EGFR Inhibition in Glioblastoma Patient-derived Xenograft Models

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Background

The epidermal growth factor receptor (EGFR), as well as its other close family members Her2/neu, HER3, and HER4, are important drivers of a variety of cellular processes including cell proliferation, differentiation, and migration. Deregulation of this receptor and its downstream signaling cascades are associated with a poor prognosis in a variety of cancers including Glioblastoma (GBM). GBM is a highly aggressive and deadly brain malignancy with a median survival after initial diagnosis of 14 months. EGFR overexpression is thought to occur in 40-60% of GBM cases and, therefore, has been considered as a promising therapeutic target. The current standard of care for GBM includes tumor resection followed by a regimen of Temozolomide (TMZ), radiation therapy (RT) and possibly concurrent or adjuvant chemotherapeutics. EGFR targeting has been used in the treatment of GBM in clinical trials but with little success. In the pursuit of understanding how EGFR targeting can be better utilized, we took advantage of the Mayo GBM Patient-derived xenograft models to assess a variety of traditional and novel small-molecule EGFR inhibitors *in vitro* and *in vivo*.

Methods

Copy Number Analysis: Determination of copy number was performed by a neuropathologist using whole exome sequencing data obtained in the Medical Genome Facility at Mayo Clinic- Rochester. DNA for sequencing was isolated from early passage, frozen or flank PDX tumor tissue. Lineage was validated against human germline or tumor, or early passage PDX flank tumor by Short Tandem Repeat (STR) analysis.

Pharmacokinetics: Male and female FVB WT and Mdr1a/b$^{-/-}$Bcrp1$^{-/-}$ mice (Taconic Biosciences, Inc., Germantown, NY) at the age of 8-14 weeks were used for pharmacokinetic (PK) studies. The dosing suspensions for subcutaneous injection were prepared in 10% DMSO and 0.25% hydroxypropyl methylcellulose (w/v) in order to achieve a dose of 1 mg/kg for each EGFR inhibitor. A single dose of each EGFR inhibitor was individually dosed in wild-type and triple knockout (*Mdr1a/b$^{-/-}$Bcrp1$^{-/-}$*) FVB mice. Blood and brain samples from mice were harvested at 1-hour and 8-hour after discrete drug administration (*N*=3-4). Concentrations of the 8 EGFR inhibitors in specimens were measured by reverse-phase liquid chromatography coupled with triple quadruple mass spectrometer (LC-MS/MS). Compounds were extracted by using 5-time volumes of ethyl acetate.

In vitro Viability Assays: *Ex vivo* cultures from GBM PDX flank tumors were grown on laminin-coated flasks in StemPro Neural Stem Cell media. Cells were plated into non-coated 96-well tissue culture plates at 500 (G12) or 2000 (G6, G10, G39) cells per well and treated the following day with individual therapeutics. Cell viability readings were obtained at Day 7 or 14 using the CellTiter GLO 3D Viability Assay. Neurosphere formation under the same conditions was assessed between Day 14 and 21 (G6, G12, G39 only).

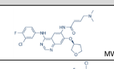
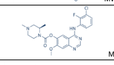
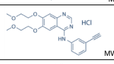
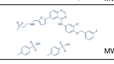
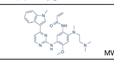
In vivo Efficacy: Cells were grown under the same stem cell conditions as above and once established, were injected intracranially into nude, immunocompromised mice at a concentration of 100,000 (GBM12) or 300,000 (GBM39) cells per mouse. Treatment was initiated on Day 7 (GBM12) or Day 14 or 15 (GBM39) and mice were followed until moribund.

EGFR Status- Mayo GBM PDX

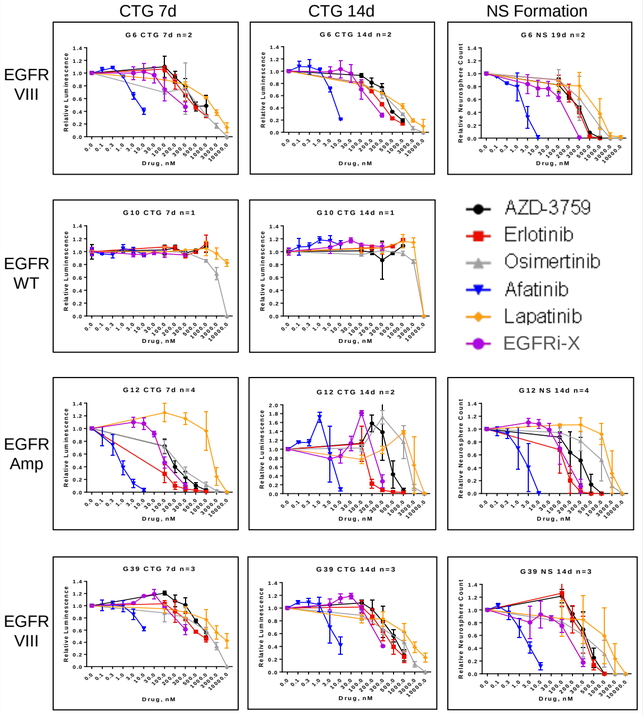
PDX #	Copy Number	Copy Number Call	EGFRvIII	Other EGFR
3	>5	Amplified	N	
5	3	Gain	N	
6	>5	Amplified	Y	
8	>5	Amplified	N	
9	2	Normal	N	
10	4	Gain	N	
12	>5	Amplified	N	
14	3	Gain	N	
15	>5	Amplified	N	
16	3	Gain	N	
22	3	Gain	N	
26	>5	Amplified	N	
28	2	Normal	N	
34	>5	Amplified	N	
36	3	Gain	N	
38	>5	Amplified	N	
39	>5	Amplified	Y	
40	>5	Amplified	N	Δ exons 25-28
43	3	Gain	N	
44	4	Gain	N	
46	>5	Amplified	N	Δ exons 14-15 (EGFRvII)
56	3	Gain	N	
59	>5	Amplified	Y	
61	2	Normal	N	
63	3	Gain	N	
64	2	Normal	N	
66	>5	Amplified	N	
67	2	Normal	N	
69	3	Gain	N	
75	>5	Amplified	Y	
76	>5	Amplified	Y	
80	3	Gain	N	
84	>5	Amplified	N	
85	2.5	Gain	N	
91	>5	Amplified	N	
102	3	Gain	N	
108	>5	Amplified	N	
110	3	Gain	N	
114	>5	Amplified	N	
115	3	Gain	N	
116	3	Gain	N	
117	>5	Amplified	N	
118	>5	Amplified	N	
120	3	Gain	N	
122	3	Gain	N	
123	>5	Amplified	****	Δ exons 14-15 (EGFRvII)
125	3	Gain	N	
126	>5	Amplified	Y	
129	3	Gain	N	
132	>5	Amplified	N	
134	3	Gain	N	
137	>5	Amplified	N	
139	3	Gain	N	
143	>5	Amplified	N	
146	>5	Amplified	Y	
147	3	Gain	N	
148	3	Gain	N	
150	3	Gain	N	
154	2	Normal	N	
155	>5	Amplified	Y	
156	>5	Amplified	****	Δ exons 25-28
157	3	Gain	N	
159	3	Gain	N	
161	3	Gain	N	
164	2	Normal	N	
167	3	Gain	N	
168	>5	Amplified	Y	
170	3	Gain	Y	
174	3	Gain	N	
177	>5	Amplified	****	Δ exons 6-7
181	>5	Amplified	Y	
182	3	Gain	N	
184	3	Gain	N	
187	>5	Amplified	N	
192	3	Gain	N	
195	>5	Amplified	N	
196	2	Normal	N	
206	3		N	
209	>5	Amplified	N	
215	3	Gain	N	
218	3	Gain	N	
229	2	Normal	N	
82	35 (43%)	35 (43%)	11 (13%)	5 (6%)

EGFR amplified (gray) and EGFRvIII (yellow) are highlighted.

EGFR Inhibitors of Interest

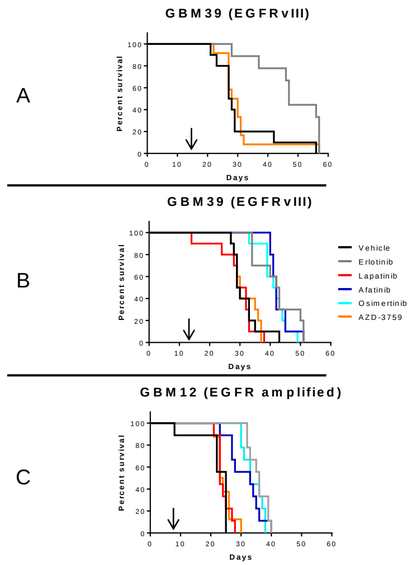
EGFR Inhibitor	Structure	Target	EGFR mutant selection	Efflux Liability	Kpuu
Afatinib	 MW: 485.94	EGFR/HER2/ HER4	L858R, L858R/T790M	P-gp, BCRP	0.046
AZD-3759	 MW: 459.9	EGFR	L858R, exon 19Del	none	2.96
Erlotinib-HCl	 MW: 429.97	EGFR		P-gp, BCRP	0.134
Lapatinib Ditosylate	 MW: 925.46	EGFR/HER2/ HER4		NA	NA
Osimertinib	 MW: 499.61	EGFR	L858R, L858R/T790M	P-gp, BCRP	0.289
EGFRi-X	NA MW: 500.48	EGFR	EGFRvIII, L858R, exon 19Del	none	~1

In Vitro Viability



In vitro viability as assessed by CTG 3D and Neurosphere Formation Assays. Individual compound doses are indicated on the X-axis

In Vivo Efficacy



***In vivo* efficacy of EGFR inhibitors**. The start of dosing is indicated by arrows. **A**. Mice with established G39 intracranial tumors were treated as follows: Vehicle, Erlotinib 100mg/kg QD PO M-Su, or AZD-3759 15 mg/kg QD PO M-Su, both until moribund. **B**. Mice with established G39 intracranial tumors were treated as follows: Vehicle, Erlotinib 80mg/kg QD PO M-Su, Lapatinib 100 mg/kg M-F BID PO and QD PO Sa-Su, Afatinib 24mg/kg QD PO, Osimertinib 25 mg/kg QD PO, or AZD-3759 20 mg/kg QD PO M-Su , all until moribund. **C**. Mice with established G12 intracranial tumors were treated as follows: Vehicle, Erlotinib 100mg/kg QD PO M-Su x2 wks and de-escalated to 80mg/kg QD PO, Lapatinib same as B, Afatinib 30 mg/kg QD PO x 2 wks and de-escalated to 24mg/kg QD PO, Osimertinib same as B, or AZD-3759 15 mg/kg QD PO M-Su, all until moribund.

Conclusions

- The Mayo GBM PDX models are representative of the GBM patient population with 43% of lines exhibiting EGFR amplification and 13% encoding the EGFRvIII deletion mutant.
- Established and novel EGFR inhibitors that have been or are currently in clinical trials for GBM differ in BBB penetrability as determined by PK analysis in WT and efflux transporter knockout mice.
- Copy Number Variation data of the GBM PDX lines is a determinant in whether an EGFR inhibitor will be potent *in vitro*. Lines with EGFR amplification and/or the vIII mutation were sensitive to the six EGFR inhibitors tested in a dose-dependent manner while a line with normal EGFR levels was not sensitive.
- While EGFR-amplified and vIII mutant intracranial tumors were sensitized to a subset of the EGFR inhibitors tested *in vivo*, BBB permeability does not seem to be the only determinant.
- Future directions include efficacy studies with novel EGFR inhibitors as well as combinations with standard of care regimens.

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