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VBI-002, a CD47xICAM-1 Bispecific Antibody for the Treatment of Hepatocellular Carcinoma, Melanoma and Non-Small Cell Lung Cancers

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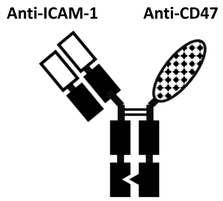
Abstract

Intercellular Cell Adhesion Molecule 1 (ICAM-1, CD54) is a type I transmembrane protein and a member of the immunoglobulin superfamily. ICAM-1 is involved in many key processes such as cell-cell interactions, signal transduction and leukocyte trans-endothelial migration. ICAM-1 is constitutively present at low levels in healthy tissues but highly expressed in some hematological cancers and solid tumors, making it an attractive tumor target.

VBI-002 is a novel human IgG1-based CD47xICAM-1 bispecific antibody with an excellent safety profile and potent single-agent activities in xenograft models of lymphoma, multiple myeloma and some solid tumors (Cancer Res 2022;82(12_Suppl):Abstract nr 3430). The bispecific design allows VBI-002 to selectively block CD47/SIRPα binding in tumor cells with high ICAM-1 expression. In contrast to the benchmark CD47 monoclonal antibody magrolimab, VBI-002 exhibits minimal red blood cell binding and does not cause hemagglutination. In a non-human primate (NHP) safety study, four weekly doses of 60 mg/kg VBI-002 were well tolerated with only mild and reversible reduction in hemoglobin and platelets.

In this report, we further investigate the anti-tumor activities of VBI-002 in solid tumors. Gene expression analysis revealed that ICAM-1 is highly expressed in non-small cell lung cancers (NSCLC), melanoma and a subset of hepatocellular carcinoma. Flow cytometry studies confirmed the frequent co-expression of ICAM-1 and CD47 in these tumors. VBI-002 has potent ICAM-1 dependent SIRPα blocking, antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) activities against cell lines of NSCLC, hepatocellular carcinoma, and melanoma. Furthermore, *in vivo* efficacy studies demonstrated that VBI-002 has potent single-agent activities in cell line-derived xenograft (CDX) models of NSCLC, hepatocellular carcinoma, and melanoma. In most models, a weekly dose of 20 mg/kg VBI-002 or less is sufficient to stop tumor growth or drive tumor regression. Notably, VBI-002 is highly effective against NSCLC models carrying Kras mutations, and it synergizes with paclitaxel to provide additional anti-tumor activity. In conclusion, VBI-002 is a versatile novel CD47xICAM-1 bispecific antibody with potent single-agent activity in a variety of solid tumors and combines well with standard of care chemotherapies for added activity. Therefore, VBI-002 warrants clinical evaluation as a single-agent and in combination with standard of care chemotherapies.

Schematic of bispecific antibody VBI-002



Anti-CD47 arm:
- Human and cyno CD47 reactive with double-digit nM affinity
- Low red blood cell and platelet bindings to minimize antigen sink effect and on-target toxicities
- Single chain Fv format

Anti-ICAM-1 arm:
- Human and cyno ICAM-1 reactive with sub-nM affinity
- Unique epitope distant from ligand binding domain

Fc:
- Human IgG1 antibody with knobs-into-holes technology
- Retain full effector functions

ICAM-1 is frequently expressed in NSCLC, melanoma, a subset of hepatocellular carcinoma and kidney cancers

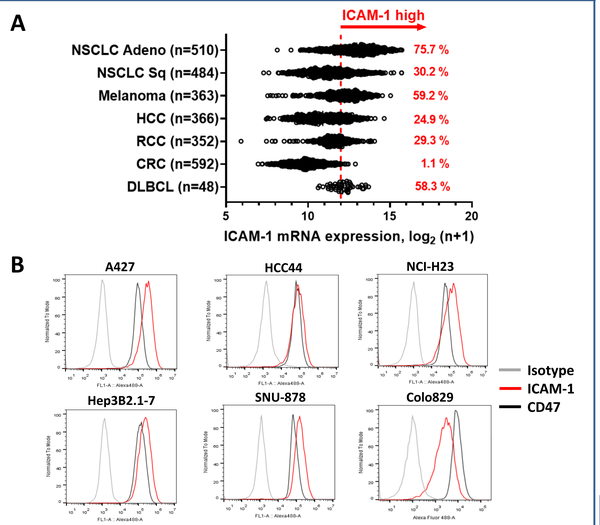


Fig. 1. (A) RNA-Seq analysis reveals high expression of ICAM-1 mRNA in some solid tumors. RNA-Seq data were from TCGA PanCancer Atlas datasets downloaded from cBioPortal [1]. CRC (colorectal cancers) and DLBCL (diffuse large B cell lymphoma), an ICAM-1 low and ICAM-1 high tumor type, respectively, served as references for setting an ICAM-1 high tumor cutoff of $\log_2$ (n+1) =12. NSCLC, non-small cell lung cancer; Adeno, adenocarcinoma; Sq, squamous; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma. **(B)** Examples of NSCLC (A427, HCC44, NCI-H23), liver (Hep3B2.1-7, SNU-878) and melanoma (Colo829) cell lines showing co-expression of CD47 and ICAM-1 by flow cytometry.

VBI-002 shows minimal binding to human RBC and platelets

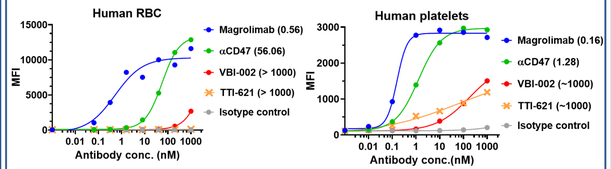


Fig. 2. Both magrolimab and bivalent CD47 antibody (αCD47) show strong binding to human RBC and platelets while bispecific antibody VBI-002 at 1 μM only binds weakly to RBC and platelets, comparable to TTI-621. Numbers in parentheses indicate the EC50 (nM) values obtained from the FACS binding assays. Expression vectors encoding magrolimab were synthesized based on the sequences from a published paper in human IgG4 S228P backbone[2]. TTI-621 sequences were from the patent (WO2017177333A1) and cloned in human IgG1. αCD47 is the bivalent parent of the anti-CD47 arm in VBI-002 with human IgG1 backbone. All antibodies were produced in CHO cells.

VBI-002 is highly potent and selective in SIRPα blocking, ADCP and ADCC activities on CD47 and ICAM-1 co-expressing cells

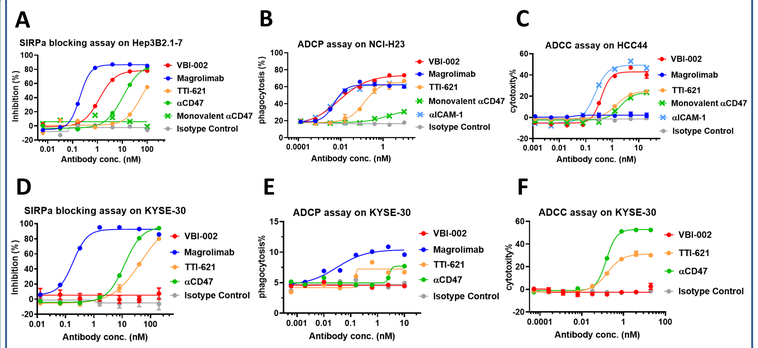


Fig.3. VBI-002 demonstrates potent and selective *in vitro* activities on CD47$^+$ ICAM-1$^+$ tumor cells (A, B and C) but is entirely inactive against CD47$^+$ICAM-1$^-$ KYSE-30 tumor cells (D, E and F) while magrolimab, TTI-621 and αCD47 show no selectivity. SIRPα blocking activities on Hep3B2.1-7 cells **(A)** and KYSE-30 cells **(D).** ADCP assays on NCI-H23 cells **(B)** and KYSE-30 cells **(E)** were performed using human monocyte-derived macrophages at an E:T ratio of 1:1 with 1.5 h of incubation. ADCC assays on HCC-44 cells **(C)** and KYSE-30 cells **(F)** were performed using human PBMC at an E:T ratio of 40:1 **(C)** or 50:1 **(F)** and 2 h incubation. Monovalent αCD47 is the same scFv anti-CD47 arm of VBI-002 in human IgG1 backbone. αICAM-1 is the bivalent parent of the anti-ICAM-1 arm in VBI-002.

Potent single-agent activities in NSCLC and melanoma CDX models

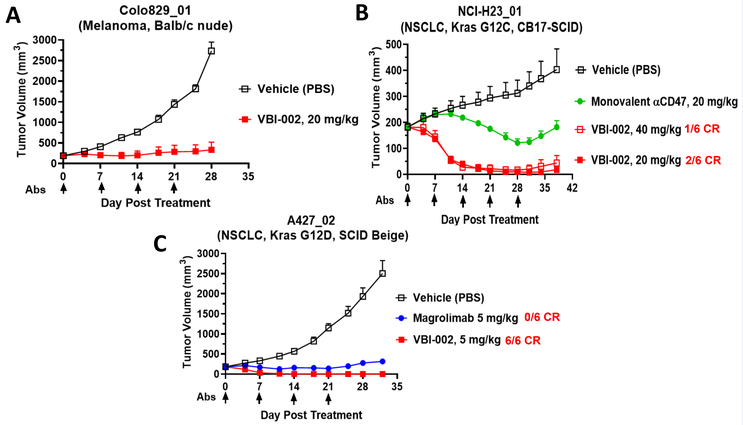


Fig.4. VBI-002 inhibited tumor growth in Colo829 (A), drove tumor regression in NCI-H23 (B) and eradicated all tumors in A427 model (C). Animals were randomized by tumor volume into 6 animals per group. Antibodies were administered through weekly bolus tail vein injections when mean tumor volumes reached 150-200 mm^3. Black arrows indicate the dosing days. Red numbers in **(B)** and **(C)** indicate the number of animals that are tumor free (CR, complete responders) at the end of studies.

VBI-002 can be combined with chemotherapies to further enhance anti-tumor activities in NSCLC and HCC

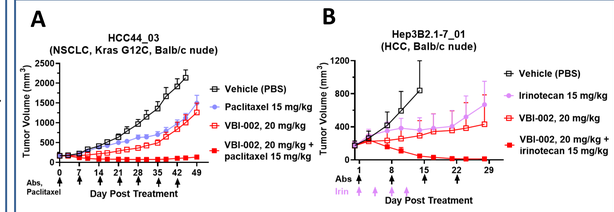


Fig. 5. Combination with paclitaxel (A) or irinotecan (B) further boosts the anti-tumor activity of VBI-002 in CDX models of NSCLC and hepatocellular carcinoma, respectively. Animals were randomized by tumor volume into 6 animals per group. Test antibodies were administered through weekly bolus tail vein injections when mean tumor volumes reached 150-200 mm^3. Paclitaxel was delivered once weekly by intraperitoneal injections **(A).** Irinotecan was given via intraperitoneal injections twice a week **(B)**. Arrows indicate the dosing days for antibodies or antibodies + paclitaxel (black) and irinotecan (purple).

Conclusions

VBI-002 is a novel human IgG1-based CD47xICAM-1 bispecific antibody. It is strategically designed to improve selectivity in targeting tumor cells where CD47 and ICAM-1 are present while sparing normal cells expressing CD47 only. In FACS binding studies, VBI-002 demonstrated minimal binding to RBCs and platelets, while the benchmark bivalent monospecific CD47 antibody, magrolimab, binds both cell types tightly with sub-nanomolar EC50s. Moreover, *in vitro* functional assays demonstrated that VBI-002 has potent SIRPα blocking, ADCP and ADCC activities on CD47$^+$ICAM-1$^+$ tumor cells but has no detectable activities on CD47$^+$ICAM-1$^-$ cells. In contrast, the benchmark magrolimab did not show any selectivity and failed to evoke ADCC on CD47$^+$ tumor cells due to its human IgG4 design. Furthermore, VBI-002 elicited potent single-agent activities against multiple CDX models of NSCLC, hepatocellular carcinoma, and melanoma. A weekly dose of VBI-002 at 20 mg/kg or less is often sufficient to halt tumor growth or drive tumor regression in most models. Notably, VBI-002 is highly effective against NSCLC CDX models carrying Kras mutations. In addition, VBI-002 can be combined with chemotherapies such as paclitaxel and irinotecan to further boost the anti-tumor activity. Due to the highly selective nature of our bispecific antibody design, we can safely use the human IgG1 Fc to capture its full effector functions. Indeed, we previously showed in a non-human primate safety study that four weekly doses of 60 mg/kg VBI-002 were well tolerated without the need for a priming dose[3].

In summary, VBI-002 is a promising novel CD47xICAM-1 bispecific antibody with potent single-agent activity in various solid tumor models and combines well with standard of care chemotherapies for added activity. The preclinical efficacy and safety data support the clinical evaluation of VBI-002 in CD47$^+$ICAM1$^+$ solid tumors as a single-agent and in combination with standard of care chemotherapies.

References

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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
3. Wang X, Wong O, Post L and Chen X. (2022) VBI-002, a CD47 x ICAM-1 Bispecific Antibody Represents a Novel Approach for Treating ICAM-1 Overexpressing Tumors. Cancer Res 2022;82(12_Suppl):Abstract nr 3430.

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