Convection enhanced delivery of EGFR-targeting antibody drug conjugates Serclutamab talirine and Depatux-M in glioblastoma patient derived xenografts

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ABSTRACT

BACKGROUND

EGFR targeted antibody-drug conjugates (ADCs) show promise as a novel treatment in a subset of glioblastoma (GBM). Two EGFR targeting ADCs include first generation Depatux-M, with an antimitotic toxin monomethyl auristatin F (MMAF), and Serclutamab talirine (Ser-T), with a DNA crosslinking agent pyrrolobenzodiazepine dimer (PBD) toxin. Due to their large molecular weight, poor drug distribution across the blood-brain barrier significantly limits the efficacy in EGFR-amplified GBM. We studied whether convection enhanced delivery (CED) can be used to safely infuse these two EGFR-targeted ADCs in patient-derived xenograft (PDX) models of EGFR-amplified GBM.

METHODS

The efficacy of Depatux-M and Ser-T was evaluated *in vitro* and *in vivo* in two EGFRvIII-amplified PDXs (GBM6 and GBM108). Immunofluorescence staining was used to evaluate drug distribution along with pharmacodynamics of the ADCs. CED was performed by stereotactic placement of an infusion catheter within the orthotopically implanted xenograft. Immunohistochemistry was used to explore mechanisms of normal cell toxicity.

RESULTS

Despite potent activity and impressive bystander killing *in vitro*, systemic administration of either ADC conferred minimal extension in survival for either GBM6 or GBM108. In contrast, CED significantly enhanced ADC delivery to tumor and peri-tumoral regions and extended survival. Dose-finding studies in orthotopic GBM6 identified 2 µg Ser-T and 60 µg Depatux-M as safe and effective associated with extended survival prolongation (>300 days and 95 days, respectively). Four Ser-T infusions every 21 days controlled tumor growth but was associated with lethal toxicity approximately 7 days after the final infusion. Limiting dosing to two infusions in GBM108 provided profound median survival extension of over 200 days. In contrast, four Depatux-M CED infusions were well tolerated and significantly extended survival in both GBM6 (158 days) and GBM108 (310 days). In a toxicity analysis, Ser-T resulted in a profound loss in NeuN+ cells and markedly elevated GFAP and γH2AX staining, while Depatux-M was associated only with modest elevation in GFAP staining. Geographic distribution of free Cys-mcMMAF following dosing of MMAF-containing ADCs was below level of detection in all mice but CED treated with Depatux-M.

CONCLUSIONS

Depatux-M is well tolerated when infused into normal brain and results in extended survival in orthotopic GBM PDXs. In contrast, Ser-T, with a distinct PBD toxin, had a much narrower therapeutic window when delivered by CED.

IN VITRO SENSITIVITY

A.

PDX line	EGFR status	Ser-T EC_{50} (ng/mL)	Depatux-M EC_{50} (ng/mL)
GBM6	Amp, vIII	0.3	0.2
GBM39	Amp, vIII	0.007	50
GBM108	Amp, vIII	2	10
GBM12	Amp, G718A	2	6,250
GBM10	Non amp, wt	1,900	12,910
GBM43	Non amp, wt	90	3,080

B.

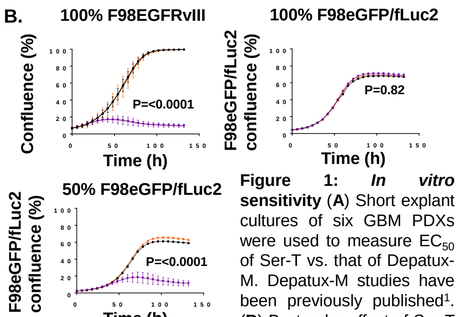

Figure 1: *In vitro* sensitivity (**A**) Short explant cultures of six GBM PDXs were used to measure EC_{50} of Ser-T vs. that of Depatux-M. Depatux-M studies have been previously published[1]. (**B**) Bystander effect of Ser-T on co-cultures of F98EGFRvIII and F98-eGFP/fLuc2 treated with 50ng/mL Ser-T. (**C**) Conditioned media from GBM6 cells treated with 100 ng/ml of the indicated drugs were collected and added 1:1 to existing media of SVG-A cultures. Cytotoxicity was calculated after 7 days.

SYSTEMIC DOSING EFFICACY

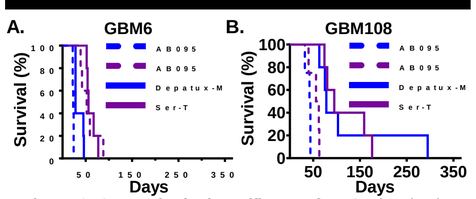

Figure 2: Systemic dosing efficacy of ADCs (**A-B**) Mice with orthotopic GBM6 (A) or GBM108 (B) treated systemically with AB095 (Isotype control; 5mg/kg in Depatux-M study, 0.1 mg/kg in Ser-T study), Depatux-M (5 mg/kg), or Ser-T (0.1 mg/kg).

CED

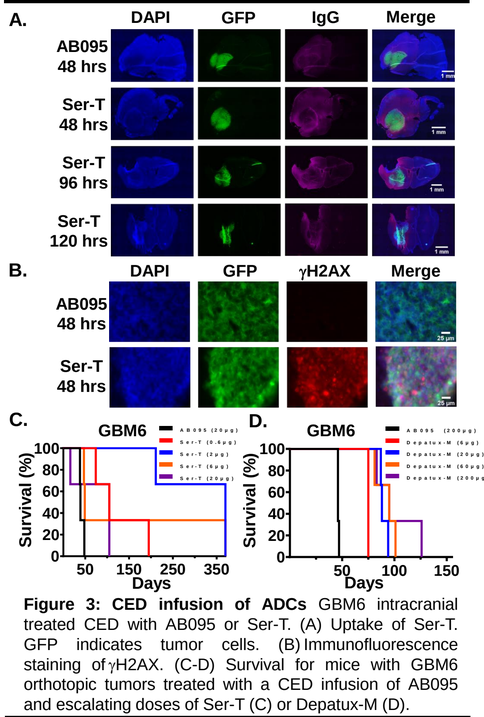

Figure 3: CED infusion of ADCs GBM6 intracranial treated CED with AB095 or Ser-T. (A) Uptake of Ser-T. GFP indicates tumor cells. (B) Immunofluorescence staining of γH2AX. (C-D) Survival for mice with GBM6 orthotopic tumors treated with a CED infusion of AB095 and escalating doses of Ser-T (C) or Depatux-M (D).

SER-T CYCLIC CED

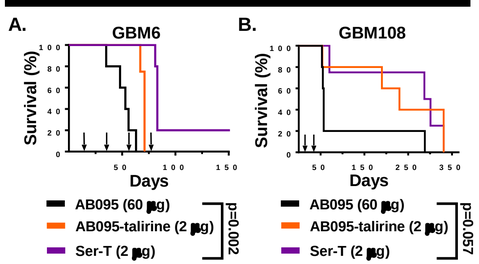

Figure 4: Cyclic CED (**A-B**) Kaplan-Meier graph of four cycles of CED infusion 21 days apart in GBM6 (A) or GBM108 (B). Arrows indicate dosing. GBM108 AB095-talirine and Ser-T only received two cycles of treatment.

DEPATUX-M CYCLIC CED

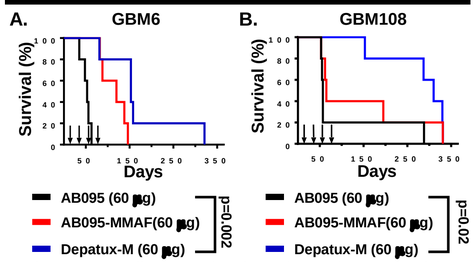

Figure 5: Cyclic CED. (**A-B**) Kaplan-Meier graph of four cycles of CED infusion 21 days apart in GBM6 (A) or GBM108 (B). Arrows indicate dosing.

NORMAL BRAIN TOXICITY

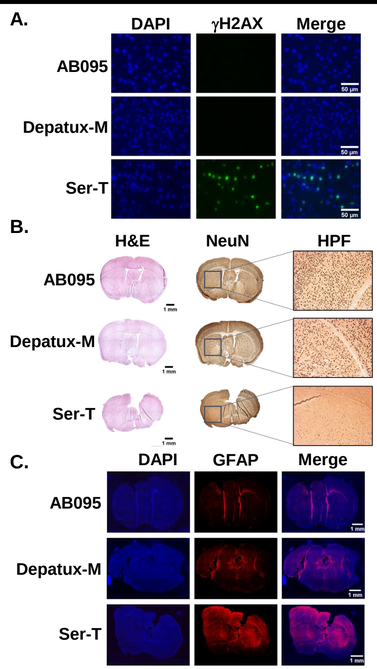

Figure 6: CED in Non-tumor bearing mice (**A-C**) γH2AX (A), NeuN (B), and GFAP (C) non-tumor bearing mice treated with CED of AB095, Depatux-M, or Ser-T.

DEPATUX-M DISTRIBUTION

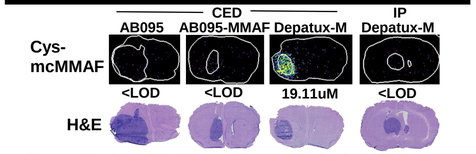

Figure 7: MALDI. Mice with established GBM6 tumors were dosed as indicated AB095 (60 µg CED), AB095-MMAF (60 µg CED) and Depatux-M (5 mg/kg IP or 60 µg CED) and were processed 48 hr later for H&E staining and MALDI-MSI. Ion images reflect the spatial distribution of the Cys-mcMMAF fragment used for quantitation. <LOD is below limit of detection.

DISCUSSION

- Circumventing the BBB through CED increases delivery and efficacy of brain impenetrable drugs like Depatux-M and Ser-T.
- Talirine has non-specific cytotoxic effects against both proliferating and quiescent cells which may contribute to the significant toxicity[2].
- Toxicity seen with AB095-talirine suggest linker cleavage in the brain microenvironment by hydrolytic enzymes, such as carboxylesterase 1[3].
- Non-specific activity of AB095-MMAF likely reflects non-specific endocytosis or IgG trafficking via Fc-γ receptors[4].

CONCLUSIONS

- CED infusion of ADCs provides robust and sustained distribution throughout the tumor and surrounding normal brain tissue.
- Depatux-M has much wider therapeutic window which supports further pre-clinical and possible clinical development of CED infusion.

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