and above: minimal individual hepatocyte necrosis, focal necrosis, and increased subacute inflammation in livers**
- **Complete recovery except for kidney tubular regeneration in one male (60 mg/kg) and one female (90 mg/kg)**
- **NOEL = 10 mg/kg**
- **NOAEL = 30 mg/kg**

Immunogenicity analysis

Plasma samples were taken from all animals on Days 0 and 7 and additional samples from recovery animals on Days 14, 21 and 28 to determine the antibody response induced in Cynomolgus monkeys. The presence of anti-VB6-845, anti-4D5 MOCB FAB, and anti-de-Bouganin antibodies were detected by an ELISA 14 days after the first VB6-845 infusion. No immune response was observed in the pre-bleed samples taken prior to dosing. Examination of the two components of VB6-845 indicated that most of the immune response (50%) was directed to the 4D5 MOCB FAB portion. A lesser immune response (up to 25%) was directed to the de-immunized Bouganin.

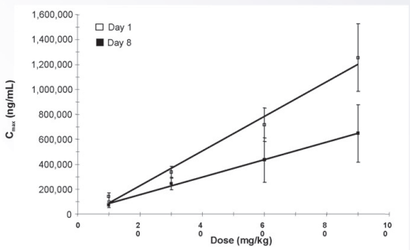
Pharmacokinetic analysis

Table 6 Pharmacokinetic analysis of intravenous injections of VB6-845 to Cynomolgus Monkeys. Plasma samples were taken (0, 0.5, 1, 2, 4, and 24 hrs) after dosing on Days 1 and 8. Data is shown for Day 1.

Dose Level	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng·h/mL)	$AUC_{0\text{-}inf}$ (ng·h/mL)	$T_{1/2}$ (h)	Cl (mL/kg/h)	Vz (mL/kg)
10	138720	801196	801600	2.4	14.533	50.928
30	334500	1783780	1784850	2.45	16.855	59.493
60	716250	3672347	3685874	2.62	16.435	61.983
90	1254500	6770403	6774564	2.48	2.48	51.183

Dose proportionality of VB6-845 was demonstrated by a 9-fold dose increase corresponding to a 9-fold C_{max} increase. The mean half-life ($T_{1/2}$) values of VB6-845 were similar between Days 1 and 8 (2.49 and 2.40 hours, respectively). C_{max} on day 8 was consistently lower than Day 1, resulting in an increase in clearance (Cl) and volume of distribution (Vz). The change in peak exposure on Day 8 was most likely due to an immune response detected in the animals by the 14th day after the first dose.

Figure 3 A comparison of average C_{max} values and VB6-845 dose levels demonstrated dose proportionality in circulating levels of VB6-845.



CONCLUSIONS

- **VB6-845 shows targeting to ovarian cancer**
- **VB6-845 administered as an IV bolus in rats resulted in elevated liver enzyme levels, edema of the paws, and slight cyanosis at the MTD 200 mg/kg**
- **VB6-845 administered as an IV infusion in Cynomolgus monkeys was well tolerated with no toxic side effects over the dose range tested**

These results clearly demonstrate that systemically administered VB6-845 will be targeted to ovarian cancer and other Ep-CAM positive solid tumors with minimal toxicity