**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 EC50 values ranging from 0.50-45.9 pM and 7.9-229.1 pM, respectively. In contrast, the other free toxins were less potent; EC50 values: Oxad, 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 SVGA cells. 40H3-MMAE had a DAR of 3 and was similarly effective in GBM108, GBM39 and GBM10 but less potent in GBM9, GBM10, and SVGA-A. Bystander cytotoxicity was evaluated in U87 cells expressing eGFP/FvIIc (U87egFvIIc) or eGFP/FvII (U87egFvII). Using two-cell imaging, U87/FvIIc cells treated with 40H3-TS or 40H3-MMAE had significantly reduced cell confluence relative to control. The same drug treatments in U87/FvIIc/FvIIc or FvIIc/FvIIc indicated a reduction in confluence of green-fluorescent cells from 50% in control across 10-20% at 48-96 h post treatment, respectively. Bystander killing of U87/FvIIc/FvIIc cells was indicated by a reduction in confluence of green-fluorescent cells from 50% in control versus 20% with 40H3-TS (p=0.001) and 26% with 40H3-MMAE (p=0.001) treatment. In GBM93 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 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-targeting ADCs to provide potent and direct cytotoxicity to GBM cell populations. Further selection and optimization of the conjugated toxins will be required to balance potency and bystander killing with toxicity for tumor-targeted ADCs.

**SUMMARY & CONCLUSIONS**

- Tesirine and MMAE conjugated ADCs have potential for 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**


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