AACR 2023
1870

VBI-003, a CD47xEpCAM Bispecific Antibody as a Potential Treatment for Colorectal and Small Cell Lung Cancers

Oi Kwan Wong, Xinhua Wang, Xiaocheng Chen and Leonard Post
Virtuoso Therapeutics, Inc., San Mateo, California, USA

Virtuoso THERAPEUTICS
www.virtuosotherapeutics.com

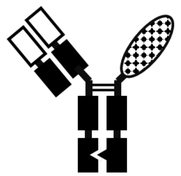
Abstract

The CD47/SIRPα axis is an important checkpoint of the innate immune system and is often exploited by tumor cells to evade host immune surveillance. In recent years, therapies such as anti-CD47 monoclonal antibodies (mAbs) and SIRPα fusion proteins that target this axis have garnered much attention. Although exciting clinical activities have been observed in hematological cancers, responses in solid tumors are less impressive and often require complex combination strategies.

We previously described a novel CD47xEpCAM bispecific antibody, VBI-003, built on a human IgG1 backbone to harness the power of blocking the CD47/SIRPα pathway while preserving the effector functions of IgG1 Fc (J ImmunoTher Cancer 2021;9:doi: 10.1136/jitc-2021-SITC2021.274). The bispecific design aims to improve selectivity, allowing effective SIRPα blocking to occur selectively on tumor cells expressing both CD47 and EpCAM. *In vitro* studies show that, in contrast to the benchmark CD47 mAb magrolimab, VBI-003 exhibits minimal red blood cell binding and does not cause hemagglutination. VBI-003 has potent single-agent activity in gastric and esophageal cell line-derived xenograft (CDX) models. Here, we further explored the anti-tumor activities of VBI-003 in areas with great unmet needs such as small cell lung cancer (SCLC) and colorectal cancer (CRC).

Gene expression analysis from public datasets reveals frequent expression of CD47 and EpCAM in SCLC and CRC tumors. Flow cytometry studies confirmed the co-expression of CD47 and EpCAM on SCLC and CRC cell lines. Furthermore, *in vitro* assays showed that VBI-003 has potent EpCAM dependent SIRPα blocking, antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) activities towards SCLC and CRC cell lines. Moreover, VBI-003 demonstrated potent single-agent activity in multiple CDX models of SCLC with tumor regression and often outperformed benchmark magrolimab. Significant tumor growth inhibition was observed in colorectal cancer models post VBI-003 treatment, with additional activity provided by combining with the standard of care chemotherapy. VBI-003 synergized with irinotecan and surpassed monotherapy groups in CDX models of CRC. Consistent with reports that some DNA damage agents induce immunogenic cell death and surface translocation of calreticulin, we observed increased surface expression of calreticulin and CD47 on CRC cells post irinotecan treatment *in vitro*. This may account for at least some of the synergy observed *in vivo*. In conclusion, VBI-003 has potent single-agent activity in SCLC tumor models and can be combined synergistically with irinotecan in CRC models. Our data support clinical investigation of VBI-003 as a treatment for CD47 and EpCAM expressing SCLC and CRC.

Schematic of bispecific antibody VBI-003



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

Anti-EpCAM arm:
- Human and cyno EpCAM reactive with low double digit nM affinity
- Single chain Fv format

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

CD47 and EpCAM co-expression in SCLC and CRC cell lines

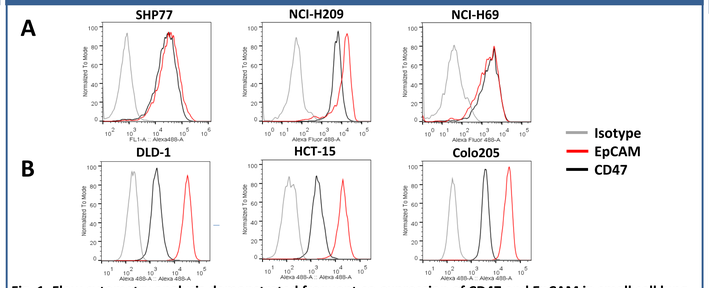


Fig. 1. Flow cytometry analysis demonstrated frequent co-expression of CD47 and EpCAM in small cell lung cancer (A) and colorectal cancer cell lines (B).

VBI-003 has reduced binding to human RBC and platelets compared to bivalent CD47 monospecific antibodies

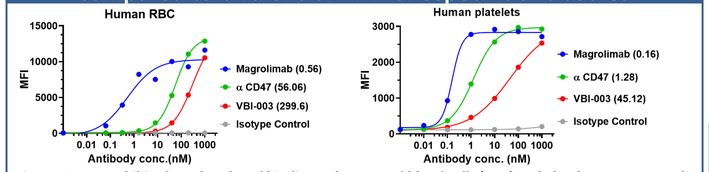


Fig. 2. VBI-003 exhibited much reduced binding to human red blood cells (RBC) and platelets as compared to the benchmark magrolimab and the parental bivalent CD47 antibody (αCD47) of VBI-003. Numbers in parentheses indicate the EC50 (nM) values obtained from the FACS binding assays. Expression vectors for magrolimab were synthesized based on the sequences from a published paper (Reference 1) in the human IgG4 S228P backbone. All antibodies were produced in CHO cells.

VBI-003 is highly potent and selective in SIRPα blocking, ADCP and ADCC activities on CD47$^+$EpCAM$^+$ tumor cells

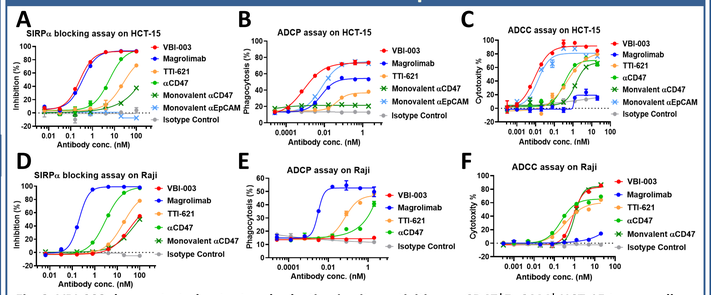


Fig. 3. VBI-003 demonstrated potent and selective *in vitro* activities on CD47$^+$EpCAM$^+$ HCT-15 tumor cells, but not on CD47$^+$EpCAM$^-$ Raji tumor cells, whereas magrolimab, TTI-621 and αCD47 exhibited no selectivity. SIRPα blocking activities on HCT-15 cells **(A)** and Raji cells **(D).** ADCP assays on HCT-15 cells **(B)** and Raji cells **(E)** were performed using human monocyte-derived macrophages at an E:T ratio of 1:1 and 1.5 h incubation. ADCC assays on HCT-15 cells **(C)** and Raji cells **(F)** were performed using human PBMC at an E:T ratio of 40:1 with 2 h of incubation. TTI-621 was generated in the human IgG1 backbone based on sequences from the patent WO2017177333A1. Monovalent αCD47 and monovalent αEpCAM share the same Fab anti-CD47 arm and the same scFv anti-EpCAM arm, respectively, of VBI-003. Both were produced in the human IgG backbone.

VBI-003 demonstrated potent single-agent activity in SCLC models outshining the benchmark CD47 mAb magrolimab

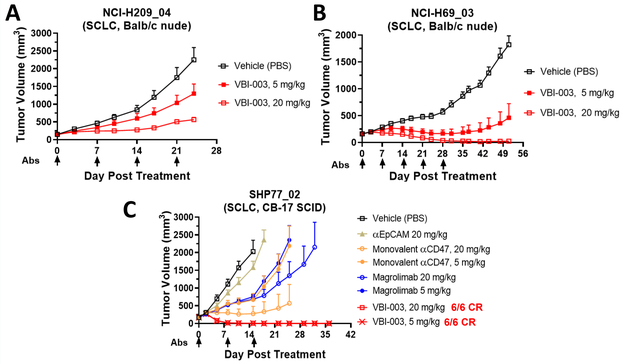


Fig.4. VBI-003 inhibited tumor growth in NCI-H209 (A), drove tumor regression in NCI-H69 (B) and eliminated all tumors in the SHP77 model (C). Animals were randomized by tumor volume into 6 animals per group. Test antibodies were administered through a weekly bolus tail vein injection when mean tumor volumes reached 150-200 mm^3. Black arrows indicate the dosing days. αEpCAM is the bivalent anti-EpCAM arm of VBI-003 in human IgG1. Red numbers in **(C)** indicate the number of tumor-free animals (CR, complete responders) at the end of the study.

Irinotecan combination further enhances the anti-tumor activity of VBI-003 in less responsive SCLC and CRC models

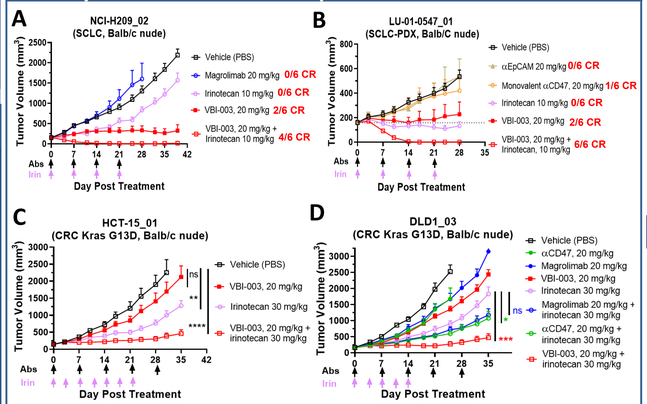


Fig. 5. Combination with irinotecan further augments the anti-tumor activity of VBI-003 in multiple CDX and PDX models of SCLC and CRC. Animals were randomized by tumor volume into 6 animals per group. Test antibodies were administered through a weekly bolus tail vein injection when mean tumor volumes reached 150-200 mm^3. Irinotecan was given by intraperitoneal injection weekly in **(A)** and **(B)** or twice a week in **(C)** and **(D)**. Arrows indicate the dosing days for antibodies (black) and irinotecan (purple). Statistical analysis was performed by one-way ANOVA. Treatment groups were compared to the vehicle group (day 31) in **(C).** In **(D)**, combo groups were compared to the irinotecan group. Significant levels: ns, non-significant; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. Red numbers in **(A)** and **(B)** indicate the number of tumor-free animals (CR) at the end of the studies.

Irinotecan induces surface translocation of calreticulin and upregulates CD47 expression *in vitro* in CRC cell lines

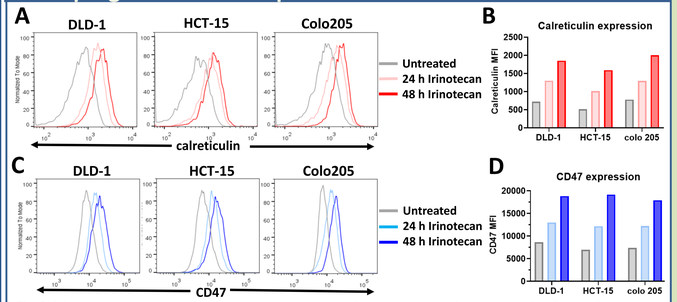


Fig.6. Irinotecan treatment induces surface translocation of "eat me signal" calreticulin and increases expression of CD47 in CRC cell lines. Cells were exposed to irinotecan at their respective GI-50 values (determined from cytotoxicity assays) for 24 h or 48 h. Irinotecan-treated and untreated cells were then harvested and stained for calreticulin and CD47. Only live cells (identified by the live-dead stain) were selected for analysis. **(A)** and **(B)** show the surface calreticulin level, whereas **(C)** and **(D)** show the CD47 expression of irinotecan-treated versus untreated cells.

Repeat dose NHP tox study demonstrated excellent safety of a closely related CD47xEpCAM bispecific antibody

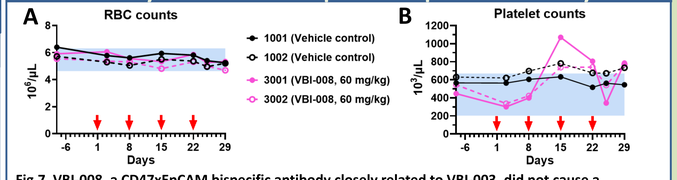


Fig.7. VBI-008, a CD47xEpCAM bispecific antibody closely related to VBI-003, did not cause a reduction in red blood cells (A) and only induced a mild and reversible decrease in platelet counts (B) in a non-human primate (NHP) toxicity study. Cynomolgus monkeys, two per group, were administered intravenously with vehicle control or 60 mg/kg VBI-008 weekly for 4 weeks (on Days 1, 8, 15, and 22 as indicated by red arrows). Blue-shaded areas represent the reported normal ranges of RBC and platelet counts (mean ± 2 x S.D.) from healthy animals (Reference 2).

Conclusions

VBI-003 is a novel CD47xEpCAM bispecific antibody with full effector functions. It is logically designed to improve selectivity in targeting tumor cells where both CD47 and EpCAM are present. Indeed, FACS binding assays showed that VBI-003 binding to RBCs and platelets is at least 300-fold weaker than the benchmark bivalent monospecific CD47 antibody, magrolimab. Moreover, *in vitro* functional assays demonstrated that VBI-003 has potent SIRPα blocking, ADCP and ADCC activities on CD47$^+$EPCAM$^+$ tumor cells but is less effective on CD47$^+$EPCAM$^-$ cells. In contrast, monospecific CD47 antibodies, including magrolimab, do not exhibit selectivity. VBI-003 has potent single-agent activity against multiple SCLC CDX and PDX models with tumor regression, and it frequently outperforms the benchmark magrolimab. Furthermore, we showed that VBI-003 synergized with irinotecan and surpassed monotherapy groups in CDX models of CRC. Consistent with reports that some DNA damage agents induce immunogenic cell death and surface translocation of calreticulin, we observed increased surface expression of calreticulin and CD47 on CRC cells post irinotecan treatment *in vitro*. This may account for at least some of the synergy observed *in vivo*. Importantly, the NHP toxicity study found that weekly administration of a closely related CD47xEpCAM bispecific antibody, VBI-008, at 60 mg/kg was safe and did not require a priming dose. In addition, VBI-008 had no effect on RBCs and only caused a mild and reversible reduction in platelets.
In conclusion, the CD47xEpCAM bispecific antibody VBI-003 represents a promising strategy to target the CD47/SIRPα pathway and merits clinical investigation as a treatment for CD47 and EpCAM expressing SCLC and CRC.

American Association for Cancer Research Annual Meeting, Orlando, Florida, April 14-19, 2023

References

1. Liu J, 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
2. Koga T, et al. (2005) Individual reference intervals of hematological and serum biochemical parameters in cynomolgus monkeys. Int J Toxicol. 24(5):377-85. doi: 10.1080/10915810500208058.