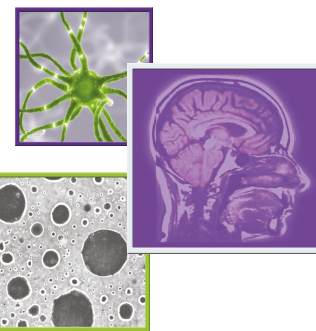




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Clinical activity of the *EGFR* tyrosine kinase inhibitor osimertinib in *EGFR*-mutant glioblastoma

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Practice points

- *EGFR* mutations are among the most common genetic alterations in glioblastoma (GBM).
- Targeted therapies against *EGFR* have been unsuccessful in GBM due to tumor heterogeneity, poor brain penetration of most targeted therapies and inadequate enrichment for *EGFR* alterations in prior targeted therapy clinical trials for GBM.
- Osimertinib is a highly brain-penetrant *EGFR* tyrosine kinase inhibitor that is approved for *EGFR*-mutant non-small-cell lung cancer.
- We present a case of a patient with multifocal GBM harboring multiple *EGFR* mutations that experienced a complete response to osimertinib in one of the patient's tumor sites.
- The patient ultimately progressed at a separate tumor site, highlighting tumor heterogeneity as a significant challenge to *EGFR*-targeted therapies in GBM.
- This case report underscores the need for further clinical evaluation of osimertinib in GBM, including identification of which *EGFR* alterations are predictive of response to this drug.

Glioblastoma(GBM) is the most common primary malignant brain tumor in adults and carries a dismal prognosis. The *EGFR* gene is among the most commonly deranged genes in GBM and thus an important therapeutic target. We report the case of a young female with heavily pretreated *EGFR*-mutated GBM, for whom we initiated osimertinib, an oral, third-generation tyrosine kinase inhibitor that irreversibly inhibits *EGFR* and has significant brain penetration. We then review some of the main challenges in targeting *EGFR*, including lack of central nervous system penetration with most tyrosine kinase inhibitors, molecular heterogeneity of GBM and the need for enhanced specificity for the *EGFR* mutations relevant in GBM.

First draft submitted: 10 July 2019; Accepted for publication: 18 September 2019; Published online: 15 November 2019

Keywords: *EGFR* • GBM • glioblastoma • osimertinib • precision oncology • tyrosine kinase inhibitor

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults and carries a dismal prognosis [1]. While use of molecularly targeted therapies is commonplace in other solid tumors, development of these agents in GBM has been challenging. Obstacles include marked intratumoral heterogeneity [2], redundancy of

signaling pathways driving tumor cell proliferation [3] and a dearth of drugs that penetrate the blood–brain barrier [4,5]. The *EGFR* gene is among the most commonly deranged genes in GBM, with over half of GBM specimens containing a mutation, rearrangement, splicing alteration and/or amplification of *EGFR* [6]. Despite extensive characterization of *EGFR* alterations [7–11] and intense efforts toward clinical development of EGFR-targeted agents in GBM [12–14], no therapies against EGFR have gained regulatory approval in this disease.

Osimertinib is an oral, third-generation tyrosine kinase inhibitor (TKI) that irreversibly inhibits EGFR and was developed specifically to target the *EGFR T790M*-resistant mutation in EGFR-mutated non-small-cell lung cancer (NSCLC) [15]. Osimertinib has significant brain penetration and has demonstrated remarkable efficacy against *EGFR*-mutant NSCLC brain metastases [16–18]. Herein, we report activity of osimertinib in a patient with progressive, *EGFR*-mutated GBM who was treated with the drug as an off-label salvage therapy.

Case report

A previously healthy young woman presented with headaches and altered mental status. A magnetic resonance imaging (MRI) scan of the brain demonstrated a heterogeneously enhancing 3.2 cm mass in the left temporal lobe. She underwent a near-total resection of the mass. Histopathologic evaluation revealed GBM and the *MGMT* promoter was unmethylated as determined by pyrosequencing. Targeted next-generation sequencing (NGS) using a 153-gene panel (Comprehensive Solid Tumor HaloPlex^{HS}, version 2.0, Agilent Technologies, CA, USA; HiSeq2500, CA, USA) was obtained at the University of Pennsylvania's (PA, USA) Clinical Laboratory Improvement Amendments-certified Center for Personalized Diagnostics Laboratory Center for Personalized Diagnostics. NGS revealed that the tumor was *IDH* wild-type and harbored both *EGFR* copy number gain and an *EGFR C628F* point mutation. A fusion gene transcript panel (custom panel, cDNA from FFPE specimen, Illumina HiSeq) was also performed and was negative for EGFRvIII. She was treated with concurrent proton radiotherapy (60 Gy) and temozolomide 75 mg/m² daily [19]. Her first postradiation MRI revealed significant increase in enhancement about the left temporal resection cavity with marked vasogenic edema. She continued to have symptomatic mass effect despite dexamethasone administration and proceeded to a repeat craniotomy. Histopathology demonstrated predominantly therapy-related changes with *foci* of recurrent/residual GBM. NGS revealed the same *EGFR C628F* mutation as her initial tumor specimen, as well as the *EGFR A289V* mutation. The RNA fusion transcript panel was again negative for EGFRvIII. Following surgery, the patient sought nontraditional therapies and was lost to follow-up. She returned 5 months later, at which time MRI revealed multifocal, bilateral tumor progression remote from her initial surgical cavity, including a right parietal lobe lesion and an anterior parasagittal lesion in the left frontal lobe. She was initiated on bevacizumab 10 mg/kg intravenously every 2 weeks. Despite modest clinical improvement after two cycles, a repeat MRI showed continued progression of both the right parietal and left frontal lobe tumors (Figure 1A). The only systemic treatment acceptable to both the patient and her treating oncologist was off-label targeted therapy against her known *EGFR* mutations. In light of its significant brain penetration and lack of other EGFR inhibitors with proven efficacy in GBM, osimertinib 80 mg daily was initiated in an off-label, off-protocol manner. An MRI scan after 1 month of uninterrupted therapy revealed a near complete response of the left anterior parasagittal frontal lobe mass (Figure 1B). However, the right parietal lobe lesion continued to progress with worsening mass effect and midline shift. Two weeks later, she underwent a third debulking surgery, this time of the right parietal mass; the left frontal tumor response continued (Figure 1C). Unfortunately, the patient continued to decline and died 2 months later. Histopathology from the final resection revealed recurrent/residual GBM. NGS revealed that the tumor was negative for *EGFR* point mutations, but continued to harbor *EGFR* copy number gain. The clinical RNA fusion transcript panel was again negative for EGFRvIII. However, prior to surgery, the patient had signed consent for research tissue banking (University of Pennsylvania Institutional Review Board Protocol 816686). Further analysis of the specimen revealed EGFRvIII positivity by digital polymerase chain reaction (Figure 2A–C), immunohistochemistry (Figure 2D) and RNA-Seq (Figure 2E). Methods for these assays are provided in the Supplementary Material. Table 1 includes a summary of molecular profiling, treatments administered and response to treatment at the time of each surgical procedure for this patient.

Discussion

Osimertinib is an oral TKI that irreversibly binds to the EGFR kinase domain by targeting the cysteine-797 residue in the ATP binding site [20]. It is currently US FDA approved for first-line treatment of metastatic NSCLC with *EGFR* exon 19 deletion or exon 21 *L858R* mutation, as well as metastatic *EGFR T790M* mutation-positive NSCLC following progression on prior EGFR TKI therapy. To our knowledge, this is the first report of clinical activity of

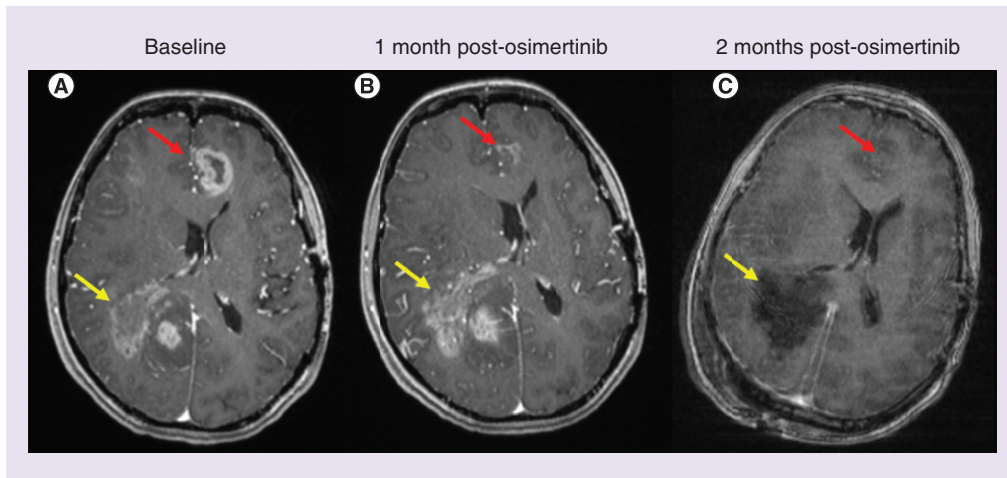


Figure 1. MRI images detailing pre- and post-treatment with osimertinib. (A) The patient started with multifocal disease prior to initiation of osimertinib, including a right parietal tumor (yellow) with satellite lesions, as well as a parasagittal tumor in the anterior left frontal lobe (red). **(B)** After 1 month of osimertinib therapy, there was a near complete response in the frontal lobe lesion and continued progression in the parietal lobe lesion. **(C)** The patient underwent resection of the progressive parietal lobe lesion and had sustained response in the frontal lobe lesion.

Table 1. Summary of tumor analyses.

Tumor specimen	Molecular profiling	Results	Treatment given	Response
1. Initial resection – 3.2 cm left temporal lobe mass	Targeted NGS panel	<i>EGFR</i> copy number gain <i>EGFR</i> C628F	Proton radiation (60 Gy) with concurrent temozolomide 75 mg/m ² /day	Disease progression in original tumor site
2. Repeat craniotomy for resection of recurrent left temporal lobe mass	Targeted NGS panel	<i>EGFR</i> C628F <i>EGFR</i> A289V	Re-resection alone, followed by bevacizumab 10 mg/kg IV every 2 weeks. Continued multifocal disease progression after 4 weeks of bevacizumab. Osimertinib 80 mg daily was started.	Complete response of left frontal lobe tumor after 4 weeks of osimertinib, but continued progression of right parietal tumor
3. Third craniotomy for resection of contralateral right parietal tumor	Targeted NGS panel Droplet digital PCR for <i>EGFR</i> vIII	<i>EGFR</i> copy number gain <i>EGFR</i> vIII	No further treatment. Patient expired 2 months following craniotomy	N/A

NGS: Next generational sequencing using 153-gene panel (Comprehensive Solid Tumor HaloPlex, version 2.0, Illumina HiSeq2500, Agilent Technology, Inc); PCR: Polymerase chain reaction.

osimertinib in human GBM. Targeted NGS performed on our patient's original tumor revealed two *EGFR* point mutations (*C628F* and *A289V*), prompting a trial of osimertinib at the time of multifocal disease relapse given its activity against *EGFR* mutations in NSCLC and significant blood–brain barrier penetration. Although there is no preclinical or clinical data suggesting sensitivity of these mutations to TKI therapy, our patient remarkably had a near complete response to osimertinib in one of her tumors, a lesion that had developed after her prior surgeries and therefore could not be sequenced for molecular analysis. Pseudo progression was considered as an alternative explanation for this response following progressive disease but was dismissed as the progression was outside the prior radiation field. A separate tumor continued to progress on osimertinib. This tumor was resected and found to be negative for *EGFR* point mutations, but positive for *EGFR*vIII.

Despite the relative abundance of activating *EGFR* mutations in GBM, attempts to target these mutations therapeutically in GBM have been disappointing [12]. One problem has been inadequate brain penetration of prior *EGFR* inhibitors. Osimertinib, on the other hand, has demonstrated significant central nervous system activity in NSCLC. This has included *EGFR*-mutant brain metastases [17,20], as well as refractory *EGFR*-mutant leptomeningeal disease [21,22]. Another issue hampering efficacy of *EGFR* TKIs in GBM has been a lack of specificity of available *EGFR* TKIs for the *EGFR* mutations typically detected in GBM [8]. These mutations involve the extracellular domain of *EGFR*, whereas *EGFR* mutations in NSCLC are found in the tyrosine kinase domain [23]. However, a recent study demonstrated significant preclinical activity of osimertinib in GBM harboring

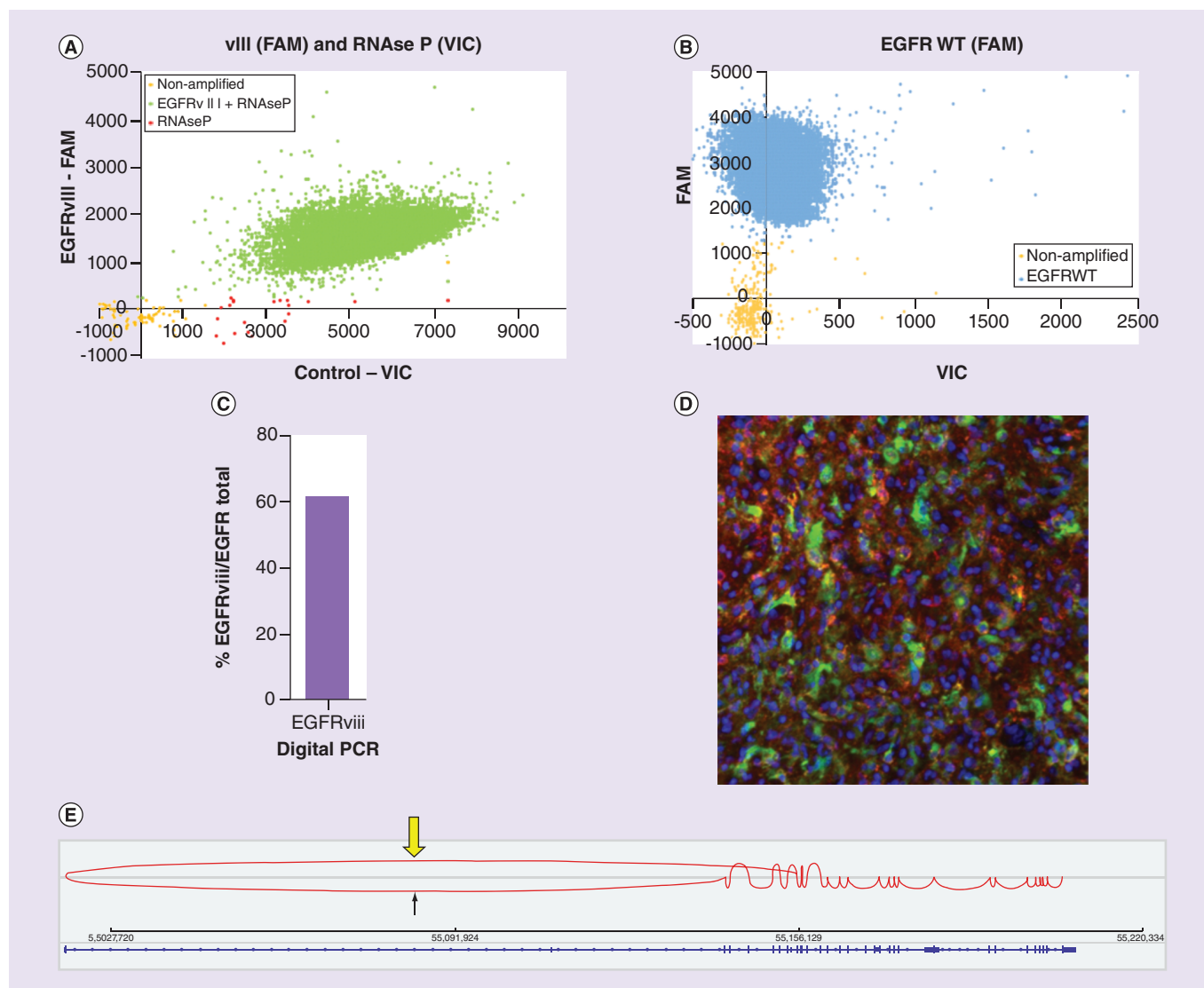


Figure 2. The patient's resected right parietal lobe tumor, which progressed during osimertinib therapy, was demonstrated to be positive for EGFRvIII by multiple methods. (A) Digital polymerase chain reaction using RNaseP internal control (VIC dye) demonstrated EGFRvIII expression (FAM dye). (B) EGFR wildtype (FAM dye) was run on a separate chip. (C) The fraction of EGFRvIII transcript as a percentage of total EGFR detection was 61.33%. (D) The specimen was positive for EGFR by immunohistochemistry (green = EGFRvIII, blue = 4',6-diamidino-2-phenylindole [DAPI], red = EGFR). (E) EGFRvIII was also detected by RNA-Seq, as displayed by a Sashimi plot showing detection of EGFRvIII splice junction (yellow arrow on red line represents the EGFRvIII exon 2–7 skipping event; black arrow on red line represents wild-type splicing; blue line includes all exons at the genomic coordinates for *EGFR* on chromosome 7).

EGFRvIII [24], the most common *EGFR* extracellular domain mutation in this disease [8]. In an EGFRvIII positive GBM stem cell model, osimertinib led to potent blockade of EGFRvIII and its downstream signaling. In addition, mice with intracranially implanted EGFRvIII-positive GBM stem cells treated with osimertinib (25 mg/kg) had a nearly 50% increase in overall survival compared with mice treated with either vehicle control or the EGFR TKI lapatinib [24]. It is unclear which *EGFR* mutation(s) in our patient's tumor, whether the *C628F* or *A289V* mutations detected in her primary tumor or a different mutation that we did not detect, sensitized one of her recurrent tumors to osimertinib. Interestingly, the tumor site in our patient that was demonstrated to harbor EGFRvIII (right parietal lobe) was also the site that actively progressed on osimertinib. It is unknown whether this progression occurred due to lack of inhibition of EGFRvIII by osimertinib in human GBM (contradicting the preclinical data); poor drug distribution within this particular tumor site related to its size, dysfunctional vasculature and elevated interstitial fluid pressure; and/or upregulation of signaling pathways other than *EGFR* that primarily drove proliferation and

survival of this particular tumor site. In light of this uncertainty, it is also possible that the patient's tumor that responded to osimertinib in fact harbored EGFRvIII, but had additional characteristics not present in the patient's progressing tumor that allowed for a therapeutic degree of EGFR inhibition.

Another major barrier to the success of EGFR TKIs in GBM is intratumoral molecular heterogeneity [2,25]. As exemplified by our case and well described in the literature, EGFRvIII and other *EGFR* mutations are both spatially [14] and temporally [9,11,26] heterogeneous in GBM. Thus, detection of these alterations is highly dependent on the part of the tumor that is sampled, when during treatment the tumor is sampled and which method of detection is used. Our patient had three separate tumors resected at different time points, with NGS revealing an *EGFR C628F* in one tumor, both *EGFR C628F* and *A289V* mutations in the second tumor and EGFRvIII in the third tumor. Furthermore, EGFRvIII was only detected in the third tumor when the tumor was tested for EGFRvIII by multiple different methods; these were pursued despite an initial negative result in light of the aforementioned preclinical data suggesting that EGFRvIII may confer sensitivity to osimertinib. Our case suggests that, even if the particular mutation(s) that sensitize human GBM to osimertinib are identified, multiple tumor samples and/or multiple detection assays may need to be tested to reveal the mutation(s) of interests. In addition, combination therapies will be needed to address all sites of disease.

In conclusion, this report demonstrates the clinical activity of osimertinib in human GBM. Our patient responded to osimertinib in one of her lesions, yet had simultaneous progression of a separate tumor, underscoring the therapeutic challenges presented by tumor heterogeneity in GBM. Future research is needed to understand which *EGFR* mutations render glioma cells sensitive to osimertinib, ultimately leading to molecularly enriched trials of this promising drug for patients with GBM.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations and that consent for this research was obtained posthumously from the patient's next of kin.

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