Design and Characterization of a TROP2-Targeting Non-Cleavable ADC with Enhanced Antitumor Activity

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Introduction

- Antibody-drug conjugates (ADCs) have emerged as a promising therapeutic strategy that combines the selectivity of monoclonal antibodies with the potent cytotoxicity of payloads, with the goal of improving antitumor efficacy while limiting systemic toxicity.
- Trop2 is an attractive ADC target because it is overexpressed in multiple epithelial malignancies and is associated with aggressive disease and poor prognosis, supporting its therapeutic relevance in solid tumors.
- However, most clinically advanced Trop2-directed ADCs have been built on cleavable-linker/topoisomerase I inhibitor platforms, which, despite their clinical activity, still face challenges related to toxicity, heterogeneous response, and resistance.
- Therefore, a Trop2-targeted ADC incorporating the non-cleavable SMCC linker and the microtubule inhibitor DM1 may offer a differentiated design strategy by enhancing plasma stability and promoting target-dependent intracellular payload release.

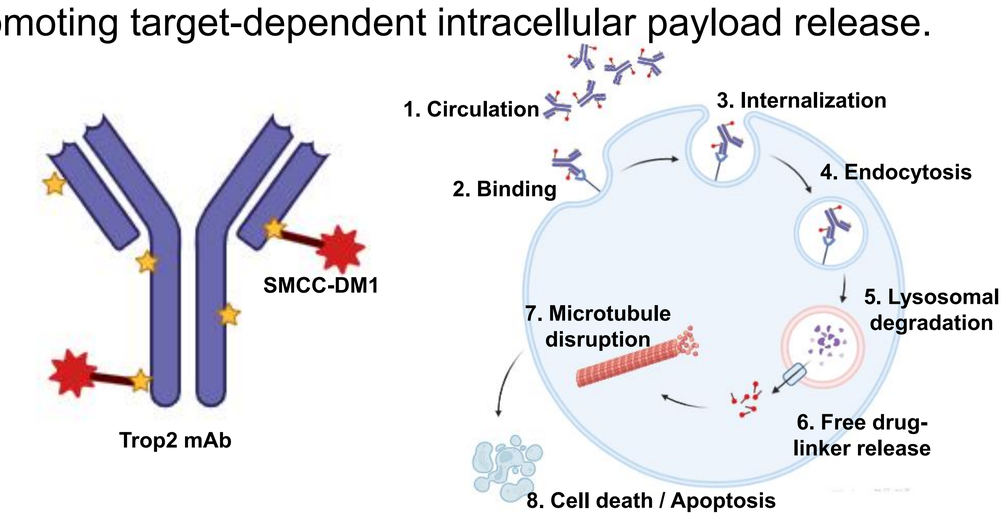


Figure 1. Trop2-Targeted ADC Design and MoA in Trop2-Positive Tumor cell

Aims

- Develop and characterize Trop2-targeting ADC with SMCC-DM1
- Evaluate the therapeutic effects of Trop2-targeting ADC *in vitro*

Results

I. Conjugation of ADC & Optimization of DAR

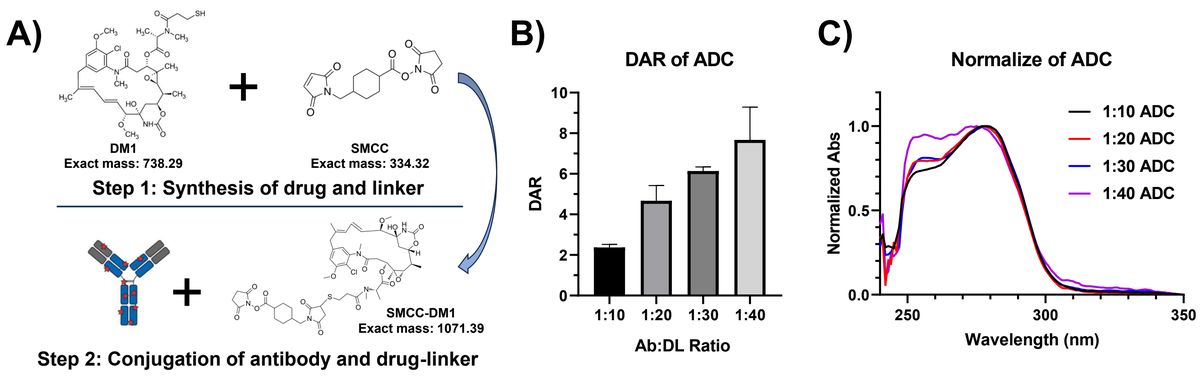


Figure 1. ADC conjugation and DAR optimization
(A) ADC synthesis scheme. Step 1: the thiol group of DM1 reacts with the maleimide group of SMCC via a thiol–maleimide Michael addition, generating the DM1–SMCC drug–linker. Step 2: the NHS ester of DM1–SMCC couples to lysine residues on the antibody to form stable amide bonds, yielding the SMCC–DM1 ADC. (B) DAR profiles obtained at different antibody-to-drug–linker ratios. (C) Absorbance spectra of ADCs normalized to an antibody absorbance of 1.0.

II. Characterization of ADC

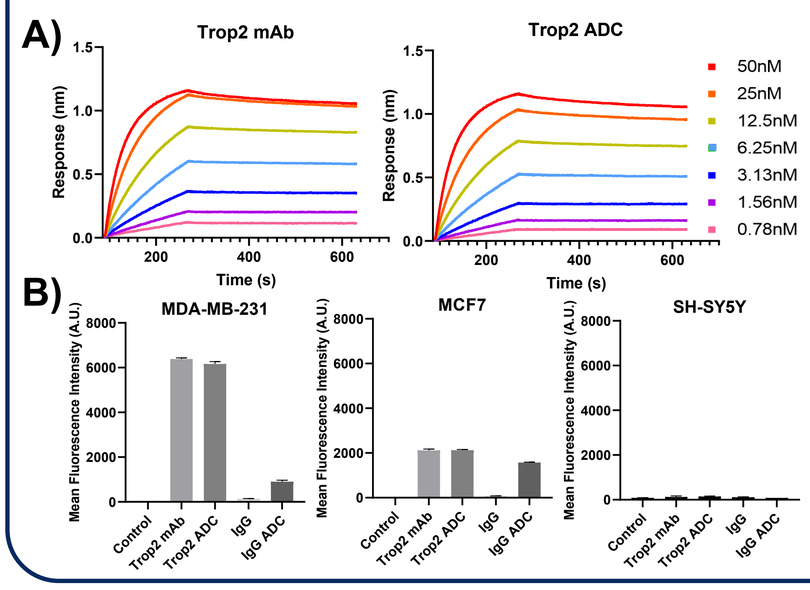


Figure 2. Binding characterization of Trop2-targeted constructs
A) BLI binding kinetics of Trop2 mAb and Trop2 ADC to Trop2 antigen with KD values of 0.40 nM and 0.39 nM, respectively. B) Binding affinity of Trop2 mAb, Trop2 ADC, IgG, and IgG ADC in three cell lines with different levels of Trop2 expression, confirming target-dependent binding of the Trop2-targeted constructs.

III. Internalization of ADC

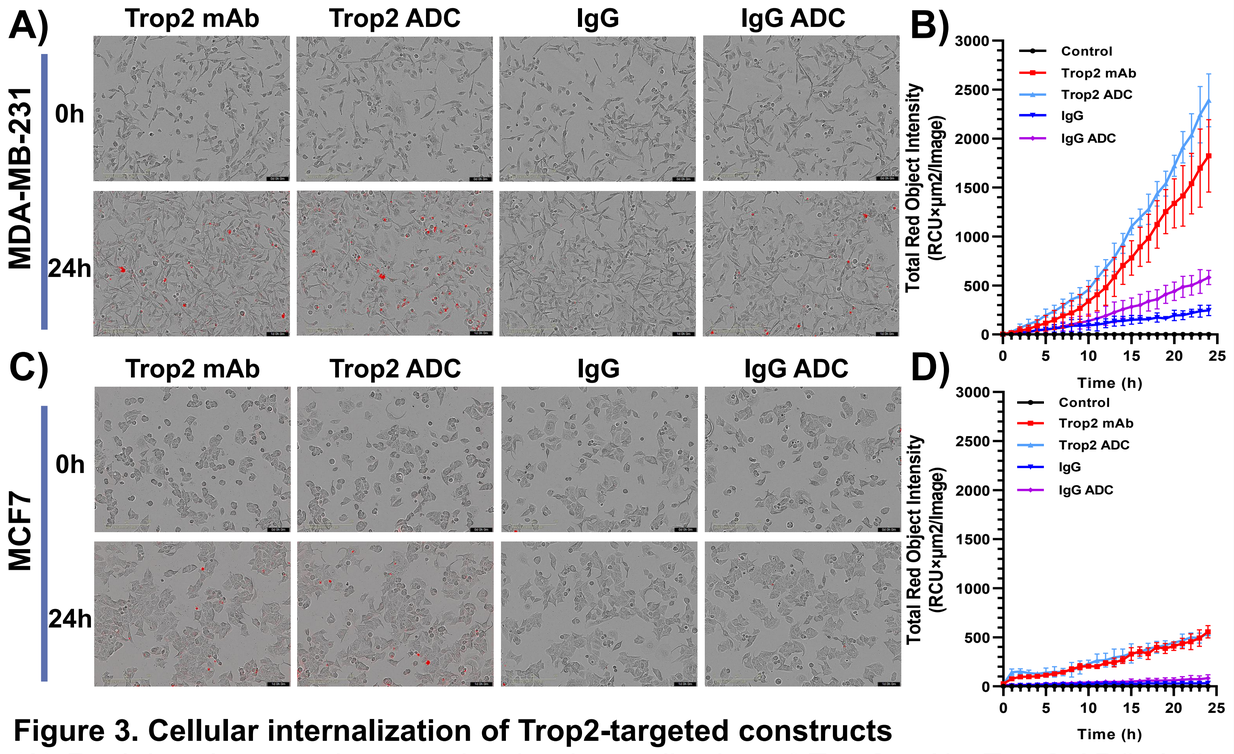
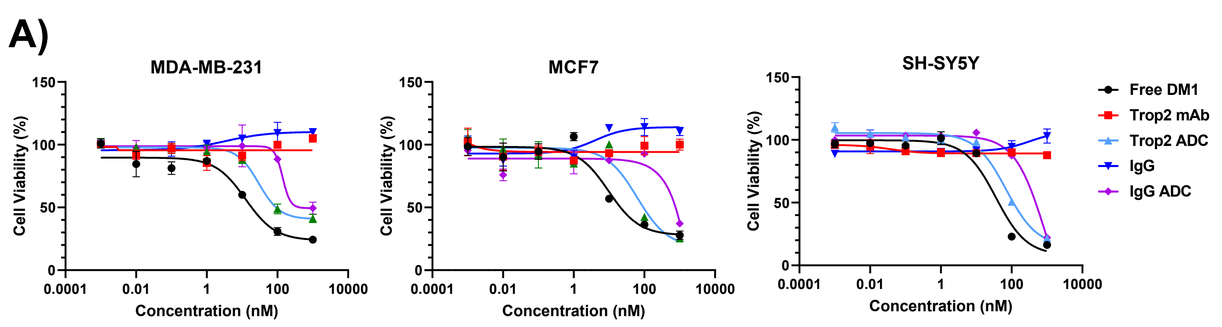


Figure 3. Cellular internalization of Trop2-targeted constructs
(A) Real-time Incucyte images showing internalization of Trop2 mAb, Trop2 ADC, IgG, and IgG ADC in MDA-MB-231 cells. (B) Quantitative analysis of fluorescence intensity over time in MDA-MB-231 cells. (C) Real-time Incucyte images showing internalization of Trop2 mAb, Trop2 ADC, IgG, and IgG ADC in MCF7 cells. (D) Quantitative analysis of fluorescence intensity over time in MCF7 cells. These results demonstrate time-dependent internalization of the Trop2-targeted antibody and ADC in both MDA-MB-231 and MCF7 cells over 1 day.

IV. Cytotoxicity of ADC



IC50 (nM)	Cell line		
	MDA-MB-231	MCF7	SH-SY5Y
Free DM1	12.5	9.2	27.7
Trop2 ADC	29.4	80.8	100.0

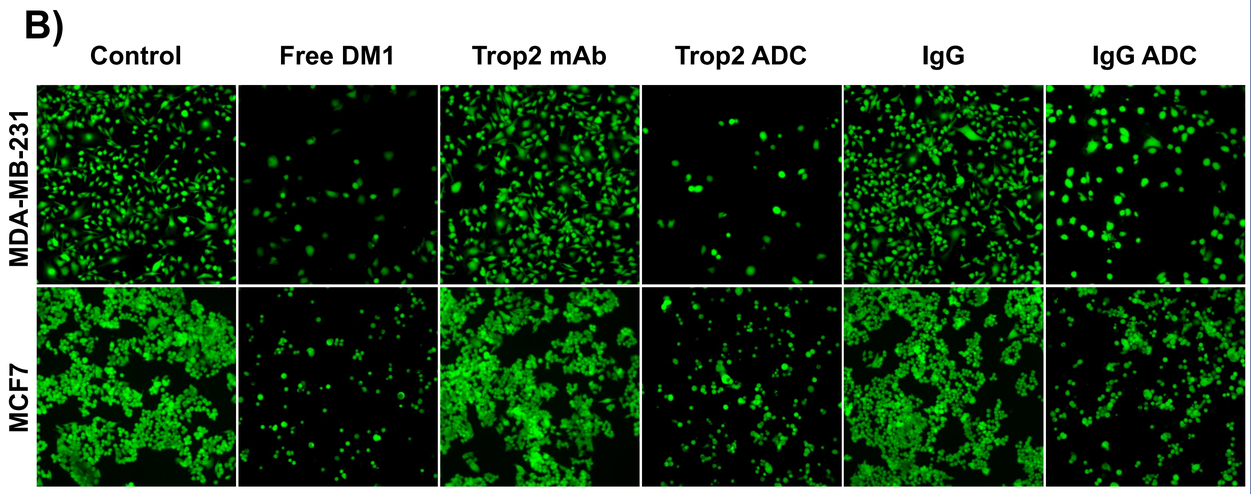


Figure 4. Cytotoxicity studies
(A) CCK-8-based cytotoxicity and IC_{50} value of free DM1, Trop2 mAb, Trop2 ADC, IgG, and IgG ADC in three cell lines (MDA-MB-231, MCF7, SH-SY5Y. (B) Representative cell viability images of MDA-MB-231 and MCF7 cells.

Conclusion

- The Trop2-targeted ADC was successfully synthesized and efficiently internalized into target cells. *In vitro* cytotoxicity assays demonstrated Trop2 expression-dependent cell killing by the ADC, supporting its potential as a Trop2-directed therapeutic candidate.
- As next steps, we will further investigate the mechanism underlying DM1-based cytotoxicity by performing cell-cycle and apoptosis assays. And then, we will evaluate the *in vivo* antitumor efficacy of this ADC in Trop2-positive tumor models, together with biodistribution, pharmacokinetic, and preliminary safety studies.

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