BG-C477, a novel topoisomerase 1 inhibitor-based ADC, exhibits antitumor activity in carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)-expressing preclinical models

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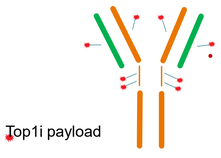
Abstract

CEACAM5 (CEA) is a cell surface glycoprotein highly expressed in many cancers, including colorectal cancer (CRC), gastric cancer (GC), lung and pancreatic cancers. BG-C477 is a novel ADC that is composed of a CEA-specific antibody conjugated to Top1i payloads via a cleavable linker. It remains stable in human plasma and mice blood circulation. BG-C477 showed specific binding to CEA-expressing MKN45 cells and stronger internalization activity compared to anti-CEA DM4 ADC. BG-C477 exhibited robust and CEA-dependent cytotoxicity in cell lines with different CEA expression levels. A potent bystander effect of BG-C477 was observed in co-culture killing assay with mixed CEA-positive MKN45 and -negative HCT116 cells, indicating the potential to treat tumors with heterogeneous CEA expression. In the animal studies, BG-C477 demonstrated potent and dose-associated single-agent efficacy in multiple CEA-expressing cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models. Moreover, BG-C477 exhibited superior antitumor efficacy in GC and CRC PDX models in which anti-CEA DM4 ADC was ineffective.

In summary, BG-C477 is a promising CEA targeting ADC with great anti-tumor activity across multiple types of tumors. The first in human study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of BG-C477 is ongoing (NCT06596473).

Molecule Design

Figure 1. BG-C477 design



CEA expression in human CRC samples

- CEA is highly expressed in CRC patients

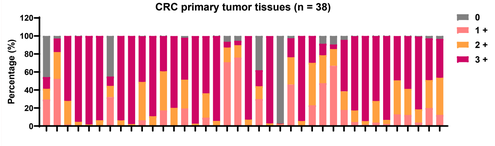


Figure 2. IHC staining of CEA in human CRC FFPE samples (n = 38)

The *in vitro* and *in vivo* stability of BG-C477 in human plasma and mice

- BG-477 remains stable when incubated in human plasma and in mice blood circulation after i.v. injection, while anti-CEA DM4 ADC shows inferior stability in both systems.

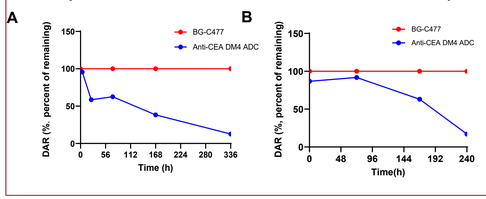


Figure 3. Stability of BG-C477 and anti-CEA DM4 ADC was assessed in A) human plasma cultured at 37℃ for up to 336 hours., and **B)** in BALB/c nude mice after i.v. injection and sampled for up to 240 h. DAR was measured using intact LC-MS.

The binding and internalization activity of BG-C477 and reference anti-CEA DM4 ADC in CEA expressing tumor cells

- BG-C477 and anti-CEA DM4 ADC showed similar binding activity to MKN45 cell (CEA positive) and no binding to HCT116 cell (CEA negative).
- BG-C477 exhibited stronger internalization activity compared to anti-CEA DM4 ADC in MKN45 cells

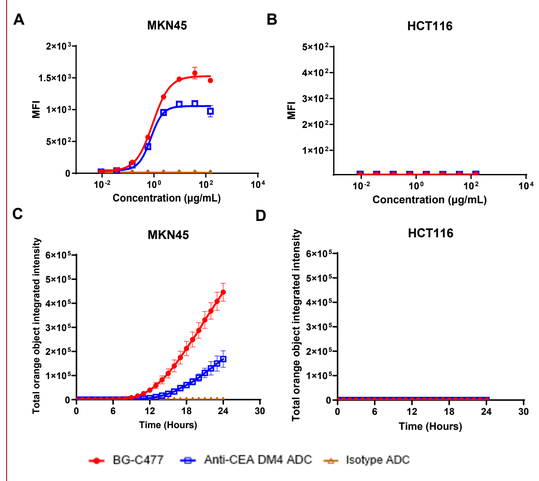


Figure 4. The binding and internalization activity of BG-C477 and reference anti-CEA DM4 ADC were assessed in MKN45 and HCT116 cells. For binding activity assessment, MKN45 **A)** and HCT116 **B)** cells were seeded into 96-well plates and incubated with BG-C477, anti-CEA DM4 ADC or isotype ADC. Fluorescence-labeled anti-human IgG $(Fab')^2$-488 was used to detect the antibody binding activity on the cell surface. For internalization activity evaluation, MKN45 **C)** and HCT116 **D)** cells were seeded into 96-well plates. Labeling reagents were mixed with BG-C477, anti-CEA DM4 ADC, or isotype ADC to allow conjugation. After adding the mixture of labeling reagents and antibodies to the cell plate, cell images were captured over the designated time course..

Conclusion

BG-C477 is a CEA selective targeting ADC carrying Top1i payload, demonstrating strong *in vitro* and *in vivo* antitumor efficacy against CEA positive tumor models

- CEA-dependent binding and potent cytotoxicity against tumor cells.
- The bystander effect of BG-C477 implies that it is also effective in tumors with CEA heterogenous expression.
- BG-C477 showed strong antitumor activity in multiple PDX / CDX models, including GC and CRC models that is resistant to anti-CEA DM4 ADC.
- BG-C477 demonstrated favorable PK and stability, with very low release of free payload in blood.

Potent cytotoxicity against CEA expressing cells

- BG-C477 showed potent and specific killing activity against CEA positive cells

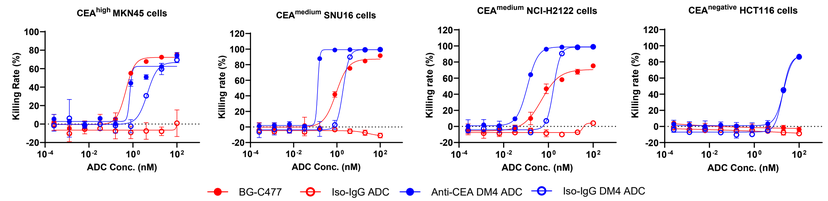


Figure 5. For cytotoxicity activity, cells were dissociated and seeded into 96-well plates and incubated with BG-C477, Iso-IgG ADC, Anti-CEA DM4 ADC, or Iso-IgG DM4 ADC. 6 days post the incubation, viable cells were measured with CellTiter-Glo®.

Bystander effect

- BG-477 showed bystander killing against HCT116-NanoLuc cells when co-cultured with MKN45 cells

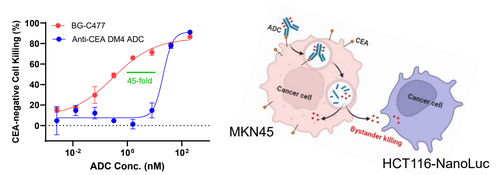


Figure 6. NanoLuc® Luciferase assay for bystander killing. MKN45 (CEA positive) and HCT116-NanoLuc (CEA negative) cells were mixed at a ratio of 1:2 and plated in ultra-low attachment plates. 6 days after the treatment, viability of CEA negative cells was evaluated by measuring the nanoluc luciferase activity.

The in vivo antitumor efficacy and PK in mice

- BG-C477 showed potent antitumor efficacy in 10 PDX and 4 CDX models and better antitumor efficacy than anti-CEA DM4 ADC in GC and CRC PDX models.
- $T_{1/2}$ in Nu/Nu mice: ~ 4 days.

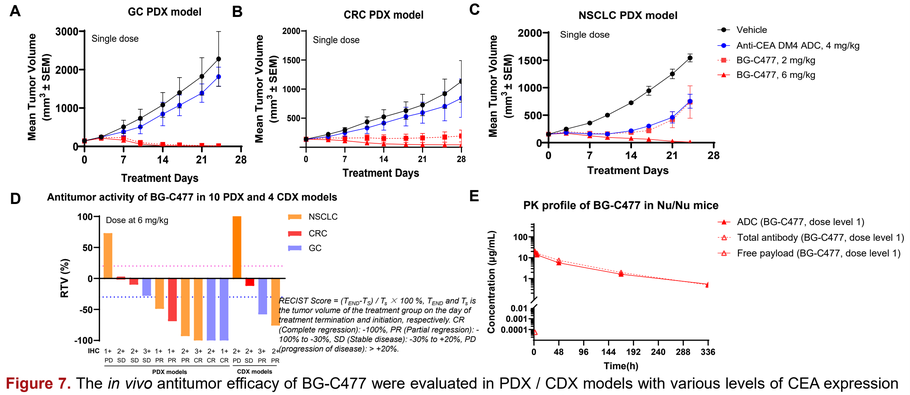


Figure 7. The *in vivo* antitumor efficacy of BG-C477 were evaluated in PDX / CDX models with various levels of CEA expression via i.v. injection **A-D)**. The serum at multiple timepoints was also collected for PK analysis. Total antibody and ADC were measured using ELISA and free payload was measured using LC-MS/MS **E)**. CR, complete regression. PR, partial regression.