Abstract # 4786

Functional and biological characterization of VB6-845, a recombinant Ep-CAM-specific Fab antibody genetically-linked with de-immunized Bouganin (de-bouganin)

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Abstract

Chemotherapeutics are highly cytotoxic agents that often represent the standard of care in the treatment of many of the solid tumor cancers. The cytotoxic action of these drugs targets rapidly dividing cells, both normal and tumor, thus creating a variety of adverse clinical side-effects. VB6-845 is a Fab antibody linked to a de-immunized form of the plant-derived toxin bouganin. Unlike chemotherapeutics which lack defined tumor-target specificity, VB6-845 restricts its cytolytic effect to Ep-CAM-positive tumor targets alone. In this study, flow cytometry analysis and cytotoxicity were measured to assess the potency and selectivity of VB6-845. Flow cytometry with VB6-845 against a large panel of tumor cells lines showed strong binding by the Fab to cell lines representative of a wide variety of cancer indications. In contrast, VB6-845 exhibited only limited cell surface binding to normal epithelial cell lines. The level of killing for VB6-845 was comparable to another Fab VB6-845 variant containing a different plant-derived toxin, gelonin. When assayed for cytotoxicity against OVCAR-3, an Ep-CAM-positive ovarian carcinoma, using a panel of standard chemotherapeutic agents, VB6-845 was shown to be more potent than 12 of the 17 drugs tested. Though 5 chemotherapeutics were more cytotoxic, they were also shown to be far more toxic in that they lacked any cell-specific killing. Of the five recommended chemotherapeutic agents for the treatment of ovarian cancer (Paclitaxel, Carboplatin, Cisplatin, Doxorubicin and Topotecan), only two (Paclitaxel and Topotecan) were more cytotoxic. While VB6-845 demonstrated highly potent cytolytic activity with an IC_{50} of 1 to 2 nM, the potent killing was restricted exclusively to the Ep-CAM-positive tumor cell line OVCAR-3. Although some Ep-CAM-negative cell lines exhibited some level of killing, the cytotoxic effect was at least 220-fold and at most >1000-fold less toxic. VB6-845 thus represents a potent antibody-directed treatment alternative to chemotherapeutics that when combined with the lower toxicity profile, holds much promise in the treatment of many different types of solid tumors.

Introduction

Most cancer patients will receive systemic chemotherapy during the course of their disease. Despite recent advances, one of the major obstacles to successful therapy is the lack of drug selectivity which gives rise to dose-limiting toxicities. Several attempts have been made to address these problems including regional drug therapy or the incorporation of the therapeutic agent into a delivery vehicle such as a liposome. To date however, high regional toxicity is still observed with the local administration and most delivery systems still require optimization and thorough testing in a clinical setting.

Viventia's approach to reducing toxicity is the use of targeted immunotoxin therapy. Viventia's immunotoxins comprise a tumor-specific fully-human Fab fragment linked to a potent toxin. One such example is VB6-845, an anti Ep-CAM Fab genetically linked to an epitope-depleted form of bouganin, a plant-derived ribosome inactivating protein (RIP). Ep-CAM is widely expressed on a wide variety of tumor cells but has only limited expression on normal epithelial cells, making it an excellent therapeutic target. In addition, bouganin is a type I RIP, which potently inhibits protein synthesis resulting in cell death. The epitope-depletion process has removed human T cell epitopes from the bouganin template and thus reduced the potential for the induction of neutralizing antibodies (HATA response) **(Figure 1)**.

The selective toxicity and mechanism of action of our immunotoxins clearly provides an excellent clinical alternative to conventional anti-cancer agents that have many unacceptable side-effects. The *in-vitro* characterization of this novel immunotoxin is described.

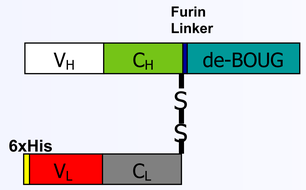


Figure 1: Structure of VB6-845. The de-immunized bouganin is fused to the recombinant Fab format of the Ep-CAM antibody.

Methods

Three aspects of the VB6-845 were assessed in this study:

Binding:

The binding of the VB6-845 to selected cell lines was analyzed by flow cytometry. A-375 (Melanoma) and CAL-27 (squamous cell carcinoma from the tongue) were used as Ep-CAM-negative and -positive cell lines, respectively. Tumor cell lines were harvested and incubated with VB6-845 and binding detected using a monoclonal mouse anti-His antibody. The cells were analyzed on a FACS Calibur following propidium iodide staining and the binding compared against an isotype-matched control antibody.

Cytotoxic Activity:

The cytotoxic activity of VB6-845 was measured by MTS assay using a panel of tumor cell lines from various indications. A-375 was used as a negative cell line control. Cells were seeded on 96-well plates and incubated at 37°C under 5% CO_2. Subsequently, VB6-845 was spiked into the wells over a range of concentrations. Following 5 days incubation, cell proliferation was evaluated by adding the MTS reagent. The plates were read at 490nm and the normalized optical density plotted vs. concentration. Cytotoxicity for each construct was expressed as an IC_{50} interpolated from each plot.

Comparison of VB6-845 with standard chemotherapeutics:

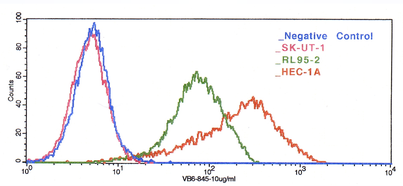
The specificity, selectivity and potency of VB6-845 were evaluated by comparing the cytotoxic activity of VB6-845 to the "standard of care" chemotherapeutic drugs on normal as well as tumor cell lines. Seventeen common chemotherapeutic drugs were compared to VB6-845 for their efficacy and selectivity against different cell lines including malignant and normal cells. The cytotoxicity of the drugs was carried out using the MTS assay as described above.

Results

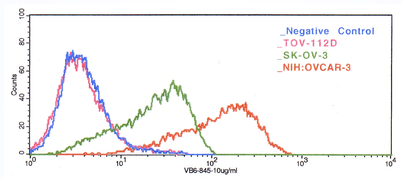
Figure 2: Cell surface binding of VB6-845 by flow cytometry.

High, medium and low binding of VB6-845 are observed in each indication when compared to the isotype-matched control antibody. A) Endometrial cancer: VB6-845 highly reacts to the HEC-1A cell line, B) Ovarian cancer: NIH:OVCAR-3 shows the highest binding of VB6-845 and C) Cervical cancer: VB6-845 has high binding to HT-3 cell line.

A. Endometrial cancer



B. Ovarian cancer



B. Cervical cancer

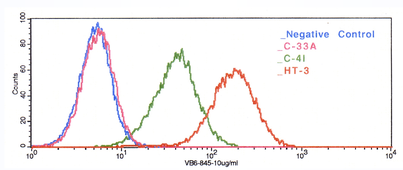


Table 1: Recapitulation of the binding of VB6-845 by flow cytometry.

Results are expressed as the mean fold-increase in median fluorescence ± SEM over the isotype-matched control. VB6-845 binds mostly to HEC-1-A, OVCAR-3 and HT-3. The melanoma cell line A-375, an Ep-CAM negative cell line shows binding equivalent to the isotype-matched control (not shown).

Indication	Cell lines	VB6-845
Endometrial	HEC-1-A	42.3±0.9
	RL95-2	4.9±0.7
	SK-UT-1	1.1±0.1
Ovarian	NIH:OVCAR-3	33.6±6.0
	SK-OV-3	4.3±1.0
	TOV-112G	1.1±0.1
Cervical	HT-3	29.1±1.2
	C-4 I	6.8±0.6
	C-33A	1.1±0.0
Melanoma	A-375	1.1±0.1
Head & Neck	CAL-27	21.26±0.01

Figure 3: Cytotoxic activity of VB6-845 by MTS assay.

Cytotoxicity of VB6-845 was tested against a variety of tumor cell lines; only representative cell lines are shown. To be cytotoxic, VB6-845 needs first to bind to the cells. The absence of binding to the A-375 shown by flow cytometry explains the lack of cytotoxicity of the VB6-845 for this cell line **(Figure 3)**.

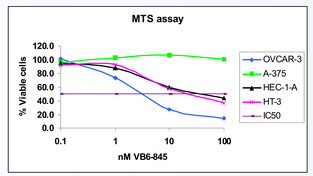


Table 2: Recapitulation of the VB6-845-mediated cytotoxicity by MTS assay.
Results are expressed as an IC_{50}. Except for the C-4I cell line, the cytotoxic activity of VB6-845 correlates well with the binding results. Again, VB6-845 exhibits a strong inhibition of the proliferation of HEC-1-A, OVCAR-3 and HT-3. Interestingly, the cervical cancer cell lines seem to be more sensitive to VB6-845 killing. A-375, the melanoma cell line does not express Ep-CAM, and therefore is resistant to VB6-845.

Indication	Cell lines	IC_{50} (nM) VB6-845 70% pure
Endometrial	HEC-1-A	43
	RL95-2	100
	KLE	>100
Ovarian	NIH-OVCAR-3	3.4
	Caov-3	1.3
	SK-OV-3	>100
Cervical	MS751	0.43
	HT-3	23
	ME-180	37
	C-4I	1.7
Melanoma	A-375	>100

Table 3: Specificity and selectivity of VB6-845 versus chemotherapeutics.

Results are expressed as an IC_{50}. VB6-845 is more potent than 12 of the chemotherapeutic drugs. However, the 5 most potent chemotherapeutic drugs are also the most toxic since they are highly cytotoxic against all cell lines including normal cells. Conversely, only the Ep-CAM positive cell line (OVCAR-3) is sensitive to VB6-845. The melanoma (A-375) as well as the lymphoma cell line (DAUDI) do not express Ep-CAM and are therefore resistant to VB6-845. HMEC, the normal mammary cell line expresses a lower level of Ep-CAM antigen; thus the cytotoxic effect was 220-fold less toxic compared to an Ep-CAM positive cell (OVCAR-3).

	IC_{50} nM			
	OVCAR-3	A-375	DAUDI	HMEC
Paclitaxel	$<10^{-6}$	$4.9x10^{-4}$	$<10^{-6}$	$<10^{-6}$
Docetaxel	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Vincristine	$4.4x10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Vinblastine Sulfate	$1.1x10^{-4}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Topotecan	0.071	1.5	0.009	4.1
VB6-845 (90% pure)	1	>1000	>1000	220
Doxorubicin	3	2.8	$16x10^{-6}$	16
Mitomycin C	28	14	2.8	50
Bleomycin Sulfate	30	170	22	600
Bleomycin A5	150	290	130	1000
Irinotecan	180	900	190	1000
Etoposide	210	280	1.7	600
Methotrexate	>1000	6	3.6	41
Chlorambucil	>1000	>1000	>1000	>1000
Fluorouracil	>1000	>1000	>1000	>1000
Cyclophosphamide	>1000	>1000	>1000	>1000
Cisplatin	>1000	>1000	>1000	>1000
Carboplatin	>1000	>1000	>1000	>1000

Conclusions

- VB6-845 demonstrated strong binding to EpCAM-positive cell lines representative of a wide range of cancer indications.
- Compared to a panel of standard chemotherapeutic agents, VB6-845 was shown to be more potent than 12 of the 17 drugs tested.
- Chemotherapeutics that were more cytotoxic than VB6-845 were also far more toxic as they lacked any cell-specific killing.
- The IC_{50} of VB6-845 against the Ep-CAM-positive OVCAR-3 was 1 to 2 nM whereas Ep-CAM-negative cell lines exhibited an IC_{50} that was 200-1000 fold less.

Summary

VB6-845 thus represents a potent, antibody-directed treatment alternative to standard chemotherapeutics. The lower toxicity profile of VB6-845 holds much promise in the treatment of many different types of solid tumors.