CD38 x ICAM-1 Bispecific Antibody Is a Novel Approach for Treating Multiple Myeloma and Lymphoma

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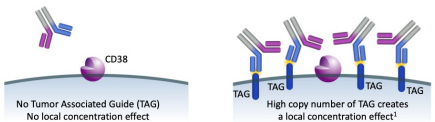
Background

Targeting cluster of differentiation 38 (CD38) with monoclonal antibodies has resulted in outstanding responses in patients with multiple myeloma (MM). However, a significant portion of patients failed to respond and nearly all patients eventually relapsed. Furthermore, daratumumab, an anti-CD38 antibody, only showed limited monotherapy activity in relapsed/refractory Non-Hodgkin lymphoma (NHL) patients in a phase 2 study. One of the mechanisms of resistance has been partially attributed to lower CD38 expression. Therefore, there are opportunities for next generation CD38 based therapies.

CD38 Bispecific Antibody Enables Specific and Potent Tumor Targeting



Intercellular Adhesion Molecule 1 (ICAM-1), an Immunoglobulin (Ig)-like Cell Adhesion Molecule, is Highly Expressed in Multiple Myeloma and Lymphoma

ICAM-1 is constitutively present at low levels on endothelial cells, but its overexpression has been observed on various types of tumors and the associated stroma[2]. ICAM-1 is highly expressed on cell surface of primary myeloma cells[3]. Antibody against ICAM-1, Bersanlimab (BI505, BioInvent), was well-tolerated, but only showed limited clinical efficacy in MM patients[4].

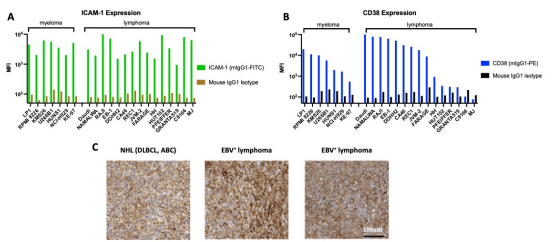


Fig 1. ICAM-1 and CD38 expression levels were evaluated using flow cytometry. ICAM-1 is highly expressed in a selected set of myeloma and lymphoma cell lines **(A)**, whereas these cell lines have a range of different CD38 expression **(B)**. Detection antibodies: Biolegend anti-human CD38 (Cat #303506) and anti-human ICAM-1 (Cat #322714). **(C)** Strong ICAM-1 IHC staining was observed in malignant lymphoma PDX samples.

VBI-001, a Novel ADCC Enhanced CD38 x ICAM-1 Bispecific Antibody

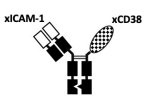


Fig 2. Schematic structure of VBI-001

Anti-CD38 arm:
- ❖Proprietary antibody
- ❖Cross binding to human and cyno CD38 with double-digit nM affinity

Anti-ICAM-1 arm:
- ❖Proprietary antibody
- ❖Cross binding to human and cyno ICAM-1 with sub nM affinity

FC:
- ❖ Afucosylated human IgG1 antibody with knob-into-hole technology

VBI-001 Showed Superior *In-Vitro* ADCC Activities

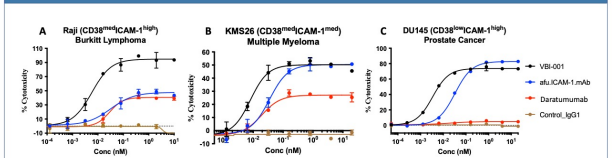


Fig 3. VBI-001 showed potent *in vitro* antibody-dependent cellular cytotoxicity (ADCC) activities on ICAM-1⁺ tumor cells with medium to low CD38 levels, where daratumumab has low or minimal effect. ADCC was tested on Raji lymphoma **(A)**, KMS26 MM **(B)** and DU145 prostate **(C)** cancer cells. The afu.ICAM-1.mAb represents the afucosylated anti-ICAM-1 antibody. ADCC activities were measured using the DELFIA time-resolved fluorescence (TRF) cytotoxicity kit (PerkinElmer cat# AD0116) following manufacturer's instruction. Tumor cells were labelled with BATDA. Serially-diluted antibodies were added to assay plate containing human PBMCs and labelled tumor cells at 50:1 effector to target ratio and incubated at 37 °C for 2 hours.

VBI-001 Showed Potent *In-Vitro* ADCP Activities

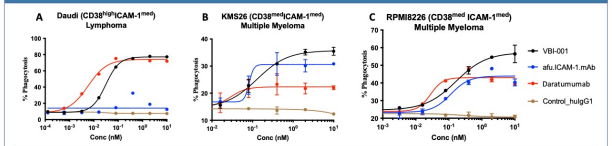


Fig 4. VBI-001 showed potent antibody-dependent cellular phagocytosis (ADCP) on Daudi lymphoma **(A)**, KMS26 MM **(B)** and RPMI8226 MM **(C)** cancer cells. Monocytes were isolated from human PBMCs and differentiated into macrophages by culturing monocytes in complete culture media (RPMI 1640 + 10% FBS) with 20 ng/ml of human Macrophage Colony-Stimulating Factor for 7 days. Target tumor cells were stained with CFSE and mixed with macrophage in 1:1 ratio, then serially diluted antibodies were added to each well and incubated for 1.5 hours. Phagocytosis was detected in a flow cytometer by the appearance of CFSE/CD11b double positive cells indicative of macrophages that engulfed the tumor cells.

VBI-001 Showed Reduced *In-Vitro* Natural killer (NK) Cell Fratricide Activity

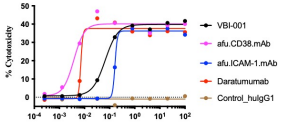


Fig 5. VBI-001 showed reduced *in-vitro* NK92 cell killing compared to daratumumab. The afu.CD38.mAb represents the afucosylated anti-CD38 antibody. The NK92 killing assays were followed the same procedures as the ADCC assay described in Fig 3 except that no additional effector cell was added.

VBI-001 Showed Reduced *Ex-Vivo* human RBC Binding

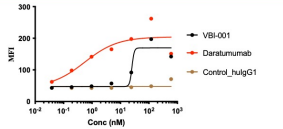


Fig 6. VBI-001 showed much weaker RBC binding compared to daratumumab. Human red blood cells (RBCs) were incubated with serial dilutions antibodies (from 600nM) at 4°C for 1 h. The binding was evaluated using flow cytometry.

VBI-001 Showed Potent Anti-Tumor Activities in Lymphoma Cell Line Derived Xenograft (CDX) Model

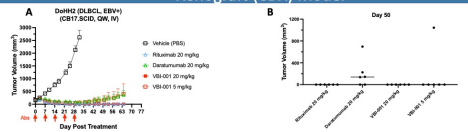


Fig 7. The DoHH2 tumor cells were cultured and harvested for tumor inoculation. Female CB17/SCID mice of 6-7 weeks in age were inoculated subcutaneously with 10^7 cells in 0.2 mL of PBS supplemented with Matrigel (1:1) for tumor development. Tumor volume was measured using a caliper device and calculated with the following formula: Tumor volume = (length x width ^{2}) / 2. When the mean tumor volume reached approximately 150 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, once per week for five doses. Tumor volume was measured twice weekly. **(A)** Average tumor volumes. **(B)** Tumor volumes of individual mouse on day 50 post treatment.

VBI-001 Showed Potent Anti-Tumor Activities in Multiple Myeloma CDX Models

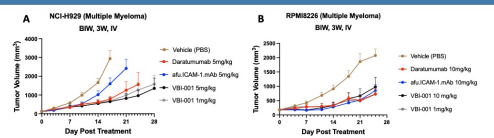


Fig 8. The multiple myeloma CDX model NCI-H929 **(A)** or RPMI8226 **(B)** cells were cultured and harvested for tumor inoculation. Female CB17/SCID mice of 6-7 weeks in age were inoculated subcutaneously with 10^7 cells in 0.2 mL of PBS supplemented with Matrigel (1:1) for tumor development. When the mean tumor volume reached approximately 150 mm^3, animals were randomized by tumor volumes into 5 animals per group. Test antibodies were administered biweekly through bolus tail vein injection at doses mentioned in the graphs. Tumor volume was measured twice weekly.

VBI-001 Showed Potent Anti-Tumor Activities in Rituximab Resistant Lymphoma Patient Derived Xenograft (PDX) Models

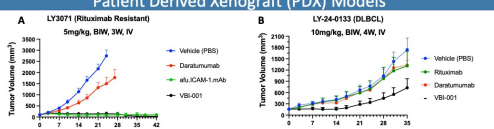


Fig 9. The Lymphoma PDX model LY3071 **(A)** or LY-24-0133 **(B)** were harvested from tumor bearing mice and cut into small chunks (approximately 2-3 mm in diameter). Female CB17/SCID mice of 6-7 weeks in age were inoculated subcutaneously with tumor chunks for tumor development. When the mean tumor volume reached approximately 100 mm^3, animals were randomized by tumor volumes into 6 animals per group. Test antibodies were administered biweekly through bolus tail vein injection at doses mentioned in the graphs. Tumor volume was measured twice weekly.

Conclusions

CD38 x ICAM-1 bispecific antibodies represent a novel approach to improve efficacy and specificity toward CD38⁺ and ICAM-1⁺ tumor cells. We developed a novel ADCC enhanced CD38 x ICAM-1 bispecific antibody, VBI-001, and demonstrated superior *in-vitro* and *in-vivo* activities for lymphoma and MM. These results support the evaluation of a CD38 x ICAM-1 bispecific antibody in clinical development for patients with relapsed/refractory lymphoma and multiple myeloma.

References

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2. Park S, Kang S, Chen X, Kim EJ, Kim J, Kim N, et al. Tumor suppression via paclitaxel-loaded drug carriers that target inflammation marker upregulated in tumor vasculature and macrophages. Biomaterials 2013;34:598–605.
3. Sherbenou, D. W. *et al.* Potent Activity of an Anti-ICAM1 Antibody–Drug Conjugate against Multiple Myeloma. *Clin. Cancer Res.* **26**, 6028–6038 (2020).
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