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Erlotinib in Glioblastoma – A Current Clinical Perspective

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1. Introduction

Glioblastoma represents the most common primary brain tumor in adults. Despite improvements of multimodal therapy, the prognosis of this disease remains unfavorable. Thus, great efforts have been made to identify therapeutic agents directed against those specific molecular targets whose presence was shown to be associated with worse clinical outcomes. The epidermal growth factor receptor (HER1/EGFR) has been identified as one such target, and different compounds were developed to inhibit HER1/EGFR and/or its mutant form, EGFRvIII. However, clinical trials did not confirm the initial enthusiasm conveyed by promising results from experimental studies. Therefore, a therapeutic approach directed at inhibiting solely HER1/EGFR does not seem to translate into a clinical benefit. In this chapter we discuss the current therapeutic situation in the setting of glioblastoma while putting the spotlight on erlotinib, a HER1/EGFR-targeted small molecule tyrosine kinase inhibitor.

The epidermal growth factor receptor belongs to the HER family of receptors and consists of an extracellular ligand-binding site, a transmembraneous part and an intracellular tyrosine kinase (TK) domain (Wells, 1999). Docking of its ligands, *e.g.*, epidermal growth factor (EGF) or transforming growth factor- α (TGF- α), to the ligand-binding site activates the intrinsic TK. Subsequently, autophosphorylation of specific tyrosine residues within the cytoplasmic catalytic kinase domain of the receptor and initiation of cytoplasmic signaling cascades such as the ras-raf-mitogen-activated protein kinase (MAPK) pathway or the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway occur (Arteaga, 2003; Scagliotti et al., 2004). As a consequence, diverse cellular functions such as proliferation or differentiation are regulated (Wells, 1999).

HER1/EGFR overexpression or ligand-independent activation was found in various epithelial malignancies (Earp et al., 2003). The causative relationship between dysregulation of the HER1/EGFR and neoplastic disorder is explained by the affection of downstream signal transduction which results in impaired apoptosis and/or stimulation of proliferation, tumori-

genesis, angiogenesis and invasion (Halatsch et al., 2006). Dysregulated HER1/EGFR signaling may be caused by different mechanisms such as gene amplification resulting in HER1/EGFR overexpression as shown for 40-50% of glioblastoma (Salomon et al., 1995). Mutational changes of the intrinsic receptor structure constitute another mechanism that may lead to pathologically altered HER1/EGFR signaling. The so-called EGFRvIII accounts for approximately 60% of all HER1/EGFR mutants and is characterized by a constitutive activation (Frederick et al., 2000; Karpel-Massler et al., 2010). The expression of EGFRvIII was shown to confer cellular transformation and enhanced tumorigenicity (Nishikawa et al., 1994).

Despite recent improvements, the clinical efficacy of existing therapeutic modalities remains disappointing. Hence, in light of accumulating evidence for HER1/EGFR-mediated promotion of tumor growth and malignant transformation, substantial interest in the realization of HER1/EGFR-targeted therapeutic strategies developed. Small molecule tyrosine kinase inhibitors such as erlotinib (Tarceva®, Genentech Inc., San Francisco, CA, U.S.A.), a combined inhibitor of both, HER1/EGFR and EGFRvIII, are the clinically most advanced HER1/EGFR-targeted agents (Karpel-Massler et al., 2009). After promising results derived from experimental studies using erlotinib in a single agent approach were not confirmed by clinical trials, hopes now are set on the identification of other targeted agents enhancing the antineoplastic activity of erlotinib in a multi-targeted approach.

2. Current standard of care for patients with glioblastoma

Glioblastoma is the most frequently encountered astrocytic brain tumor in adults and accounts for more than 50% of all gliomas (Reardon, D.A. & Wen, 2006). The tumor rapidly infiltrates normal surrounding brain tissue. Patients with glioblastoma typically encounter tumor progression or recurrence, and median survival is only 14.6 months (Stupp et al., 2005). Neurologically safe, maximal surgical tumor resection is generally considered the first therapeutic measure for the treatment of newly diagnosed glioblastoma. However, localization of the tumor in or near eloquent brain areas will impose considerable restrictions on the radicality of the surgical procedure in order to avoid severe postoperative neurological deficits. Radiotherapy in combination with concomitant and adjuvant chemotherapy with temozolomide (Temodar®/Temodal®, Schering Corporation, Kenilworth, NJ, U.S.A.) is a viable postoperative treatment option. Whole brain irradiation (50-60 Gy) was shown by several randomized studies to increase survival by 14-36 weeks (Walker et al., 1980). While the chemotherapeutics that were initially used for the adjuvant treatment of glioblastoma were only of minor benefit, a randomized controlled trial in patients with newly diagnosed glioblastoma showed that administration of temozolomide concomitantly with and subsequently to radiation therapy significantly increased two-year survival from 10.4% to 26.5% and median survival from 12.1 to 14.6 months when compared to adjuvant radiation therapy alone (Stupp et al., 2005). With this study, a new therapeutic standard was established. Nevertheless, a progression-free survival and overall survival of only 6.9 and 14.6 months, respectively, strongly emphasize that further improvement of glioblastoma therapy is urgently needed.

Currently, a standard of care for the treatment of *recurrent* glioblastoma does not exist. In general, repeated gross tumor resection should be attempted. However, this strategy might not always be appropriate, especially when considering the fact that progressive tumor invasion may significantly increase the risk of provoking neurological deficits. Chemotherapeutics that were especially used before the temozolomide era upon tumor relapse include nitrosoureas such as carmustine (BCNU) or lomustine (CCNU) and alkylating agents such as procarbazine. However, the antineoplastic activity of these agents in clinical trials was shown to be rather modest (Rodriguez et al., 1989; Newton et al., 1990; Brandes et al., 2004). Irinotecan (Camptosar[®], Pfizer Pharmaceuticals, New York, NY, U.S.A.), an inhibitor of topoisomerase I (Raymond et al., 2003; Reardon, DA et al., 2005), or bevacizumab (Avastin[®], Genentech Inc., San Francisco, CA, U.S.A.), a humanized monoclonal antibody targeted to vascular endothelial growth factor (VEGF), represent two compounds that have been introduced more recently for the treatment of recurrent glioblastoma and that showed anti-glioblastoma activity (Stark-Vance, 2005).

3. Why interfering with HER1/EGFR or EGFRvIII-mediated signaling?

Given the poor therapeutic efficacy of current treatment measures for glioblastoma, the need for different therapeutic strategies is evident. HER1/EGFR is the most frequently amplified gene in glioblastoma, and its overexpression was found in more than half of these tumors which renders HER1/EGFR an outstanding therapeutic target (Salomon et al., 1995). Experimental studies show that HER1/EGFR stimulates tumor growth, invasion and migration (Lund-Johansen et al., 1990). In addition, data from clinical studies suggest that HER1/EGFR amplification is related to decreased overall survival and worse prognosis in patients with glioblastoma (Lund-Johansen et al., 1990; Shinojima et al., 2003).

EGFRvIII represents the most common mutant form of HER1/EGFR and is characterized by constitutive TK activity independent of ligand-binding (Batra et al., 1995; Frederick et al., 2000). Analysis of the expression of HER1/EGFR and EGFRvIII in bioptic glioblastoma specimens suggests concurrent overexpression of both EGFRvIII and HER1/EGFR in most of the tumors (Biernat et al., 2004). Moreover, in an experimental study using a murine model of human glioma xenografts, EGFRvIII expression was found to be related to increased proliferation, inhibition of apoptosis, and tumor formation (Nishikawa et al., 1994; Nagane et al., 1996). Other studies showed similar results and identified activation of the MAPK/ERK1/2 and PI3-K/Akt pathways as driving forces of cellular proliferation and tumor progression (Moscatello et al., 1998; Klingler-Hoffmann et al., 2001; Klingler-Hoffmann et al., 2003). In addition, in a murine orthotopic xenograft model of glioblastoma, administration of a monoclonal antibody targeting EGFRvIII (mAb 806) was shown to cause a significant decrease of tumor growth, increase of apoptosis and prolongation of survival (Mishima et al., 2001).

The tumor-specific properties of EGFRvIII have also lead to the development of EGFRvIII-targeted vaccines in order to provoke an immunologic response against EGFRvIII-bearing glioblastoma cells. Potential antitumor efficacy of EGFRvIII-targeted vaccines had been shown

by experimental studies. Immunization of mice with transfected allogenic 300.19/EGFRvIII cells was reported to induce a major histocompatibility complex class I-restricted response against EGFRvIII-bearing syngeneic B16-F10 melanoma or 560 astrocytoma cells that were implanted intracranially (Ashley et al., 1997). In addition, vaccinated animals were shown to have a significantly longer median survival upon intracranial tumor challenge when compared to controls. Similar findings were reported for mice that were vaccinated with PEP-3-KLH (rindopepimut, CDX-110, Celldex Therapeutics, Needham, MA, U.S.A.), a conjugate of a peptide comprising the tumor-specific mutated segment of EGFRvIII (PEP-3) and keyhole limpet hemocyanin (KLH) (Heimberger et al., 2003). In this study, C3H mice received vaccination with 100 µg of PEP-3-KLH 8, 6 and 2 weeks prior to intracerebral administration of K1735 murine melanoma cells that were transfected with a murine homologue of the human EGFRvIII, and additional vaccination 4 days after intracranial implantation of the tumor cells. A more than 173% longer survival time was shown for mice vaccinated with PEP-3-KLH when compared to mice receiving only KLH. Moreover, mice with already established intracranial tumors that were treated with a single dose of the PEP-3-KLH vaccine 4 days after administration of the transfected K1735 cells had a 26% increase of median survival. Based on these promising preclinical data, several clinical trials were conducted. In two phase II trials, vaccination with PEP-3-KLH was examined in patients with EGFRvIII-expressing newly diagnosed glioblastoma. In the ACTIVATE trial, 18 patients underwent gross-total tumor resection prior to radiotherapy and concurrent chemotherapy with temozolomide followed by vaccination with PEP-3-KLH bi-weekly for 3 doses and continued monthly until progression (Sampson et al., 2010). The data were compared to a matched historical control group (n=17). The median progression-free survival and overall survival were 14.2 months and 26 months, respectively, versus 6.3 months and 15 months, respectively, in the control group. Notably, the patients who developed an EGFRvIII-specific antibody response had an overall survival of 47.7 months (n=6) compared to an overall survival of 22.2 months in patients lacking a specific antibody response (n=8). In the ACT II trial, 22 patients who met the same inclusion criteria as for the ACTIVATE trial received the same therapeutic regimen except for an additional treatment with temozolomide either at a dose of 200 mg/m² for 5 days of a 28-day cycle or at a dose of 100 mg/m² for 21 days of a 28-day cycle in conjunction with the vaccination therapy (Heimberger et al., 2009). Combination therapy of PEP-3-KLH and temozolomide was well tolerated, and a favorable median overall survival of 20.5 months was reported. An additional phase II study (ACT III) was conducted by Celldex Therapeutics. Sixty-five patients with newly diagnosed EGFRvIII-positive glioblastoma were enrolled in this single-arm multicenter study which was initially planned as a phase IIb/III randomized two-arm trial but had to be transformed into a single-arm design due to withdrawal of consent to participate in this study by 14 of the 16 patients that were randomized to the control group. In this study, a median overall survival of 21.8 months was reported which encouraged Celldex to launch two more studies: ACT IV, a randomized controlled phase III study in patients with newly diagnosed EGFRvIII-positive glioblastoma and ReACT, a phase II study in patients with EGFRvIII-positive recurrent glioblastoma. The final results of these studies are pending. However, what needs to be taken into account is the fact that only a part of the glioblastomas

express EGFRvIII. For this subset of patients, however, vaccination with PEP-3-KLH might confer a significant clinical benefit.

4. Erlotinib for the treatment of glioblastoma

HER1/EGFR TK inhibitors such as erlotinib compete with adenosine triphosphate and reversibly bind to the intracellular catalytic TK domain of HER1/EGFR or EGFRvIII thus inhibiting autophosphorylation of the receptor as well as further downstream signaling (Halatsch et al., 2006). In preclinical studies, erlotinib was shown to exert a variety of relevant antineoplastic effects in the setting of glioblastoma. Lal *et al.* showed that exposure of transformed D54-MG glioblastoma cells (D54-EGFRvIII) to 20 μ M of erlotinib resulted in significant downregulation of certain genes encoding pro-invasive proteins and in significant inhibition of the invasiveness of D54-EGFRvIII cells (Lal et al., 2002). In a different study, erlotinib was shown to significantly reduce cellular viability of six human glioblastoma-derived tumor-initiating cell lines when given at a concentration of 5 μ M (Griffero et al., 2003). This effect was shown to be in concordance with decreased EGF-induced phosphorylation of HER1/EGFR and subsequent inhibition of the MAPK signaling pathway by reduced phosphorylation of ERK1/2. Moreover, Halatsch *et al.* showed that the extent of erlotinib-mediated inhibition of anchorage-independent growth of glioblastoma-derived cell lines correlates inversely with the cellular capability to induce HER1/EGFR mRNA, emphasizing the important role of HER1/EGFR in the pathogenesis of glioblastoma (Halatsch et al., 2004).

Based on the positive findings reported by preclinical studies, much hope was set on the clinical application of erlotinib in glioblastoma patients. To date, several published studies have examined the effects of erlotinib on patients with recurrent or newly diagnosed glioblastoma. In phase I trials, erlotinib exhibited a reasonable safety profile and was generally well tolerated (Krishnan et al., 2006; Prados et al., 2006). In addition, EIAEDs were shown to accelerate drug metabolism of erlotinib which requires dose modification of erlotinib or a change in the antiepileptic drug regimen (Stupp et al., 2006). In terms of clinical efficacy, Raizer *et al.* examined the effects of erlotinib applied at a dose of 150 mg/d on 42 patients with recurrent glioblastoma and 43 patients with non-progressive glioblastoma following radiotherapy in a phase II trial (Raizer et al., 2010). For the patients with recurrent glioblastoma, median overall survival was reported as 6 months and median progression-free survival as only 2 months. Median overall survival and the 12-month overall survival were reported as 14 months and 57%, respectively, for the patients with non-progressive glioblastoma after radiotherapy. Thus, this study did not show a significant improvement of the clinical outcome attributable to the treatment with erlotinib in patients with recurrent glioblastoma or non-progressive glioblastoma after radiotherapy. However, Yung *et al.* showed that median overall survival and 6-month progression-free survival of 48 patients with recurrent glioblastoma who were treated with erlotinib reached or exceeded historical values for patients receiving chemotherapy for recurrent glioblastoma (Yung et al., 2010). Notably, this study was discontinued due to an insufficient number of responses after a planned interim analysis, and a control group was not included. Van den Bent *et al.* showed in a randomized controlled phase II trial that only 11.4%

of 54 patients with recurrent glioblastoma who were treated with erlotinib remained free of progression after 6 months compared to 24.1% of patients in the control group who received either temozolomide or BCNU (van den Bent *et al.*, 2009). Moreover, median overall survival was shown to be similar across the treatment groups (7.7 months for the erlotinib group versus 7.3 months for the temozolomide/BCNU group).

Thus, taking erlotinib to clinical application in a monotherapeutic approach has so far fallen short of expectations. As a logical consequence, the question rose if erlotinib might provide a therapeutic benefit when combined with conventional radiochemotherapy. As outlined in detail in the following, the addition of erlotinib to a combined regimen of temozolomide and radiotherapy did not meet enthusiastic expectations and even raised the suspicion of inducing serious toxic side effects. In a phase I/II trial, Brown *et al.* studied the clinical efficacy of a combined treatment with erlotinib, temozolomide and radiotherapy in 89 patients with newly diagnosed glioblastoma (Brown *et al.*, 2008). Erlotinib was administered at a dose of 150 mg/d starting 1 week prior to fractionated radiotherapy (60 Gy) and chemotherapy with temozolomide at a dose of 75 mg/m²/d. After radiotherapy, treatment with erlotinib was continued and accompanied by up to six cycles of temozolomide at a dose of 200 mg/m²/d for 5 days every 4 weeks. Median overall survival was reported as 15.7 months, and comparison to the “radiotherapy plus temozolomide arm” from the European Organisation for Research and Treatment of Cancer 26981/22981-National Cancer Institute of Canada trial revealed no significant difference (Mirimanoff *et al.*, 2006). In contrast, Prados *et al.* showed in another phase II trial which included 65 patients with newly diagnosed glioblastoma or gliosarcoma receiving treatment with erlotinib and fractionated radiotherapy with concomitant and adjuvant temozolomide a marked improvement of median progression-free and overall survival (8.2 months and 19.3 months, respectively) when compared to a combined historical control (Prados *et al.*, 2009). Rather disturbing results were reported from a phase II study published by Peereboom *et al.* (Peereboom *et al.*, 2010). Twenty-seven patients with newly diagnosed glioblastoma were treated with a maximum dose of 150 mg/d erlotinib and radiotherapy (60 Gy in 30 fractions) with concurrent (75 mg/m²/d for 42 days) and subsequent (12 four-week cycles comprising each 5 days of 150–200 mg/m²/d) temozolomide. This trial was terminated preterm because of unacceptable toxicity and lack of efficacy. Median progression-free and overall survival were 2.8 months and 8.6 months, respectively. Twenty-two patients (67%) had progressive disease, and 4 patients (15%) had an adverse event. Three deaths occurred that were reported to be treatment-related. One patient died of pneumocystis carinii pneumonia despite treatment with pentamidine. Similarly, Brown *et al.* reported two cases of fatal pneumocystis carinii pneumonia (Brown *et al.*, 2008). Again high expectations were disappointed.

5. Future perspectives

None of the therapeutic strategies evaluated so far involving erlotinib either alone or in combination with conventional adjuvant therapies represent a major success for the treatment of glioblastoma. Therefore, changing the general strategy towards a combined approach with

HER1/EGFR TK inhibitors and other targeted agents might provide a more pronounced clinical benefit for patients suffering from this disease.

In experimental studies, favorable effects were observed for the inhibition of downstream key regulators such as mammalian target of rapamycin (mTOR) and PI3-K in addition to the treatment with HER1/EGFR TK inhibitors. For example, phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-deficient U87MG and SF295 glioblastoma cells that were subjected to a combined treatment with erlotinib and rapamycin, an mTOR inhibitor, showed significantly increased antiproliferative effects when compared to cells receiving erlotinib alone (reduction of proliferation by 38% versus 14%, respectively, in PTEN-deficient SF295 cells) (Wang et al., 2006). Another experimental study showed similar findings (Fan et al., 2007). In this study, additional inhibition of PI3-K using a dual mTOR/PI3-K inhibitor (PI-103) resulted in even more pronounced antiproliferative efficacy in PTEN-mutant glioma cells when combined with erlotinib in comparison to erlotinib combined with either mTOR or PI3-K inhibition. In a clinical pilot study including 22 patients with recurrent glioblastoma, Doherty *et al.* showed that patients treated with erlotinib or gefitinib in combination with sirolimus (rapamycin, Rapamune®, Wyeth Pharmaceuticals Inc., Ayerst, PA, U.S.A.) had a 6-month progression-free survival of 25% (Doherty et al., 2006). In addition, 32 patients with recurrent glioblastoma were treated with 150 mg/d (450 mg/d when on EIAEDs) of erlotinib and 5 mg/d (10 mg/d when on EIAEDs) of sirolimus in a phase II clinical trial (Reardon, DA et al., 2010). In this study, however, antitumor activity was negligible with no complete or partial responses and a median progression-free survival and a median overall survival of 6.9 weeks and 33.8 weeks, respectively.

Carboplatin was shown to have some antineoplastic activity in patients with recurrent glioblastoma and anaplastic astrocytoma (Prados et al., 1996). De Groot *et al.* examined the therapeutic efficacy of a combined regimen of the cytotoxic agent carboplatin and erlotinib in recurrent glioblastoma (de Groot et al., 2008). In this phase II study, 43 patients were treated with erlotinib at a dose of 150 mg/d that was escalated up to 200 mg/d as tolerated in combination with carboplatin administered once every 4 weeks at doses modified according to renal function. While this regimen was well tolerated, antineoplastic activity was modest. Median progression-free survival and overall survival were 9 weeks and 30 weeks, respectively, and only one partial response was achieved.

Tumor angiogenesis has been shown to be a crucial process for the growth and metastasis of solid tumors (Heath & Bicknell, 2009). The combined treatment with bevacizumab and HER1/EGFR-targeted agents has been evaluated by two recent phase II studies in the setting of recurrent high-grade glioma. Forty-three patients with recurrent glioblastoma were treated with 10 mg/kg bevacizumab, 125 or 340 mg/m² irinotecan (dose depending on EIAED comedication), and cetuximab, a monoclonal antibody targeted at HER1/EGFR (loading dose of 400 mg/m² followed by weekly administration of 250 mg/m²) (Hasselbalch et al., 2010). Two complete responses (5%) and 9 partial responses (21%) were observed. Stable disease was achieved in 17 patients (40%). Median overall survival and progression-free survival were 30 weeks and 16 weeks, respectively. In the second phase II trial, twenty-five patients with recurrent primary glioblastoma were treated with 10 mg/kg bevacizumab every 2 weeks and

concomitantly with 200 or 500 mg/d erlotinib (dose depending on EIAED comedication) (Sathornsumetee et al., 2010). Median overall survival and 6-month progression-free survival were reported as 42 weeks and 28%, respectively. Moreover, radiographic response was observed for 48% of the glioblastoma patients. Unfortunately, appropriate control groups were not included in this study. However, comparison to historical data of patients treated with bevacizumab only showed a similar progression-free survival and radiographic response. Thus, additional inhibition of HER1/EGFR does not appear to greatly increase the clinical efficacy when combined with bevacizumab in recurrent glioblastoma.

Overall, while promising results were reported by some early phase clinical trials evaluating the therapeutic efficacy of a combined treatment with HER1/EGFR TK inhibitors and other agents, further studies with a randomized controlled design and a larger patient population will be needed to make a final judgment.

Considering the fact that a multitude of different converging and diverging signaling pathways are involved in the maintenance and progression of glioblastoma, the failure of targeting of a single molecular determinant such as HER1/EGFR does not come as a surprise. Moreover, limiting the focus on therapeutic strategies targeted at already known oncogenic signaling pathways might impede further progress. Therefore, the search for novel targets is crucial in order to allow for a more efficient treatment of glioblastoma. A bioinformatic approach might help to identify molecules that are potentially relevant as tumor-driving forces. Halatsch *et al.* identified a panel of genes overexpressed in glioblastoma cells with an erlotinib-resistant phenotype by RNA microarray analysis of which some have been confirmed as promising co-targets *in vitro* (Halatsch et al., 2009; Karpel-Massler et al., 2013). Hopefully, in the future, broad molecular tumor screening will lead to the identification of individual molecular signatures amenable to successful multitargeting. Since these molecular signatures are likely to change during therapy, repeated therapeutic adjustments will be necessary based on updated molecular characteristics of the tumor (Cloughesy & Mischel, 2011). This kind of dynamic personalized therapy of glioblastoma will likely involve HER1/EGFR-targeted therapeutics such as erlotinib at one point.

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