EGFRvIII-Targeted Alpha Therapy Shows Significant Therapeutic Efficacy as Both a Single-Agent and in Combination with Standard of Care Against Preclinical GBM Models

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AACR Poster # **LB313**

Background

Targeted alpha therapy (TAT) is a rapidly advancing class of radiotherapeutics that can effectively deliver potent and local radiation to cancer cells while sparing the surrounding normal cells. TATs hold great promise for treatment-resistant tumors such as glioblastoma multiforme (GBM) due to the extensive DNA damage and cell death induced by alpha particles. GBM is an aggressive and lethal primary adult brain tumor that is highly resistant to external beam radiation and chemotherapy.

Herein, we present the preclinical evaluation of a novel TAT for treatment of GBM targeting the most common tumor-specific mutant, epidermal growth factor receptor variant 3 (EGFRvIII). Our EGFRvIII TAT consists of a humanized EGFRvIII monoclonal antibody (IgG4 isotype), a proprietary bifunctional chelate, and the alpha-emitting radionuclide, actinium-225 [^{225}Ac]. *In vivo* biodistribution and efficacy of our EGFRvIII TAT was evaluated in two aggressive orthotopic EGFRvIII-expressing GBM patient-derived xenograft models (PDXs; G06 and G39) with varying degrees of blood-brain tumor barrier (BBTB) permeability.

EGFRvIII-Expressing PDXs Have Varying Degrees of Blood-Brain Tumor Barrier (BBTB) Permeability

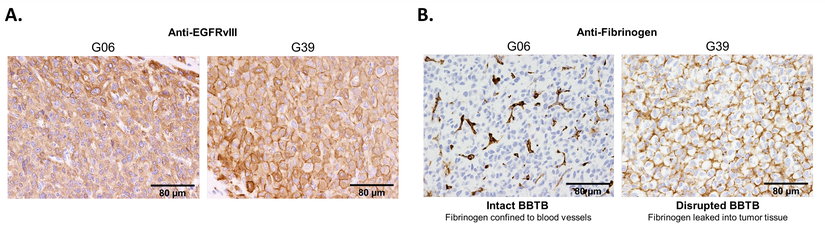


Figure 1. Immunohistochemical staining for EGFRvIII *(A)* and mouse fibrinogen *(B)* in G06 and G39 PDXs.

[^{111}In]-anti-EGFRvIII Shows High Tumor Uptake in a PDX Model with a Leaky BBTB

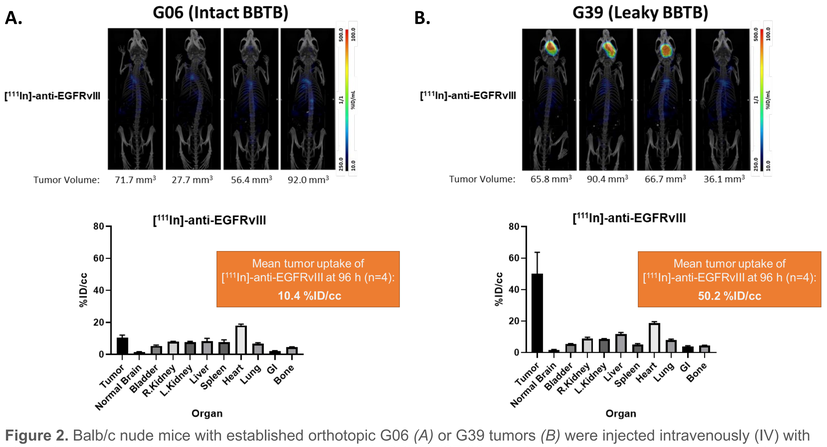
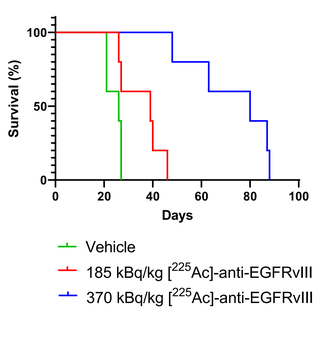
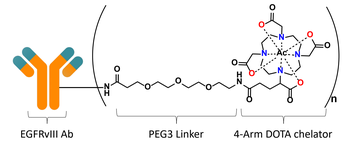


Figure 2. Balb/c nude mice with established orthotopic G06 *(A)* or G39 tumors *(B)* were injected intravenously (IV) with 185 MBq/kg [^{111}In]-anti-EGFRvIII. Mice were imaged via SPECT/CT 96 h post-injection. Tumor volumes were determined by volumetric analysis of 3D bioluminescence tomography images (*images not shown*).

Single-Dose [^{225}Ac]-anti-EGFRvIII Significantly Extends Survival in an Orthotopic GBM PDX Model with an Intact BBTB



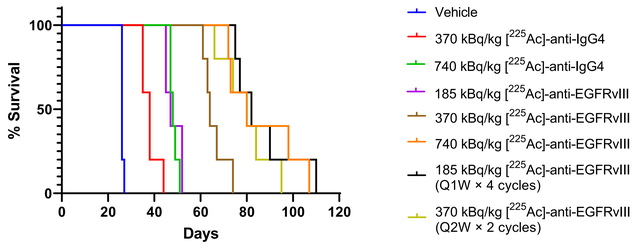


Treatment group	n	Median survival (days)	Survival Benefit	p-value (vs. vehicle)
Vehicle	5	26	1.0	
185 kBq/kg [^{225}Ac]-anti-EGFRvIII	5	39	1.5	0.04
370 kBq/kg [^{225}Ac]-anti-EGFRvIII	5	80	3.1	0.003

Single-dose 370 kBq/kg [^{225}Ac]-anti-EGFRvIII results in a 3.1-fold increase in survival compared to vehicle control

Figure 3. Balb/c nude mice with established G06 orthotopic PDX tumors were treated with a single IV dose of vehicle,185 kBq/kg [^{225}Ac]-anti-EGFRvIII, or 370 kBq/kg [^{225}Ac]-anti-EGFRvIII. The log rank test was used to statistically compare the survival between groups and determine p-values. The structure of [^{225}Ac]-anti-EGFRvIII is shown top right, where *n* represents the chelate to antibody ratio (CAR).

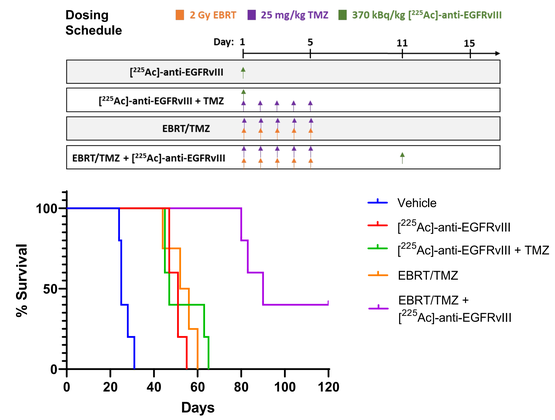
Fractionated Dosing of [^{225}Ac]-anti-EGFRvIII has Equivalent Therapeutic Benefit to Single High-Dose



Treatment group	n	Median survival (days)	Survival Benefit	p-value (vs. vehicle)
Vehicle	5	26	1.0	
370 kBq/kg [^{225}Ac]-anti-IgG4	5	38	1.5	0.002
740 kBq/kg [^{225}Ac]-anti-IgG4	5	48	1.9	0.002
185 kBq/kg [^{225}Ac]-anti-EGFRvIII	5	47	1.8	0.002
370 kBq/kg [^{225}Ac]-anti-EGFRvIII	5	64	2.5	0.002
740 kBq/kg [^{225}Ac]-anti-EGFRvIII	5	80	3.1	0.002
185 kBq/kg [^{225}Ac]-anti-EGFRvIII (Q1W × 4 cycles)	5	82	3.2	0.002
370 kBq/kg [^{225}Ac]-anti-EGFRvIII (Q2W × 2 cycles)	5	80	3.1	0.002

Figure 4. Mice bearing orthotopic G39 tumors were administered [^{225}Ac]-anti-EGFRvIII at a single IV dose of 185 kBq/kg, 370 kBq/kg, or 740 kBq/kg, or a total cumulative radiochemical dose of 740 kBq/kg fractionated as follows: 185 kBq/kg dosed once weekly for 4 cycles (Q1W × 4 cycles) or 370 kBq/kg dosed every 2 weeks for 2 cycles (Q2W × 2 cycles). Control groups were administered a single IV dose of either 370 kBq/kg or 740 kBq/kg of [^{225}Ac]-anti-IgG4. The log rank test was used to statistically compare the survival between groups and determine p-values.

Combined Treatment of [^{225}Ac]-anti-EGFRvIII + EBRT/TMZ Shows Improved Efficacy vs. EBRT/TMZ Alone



Treatment group	n	Median survival (days)	Survival Benefit	p-value (vs. vehicle)
Vehicle	5	25	1.0	
[^{225}Ac]-anti-EGFRvIII	5	51	2.0	0.002
[^{225}Ac]-anti-EGFRvIII + TMZ	5	47	1.9	0.002
EBRT/TMZ	5	52	2.1	0.005
EBRT/TMZ + [^{225}Ac]-anti-EGFRvIII	5	90	3.6	0.002

Figure 5. Mice bearing orthotopic G06 tumors were administered a single IV dose of vehicle, 370 kBq/kg [^{225}Ac]-anti-EGFRvIII, or 370 kBq/kg [^{225}Ac]-anti-EGFRvIII in combination with 25 mg/kg temozolomide (TMZ) administered orally once daily for 5 days. External beam radiation therapy (EBRT) was delivered as 2 Gy once daily for 5 consecutive days (2 Gy × 5) in combination with 25 mg/kg TMZ (orally, once daily for 5 days). The combination group (EBRT/TMZ + [^{225}Ac]-anti-EGFRvIII) received a single IV dose of 370 kBq/kg [^{225}Ac]-anti-EGFRvIII 6 days after completion of EBRT/TMZ treatments. The log rank test was used to statistically compare the survival between groups and determine p-values.

Summary and Conclusions

- [^{225}Ac]-anti-EGFRvIII shows high tumor uptake in GBM models with leaky vasculature
- Single-dose IV administration of 370 kBq/kg [^{225}Ac]-anti-EGFRvIII demonstrates good efficacy in GBM PDX models with both leaky and intact BBTB, suggesting that even low tumor uptake has potent anti-tumor effects
- Fractionated dosing of [^{225}Ac]-anti-EGFRvIII showed equivalent therapeutic benefit to single high-dose administration. A fractionated dosing schedule may help to minimize toxicity while maximizing the therapeutic benefit in the clinic
- [^{225}Ac]-anti-EGFRvIII in combination with EBRT/TMZ was well-tolerated in mice and offered a significant survival benefit in comparison to EBRT/TMZ or [^{225}Ac]-anti-EGFRvIII alone. This suggests it may be safe to combine our EGFRvIII TAT with standard of care (SoC) in the clinic with the potential for a significant additive therapeutic benefit
- Overall, our data suggests that EGFRvIII-targeted alpha therapy (TAT) has potent anti-tumor effects and may be a promising new approach for the treatment of EGFRvIII-expressing GBM tumors