Bystander effects of EGFR-targeting 40H3 antibody-drug conjugates in glioblastoma with heterogeneous EGFR expression

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ABSTRACT

BACKGROUND: An important aspect to determine the efficacy of a targeted macromolecule in treating brain tumors is penetration and activity in tumor across a heterogeneously intact blood-brain barrier.

OBJECTIVE: In this study, novel epidermal growth factor receptor (EGFR)-targeted antibody drug conjugates (ADCs) were constructed with a focus on cytotoxicity in glioblastoma (GBM) with an EGFR heterogeneous cell population.

RESULTS: Initially, the cytotoxicity of free payloads with multiple mechanisms of cell kill was assessed. Across four GBM PDXs with varied EGFR expression, free TS and MMAE were consistently potent with EC_{50} values ranging from 0.50-45.9 pM and 7.9-229.1 pM, respectively. In contrast, the other free toxins were less potent; EC_{50} values: Dxd, 0.21-13.1 nM; SN38, 0.87-5.6 nM; DM1, 2.1-19.9 nM. Based on these results, the 40H3 EGFR-specific IgG was used to construct ADCs with TS and MMAE. 40H3-TS had a drug:antibody ratio (DAR) of 2.5 and was potently cytotoxic in GBM6, GBM39 and GBM108, while minimal cytotoxicity was observed in GBM10 or normal astrocyte SVG-A cells. 40H3-MMAE had a DAR of 3 and was similarly effective in GBM6 and GBM39 but less potent in GBM108, GBM10, and SVG-A. Bystander cytotoxicity was evaluated in U87 cells expressing eGFP/fLuc2 (U87eGFP/fluc2) or EGFRviii (U87EGFRviii). Using live-cell imaging, U87EGFRviii cells treated with 40H3-TS or 40H3-MMAE had significantly reduced cell confluence relative to control. The same drug treatments in U87eGFP/fLuc2 cells had no effect on growth/confluence. However, in a 1:1 mixed culture, overall confluence was reduced from 89% in control to 35% and 32% after 40H3-TS (p<0.0001) or 40H3-MMAE (p<0.0001) treatment, respectively. Bystander killing of U87eGFP/fLuc2 cells was indicated by a reduction in confluence of green-fluorescent cells from 50% in control versus 29% with 40H3-TS (p<0.0001) and 26% with 40H3-MMAE (p<0.0001) treatment. In GBM39 orthotopic tumors, a single infusion of 10 or 20 µg 40H3-TS via convection enhanced delivery (CED) reduced the bioluminescence signal 7 days post treatment by ~10-fold (p=0.03) as compared to 40H3 control. However, 50% and 80% mortality was observed within a week of infusing 10 and 20 µg 40H3-TS, respectively. Neurotoxicity was associated with neuron loss in treated hemisphere as determined by NeuN staining.

CONCLUSIONS: In summary, these data highlight the potential for novel EGFR-targeted ADCs to provide potent direct and bystander cytotoxicity to GBM cells. However, further selection and optimization of the conjugated toxins will be required to balance potency and bystander killing with toxicity for EGFR-targeted ADCs.

FREE TOXIN CYTOTOXICITY

A

GBM#	EGFR status	TMA stain
6	Amplified, viii	
39	Amplified, viii	
108	Amplified, viii	
10	Not amplified, wt	

B

Payload	Toxin target
Tesirine (TS, SG-3199)	DNA minor groove
Monomethyl auristatin E (MMAE)	Microtubules
Mertansine (DM1)	Microtubules
Exatecan derivative (Dxd1)	Topoisomerase I
SN-38	Topoisomerase I

C

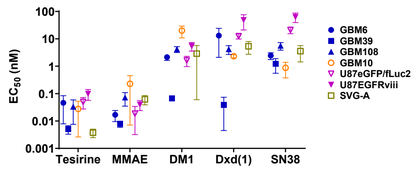

Figure 1. A – EGFR expression in four GBM PDX lines. **B –** Table listing details of 5 toxin payloads with their targets. **C –** *In vitro* potency of toxins (EC_{50}) determined by CellTiterGlo assay in GBM PDX cells, established glioma line (U87) and normal human astrocyte cells (SVG-A).

40H3-ADC CYTOTOXICITY

A

ADCs	Drug to antibody ratio (DAR)	Linker	Cleavage mechanism	Bystander effect
40H3-TS	2.5	MC-Val-Ala	Cathepsin B	Yes
40H3-MMAE	3.0	MC-Val-Cit-PAB	Cathepsin B	Yes

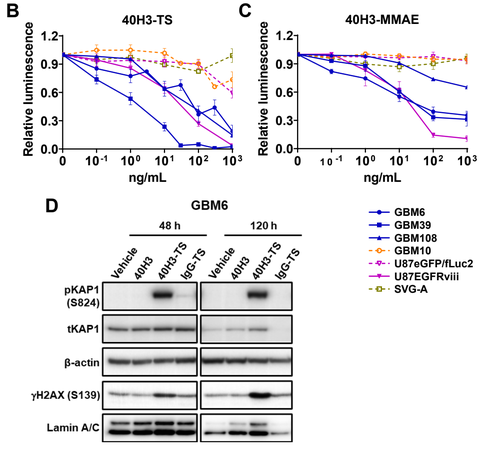

Figure 2. A – Characteristics of the tested ADCs. **B,C –** *In vitro* cytotoxicity of 40H3 antibody conjugated with either tesirine (40H3-TS, B) or MMAE (40H3-MMAE, C) in GBM PDXs, U87 and SVG-A cells. **D –** Expression of DNA damage signaling proteins in the lysates of GBM6 cells treated with 100 ng/mL 40H3, IgG-TS or 40H3-TS for 48 h and 120 h.

BYSTANDER KILLING

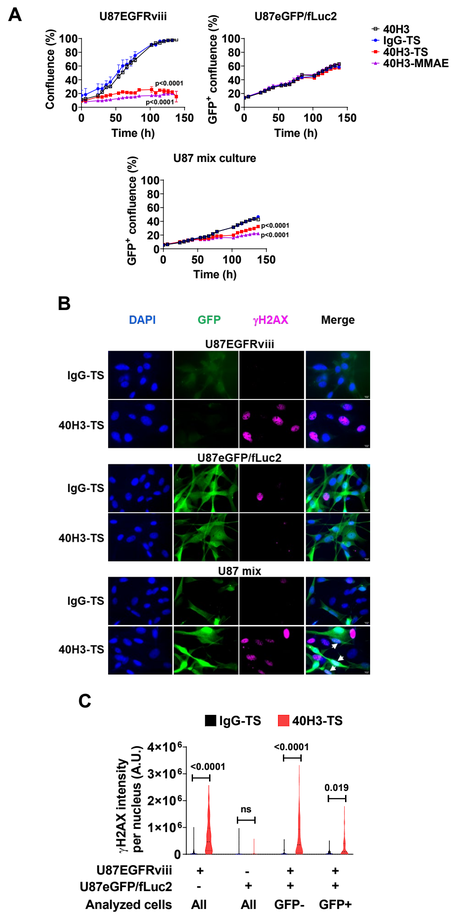

Figure 3. A – Bystander cytotoxicity of 100 ng/mL 40H3, IgG-TS, 40H3-TS or 40H3-MMAE was measured as percent confluence using white light or green fluorescence live-cell imaging. Data is representative of three independent experiments and *p*-values are determined by Student's *t*-test at 132 h post treatment. **B –** γH2AX staining of U87 mix cultures (1:1) after 48 h treatment with 100 ng/mL of either IgG-TS or 40H3-TS determined by immunofluorescence microscopy. White arrows represent bystander DNA damage in non-targeted cells. **C –** Intensity of γH2AX per nucleus of green fluorescent (U87eGFP/fLuc2) and non-fluorescent (U87EGFRviii) cells are calculated using ImageJ software. *p*-values are calculated using Mann-Whitney test.

BYSTANDER EFFECTS IN NORMAL ASTROCYTE CELLS

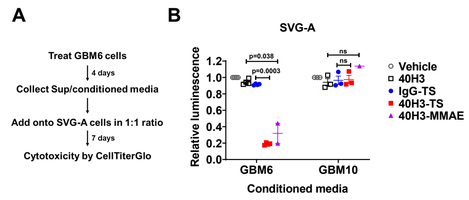

Figure 4. A – Schematic of the experimental design. **B --** Conditioned media collected from GBM6 or GBM10 cells treated with 100 ng/mL of the indicated drugs were added 1:1 to existing media of SVG-A cultures and cytotoxicity assessed seven days later by CellTiterGlo. Data represents mean±SEM from repeated independent studies and each dot is data from single experiment. *p*-values are determined by Student's t-test.

IN VIVO EFFICACY & TOXICITY

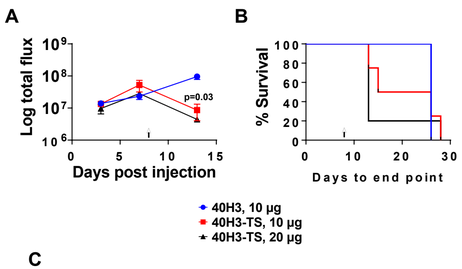

Figure 5. A,B – Intracranial tumor growth of GBM39-eGFP/fLuc2 orthotopic tumors, as measured by bioluminescent imaging (BLI, A) and Kaplan-Meier survival (B) for tumor bearing mice treated with a single CED infusion (20 µL) of 40H3 or escalating doses of 40H3-TS. Arrow indicates time of dosing. **C –** Brain sections from mice (n=4) treated with 20 µg 40H3-TS stained with H&E and NeuN staining. Arrow indicates brain hemisphere which received tumor injection and CED infusion.

SUMMARY & CONCLUSIONS

- Tesirine and MMAE are found to be most potent payloads to make antibody-drug conjugates to target GBM.
- Tesirine and MMAE conjugated ADCs have potential of bystander killing of neighboring cells with low or no EGFR expression thus expected to have better efficacy.
- Further investigation is warranted to develop novel EGFR-targeting ADCs with toxins which have balanced bystander potential and low toxicity.

FUTURE DIRECTIONS

- *In vivo* efficacy studies with 40H3-TS will be expanded to other GBM PDX lines and dose optimization will be done.
- 40H3-MMAE will be tested *in vivo* in multiple PDX models.
- Pharmacodynamics and pharmacokinetics will be analyzed in intracranial PDX model after treatment with 40H3-TS and/or 40H3-MMAE.

REFERENCES

1. Ho *et al*. Antibody drug conjugates, targeting cancer-expressed EGFR, exhibit potent and specific antitumor activity. 2023. PMID: 36459711
2. Marin *et al*. Heterogeneous delivery across the blood-brain barrier limits the efficacy of an EGFR-targeting antibody drug conjugate in glioblastoma. Neuro-Oncology. 2021. PMID: 34050676
3. Ho *et al*. Characterization of monoclonal antibodies generated to the 287–302 amino acid loop of the human epidermal growth factor receptor. Antibody Therapeutics. 2019. PMID: 31934685

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