VBI-002, a CD47 x ICAM-1 Bispecific Antibody Represents a Novel Approach for Treating ICAM-1 Overexpressing Tumors

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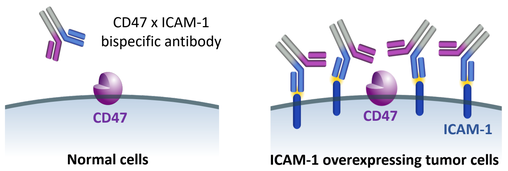
Background

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[1]. 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. Encouraging clinical trial results from several CD47 targeting agents suggest that blocking CD47 can show clinical benefit in difficult-to-treat tumors. However, the wide expression of CD47 on normal cells could cause antigen sink and lead to safety issues, such as anemia and thrombocytopenia. Herein, anti-CD47 bispecific antibody specifically targeting tumors is an ideal approach for cancer treatment with improved safety profile. The intercellular adhesion molecule-1 (ICAM-1, CD54) is a type I transmembrane glycoprotein, and a ligand for leukocyte integrins a_Lb_2 (LFA-1, CD11a/CD18) and a_Mb_2 (Mac-1, CD11b/CD18). ICAM-1 is constitutively present at low levels on endothelial cells, but highly expressed in myeloma, lymphoma and certain type of solid tumors[3]. Antibody against ICAM-1, Bersanlimab (BI505, BioInvent), was well-tolerated, but only showed limited clinical efficacy in MM patients[4]. Here we describe a novel anti-CD47 x ICAM-1 bispecific antibody with human IgG1 Fc, VBI-002, which selectively targets CD47 on ICAM-1$^+$ tumors and shows superior *in vitro* and *in vivo* activities as well as improved safety profile in NHP study.



Intercellular Adhesion Molecule 1 (ICAM-1) is Highly Expressed in Myeloma, Lymphoma and a Subset of Solid Tumors

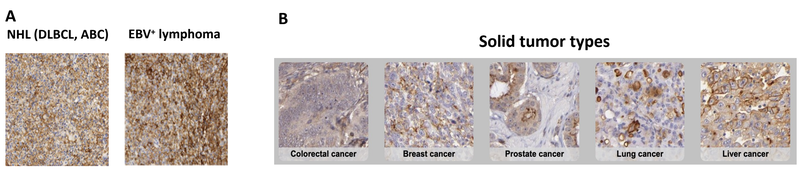
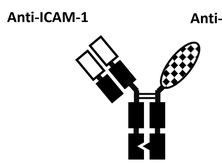


Fig 1. ICAM-1 is highly expressed in different type of tumors (A) Strong ICAM-1 IHC staining was observed in malignant lymphoma PDX samples. **(B)** ICAM-1 expression in different solid tumor types from The Human Protein Atlas.

Assembly of VBI-002, a Novel CD47 x ICAM1 Bispecific Antibody



Anti-CD47 arm:
- Generated from hybridoma discovery
- Cross binding to human and cyno CD47 with double-digit nM affinity

Anti-ICAM-1 arm:
- Generated from rodent animal B cell cloning
- Cross binding to human and cyno ICAM-1 with sub-digit nM affinity

Fc:
- Full human IgG1 antibody with knobs-into-holes technology

Fig 2. Schematic structure of CD47 x ICAM-1 bispecific antibody VBI-002.

VBI-002 Shows Potent, Selective SIRPα Blocking on ICAM-1$^+$ Tumor Cells

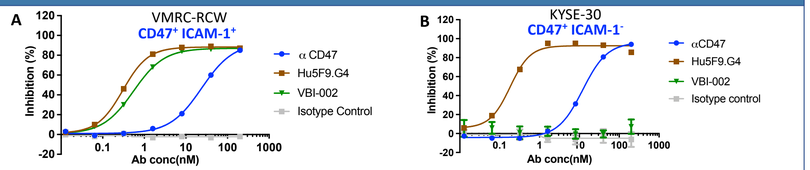


Fig 3. VBI-002 showed potent and selective blocking activity on FACS based SIRPα blocking assay. IC_{50} of VBI-002 on ICAM-1$^+$ VMRC-RCW kidney tumor cells is < 1 nM, whereas no SIRPα blocking on ICAM-1$^-$ KYSE-30 esophageal tumor cells. VMRC-RCW tumor cells **(A)** and KYSE-30 tumor cells **(B)** were incubated with serial diluted biospecific or bivalent anti-CD47 antibodies and a constant amount of hu SIRPα-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 in human IgG1 backbone.

VBI-002 Shows Potent ADCP and ADCC Activities

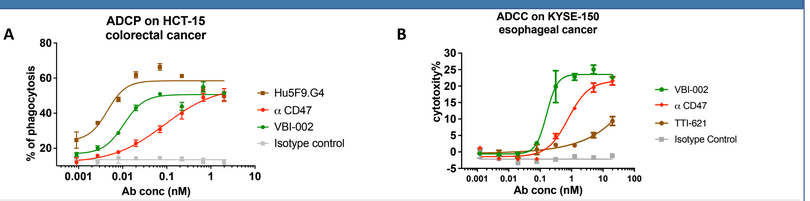


Fig 4. VBI-002 showed potent ADCP and ADCC activity *in vitro*. (A) FACS based antibody-dependent cellular phagocytosis (ADCP) assay. Monocytes were isolated from human peripheral blood mononuclear cells (PBMCs) and differentiated into macrophages. Target tumor cells were stained with CFSE and mixed 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-002 Shows Superior Single Agent Anti-tumor Activity

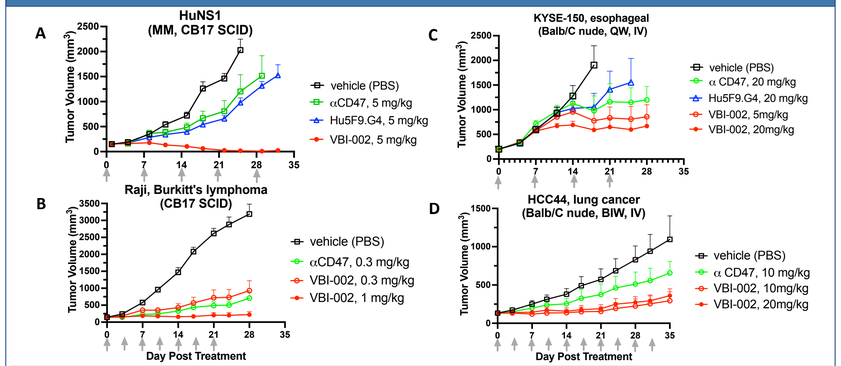


Fig 5. Single agent treatment of VBI-002 drove tumor free in HuNS1 CDX model and significant inhibition of tumor growth in Raji, HCC44 and KYSE-150 CDX model. The HuNS1 **(A)**, Raji **(B)**, HCC44 **(C)** and KYSE-150 **(D)** tumor cells were cultured and harvested for tumor inoculation. Female CB17/SCID or Balb/C nude 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. 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. Grey arrows indicate the dosing day.

VBI-002 is a Safer Approach to Target CD47 Compared to CD47 Bivalent Antibodies

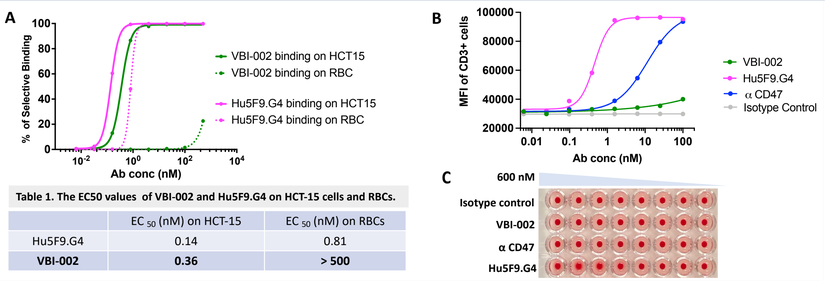


Table 1. The EC50 values of VBI-002 and Hu5F9.G4 on HCT-15 cells and RBCs.

	EC $_{50}$ (nM) on HCT-15	EC $_{50}$ (nM) on RBCs
Hu5F9.G4	0.14	0.81
VBI-002	**0.36**	**> 500**

Fig 6. VBI-002 selectively binds to ICAM-1$^+$ tumor cells over normal human red blood cells (RBCs) or T cells and no hemagglutination was observed even at 600 nM 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 600 nM) 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 did not settle to the bottom of the well, in contrast to non-hemagglutinated RBCs.

VBI-002 Shows Superior Safety Profile in Repeat Dose Non-Human Primate Study

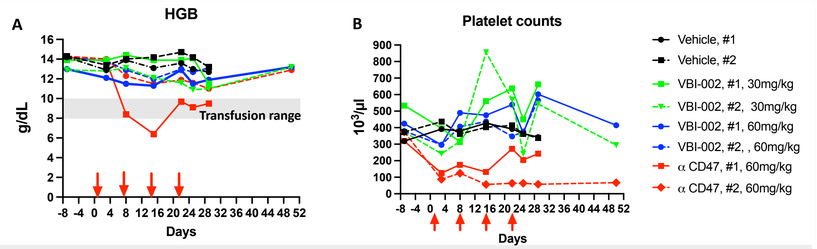


Fig 7. VBI-002 showed mild and reversible decreases in hemoglobin (HGB) and platelets in repeat dose NHP study. Cynomolgus monkeys were randomly assigned to different groups, two monkeys per group, and were treated intravenously every week for 4 weeks (on Day 1, 8, 15, 22) with vehicle, indicated doses of VBI-002 or α CD47 antibody. Hemoglobin **(A)** and Platelets **(B)** were calculated in certain time points, the last time point was either on Day 29 or on Day 50 before necropsy.

Conclusions

Our findings suggest that VBI-002, a novel CD47 x ICAM-1 bispecific antibody selectively binds to CD47 and blocks CD47/SIRPα binding on ICAM-1 overexpressing tumor cells. Its human IgG1 backbone allows engagement of both ADCP and ADCC for potent single agent anti-tumor activity. VBI-002 has minimum RBC binding compared to the bivalent CD47 monospecific antibodies and was well-tolerated in NHP safety study. In conclusion, VBI-002 shows specificity toward ICAM-1 positive tumor cells and robust single agent *in vitro* and *in vivo* activity. It is expected to induce less hematological toxicities compared to conventional bivalent CD47 antibodies. The CD47 x ICAM-1 bispecific antibody represents a novel approach for treating ICAM-1 positive tumors.

References

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