Complete regression of ovarian cancer xenografts following treatment with the recombinant immunocytotoxic protein, VB6-845

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ABSTRACT

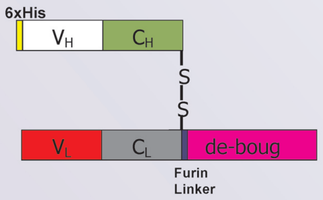
VB6-845 is a recombinant fusion construct consisting of a tumor-targeting Fab linked to de-immunized Bouganin, a type 1 ribosome-inactivating protein (RIP). VB6-845 specifically targets the epithelial cell adhesion molecule (Ep-CAM) that is highly expressed on many different epithelial carcinomas, including ovarian cancer. Ovarian cancer is the fourth most common cause of cancer mortality in women. Advances in surgery and treatment modalities have improved survival, but prognosis remains poor with a five-year survival rate of about 30% indicating the need for more effective treatment. Immunohistochemistry (IHC) determined a high level of VB6-845 immunoreactivity in ovarian primary (>90%) and metastatic (90%) carcinomas. VB6-845 exhibited potent 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 of VB6-845 was demonstrated in the OVCAR-3 subcutaneous implanted xenograft model in SCID mice. I.V. administration of 10 & 20 mg/kg of VB6-845 resulted in 100% survival as compared to 27% survival in the untreated group on Day 75. The majority of the mice (80%) dosed at 20 mg/kg were tumor free ($p < 0.001$) at the end of the study. *In vivo* efficacy of VB6-845 also was demonstrated in the OVCAR-3 intraperitoneal (I.P.) implanted xenograft model in Balb/c *Nude* mice. I.P. administration of 10 mg/kg of VB6-845 resulted in 93% survival as compared to 0% in the untreated group on Day 84 ($p < 0.0001$) and none of the treated mice had tumor masses or ascites. CA125 levels of mice treated with VB6-845 were not different from tumor-free mice ($p = 0.9889$) whereas untreated mice had CA125 levels significantly higher than treated mice ($p < 0.0001$). Efficacy also was demonstrated in another Ep-CAM positive MCF-7 model, whereas VB6-845 had negligible effect on the Ep-CAM negative A-375 model.

INTRODUCTION

VB6-845 is a recombinant fusion protein consisting of a Fab version of a single chain variable fragment (scFv) specific for the Epithelial Cell Adhesion Molecule (Ep-CAM) antigen linked to a de-immunized Bouganin, a type I ribosome inactivating protein (RIP) that is missing the antigenic domains. Ep-CAM is a cell surface marker that is highly expressed on carcinoma cells of epithelial origin, but has limited expression on normal cells.

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. Only one ovarian epithelial cancer tumor marker has been identified: CA-125, a membrane glycoprotein. Approximately 90% of advanced stage epithelial ovarian cancer patients have elevated concentrations of CA-125, compared to 50% of stage I patients that display elevated CA-125 levels. Expression of CA-125 has been identified as a marker for patient prognosis, disease progression and relapse, and response to chemotherapy. 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.

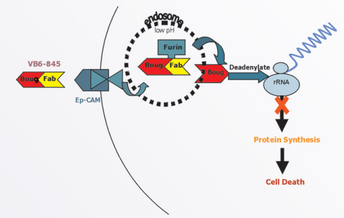
Figure 1 Schematic Representation of VB6-845



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



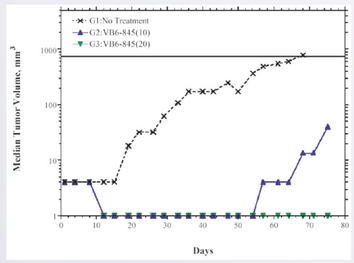
Immunohistochemistry (IHC) examination of VB6-845 in various tissue types showed a high level of immunoreactivity in ovarian primary (>90%) and metastatic (90%) carcinomas. VB6-845 exhibited potent activity against the ovarian Ep-CAM-positive cancer cell line, OVCAR-3 as determined using an MTS assay, with an IC_{50} of 0.6 nM (0.26 μg/mL). In vivo efficacy studies were conducted to examine the cytotoxic effect of VB6-845.

RESULTS

Subcutaneous Implanted Xenograft Model

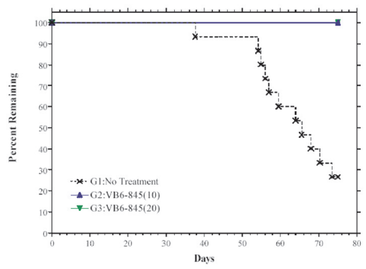
Figure 2 VB6-845 mediated tumor regression and growth inhibition against a subcutaneous ovarian tumor (OVCAR-3) xenograft model.

Female SCID mice (15 per treatment group)
Dose: IV 10 or 20 mg/kg
Schedule: 5 days followed by 2 days off for 3 cycles, twice weekly for 4 weeks



End of Study 0 mg/kg: 73% reached 750 mm^3 tumor endpoint volumes
10 mg/kg: 20% complete regression, average tumor 40 mm^3
20 mg/kg: 80% complete regression, negligible tumor growth
Highly significant compared to 0 mg/kg ($p < 0.001$)

Figure 3 Treatment with VB6-845 resulted in long-term survival of mice with subcutaneous implanted tumor tissue.



End of Study 0 mg/kg: 27% survival
10 mg/kg: 100% survival
20 mg/kg: 100% survival

Intraperitoneal Implanted Xenograft Model

Table 1 Efficacy in an intraperitoneal ovarian tumor (OVCAR-3) xenograft model.

Female Balb/c *Nude* mice (15 per treatment group)
Dose: IV 10 or 20 mg/kg
IP 10 or 20 mg/kg
Schedule: 5 days followed by 2 days off for 3 cycles, twice weekly dosing for 4 weeks (modified due to IP toxicity).

Group	Treatment	T/C %	Mean survival time Days ± SD	Log-Rank test as compared to Group 2	Median survival time
1	No tumor	-	-	$p < 0.0429$	-
2	No treatment	NA	53.3 ± 10.9	-	56
3	IP 10mg/kg	150	80.1 ± 15.0	$p < 0.0001$	84
4	IV 10mg/kg	150	77.1 ± 13.0	$p < 0.0001$	84
5	IP 20mg/kg	150	56.3 ± 35.2	$p < 0.0504$	84
6	IV 20mg/kg	150	78.1 ± 17.0	$p < 0.0001$	84

T is the median survival time of treated animals and C the median survival time of untreated animals. Significant survival is indicated when T/C% exceeds 125 %, the threshold value for anti-tumor effectiveness.

End of Study At equal doses the IV route better tolerated than IP route
0 mg/kg: 100% animals reached tumor endpoint volume
IV: significant survival at 10 & 20 mg/kg. Efficacy at 20 mg/kg
IP: toxicity at 20 mg/kg. Efficacy at 10 mg/kg

Figure 4 Analysis of CA125 plasma levels corroborates the anti-tumor effectiveness of VB6-845. VB6-845 treatment of OVCAR-3 xenograft mice resulted in a statistically lower CA125 plasma level compared to untreated, tumor bearing mice. Animals treated IP 10 & 20 mg/kg and IV 20 mg/kg (groups 3, 5 & 6) were not statistically different compared to the negative control animals (ungrafted and untreated).

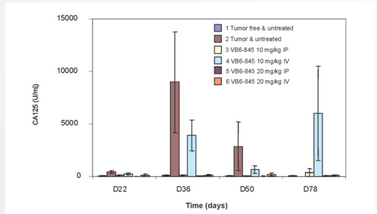


Figure 5 Representative pictures of mice from the OVCAR-3 intraperitoneal study demonstrating the difference between untreated animals and animals receiving treatment at efficacious doses of VB6-845. Pictures taken at the end of the study or last day of survival.

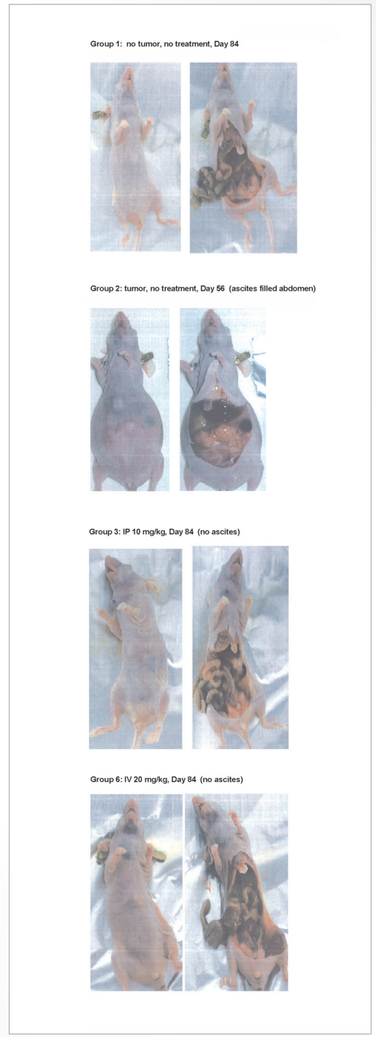
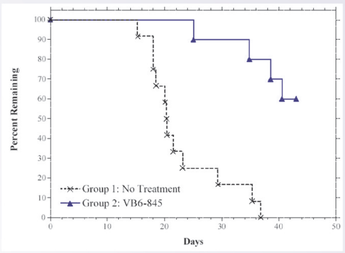


Figure 6 VB6-845 efficacy in another Ep-CAM-positive breast tumor (MCF-7) xenograft model.

Female HRLN *nu/nu* mice (12 per treatment group)
Dose: IV 20 mg/kg
Modified Schedule: 5 days followed by 2 days off for 3 cycles



End of Study 0 mg/kg: 100% animals reached 1000 mm^3 tumor endpoint volume
20 mg/kg: 30% complete response, ($p < 0.001$)

Table 2 Demonstration of VB6-845 specificity using an Ep-CAM-negative melanoma (A-375) xenograft model.

Dose Level	Schedule	Median tumor volume on day 10	% Tumor growth inhibition	Log-Rank Test (comparison of survival curves)
No treatment	-	126	-	-
20 mg/kg	5/2/4	82	35%	Non significant

End of Study There was no difference in survival or tumor growth delay between treated and untreated animals.

CONCLUSIONS

1. ***Increased survival and tumor growth inhibition were demonstrated in Ep-CAM-positive tumors***
   - Subcutaneous OVCAR-3 implantation
     - IV administration 20 mg/kg
   - Intraperitoneal OVCAR-3 implantation
     - IP administration 10 mg/kg
     - IV administration 20 mg/kg
2. ***The anti-cancer effect of VB6-845 was Ep-CAM specific***
   - Treatment effective only against Ep-CAM-positive tumors
     - Ovarian (OVCAR-3) and breast (MCF-7) models
   - Treatment had no effect against Ep-CAM negative tumors
     - melanoma (A-375) model

These results clearly demonstrate that VB6-845 is potently cytotoxic and specific against ovarian cancer and other Ep-CAM positive solid tumors