LB215

A first-in-class anti-TROP2/EGFR bispecific antibody-drug conjugate, DM001, exhibits potent anti-tumor efficacy

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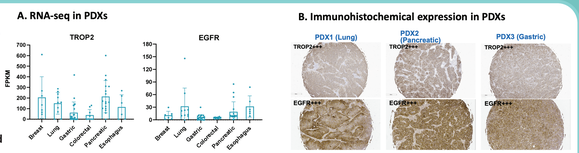
ABSTRACT

EGFR is a well-established target for the treatment of many cancers. However, limitations encountered with current therapies, such as drug resistance and low cytotoxicity, indicate a need for alternative treatments. Antibody-drug conjugates (ADCs) are a promising new therapeutic strategy, because of their potent killing effects and high target specificity. However, the toxicity of the ADC payload can often cause safety concerns, so their efficacy and safety must be carefully evaluated. With these challenges in mind, we developed a bispecific ADC (BsADC) targeting EGFR and a second tumor-associated antigen with the goal to improve tumor specificity, thereby limiting the occurrence of on-target off-tumor effects. TROP2 and EGFR are co-expressed in multiple types of solid tumors, including head and neck, esophageal, lung, and pancreatic cancers, suggesting that this target combination could provide therapeutic benefit for a wide range of tumors.
Herein, we developed a novel BsADC, DM001, targeting TROP2 and EGFR, conjugated with monomethyl auristatin E (MMAE) via a protease-cleavable linker. *In vitro*, DM001 showed similar levels of internalization and tumor killing activity compared with its parental monoclonal anti-TROP2 and anti-EGFR antibodies in TROP2^{+} EGFR^{+} cells. Compared with single positive cells, DM001 can selectively bind and better kill double positive cells. Mechanistically, DM001 delays progression of the cell cycle and increases the frequency of apoptosis *in vitro* in an antigen-dependent manner. Pharmacokinetic analyses in mice with humanized FcRn (B-hFcRn) demonstrated a similar half-life of DM001 to isotype controls. Importantly, DM001 demonstrated strong anti-tumor activity in several cell line-derived and patient-derived xenografts, including lung and pancreatic tumors. Notably, the efficacy of DM001 was superior to benchmark ADCs in A431 and Panc.02.03 xenografts. Interestingly, the efficacy of DM001 was superior to its parental ADCs in BP0508 lung cancer and BP0209 pancreatic cancer PDX models, but not obvious in Panc.02.03 CDX models, indicating that DM001 may effectively target heterogeneous tumors, which better mimic the tumor microenvironment in patients. In summary, DM001 is a novel bispecific ADC with promising therapeutic potential that can be further exploited to treat TROP2 and EGFR co-expressing tumors.

Co-expression of TROP2 and EGFR in PDXs

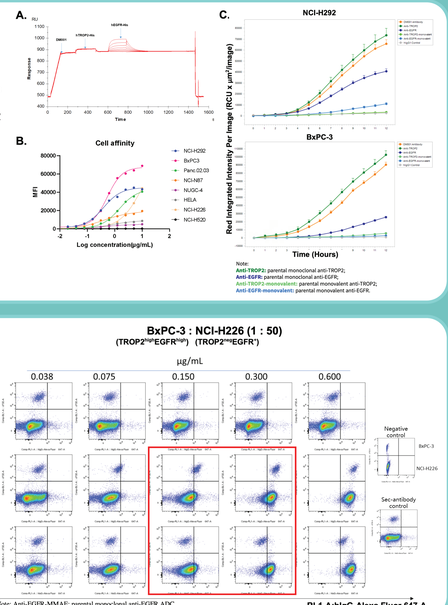


TROP2 and EGFR are highly co-expressed in patient-derived xenografts (PDX).
(A) RNA-seq data from several PDXs (in-house) were analyzed for expression of TROP2 and EGFR. Both were highly expressed in lung, pancreatic and esophagus cancer tissues.
(B) TROP2 and EGFR were also co-expressed in some of PDX samples as assessed by IHC.
(C) Co-expression of TROP2 and EGFR in populations identified by single cell (scRNAseq) was also observed in these PDX samples.

DM001 bsAb showed high affinity and internalization activity

Binding and internalization activity of DM001 BsAb. (A) Continuous binding on hTROP2 and hEGFR antigens of DM001 via SPR. **(B)** DM001 affinity measurements by flow cytometry. Expression of TROP2 and EGFR in the cell lines are as follows:
TROP2^{high} EGFR^{high}: NCI-H292, Panc.02.03 and BxPC-3;
TROP2^{high} EGFR^{low}: NCI-N87;
TROP2^{low} EGFR^{low}: NUGC-4 and HELA;
TROP2^{neg} EGFR^{+}: NCI-H226;
TROP2^{neg} EGFR^{neg}: NCI-H520.
(C) Internalization of DM001 BsAb in NCI-H292 and BxPC-3 cells measured by Incucyte.

Results: DM001 showed high affinity and increased internalization in several cancer cell lines compared with its 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.

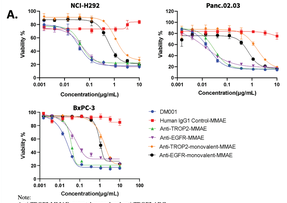
Selectivity of DM001 bsADC for TROP2^{+}EGFR^{+} cells

DM001 preferentially binds cells that highly express both TROP2 and EGFR. BxPC-3 (TROP2^{high}EGFR^{high}) and NCI-H226 (TROP2^{neg}EGFR^{+}) cells were co-cultured at a ratio of 1:50, respectively, then treated with DM001 and anti-EGFR-MMAE to assess the binding selectivity.

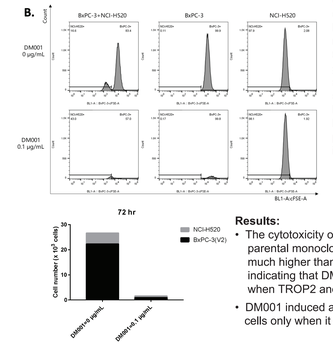
Results: DM001 selectively binds double positive BxPC3 cells at lower concentrations (red box). As the concentration increased, DM001 then began to bind single positive NCI-H226 cells. Anti-EGFR-MMAE was used as control.

Note: Anti-EGFR-MMAE: parental monoclonal anti-EGFR ADC

DM001 induces cytotoxicity in TROP2^{+}EGFR^{+} cells & bystander killing in TROP2^{neg}EGFR^{neg} cells



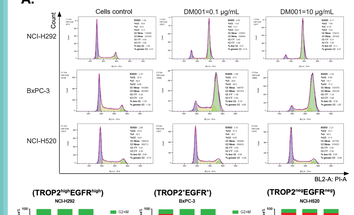
Note:
Anti-TROP2-MMAE: parental monoclonal anti-TROP2 ADC;
Anti-EGFR-MMAE: parental monoclonal anti-EGFR ADC;
Anti-TROP2-monovalent-MMAE: parental monovalent anti-TROP2 ADC;
Anti-EGFR-monovalent-MMAE: parental monovalent anti-EGFR ADC.

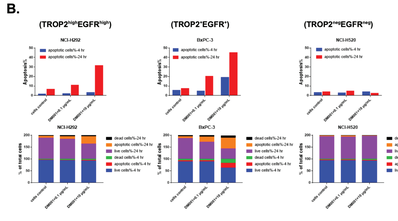


Cytotoxicity and Bystander effect of DM001 *in vitro*. **(A)** Cytotoxicity of DM001 on several cell lines.
(B) DM001 induces bystander effects. 0.1 μg/mL DM001 was added to 3 groups: BxPC-3 + NCI-H520, BxPC-3 and NCI-H520. While DM001 induced cytotoxicity of the double positive BxPC-3 cells, double negative NCI-H520 cells were only killed in the presence of BxPC-3 cells. Error bars indicate SD.

Results:
- The cytotoxicity of DM001 was comparable to its parental monoclonal TROP2 or EGFR ADCs, and much higher than its parental monovalent ADCs, indicating that DM001 has robust killing activity only when TROP2 and EGFR were both expressed.
- DM001 induced a bystander effect by killing NCI-H520 cells only when it was co-cultured with BxPC-3.

DM001 delays progression of the cell cycle and induces apoptosis

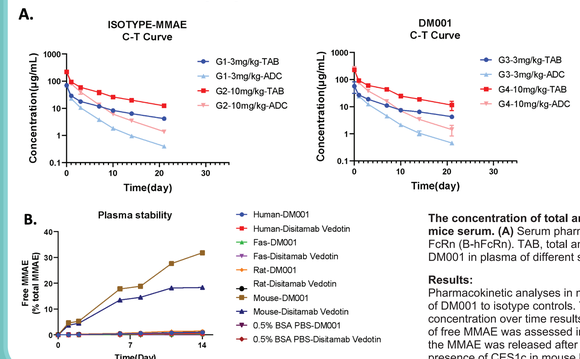




Mechanism of action of DM001. DM001 delayed cell cycle **(A)** and induced apoptosis **(B)** only in cell lines expressing TROP2 and EGFR. Cell Apoptosis Kits with Annexin V were used for cell cycle analysis. Cell Cycle Assay Kit (red fluorescence) was used for apoptosis assessments.

Results:
DM001 delayed the progression of the cell cycle and increases the frequency of apoptosis *in vitro* in an antigen-dependent manner.

Pharmacokinetic properties of DM001 in humanized B-hFcRn mice



		T1/2 (day)	
		ADC	Tab
ISO-MMAE	3mpk	5.069	11.365
	10mpk	5.088	10.335
DM001	3mpk	5.053	13.318
	10mpk	4.818	10.581

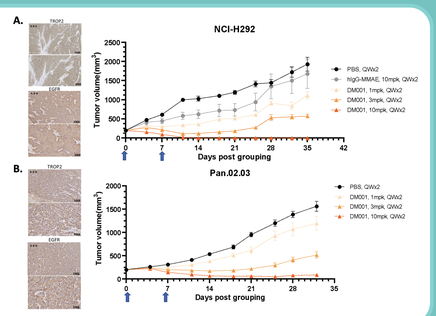
The concentration of total antibody (TAB) and DM001 ADC in B-hFcRn mice serum. (A) Serum pharmacokinetic analyses in mice with humanized FcRn (B-hFcRn). TAB, total antibody. Error bars indicate SD. **(B)** Stability of DM001 in plasma of different species.

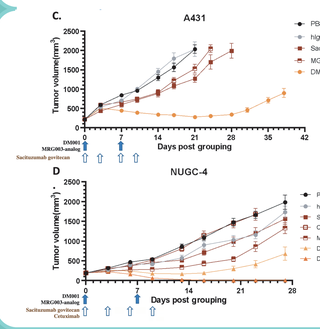
Results:
Pharmacokinetic analyses in mouse serum demonstrated a similar half-life of DM001 to isotype controls. To determine whether the reduction in ADC concentration over time results in increased MMAE release, the percentage of free MMAE was assessed in plasma, which indicates that about 30% of the MMAE was released after 14 days in mice. This may be due to the presence of CES1c in mouse blood.

DM001 exhibits dose-dependent anti-tumor efficacy in CDX models

Anti-tumor efficacy of DM001 in CDX. DM001 efficacy was assessed in NCI-H292 (TROP2^{high}EGFR^{high}) (A) and (B) Panc.02.03 (TROP2^{high}EGFR^{high}) CDX models. Tumor samples were analyzed by immunohistochemistry. Different concentrations of DM001 (1mpk, 3mpk, 10mpk) were administered intravenously once a week as indicated (blue arrows). Error bars indicate SEM.

Results:
DM001 showed strong and dose-dependent anti-tumor efficacy in NCI-H292 and Panc.02.03 CDX models. At the 10 mpk dose, DM001 completely abolished NCI-H292 tumor growth after Day 14.

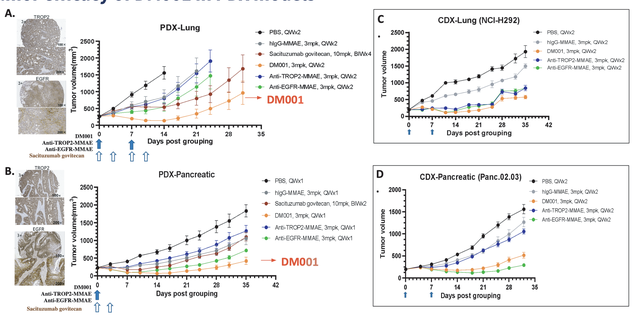




DM001 efficacy assessments in A431 (TROP2^{high}EGFR^{high}) (C) and NUGC-4 (TROP2^{low}EGFR^{low}) (D) CDX models. DM001 was administered intravenously once a week (2x total) as indicated. Sacituzumab govitecan was intravenously administered twice a week or once per 3 days (4x total) as a benchmark for TROP2 ADC. Cetuximab was intravenously administered twice a week or once per 3 days (4x) as a benchmark for EGFR. MRG003-analog was intravenously dosed once a week (2x total) as a benchmark for EGFR ADC. Error bars

Results:
DM001 showed strong and superior anti-tumor efficacy in A431 and NUGC-4 CDX models compared to the benchmarks Sacituzumab govitecan, Cetuximab and MRG003-analog.

Anti-tumor efficacy of DM001 in PDX models



Anti-tumor efficacy of DM001 in PDX models. DM001 was assessed in lung (TROP2^{high}EGFR^{high}) **(A)** and pancreatic (TROP2^{high}EGFR^{high}) **(B)** PDX models. DM001 was intravenously administered once a week (1x total) at a dosage of 3 mpk. Sacituzumab govitecan (a benchmark of TROP2 ADC) was intravenously administered twice a week (2x total) at a dosage of 10 mpk. **(C-D)** Efficacy of DM001 in lung (TROP2^{high} EGFR^{high}) and pancreas (TROP2^{high} EGFR^{low}) CDX models was similarly assessed. Error bars indicate SEM.

Results: DM001 showed strong anti-tumor efficacy in these two PDX models at a dosage of 3 mpk. The efficacy was more potent than benchmark Sacituzumab govitecan and its parental ADCs.

SUMMARY

***In vitro* efficacy of DM001** :
DM001 showed high affinity and cytotoxicity in multiple TROP2^{+} EGFR^{+} cancer cell lines.
DM001 delayed cell cycle progression and induced apoptosis in cancer cells.
DM001 bsADC showed preferential binding to cells expressing both TROP2 and EGFR, indicating potentially better safety in single positive cells.

***In vivo* efficacy of DM001** :
DM001 exhibited potent and dose-dependent anti-tumor efficacy in multiple CDX and PDX models. DM001 showed stronger efficacy than benchmarks Sacituzumab govitecan, Cetuximab and MGR003-analog.
While the efficacy of DM001 was higher than its parental ADCs in PDX models, it was not obvious in CDX models, indicating that DM001 may be more effective in targeting heterogeneous tumors, which better mimics the tumor microenvironment in patients.