Targetting the EGFR Pathway in Glioblastoma Multiforme: A Review of Current Pre-clinical and Clinical trials with Tyrosine Kinase Inhibitors.

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Abstract

With a median overall survival expectancy of 15 months or less [FC17], Glioblastoma Multiforme (GBM) is the most common type of primary brain tumor [SA18]. Despite extensive research on the pathophysiology and clinical course of GBM, the malignancy remains one of the most lethal cancers to date as the 10 year survival rate is 0.71 percent [TT18]. While established methods of treatment such as resection, radiotherapy, and chemotherapy are effective in prolonging survival time, they are not effective in preventing recurrence [HL06] which occurs in almost every patient [OM14]. To better combat the dismal outcomes of GBM, novel approaches are necessary given the increase in incidence as well as the increase in tumor burden globally [GN20]. Gene therapy may serve as a promising novel therapeutic, with initial clinical studies indicating promising results [PK05]. This review will outline the most recent treatment protocols for differing GBM subtypes, characterize the tyrosine kinase epidermal growth factor receptor (EGFR) and its downstream signaling pathway, and analyze currently on-going and recently completed clinical trials involving tyrosine kinase inhibitors in GBM.

1 Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults accounting for over 45 percent of all malignant primary CNS tumors. The disease occurs in older adults with a median diagnostic age of 64 years and peak incidence between 75-84 years. Incidence is higher in males than in females as well as in white, non-Hispanics. GBM remains an incurable tumor, with a median survival time of 15-20 months and 5-year survival rate of approximately 5 percent due to the heterogeneous and complex nature of the disease. Approximately 80 percent of GBM tumors are primary, rapidly developing de novo without precursor lesions such as lower-grade gliomas that

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are common in secondary tumors. Of primary GBM tumors, 57 percent contain EGFR gene amplification, encoding the epidermal growth factor receptor (EGFR). EGFR is a transmembrane receptor tyrosine kinase that contains an extracellular region composed of four domains and an intracellular region composed of a tyrosine kinase domain as well as C-terminal tail. Upon binding of the epidermal growth factor ligand, EGFR dimerizes and autophosphorylates its C-terminal tail, which serves as a docking site for several secondary messengers that induce cellular proliferation and resist apoptosis. Prominent downstream pathways of EGFR include the RAS-RAF-MEK-ERK MAPK as well as PI3K-AKT-mTOR pathways. Interestingly, approximately 26 percent of primary GBM tumors contain EGFR activating mutations. The most common EGFR variant EGFRvIII, occurring in approximately 50 percent of all EGFR-amplified GBM cases, involves the deletion of amino acids 6-273, encompassing exons 2-7. This mutation results in an EGFR that contains a modified extracellular domain which allows for constitutive activation of the receptor. Clinically, patients with either increased EGFR expression or mutation are likely to have increased tumor invasion with lower overall survival rates at 6 months, as compared to the median overall survival rate of 15 months for GBM patients [BZ18]. Other common mutations in GBM patients include specific genes that lead to increased development of malignancy, and guide prognosis. Mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are oncogenic, promoting methylation in cancers as well as production of oncometabolites such as 2-hydroxyglutarate (2-HG) [CA13, TZ14]. While the mutations themselves promote undifferentiated cell proliferation, they are also associated with better prognosis due to targeting therapies [CA13]. In addition, O6-Methylguanine-DNA-methyltransferase (MGMT) promoter methylation status. The methylation of this enzyme promoter makes tumor cells susceptible to DNA damage caused by alkylating agents, such as temozolomide (TMZ) [HM05]. Activation of the EGFR receptor leads to homodimerization and autophosphorylation of several tyrosine residues on the C-terminal domain, eliciting downstream activation of secondary messengers including protein kinase B (Akt) and mammalian target of rampamycin (mTOR). Studies have found that the amplification of EGFR is often seen in tandem with increased abundance and phosphorylation of pleckstrin homology-like domain family A member proteins (PHLDA1 and PHLDA3), transcription factor SOX9, cell adhesion protein CTNND2 (-catenin), and cell cycle proteins CDK6 and CDKN2C15. Patients with increased EGFR expression are likely to have increased tumor invasion with lower overall survival rates at 6 months, as compared to the median overall survival rate of 15 months for GBM patients [BZ18]. In this review, we will cover the most recent pre-clinical and clinical studies concerning modulation of the EGFR using tyrosine kinase inhibitors and discuss potential synergistic strategies to possibly decrease the high tumor burden of GBM.

2 Initial Diagnosis

Patients with suspected GBM typically present with progressive neurological symptoms such as headaches, seizures, and memory loss [BF15]. In patients with suspected GBM, contrast-enhanced MRI scans are conducted to examine areas of microvascular proliferation and focal necrosis that may represent the histological characteristics of the disease [TA20]. Screening for systemic malignancies are often not necessary when radiographic suspicion is high for high-grade glioma. Full diagnosis is only achieved upon biopsy, which is collected after maximum tumor resection or, in patients where tumor resection presents itself to be unamenable, in a biopsy procedure [HM19]. In addition to scans and tissue pathology, the detection of certain genetic mutations through fluorescence in situ hybridization (FISH), such as EGFR, may also aid in the diagnosis of the disease [MC14].

3 Current Treatment Protocols

Treatment of GBM is typically a combined approach involving surgical resection and adjuvant therapy and can diverge into multiple different approaches based on several factors, including age. However, clinicians typically start off with maximum resection surgery, unless this procedure is contraindicated due to tumor location or patient status [KD11, RC14]. After resection, adjuvant therapy is based on patient age, Karnofsky Performance Status Scale (KPS score), and methylation status of O6-methylguanine-DNA methyltransferase (MGMT). Patients 70 years of age, KPS score 60, and methylated MGMT receive radiotherapy (60 Gray, in 30 fractions) along with daily temozolomide (TMZ) (75 mg/m2/day for 6 weeks), followed by 6 maintenance cycles of TMZ (150–200 mg/m2/day for the first 5 days of a 28-day cycle) [FC17, TA20]. Patients 70 years of age, KPS score ¡60, and methylated MGMT receive hyperfractionated radiotherapy (HFRT) as the preferred line of treatment to reduce toxicity. HFRT can also be administered along with adjuvant TMZ, to increase efficacy of overall treatment, but clinicians may also choose to just use TMZ alone, or simply provide best supportive care [TA20]. Interestingly, recent clinical trials indicate maintenance TMZ may be accompanied by tumor-treating fields (TTFields), a treatment employing non-invasive delivery of low-intensity (1–3 V/cm), intermediate-frequency (100–300 kHz), alternating electric fields [DA13] that target polymerization and depolymerization of microtubules in the mitotic spindle [FD19]. This combination has been shown to increase overall survival and disease-free progression [**?**] across multiple clinical trials, with one resulting in patients who had completed initial radiotherapy and TTFields plus TMZ having median progression-free survival of 6.7 months, as compared to 4.0 months in TMZ-alone group [GG19]. If the patient is ¿70 years of age, KPS score 60, and methylated MGMT, then HFRT, along with TMZ, is given (dosage dependant on number of fractions, and TMZ over the course of radiation), followed by maintenance TMZ. A second option is the use of standard radiotherapy com-

bined with TMZ, followed by maintenance TMZ and TTFields [TA20]. If the patient has poor functional status and a KPS ¡ 60, then HRFT alone, or TMZ alone, is given. Patients who contain unmethylated MGMT are generally resistant to TMZ adjuvant therapy [AI20]. In such cases, standard radiotherapy is administered, given the patient has a KPS score 60 [TA20]. At tumor recurrence, the most preferred line of therapy is surgery, as research has shown that reoperation improves overall survival1, though there is no standard line of adjuvant treatment for recurring tumors [TA20]. Re-radiation, with a median total dose of 30–36 Gy, may be an alternative treatment option [WM13] however, it is not as highly recommended as surgery or systemic therapy, due to potential for increased toxicity [TA20,O.15]. Systemic therapy involves administering chemotherapeutic as well as immunotherapeutic agents such as TMZ and bevacizumab as well as alkylating agents like carmustine or other blood-brain barrier (BBB) penetrant nitrosoureas. Unfortunately, systemic therapy during tumor recurrence though results of studies testing the effectiveness of such drugs with recurrence have been discouraging [O.15]. The attending physician typically chooses the treatment method based on several factors including the patient's KPS score, tumor burden, methylation status of MGMT, epidermal growth factor receptor (EGFR) status, and IDH status. TTFs may also be used, though studies have shown that majority of patients still do not survive for over two years, which is why supportive care may present itself as the best option, as it emphasizes improving quality of life and managing discomforting symptoms [FC17, TA20].

4 Recent Tyrosine Kinase Inhibitors

4.1 CM93

CM93, a novel covalent-bonding TKI, has shown to contain comparable efficacy to Osimertinib (IC50 3.66nM vs. 12.03nM, respectively) in several cancer cell lines harboring EGFR mutations [ea20]. Furthermore, CM93 displays a 20-fold greater brain-to-plasma ratio at estimated steady states. In addition, CM93 reduced 293-EGFRvIII cell viability with an IC50 of 1.48 M which was lower than Erlotinib (IC50 4.83 M), gefitinib (IC50 15.67M) and Osimertinib (IC50 2.19 M). In vitro, CM93 reduced EGFRvIII phosphorylation in two tyrosine kinase sites in HEK293-EGFRvIII cells. Further titration revealed that CM93 had an IC50 value of 0.19 M on EGFRvIII phosphorylation [Ni21]. In mice, CM93 had comparable efficacy to Osimertinib; both had significantly inhibited tumor growth with a 25mg/kg dose. With a 10mg/kg and 30mg/kg dose of both CM93 and Osimertinib tumor count significantly reduced tumor cell count with no statistically significant difference between the two drugs. In an NSCLC brain metastasis model, mice were given CM93 at 25mg/kg and 50mg/kg and Osimertinib at 25mg/kg. The median survival time of mice taking 25mg/kg of CM93 was 80 days, and mice taking 50mg/kg had a median survival time of 100 days. Mice taking Osimertinib reached an endpoint after four weeks due to body

weight loss and skin lesions when dosed at 25mg/kg. They had a brain to plasma drug concentration ratio was 6:1 in males and 7:1 in females; whereas mice taking CM93 had a brain to plasma drug concentration ratio of 14:1 in males and 15:1 in females, suggesting CM93's ability to penetrate through the blood brain barrier and, therefore, showing efficacy in the brain [ea20]. Another preclinical trial demonstrates similar results comparing CM93 to Gefitnib, another EGFR TKI. After a pilot comparative assessment seven hours after a single dose of 30mg/kg of CM93 or 50mg/kg of Gefitinib was administered, CM93 had a kp value of 28.3; whereas, Gefitinib had a kp value of 0.55 [Ni21]. Unlike Osimertinib, CM93 had little adverse effect on mouse skin; with Osimertinib, mice lost more than 20 percent of their body weight reaching their endpoint and had severe hair loss after three weeks. Mice treated with CM93, however, showed no hair loss; this demonstrates CM93's potential to improve patient quality of life [ea20]. Another preclinical trial further examined CM93's efficacy in vivo using genetically engineered mice with GBM. Mice taking CM93 had a medium survival of 33 days while the control group had a medium survival of 25.5 days. In this model too, there was no significant hair or loss of body weight observed in the CM93 Group [Ni21].

4.1.1 ERAS-801

Currently in phase one and in a nonrandomized sequential open label designed study format, the next trial includes patients with a diagnosis of GBM IDH wildtype. Patients with prior EGFR inhibitor treatment for GBM are excluded. This clinical trial's intervention is ERAS-801, a new EGFR Tyrosine kinase inhibitor ERAS-801 targets the RAS/MAPK pathway and inhibits EGFR [ERAb]. Targeting wildtype EGFR and mutant variants of EGFR by small molecules and antibodies has been shown to improve patient outcomes in NSCLC, CRC, and HNSCC; however, in CNS tumors the ability to target wtEGFR and mutant EGFR remains an unmet need. The two main reasons why current EGFR inhibitors lack efficacy is their lack of ability to penetrate the blood brain barrier and are week inhibitors of EGFRvIII mutant protein. ERAS-801, however, differs as it is designed to be selective, reversible, orally available, and has a 3:7 brain to plasma ratio in mice demonstrating CNS penetrability. ERAS-801 is also able to target EGFR alterations such as EGFRvIII . When a single oral dose of 10mg/kg of ERAS-801 was administered to mice, ERAS-801's kp value was 3.7, which was higher than Osimertinib's (0.99) , Afatinib's (0.25) , Erlotinib's (0.06) , Gefitinib's (0.36) , and Dacomitinib's (0.61) ; all of the other named drugs are other EGFR TKI's. Taken together the evidence suggests that ERAS-801 out performs other inhibitors in terms of CNS penetration. In preclinical studies, ERAS-801 showed efficacy against EGFR through an IC50 of 0.3nM and high selectivity for EGFR based on a biochemical screen of 484 kinases where ERAS-801 at 10 µM inhibited two non EGFR family kinases at greater than 90 percent. In vitro cell based assays, ERAS-801 had an IC50 of 1.1nM against wildtype EGFR an IC50 of 0.7 nM against EGFRvIII, and an IC50 value of less than 3 µM in a 31 patient derived glioma

cell panel where 65 percent of glioma cell growth was inhibited by ERAS-801. The patient derived glioma cell panel had the most common types of EGFR alterations which include amplification, EGFRvIII, extracellular domain mutations, and chromosome 7 polysomy. ERAS-801 also showed no activity against astrocytes, the most common cell in the human brain. This suggests that ERAS-801 selectively inhibits EGFR without disturbing normal brain cells that were not dependent on EGFR signaling. In vivo, ERAS-801's high CNS penetration resulted in survival benefit. In an EGFRvIII mutant patient-derived GBM model, the medium survival time was 40 days for the control group, between 60 and 70 days for the 10mg/kg dose of ERAS-801, around 80 days for the 25mg/kg of ERAS-801 group, and around 80 days for the 75mg/kg of ERAS-801 group. In four additional patient-derived glioma models that harbor EGFRvIII, EGFR amplified, or chromosome 7 polysomy mutations, ERAS-801 showed TGI in 93 percent of 14 patient derived models. Taken together, the evidence suggests ERAS-801's efficacy in combatting GBM [ERAa].

4.1.2 AZD9291 (Osimertinib)

The next clinical trial follows a single group assignment format and is in phase II. Including those who have supratentorial contrast enhancing progressive or recurrent tumors and an EGFR mutation or amplification and excluding those with p53 mutations and prior exposure to EGFR targeted treatments, the trial tests Osimertinib, also known as AZD9291. Osimertinib is a small molecule TKI inhibitor, antineoplastic agent used in therapy of selected forms of NSCLC. Its common side effects include diarrhea, rash and dry skin, and nail toxicity. Its severe but uncommon side effects include interstitial lung disease, prolongation of QTC interval, and cardiomyopathy [Osia]. Evidence from three preclinical studies show the drug's promise and its limitations. In athymic mice, Osimertinib showed CNS penetration with a concentration of 3,695 $\pm$ 425 nM Osimertinib in the brain compared to 314nM of the drug found in plasma, giving Osimertinib a brain to plasma ratio greater than 10. In vitro, when Osimertinib's efficacy is tested against D317 cells which express high levels of EGFRvIII, Osimertinib inhibited EGFR phosphorylation at an IC50 of 50nM. Although there was no effect on the total level of EGFR, Osimertinib leads to a blockade of EGFRvIII's intracellular signaling. In vitro, the quantification of Osimertinib's inhibition of D317 cells' growth using WST-1 cell proliferation assay led to an IC50 of 476 $\pm$ 163 nM, indicating Osimertinib's ability to inhibit EGFRvIII+ growth at concentrations attainable in the brain [Cha20]. Another preclinical trial demonstrates its efficacy by comparing osimertinib to six other EGFR inhibitors in 22 patient-derived GBM cell samples. Osimeritinib showed efficacy in 10 of the 22 samples tested with a 50 percent growth inhibition at the concentration of three micro moles [Pet16]. A different preclinical trial demonstrated that Osimeritinb can inhibit wild-type EGFR with weaker binding than that of T790 mutant EGFR with IC50 values of 184 and 1 nanomoles respectively [LX19]. In GBM cell lines, Osimertinib inhibited the growth of six cell lines in a dose dependent manner with IC50 values ranging from 1.25 to 3 mi-

cromoles; first generation EGFR inhibitors had IC50 values of 10 micromoles in the same setting, suggesting that Osimertinib has greater efficacy compared to first generation EGFR TKIs [Pet16]. To examine whether Osimertinib inhibits GBM cell growth due to off-target effect, two U87 cell lines stably expressing wild-type or Cys797 mutant EGFR were constructed to reveal that Cys797 residue in the catalytic domain of EGFR is key to the inhibitory effect of Osimertinib. While treatment significantly inhibited growth of cells expressing wild-type EGFR, effects on growth were nearly abolished with Cys797 mutant EGFR. EdU-positive assay to evaluate Osimeritinib's inhibitory effect on GBM proliferation showed that proliferation in U87 and U251 lines were reduced to 25.59 percent and 37.37 percent respectively, suggesting Osimertinib's strong inhibition of GBM cell proliferation in a dose dependent manner. Furthermore, a colony formation assay revealed that the number of colonies as reduced 767.82 percent by Osimertinib, and a Methylcellulose colony confirmed these results suggesting Osimertinib's ability to significantly inhibit GBM cell colony formation. Flow cytometry also revealed that Osimertinib's mechanism of GBM cell proliferation inhibition, was that the cell cycle distribution and progression was arrested in in G1 phase in both cell types tested in the assay (u87 and U251). Western blot analysis to test inhibition of the EGFR/ERK pathway activation in which Osimertinib's effect was tested on EGFR, AKT, STAT3, and ERK phosphorylation in GBM cells. Different concentration of Osimertinib treatment U87 and U251 GBM cells tested had no significant changes in total EGFR expression; however, phosphorylated EGFR numbers gradually reduced with increasing Osimertinib concentrations which also lowered the level of ERK and had no effect on AKT and Stat3 level. In erlotinib, a well known TKI, inhibited ERK phosphorylation for 24-48 hours after which ERK reactivation was observed. Osimertinib, on the other hand, can continuously suppress EGFR and ERK phosphorylation and may therefore inhibit the growth of GBM cell continuously by blocking the EGFR/ERK pathway. Also, when Osimertinib is combined with ERK inhibitor PD098059, anti-proliferations and anti-invasion activities of Osimertinib are enhanced. Results from EdU assays show that both Osimertinib and PD098059 inhibited the proliferation of GBM cells; however, compared to the monotherapies, the combination was observed to be more effective. PD098059 also enhanced the inhibitory effect of Osimertinib on GBM cell invasion. Combined with another ERK inhibitor SCH772984, however, Osimertinib showed effects on proliferation of GBM cells but not on cell invasion. This data suggests that ERK inhibition could increase the sensitivity of GBM cells to Osimertinib [LX19]. In vivo, orthotopic and heterotopic mice models, tumor growth in the Osimertinib treated group was slower with a T/C of 0.0241, which is significant because any value less than 0.4 is considered significant inhibition. Osimertinib was effective in slowing the growth of intracranial tumors and the median survival of untreated mice, 26 days, was increased to 42 days in the treated mice [Cha20]. Another preclinical trial used in situ GBM nude mice models treated with an intraperitoneal injection and an oral administration of osimertinib to observe that immunofluorescence stainin of GBm sections in the Osimertinib treatment group were significantly higher than those in the control

group, suggesting that Osimertinib inhibited proliferation and promoted GBm cell apoptosis in vivo [LX19]. A completed clinical trial including patients with IDH1 or IDH2 wildtype GBM involved paitents taking 80mg of Osimertinib orally once a day until unacceptable side effects, death, or medical complications occurred. Four out of the six patients were assessed for response. Out of four patients, one showed partial response, two had received stable disease, and the last was refractory to treatment. Transient improvement in imaging was not without side effects: two patients had Thrombocytopenia, one developed grade 1 diarrhea and pneumonia, and the other developed grade one mucositis [Abo10]. Because Osimertinib penetrated the blood brain barrier effectively, had in vitro and in vivo data to support its efficacy, and inhibits multiple intracellular pathways, it may be a better treatment option than previously tested EGFR-TKI's for GBM patients. Osimeritinib is also irreversible and can lead to prolonged survival and continuous ERK inhibition. Results show that the combination of an EGFR inhibitor and an AKT/STAT3 pathway may be more effective than a monotherapy [LX19]. The clinical trial also shows that Osimeritnib may benefit select patients with recurring MG and EGFR alterations underscoring the importance of characterizing EGFR alterations before considering Osimertinib treatment for a certain patient [Abo10].

4.2 BDTX-1535

The next ongoing clinical trial investigates the potential of BDTX-1535 monotherapy. Currently in phase I, the trial's includes patients diagnosed with wildtype IDH GBM and astrocytoma with molecular features of GBM; both must be recurrent cancers. Its exclusion criteria include known resistant mutations in tumor tissue or ctDNA, prior treatment with EGFR inhibitors, and brain metastases or spinal cord compression requiring intervention. BDTX-1535, the intervention, is selective, highly potent, and an irreversible inhibiter of EGFR alterations including amplification, mutations, and splice variants seen in GBM. A report summarizes more information about the drug and some key preclinical trials that offer some descriptions of BDTX-1535. If BDTX-1535 could overcome Osimertinib resistance, it could address a pressing rising need in EGFR mutant non-small lung cell cancer. BDTX is optimized against a broad spectrum of EGFR mutations and a Goldilocks wild type selectivity profile. Results have shown that in mice harboring NSCLC with C797S mutation, BDTX-1535 induced a dose dependent tumor shrinkage without a loss of body weight. The mice treated with Osimertinib, however, looked like the untreated control group. BDTX-1535 could penetrate the blood brain barrier addressing brain metastases and CNS tumors [BDT21].

4.3 HMPL-813 (Epitinib Succinate)

Epitinib has the potential to cross the brain-blood barrier and display its effectiveness in brain metastasis tumors. Another phase I clinical trial involving epitinib in patients with non-small-cell lung cancer has been conducted with

72 patients enrolled, all of which had EGFR-mutant advanced non-small-cell lung cancer with brain metastases. Patients were given 120mg or 160 mg orally with safety and tolerability being the primary outcomes. Treatment related toxicities occurred in 13 (43.3 percent) of the patients in the 120 mg group and 21(50 percent) of the patients in the 160mg group. The drug had an objective response rate of 53.6 percent in 120 mg group and 40.5 percent in the 160 mg group. The median duration of response was 7.4 and 9.1 months in the 120 and 160 mg groups respectively, while the median progression-free survival was 7.4 months for both groups. Taken together, the data suggests epitinib in 160 mg showed promising efficacy and was well tolerable; this was also taken as the recommended phase II dose [ea22]. Another clinical trial testing the safety of Epitinib in patients with EGFRm+ NSCLC recruited 36 patients in a dose escalation phase at 7 dose levels up to 240mg starting at 20 mg. Dose escalation was followed by a 3+3 design. The most common adverse effects seen were: rashes which occurred in 60 percent, diarrhea (34.2 percent), elevated AST(34.3 percent), and hyperbilirubinemia (28.6 percent). Drug exposure increased proportionally until it plateaued at 160 mg and above. Out of 12 patients treated with 160 mg of eptitinib, 5 all reached PR and showed tumor shrinkage. 2 progression events, in the liver and brain, were observed. With this evidence taken together, further development of this drug was supported [ZQ16].

4.4 Anlotinib

Currently in phase II, anlotinib is a multitarget TKI that blocks the migration and proliferation of endothelial cells, reduces the tumor microvascular density by targeting VEGFRs, FGFRs, and PDGFRs [Anl]. A preclinical trial attempting to test if Osimertinib overcomes acquired resistance to EGFR TKI's in patients with EGFR mutant non-small cell lung cancer was conducted. The researchers evaluated the antitumor effects of gefitinib + anlotinib in gefitinib resistant lung adenocarcinoma models in vitro and in vivo and investigated the treatment of an EGFR TKI + Anlotinib in 24 patients with advanced EGFR mutant NSCLC after EGFR TKI acquired resistance. The results show that Anlotinib reversed gefitinib resistance adenocarcinoma models by enhancing antiproliferative and proapoptotic effects of gefitinib. Similarly, EGFR-TKI+ Anlotinib therapy showed an objective response rate of 20.8 percent and a disease control rate of 95.8 percent. While median progression free survival was 11.53 plus of minus 2.41 months, overall median survival could not be reach. In the clinical trial, one adverse event in grade 3 was noted, but there were not grade 4 or 5 adverse events. The researchers conclude by stating that EGFR TKI + Anlotinib demonstrates powerful antitumor activity in vitro and in vivo. Using anlotinib can overcome resistance to EGFR-TKI in advanced EGFR mutant NSCLC patients [Zha21]. Another preclinical trial examined the effects of anlotinib with temozolomide and the molecular mechanisms of anlotinib in Glioblastoma. Through a Cell Counting Kit-8 and colony forming assays, the researchers examined cell viability. Cells treated with anlotinib in 0, 1.25, 2.5, 5, 10, and 20 micro moles were tested to reveal that anlotinib could induce cell

death when concentrated and in a dose dependent manner in all GBM cell lines tested. To see long term effects, the researchers used colony formation assay and found that the size of independent colonies in anlotinib treated group were much smaller and were significantly reduced, indicating that anlotinib inhibited the proliferation of GBM cells in a dose dependent manner. Then the migratory ability of GBM cells was tested through wound healing. The migratory ability of GBM cells compared to untreated control cells was decreased by anlotinib. Following that, Transwell migration and Matrigel invasion assays revealed that GBM cell migration and invasion capacities were reduced when treated with anlotinib, so anlotinib suppressed the migration and invasion of glioblastoma cells in a concentration-dependent manner. Then flow cytometry was used to analyze anlotinib treatment's effect on the cell cycle profile. After pretreatment with 0, 2, and 4 micromoles of anlotinib for 24 hours the percentage of cells in the G2/M phase increased in a dose dependent manner suggesting that anlotinib could induce a G2/M phase arrest [XP22]. Since previous studies have indicated that arresting the cell cycle initiates an apoptotic program, anlotinib's effect was examined to reveal that the percentage of apoptotic cells was elevated in three human GBM cell lines. Compared to the cell group, anlotinib was able to induce apoptosis. Researchers also observed that anlotinib induced autophagy related proteins according to western blotting suggesting that anlotinib started autophagic programs in GBM. JAK2/STAT3 signaling pathways plays a key role in angiogenesis; VEGFA, which anlotinib has also known to target, is a downstream target gene of JAK2/STAT3 which promotes angiogenesis. A tubular formation assay was performed to evaluate anlotinib's effects on new capillaries sprouting. The human umbilical endothelial tumor formation was inhibited by u87/anlotinib supernatant which was enhanced by S31-201. Because VEGFA plays a crucial role in tumor angiogenesis and anlotinib was able to decrease VEGFA levels secreted by U87 cells, the researchers decided to further explore underlying molecular mechanisms in GBM cell treatment with anlotinib. After a western blot analysis, the researchers found several key signaling pathway proteins, and after Anlotinib treatment, cell motility related proteins and proliferation related protein expression decreased after 2 micromoles of treatment which was later enhanced by S31-202 in 100 micromoles. These findings showed that anlotinib's influence on the JAK2/STAT/VEGFA signaling pathway could affect its influence on the anti-angiogenic and anti-glioblastoma effects in GBM. When put together with temozolomide, a wound-healing assay showed that the combination of the drugs increased the cell migration inhibition compared to each drug used alone. Flow cytometry was used to test whether the enhanced cytotoxicity was due to cellular apoptosis, but the drugs alone increased apoptosis with greater efficacy than the combination of drugs [XP22]. Changes to components of the JAK2/STAT3/VEGFA signaling pathway were assessed to reveal that the combination of drugs were more effective than either drug alone to suppress JAK2/STAT3/VEGFA signaling. The researchers proceeded to perform in vivo, nude mice, bioluminescence imaging every seven days suggesting that anlotinib delayed tumor growth compared to the control group. Staining also revealed that anlotinib reduced the positivity of the proliferation index. Western

blotting further revealed that anlotinib reduced p-JAK2, p-STAT3, and VEGFA in vivo, indicating that anlotinib was able to inhibit proliferation in vivo. The researchers conclude that because anlotinib can suppress proliferation, migration, invasion and angiogenesis of GBM cells in a dose-dependent manner, anlotinib offers promise. Furthermore, its cooperative effect with temozolomide to further enhanced cytotoxicity and anti-angiogenesis offers only stronger evidence of its promise. While the previous trial did characterize anlotinib in terms of a VEGF inhibitor, the next trial examines anlotinib combined with cranial radiotherapy to address cancer patients with brain metastasis. By analyzing the clinical effects of anlotinib + Cranial Radiotherapy (CRT) versus CRT alone in NSCLC patients with brain metastasis, the researchers found no significant clinical features between the two groups of patients where 45 received CRT alone and 28 received CRT + anlotinib. The researchers also analyzed the overall survival of anlotinib + CRT compared to CRT alone. After evaluating clinical characteristics to establish a baseline, prognostic factor for intracranial progression free survival and overall survival underwent univariate and multivariate analysis. Compared to the CRT group, the combined group had greater median intracranial progression-free survival of 3 months and 11 months respectively; however, there were no significant differences in overall survival, extracranial progression free survival, and systemic progression free survival. Univariate and multivariate analysis further revealed that the addition of anlotinib to treatment was an independent advantage predictor while an age greater than 57 years and a KPS score less than or equivalent to 80 were independent disadvantage predictors of overall survival [He21]. While the difference was not statistically significant, those with anlotinib and Local CRT treatment had the longest intracranial Progression free survival of 27 months and overall survival of 36 months, and the mi progression free survival and m overall survival values for the local CRT group had values of 11 months and 18 months respectively for shorter values of the brain. The research concludes by saying that anlotinib can improve intracranial lesion control and survival prognosis of NSCLC patients with CRT [He21].

5 Conclusion

With its comparable efficacy to Osimertinib in T790M mutations (ic50 4.39nM), CM93 offers the most promise out of all the other drugs listed above. Although its inhibition of wt-EGFR (ic50 3300nM) is lacking, it is a selective inhibitor of EGFR and effectively inhibits EGFRvIII (IC50 0.19 mu moles), the most common EGFR mutation. CM93's higher median survival of mice and high brain-to-plasma concentration suggest potentially improved prognosis and efficacy in patients. The mice's lack of skin lesions and body weight loss suggests improved quality of life for patients and its ability to be tolerated in higher doses gives makes this drug a promising drug for the future. Epitinib offers the least promise of the drugs listed. Despite its efficacy and ability to penetrate the BBB, its toxicity and adverse side effects in patients (rashes, diarrhea, elevated AST, hyperbilirubinemia) suggest its limited effectiveness. The two progression cases

in the liver and brain observed in the clinical trial evaluating Epitinib lowers the drug's promise as it adds a risk factor to the drug. The scarcity of preclinical information available about this drug also puts limits its promise as it comes with many unknowns. After CM93, BDTX-1535 and WSD0922-FU offer promise in terms of improving patient quality of life. BDTX-1535 reported no body weight loss in vivo and WSD0922-FU reported no dose-related toxicities in vivo studies. Both show potential to overcome resistance to widely used Tyrosine Kinase inhibitors (Oismeritibinib for BDTX-1535 and Cetuximab for WSD0922-FU). WSD0922's low IC50 values for EGFRm and EGFRvIII inhibition, show its promise to inhibit different types of EGFR mutations while BDTX's inhibition of various EGFR mutations irreversibly offers similar promise. Both have the ability to penetrate the blood-brain barrier and increase the median survival time in vivo. With similar efficacy and safety profiles, the lack of information regarding both drugs introduces many unknowns giving it less promise than CM93 which not only offers more specific reduced negative effects toxicities on the mice but also specific inhibition values for various EGFR mutations/variants. With similar efficacy to CM93, ERASS-801 shows great potential to penetrate the BBB and inhibit EGFR with low IC50 values (1.1 nM against wild-type and EGFR, 0.3 nM against EGFRvIII) suggesting strong efficacy. Its selectivity and lack of interference with astrocytes suggest fewer negative effects or impacts on the other parts of the brain. Its efficacy and selectivity, while offering promise, do not mention the effects or potential toxicities on patients placing it below CM93 in terms of the promise. Similar to ERAS-801, Anlotinib, while showing strong efficacy with its potential to arrest the G2/M phase in cells, inhibit in vivo proliferation, and 11.53 months survival progression time shows no evidence of potential to improve patient quality of life. Its high median survival time, suggests improvements in prognosis; however, if Anlotinib, like Epitinib, comes with strong dose-related toxicities, it is likely that those toxicities may inhibit or hinder improvements in a patient's condition, limiting its promise. Osimeritib, while offering strong efficacy through its high kb value (greater than 10) and its low IC50 values (184nM for wt-EGFR, 1nM for t790M mutations, and 1.25-3 micromoles in GBM cell lines), shows limited promise despite its ability to increase the median survival time of mice by 16 days. Osimeritnib's toxic side effects and severe side taken with the results from the clinical trial evaluating the drug's effects on four patients suggest that the drug's toxicities could potentially inhibit/hinder treatment/recovery. Its negative effects lower patient quality of life while drugs such as CM93 show the potential to increase patient quality of life. Taken together, the preclinical/clinical profiles of these EGFR Tyrosine Kinase inhibitors suggest that CM93 shows the most promise followed by BDTX-1535 and WSD0922-FU, ERAS-801, and Anlotinib. Epitinb and Osimeritnib, while efficacious, lower patient quality of life, giving them less promise.

Figure 1: Enter Caption

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