DM001, a Novel TROP2xEGFR Bispecific ADC, Demonstrates Potent Tumor Growth Inhibition in Preclinical Models and Favorable Safety Profile in Cynomolgus Monkey

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INTRODUCTION

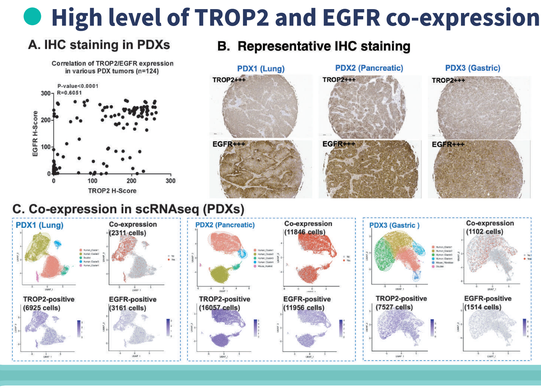
EGFR and TROP2 are validated therapeutic targets co-expressed on multiple tumor types. Single-targeting ADCs have shown clinical benefits, however, on-target off tumor toxicities were observed due to their expression in normal tissues. Such toxicities would limit dose escalation and subsequently efficacy. Bispecific ADCs (bsADCs), by targeting EGFR and TROP2 simultaneously, potentially allow enhanced efficacy, reduced resistance, and improved safety profiles.

We screened pools of anti-EGFR and anti-TROP2 monoclonal antibodies, and selected clones to construct a bispecific ADC targeting EGFRxTROP2 that provided optimized internalization profile. *In vitro*, unconjugated DM001 (DM001 bsAb) demonstrated enhanced internalization as opposed to its parental monoclonal antibodies (mAbs). For a proof of concept, the TROP2xEGFR bsAb was initially conjugated to monomethyl auristatin E (MMAE) via a protease-cleavable linker (vcMMAE). The ADC, DM001-vcMMAE, demonstrated robust inhibition of growth of two PDX models, with the bsADC being more potent than its parental single-targeting ADCs.

To further improve upon the linker/payload system, we chose to conjugate DM001 bsAb to BLD1102, Biocytogen's novel, proprietary linker/payload system composed of a DNA topoisomerase I inhibitor payload (BCPT02) and a very hydrophilic, protease-cleavable linker, with a drug-to-antibody ratio (DAR) of 8. The resulting ADC, DM001-BLD1102 or DM001, was highly stable in human and monkey plasmas and in mouse circulation. BLD1102 also showed a stronger bystander killing effect than Dxd when conjugated to DM001 bsAb. *In vivo*, DM001 showed potent anti-tumor activity in PDX including those less sensitive to vcMMAE-conjugated ADC. DM001 showed robust efficacy in a number of PDX models, including triple negative breast cancer (TNBC), esophageal squamous cell carcinoma (ESCC), head and neck squamous cell carcinoma (HNSCC), colorectal cancer (CRC), gastric cancer (GC), and non-small cell lung cancer (NSCLC) with and without EGFR mutations. In a dose range finding study, DM001 (DM001-BLD1102) was well tolerated in cynomolgus monkeys when IV dose was escalated to 20 mg/kg Q3W x2 after initial cycle of 5 mg/kg Q3Wx1. Further toxicity study is planned.

In summary, DM001 is a novel bispecific ADC composed of an improved bispecific targeting moiety against EGFRxTROP2 and a highly stable and cytotoxic linker/payload system with an excellent bystander killing effect. DM001 showed potent anti-tumor activity, had a favorable safety profile in cynomolgus monkeys, and is currently in preclinical development toward IND filing.

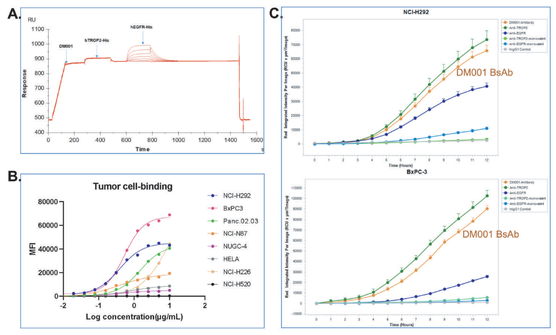
High level of TROP2 and EGFR co-expression in multiple solid tumor PDX models



TROP2 and EGFR are highly co-expressed in PDXs.
(A) Immunohistochemical (IHC) scores of TROP2 and EGFR in 124 PDX models.
(B) Representative IHC staining of PDXs for TROP2 and EGFR expression.
(C) Single-cell RNA sequencing (scRNA-seq) analysis for expression of TROP2 and EGFR.

Results: TROP2 and EGFR are co-expressed in a wide range of tumors, and on individual tumor cells in multiple PDX models.

DM001 bsAb demonstrated robust affinity, tumor cell binding, and internalization activity

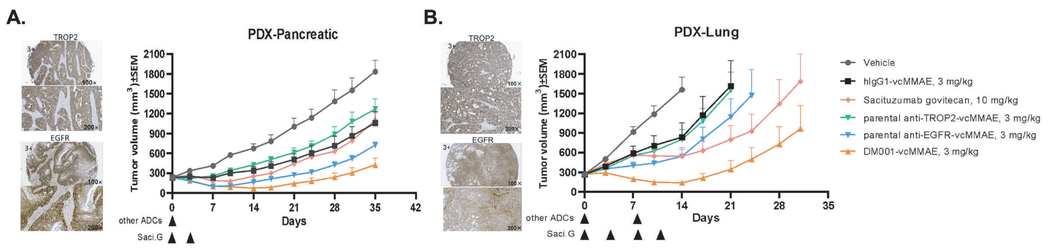


Binding and internalization capacity of DM001 BsAb.
(A) Binding of hTROP2 and hEGFR by DM001 bsAb with SPR.
(B) DM001 bsAb cellular binding to several cancer cell lines by flow cytometry. TROP2 and EGFR expressions in the cell lines are: NCI-H292, BxPC-3, Panc.02.03, NUGC-4, Hela and NCI-N87: TROP2^{+}EGFR^{+}; NCI-H226: TROP2^{-}EGFR^{+}; NCI-H520: TROP2^{-}EGFR^{-}.
(C) Internalization of DM001 bsAb in NCI-H292 and BxPC-3 cells measured by Incucyte.

Results: DM001 bsAb displayed robust affinity for both TROP2 and EGFR, and bound well to a number of cell lines. DM001 bsAb was internalized efficiently and more so as opposed to the parental TROP2 or EGFR monovalent antibodies.

Note:
Anti-TROP2: parental monoclonal anti-TROP2;
Anti-EGFR: parental monoclonal anti-EGFR;
Anti-TROP2-monovalent: parental monovalent anti-TROP2;
Anti-EGFR-monovalent: parental monovalent anti-EGFR.

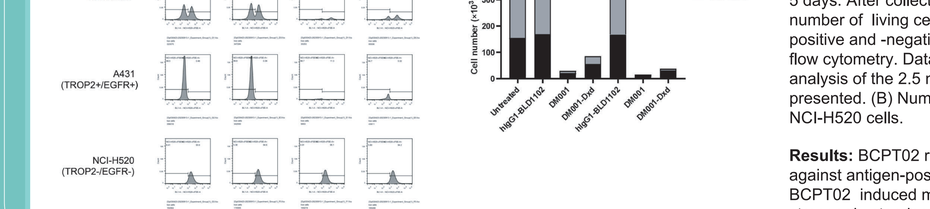
Enhanced anti-tumor activity of DM001-vcMMAE in two PDX models



Results: DM001-vcMMAE showed strong anti-tumor activity in these PDX models. BsADC demonstrated superior efficacy to either of its parental mAb ADCs.

DM001-BLD1102: DM001 bsAb conjugated to a novel topoisomerase I inhibitor payload

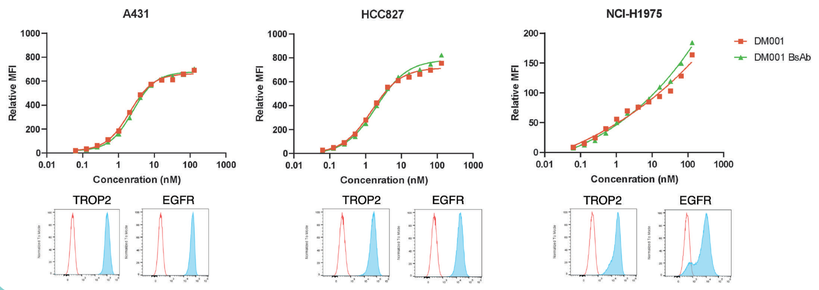
DM001-BLD1102 (DM001) has excellent hydrophilicity



DM001 bsAb was conjugated to BLD1102, a Biocytogen's novel proprietary linker/payload platform, with DAR4 or DAR8, and characterized with SEC-HPLC for determination of aggregation and HIC-HPLC/RP-HPLC for hydrophilicity.

Results: DM001-BLD1102 showed no aggregation in either DAR4 or DAR8 and exhibited strong hydrophilicity similar to parental DM001 bsAb.

BLD1102 conjugation did not affect DM001 binding



The cellular binding properties of DM001 (DM001-BLD1102, DAR 8) before and after conjugation to tumor cells determined by flow cytometry.

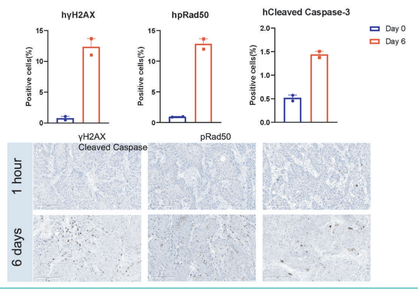
Results: The conjugation of BLD1102 did not affect binding of DM001 to its antigens.

BCPT02 showed a potent bystander killing effect *in vitro*

Bystander killing effect *in vitro*. (A) Unlabeled A431 and CFSE-labeled NCI-H520 cells were cocultured and treated with 2.5/10 nM ADCs for 5 days. After collection of adherent cells, the number of living cells and the ratio of CFSE-positive and -negative cells were determined by flow cytometry. Data from the flow cytometric analysis of the 2.5 nM treatment group were presented. (B) Numbers of viable A431 and NCI-H520 cells.

Results: BCPT02 rendered potent cytotoxicity against antigen-positive cells. Data suggests that BCPT02 induced more killing of NCI-H520 cells, or stronger bystander effect, than Dxd.

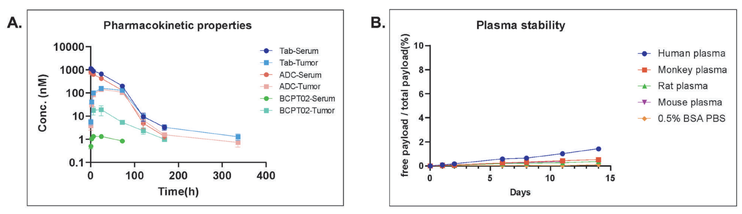
Induction of DNA damage and apoptosis in cancer cells by DM001



Induction of DNA damage and apoptosis in cancer cells by DM001. DM001 was administered intravenously at 10 mg/kg to mice bearing grown A431 tumors. Tumor tissues were harvested after 1 hour of dosing and on day 6 to assess induction of DNA damage and apoptosis. The levels of γH2AX and pRad50 were evaluated as markers of DNA damage and the level of cleaved caspase 3 was evaluated as a marker of apoptosis.

Results: BLD1102 on DM001 induced phosphorylation of H2AX and Rad50 and the cleavage of caspase 3, indicating a cytotoxic mechanism against cancer cells.

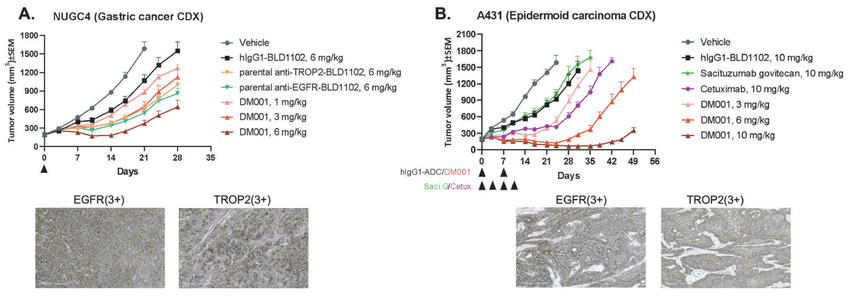
Pharmacokinetics (PK) in A431 model and *in vitro* plasma stability of



Pharmacokinetics (PK) and *in vitro* plasma stability of DM001. (A) The kinetics of concentrations of total antibody (Tab), DM001 (ADC) and free payload BCPT02 in serum and tumor in A431 tumor-bearing B-NDG mice after a single 10 mg/kg injection at time 0. (B) Stability of DM001 in the plasma of different animal species.

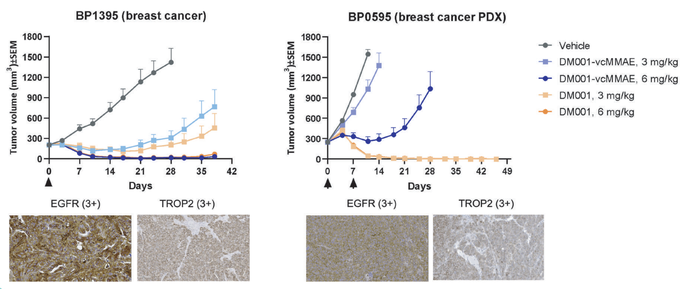
Results: (A) Serum and tumor distribution of DM001 *in vivo*. (B) Plasma stability test. Release of free BCPT02 from DM001 was between 0.5% to 1.4% on day 14 in monkey and human plasma, indicating stable conjugation of BLD1102 in these plasmas.

DM001 demonstrated superior, dose-dependent anti-tumor activity in CDX models



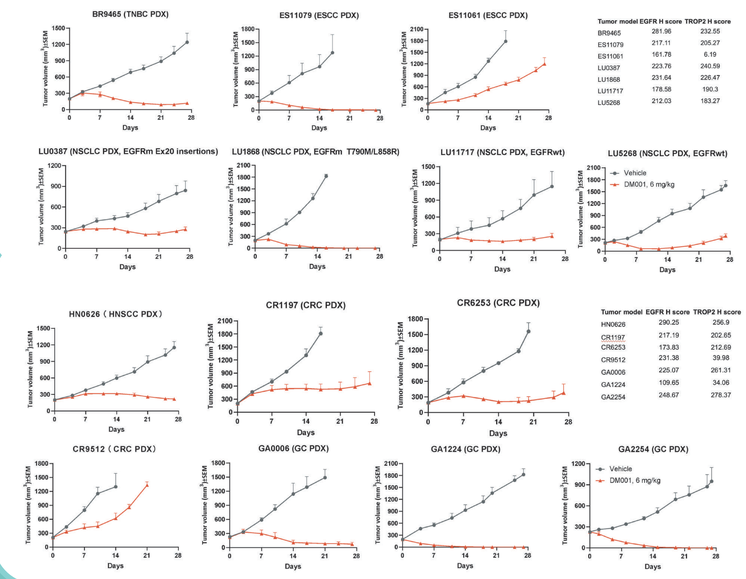
Results: DM001 showed potent anti-tumor activity in CDX models. In the NUGC-4 model, DM001 was more efficacious than its parental ADCs. In the A431 model, DM001 showed superior activity than marketed anti-TROP2 ADC sacituzumab govitecan (Saci G). Dose-dependent anti-tumor activities were observed in both CDX models.

DM001 demonstrated stronger anti-tumor activity vs vcMMAE conjugated ADC in PDX models



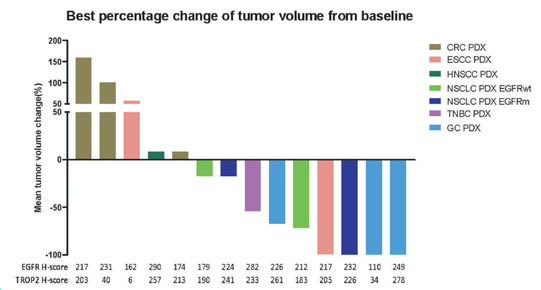
Results: DM001 showed stronger activity in both breast cancer PDX models compared with that of vcMMA conjugated ADCs, including PDX that was only moderately sensitive to the vcMMAE linker/payload.

DM001 inhibited a variety of PDX models, including both EGFR-mutant and wild-type NSCLC PDX



Mice in all groups were treated with a single intravenous injection of 6 mg/kg DM001 on day 0.

Summary of DM001 anti-tumor activities in PDX models



Summary of best percent change of tumor volumes in PDX models with 6 mg/kg DM001 injected IV at day 0. DM001 was efficacious across multiple cancer types, notably three out of three gastric cancer PDX models. It was less effective against colorectal PDX models (two out of three showed progression). No obvious correlation of expression levels and degree of tumor growth inhibition was observed in this small group of PDX models.

Preliminary DM001 safety study in Cynomolgus Monkeys showed no general toxicities

Species	Cynomolgus Monkeys
General observations	-
Body Weight / Food Consumption	-
Hematology	↓Reticulocytes ↓WBC (Neutrophils, lymphocytes, monocytes)
Serum Chemistry	Transient ↓ in Na, Cl, P in individual animals
coagulation	-
urinalysis	-
gross necropsy	-

< No treatment-related effects was noted after administration (once every 3 weeks for 2 doses)

- DM001 was well tolerated in cynomolgus monkeys dosed IV at 20 mg/kg Q3W x2 after initial 5 mg/kg QWx1.
- No treatment-related general toxicities were observed.
- No visible lesions of tissues/organs including skin or lung were observed
- White blood cell (WBC) and reticulocyte (RET) counts transiently decreased and subsequently recovered during cycles.
- Further safety study is planned

SUMMARY

- EGFR and TROP2 are extensively co-expressed in many solid tumors, allowing dual targeting them with bsADCs.
- DM001 bsADCs simultaneous targeting both EGFR and TROP2 showed superior efficacy compared with parental single-targeting ADCs in PDX models.
- The new topoisomerase I inhibitor linker/paylopad (BLD1102) exhibits excellent hydrophilicity and is highly stable in human, monkey, and mouse plasmas and *in vivo* in mice.
- BLD1102 has strong bystander killing effect.
- DM001 (DM001-BLD1102) demonstrated superior anti-tumor activity to DM001-vcMMAE in PDX models, including in MMAE-resistant models.
- DM001 (DM001-BLD1102) inhibited the growth of NSCLC PDX models with or with out EGFR mutations, as well as growth of TNBC, HNSCC, CRC, ESCC and GC PDXs.
- Pilot safety study of DM001 (DM001-BLD1102) did not show general toxicities in cynomolgus monkeys. Further study is planned.