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A novel topoisomerase I inhibitor-based anti-ICAM-1 antibody drug conjugate for the treatment of hematologic malignancies and solid tumors

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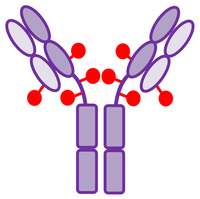
Abstract

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 lymphoma, myeloma and some types of solid tumors including melanoma, non-small cell lung cancers and liver cancers. Previous attempts to target ICAM-1 with a monoclonal antibody bersanlimab (BI505, BioInvent) revealed that it was well tolerated but had limited clinical efficacy. To improve the effectiveness of targeting ICAM-1, we developed a novel ICAM-1 antibody drug conjugate (ADC) consisting of a humanized anti-ICAM-1 IgG1 antibody conjugated with a novel topoisomerase I inhibitor via a cleavable linker (VC001) at a drug-to-antibody ratio (DAR) of about 8. The antibody moiety of the ADC targets a unique epitope on ICAM-1 that is distant from its ligand binding domain. The ICAM-1 ADC, called ICAM-1-VC001, exhibited potent *in vitro* cytotoxicity on a range of hematologic and solid tumor cell lines with single-digit nanomolar EC50s. In preclinical studies, administration of one or two doses of ICAM-1-VC001 at 5 mg/kg was sufficient to induce tumor regression in various cell line-derived xenograft (CDX) models, including lung and liver cancers. In all models tested thus far, our topoisomerase I inhibitor ADC, ICAM-1-VC001, has demonstrated at least comparable, if not superior, efficacy to ICAM-1-DXd, which is the same ICAM-1 antibody conjugated with the benchmark topoisomerase I inhibitor linker payload deruxtecan. In addition, in a repeat dose exploratory non-human primate safety study, the benchmark conjugate ICAM-1-DXd (DAR 8) was well tolerated at the highest dose of 41 mg/kg. In conclusion, ICAM-1 is an appealing target for topoisomerase I inhibitor-based ADCs and warrants further investigation.

A novel topoisomerase I inhibitor-based ICAM-1 ADC



ICAM-1-VC001

ICAM-1 antibody
- Humanized ICAM-1 antibody with human and cyno ICAM-1 affinity of about 0.5 nM
- Unique epitope distant from ligand binding domain

Fc
- Human IgG1 backbone with full effector functions

Warhead
- Proprietary Topo-I inhibitor-based linker payload (VC001) at a drug-to-antibody ratio of 8

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

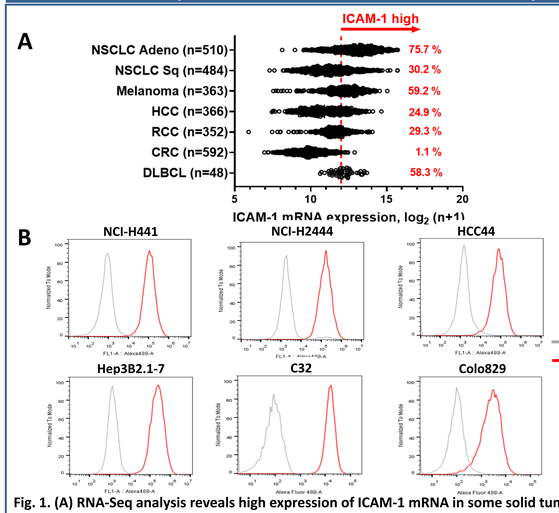


Fig. 1. (A) RNA-Seq analysis reveals high expression of ICAM-1 mRNA in some solid tumors. RNA-Seq data were from TCGA PanCancer Atlas datasets downloaded from cBioPortal [1]. CRC (colorectal cancers) and DLBCL (diffuse large B cell lymphoma), an ICAM-1 low and ICAM-1 high tumor type, respectively, served 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 (NCI-H441, NCI-H2444, HCC44), liver (Hep3B2.1-7) and melanoma (C32, Colo829) cell lines showed expression of ICAM-1 by flow cytometry.

ICAM-1-VC001 has strong cytotoxicity against hematologic and solid tumor cell lines, often surpassed ICAM-1-DXd

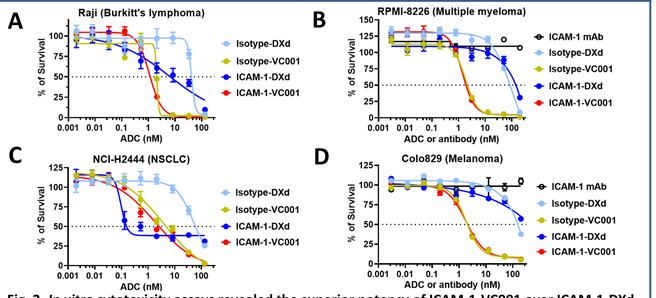


Fig. 2. *In vitro* cytotoxicity assays revealed the superior potency of ICAM-1-VC001 over ICAM-1-DXd, the benchmark deruxtecan conjugate, against various heme and solid tumor cell lines. Cells were incubated with serial dilutions of ADCs or ICAM-1 unconjugated antibody (ICAM-1 mAb) for 6 days. CellTiter-Glo was used to measure the number of surviving cells at the end of the assays. Isotype-DXd and isotype-VC001 are human IgG1 isotype antibodies conjugated with the benchmark deruxtecan or our linker-payload VC001, respectively, at DAR 8.

ICAM-1-VC001 is highly effective in lymphoma and multiple myeloma models, superior to the benchmark ICAM-1-DXd

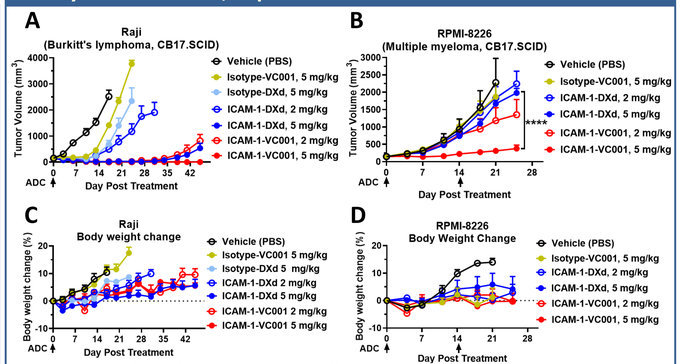


Fig.3. One or two doses of ICAM-1-VC001 were sufficient to induce tumor regression in Raji (A) or stop tumor growth in RPMI-8226 (B) model, respectively, outperforming the benchmark conjugate ADC ICAM-1-DXd. Animals were randomized by tumor volume into 6 animals per group. ADCs were administered through weekly bolus tail vein injections when mean tumor volumes reached 150-200 mm3. Animals with tumor volume exceeding 3000 mm^3 were humanely euthanized. All ADCs were well tolerated with no significant body weight loss observed in all animals **(C and D)**. Black arrows indicate the dosing days. Statistical analysis was performed with unpaired t-test. ****, P<0.0001.

ICAM-1-VC001 has potent anti-tumor activities against melanoma, HCC and NSCLC models, and has repeatedly outperformed ICAM-1-DXd

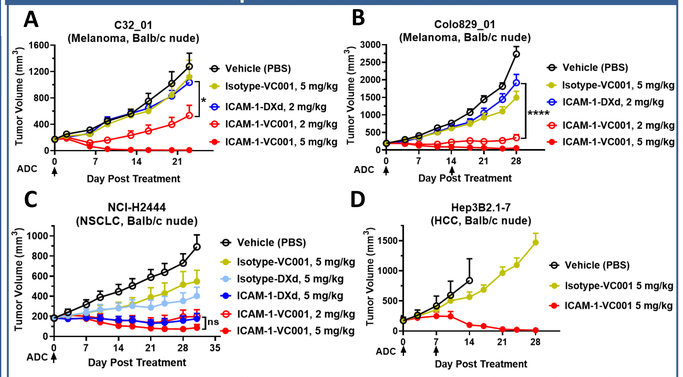


Fig.4. ICAM-1-VC001 is extremely effective in some solid tumor models, including melanoma (A and B), NSCLC (C) and HCC (D). ICAM-1-VC001 was often more effective than ICAM-1-DXd in driving tumor regression with one or two doses. Animals were randomized by tumor volume into 6 animals per group. ADCs were administered through weekly bolus tail vein injections when mean tumor volumes reached 150-200 mm3. All ADCs were well tolerated with no significant body weight loss observed in ADC-treated animals. Black arrows indicate the dosing days. Statistical analysis was performed with unpaired t-test. *, P<0.05; ****, P<0.0001; ns, not significant.

Pilot NHP tox study established that ICAM-1 can be safely targeted with a topoisomerase I ADC

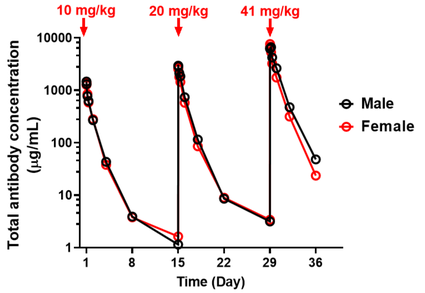


Fig. 5. Intra-animal dose escalation non-human primate (NHP) toxicity study showed that ICAM-1 antibody conjugated with the benchmark linker-payload, DXd, at DAR 8 was well tolerated with good antibody exposure. It demonstrated that ICAM-1 can be a safe target for topoisomerase-I-based ADC. Two cynomolgus monkeys, one per sex, were administered intravenously with 10 mg/kg, 20 mg/kg and 41 mg/kg ICAM-1-DXd on Days 1, 15, and 29, respectively (as indicated by red arrows). Blood samples were taken at various timepoints for the determination of total antibody concentration in plasma. All animals survived until the end of the study without any appreciable weight loss or sign of distress. No concerning findings observed in hematology, clinical chemistry and coagulation analyses. No abnormalities observed in gross and microscopic examination of tissues and organs. A NHP study with ICAM-1-VC001 is planned.

Conclusions

ICAM-1-VC001 is a versatile novel topoisomerase I inhibitor-based ADC. It comprises a humanized anti-ICAM-1 IgG1 antibody, which is conjugated with a novel topoisomerase I inhibitor via a cleavable linker at a DAR of about 8. *In vitro*, ICAM-1-VC001 demonstrated potent cytotoxicity against various hematologic and solid tumor cell lines with EC50s in the single-digit nanomolar range. It demonstrated high cytotoxicity even against cell lines that were insensitive to ICAM-1-DXd (DAR 8), the benchmark deruxtecan conjugate. Consistent with the *in vitro* results, ICAM-1-VC001 exhibited robust anti-tumor activity across a broad range of hematologic and solid tumor CDX models, including NSCLC, hepatocellular carcinoma, melanoma, lymphoma, and multiple myeloma. Administration of one or two doses of ICAM-1-VC001 at 5 mg/kg was sufficient to induce tumor regression. In contrast, ICAM-1-DXd was not consistently effective in the tumor models tested. Although the negative control ADC (isotype-VC001) exhibited significant activity in the *in vitro* cytotoxicity assays, it had minimal anti-tumor effect *in vivo*, comparable to that of isotype-DXd. These findings indicate that ICAM-1-VC001 enables target-mediated killing *in vivo*. Notably, in a repeat dose exploratory non-human primate safety study, the benchmark conjugate ICAM-1-DXd (DAR 8) was well tolerated at the highest dose of 41 mg/kg. These results indicate that targeting ICAM-1 with topoisomerase I ADC can be safe.

In conclusion, ICAM-1 is an attractive target for topoisomerase I inhibitor-based ADCs. A NHP toxicity study to evaluate the safety of ICAM-1-VC001 is planned.

Reference

1. Cerami et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery. May 2012 2; 401.

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