EGFR Inhibition in Glioblastoma Patient-derived Xenograft Models

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The epidermal growth factor receptor (EGFR), as well as its other close family members HER2, HER3, and HER4, are important drivers of a variety of cellular processes including cell proliferation, differentiation, and migration. Dereulation 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 neurooncologist using whole exome sequencing data obtained in the Medical Genome Facility at Mayo Clinic. DNA for sequencing was isolated from early passage, frozen or fresh PDX tumor tissue. Lineage was validated against human germline or tumor, or early passage PDX flank-tumor by Short Tandem Repeat (STR) analysis.

Pharmacokinetic: Male and female FVB WT and Har+/-; B6C3F1 +/- mice (Taconic Biosciences, Inc., Germantown, NY) at the age of 8-14 weeks were used for pharmacokinetics (PK) studies. The dosing suspensions for subcutaneous injection were prepared in 10% DMSO and 0.2% hydroxypropyl methylcellulose (HPMC) 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 (M6/M6-/-; B6C3F1) mice. Blood and brain samples from mice were harvested at 1 hour and 8-hour after discrete drug administration (Ex-4). Concentrations of the 8 EGFR inhibitors in specimens were measured by reverse-phase liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS). Compounds were extracted by using 5-volume times of ethyl acetate.

In vitro Viability Assessment: Fluorescence from GBM PDX flank tumors were grown on laminin-coated dishes in StemPro Neural Stem Cell media. Cells were treated with inhibitor for 24 hours in serum-free low serum conditions at 500 (D13), 1500 (D14), or 3000 (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 (G10, G39) only.

In vivo Efficacy: Cells were grown under the same same 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 mortality.

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

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

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 effux transporter knockout mice.
- Copy Number Variation data of the GBM PDX lines is a determinant in whether an EGFR inhibitor will be potent in vivo.
- Lines with EGFR amplification and/or the vIII mutation 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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