Virtuoso THERAPEUTICS

SITC #274

VBI-003, a CD47 x EpCAM Bispecific Antibody Represents a Novel Approach for Treating EpCAM Overexpressing Solid Tumors

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Background

CD47 conveys a "don't eat me" signal through the interaction with its ligand signal regulatory protein-alpha (SIRPα) on myeloid cells and blocks macrophage mediated phagocytosis. Tumor cells, which express high level of CD47, exploit this mechanism to evade from immune surveillance. CD47/SIRPα axis is an important checkpoint of innate immune system and CD47 is considered a prominent target for cancer treatment. However, the wide expression of CD47 on normal cells could cause antigen sink and lead to safety issues, such as anemia and thrombocytopenia. EpCAM is highly expressed in many epithelial cancers. Targeting EpCAM with antibodies has been demonstrated safe in clinical trials but with limited efficacy. Here we describe a novel anti-CD47 x EpCAM bispecific antibody with human IgG1 Fc, VBI-003, which selectively targets CD47 on EpCAM+ tumors and shows superior *in vitro* and *in vivo* activities.

CD47 Antagonists are Emerging as The Next Frontier In Immunotherapy

- CD47 is the "don't eat me" signal that inhibits macrophage mediated phagocytosis
- CD47 is overexpressed in multiple tumor types
- Encouraging clinical trial results from magrolimab (Gilead) and several other CD47 targeting agents suggest that blocking CD47 can show clinical benefit in difficult-to-treat tumors
- However, CD47 is widely expressed on normal cells, highly expressed on RBC and platelet, which could lead to poor PK and cause hematological toxicity

EpCAM is Highly Expressed in Epithelial Cancers

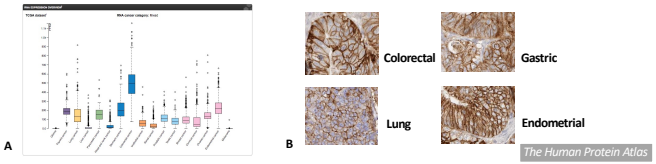

Fig 1. EpCAM shows high expression in different type of tumors. (A) RNA seq data shows EpCAM is highly expressed in different solid tumor types. **(B)** IHC staining shows high expression of EpCAM in colorectal, gastric, lung and endometrial cancer.

- EpCAM is highly expressed in many epithelial cancers, particularly in CRC, gastric, endometrial and lung cancer
- Antibodies against EpCAM like edrecolomab (J&J) and adecatumumab (Micromet/Amgen) were well-tolerated, but only showed limited clinical efficacy
- Removab, an anti-EpCAM trispecific antibody (T cell engager), obtained market authorization in Europe in 2009, but it was withdrawn from the US market after 4 years for commercial reasons
- Multiple efforts are on-going to evaluate 2nd gen EpCAM based T cell engager and EpCAM based CARTs in clinical trials for advanced solid tumors

Assembly of VBI-003, a Novel CD47 x EpCAM Bispecific Antibody

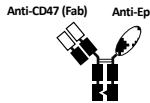

Fig 2. Schematic structure of CD47 x EpCAM bispecific antibody VBI-003.

Anti-CD47 arm:
- Generated from hybridoma discovery
- Cross binding to human and cyno CD47 with double-digit nM affinity
- Full human IgG1 antibody with knobs-into-holes technology

Anti-EpCAM arm:
- Generated from hybridoma discovery
- Cross binding to human and cyno EpCAM with single-digit nM affinity

VBI-003 Shows Potent, Selective SIRPα Blocking on EpCAM+ Tumor Cells

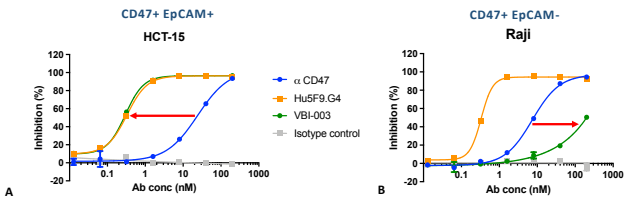

Table 1. The IC50 of VBI-003 and control anti-CD47 molecules on HCT-15 and Raji cells.

	IC 50 (nM) on HCT-15	IC 50 (nM) on Raji
Hu5F9.G4	0.33	0.33
VBI-003	**0.30**	**>200**
α CD47	24.18	7.40

Fig 3. VBI-003 showed potent and selective blocking activity on FACS based SIRPα blocking assay. IC_{50} of VBI-003 on EpCAM+ HCT15 tumor cells is < 1nM, whereas IC_{50} on EpCAM- Raji tumor cells is more than 200nM. HCT-15 tumor cells **(A)** and Raji cells **(B)** were incubated with serial diluted bispecific or bivalent anti-CD47 antibodies and a constant amount of huSIRPα-mIgG2a fusion protein in FACS buffer for 1h at 4°C. The plates were washed and incubated with Alexa Fluor 488 donkey anti-Mouse IgG(H+L) secondary antibody for 1 h at 4°C. Samples were washed before analyzed by flow cytometry. Hu5F9.G4 was synthesized based on the sequences from the published paper (Reference #2) in huIgG4 S228P backbone, then produced in house in CHO cell line. α CD47 is the parental bivalent anti-CD47 antibody.

VBI-003 is a Safer Approach to Target CD47 Compared to CD47 Bivalent Antibodies

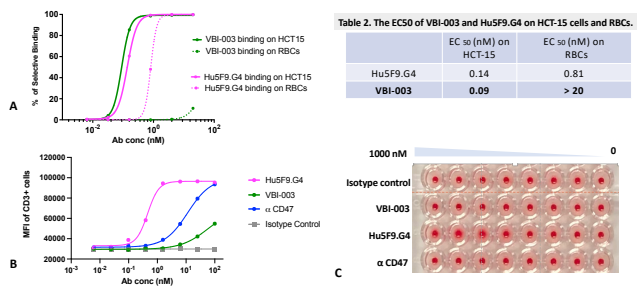

Table 2. The EC50 of VBI-003 and Hu5F9.G4 on HCT-15 cells and RBCs.

	EC 50 (nM) on HCT-15	EC 50 (nM) on RBCs
Hu5F9.G4	0.14	0.81
VBI-003	**0.09**	**> 20**

Fig 4. VBI-003 selectively binds to EpCAM+ tumor cells over normal cells (RBCs or T cells) and no hemagglutination was observed even at 1000nM concentration. (A) Binding of antibodies on mixed tumor cells and RBCs by FACS. Antibodies were incubated with mixed HCT-15 tumor cells and RBCs(x20 fold of tumor cells numbers) for 1 h at 4°C. The binding of antibodies on HCT-15 cells and RBCs were measured by using different FACS markers. **(B)** Binding of antibodies on CD3+ T cells from PBMCs by FACS. (C) Hemagglutination assay. Human red blood cells (RBCs) were incubated with serial diluted CD47 antibodies (from 1000nM) at 37°C for 2 h in a round bottom 96 well plate. Hemagglutination was demonstrated by the presence of crosslinked RBCs, which appeared as a haze because they do not settle to the bottom of the well, in contrast to non-hemagglutinated RBCs.

VBI-003 Shows Potent ADCP and ADCC Activities

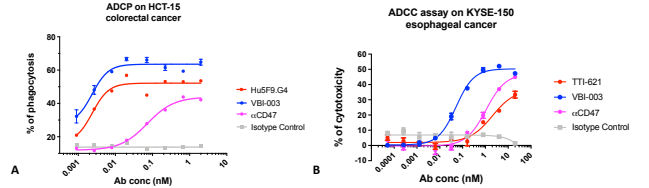

Fig 5. VBI-003 showed potent ADCP and ADCC activity *in vitro*. (A) FACS based antibody-dependent cellular phagocytosis (ADCP) assay. Monocytes were isolated from human peripheral blood mononuclear cells (PBMCs) and differentiated into macrophages. Target tumor cells were stained with CFSE and mixed with macrophages at 1:1 ratio. Serial diluted antibodies were then added to each well and incubated for 1.5 h. Phagocytosis was detected with a flow cytometer by the appearance of CFSE/CD11b double positive cells indicative of macrophages that engulfed the tumor cells. **(B)** Antibody-dependent cellular cytotoxicity (ADCC) was tested on KYSE-150 esophageal cancer cells. Serial diluted antibodies were added to assay plate containing human PBMCs and BATDA labelled tumor cells at 40:1 effector to target ratio for 2 h. ADCC assays were performed using DELFIA® EuTDA Cytotoxicity Reagents following manufacturer' instruction. TTI-621 was synthesized based on the sequence from the published patent (WO2017177333A1), then produced in house in CHO cell line.

VBI-003 Shows Potent Single Agent Anti-tumor Activity and is Superior to Monospecific Anti-CD47 Molecules

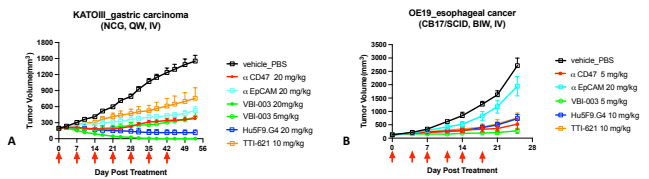

Fig 6. Single agent treatment of VBI-003 drove tumor free in KATOIII CDX model and inhibited tumor growth completely in OE-19 CDX model. The KATOIII tumor cells **(A)** or OE-19 tumor cells **(B)** were cultured and harvested for tumor inoculation. Female NCG or CB17/SCID mice of 6-7 weeks in age were inoculated subcutaneously with 10^7 cells in 0.2 mL of PBS supplemented with Matrigel (1:1) for tumor development. On day 6-8 post tumor inoculation when the mean tumor volume reached approximately 175 mm^3, animals were randomized by tumor volumes into 6 animals per group. Test antibodies were administered through bolus tail vein injection at doses mentioned in the graphs. Red arrows indicate the dosing day. α CD47 and α EpCAM antibodies represent the parental bivalent anti-CD47 and anti-EpCAM antibodies for VBI-003.

Conclusions

Our findings suggest that VBI-003, a novel CD47 x EpCAM bispecific antibody selectively binds to CD47 and blocks CD47/SIRPα binding on EpCAM overexpressing tumor cells. Its human IgG1 backbone allows engagement of both ADCP and ADCC for potent single agent anti-tumor activity. VBI-003 has minimum RBC binding compared to the bivalent CD47 monospecific antibodies and thus is expected to induce less hematological toxicities. In conclusion, VBI-003 shows specificity toward EpCAM positive tumor cells and robust single agent *in vitro* and *in vivo* activity. The CD47 x EpCAM bispecific antibody represents a novel approach for treating EpCAM positive tumors.

References

1. Chao M, Weissman I, Majeti R. The CD47-SIRPa Pathway in Cancer Immune Evasion and Potential Therapeutic Implications. Curr Opin Immunol. 2012; 24(2): 225–232.
2. Liu J, Wang L, Zhao F, Tseng S, Narayanan C, Shura L, et al. (2015) Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. PLoS ONE 10(9): e0137345. doi:10.1371/journal.pone.0137345