**ABSTRACT**

**BACKGROUND:** Key aspects that determine the efficacy of a targeted macromolecule in treating brain tumors are: 1) penetration across a heterogeneously intact BBB, and 2) activity in (or most of) the tumor cells encountered.

**OBJECTIVE:** Epidermal growth factor receptor (EGFR)-amplified glioblastoma (GBM) are highly plastic, heterogeneous brain tumors, and in this study, novel EGFR-targeted antibody drug conjugates (ADCs) were constructed with a focus on cytotoxicity in GBM with an EGFR heterogeneous cell population.

**RESULTS:** Initially, the cytotoxicity of free payloads (no antibody) with multiple mechanisms of cell kill was assessed. Across four GBM PDXs that vary in expression of EGFR, free TS and MMAE were consistently potent with EC_{50} values ranging from 0.5 to 4.9 nM and 7.9–21.1 nM, respectively. In contrast, the other free toxins were less potent: EC_{50} values: Dtx, 0.21–13.1 nM; SN38, 0.87–5.6 nM; D1M, 2.1–19.9 nM. Based on these initial results, the 40H3 EGFR-specific IgG was used to construct ADCs with TS and MMAE. 40H3-TS was potently cytotoxic in GBM6, GBM39 and GBM108, while minimal cytotoxicity was observed in GBM10 or normal astrocyte SVG-A cells. 40H3-MMAE 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-Luc2 (U87/eGFP-Luc2) and in U87 cells expressing EGFRviii (U87/EGFRviii). Using live-cell imaging, U87/eGFP-Luc2 cells treated with 40H3-TS or 40H3-MMAE had significantly reduced cell confluence relative to control. The same treatment in U87/EGFRviii cells had no effect on growth/confluence. However, in a 1:1 mixed cell 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. This bystander killing of U87/eGFP-Luc2 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. Extending to in vivo studies using GBM39 orthotopic tumors, a single infusion of 10 or 20 ug 40H3-TS via convection enhanced delivery (CED) reduced the bioluminescence signal 7 days post treatment by approximately 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.

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**REFERENCES**


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