1. Introduction

In adults, glioblastoma multiforme (GBM) represents the most common malignant brain tumor (Karpel-Massler et al., 2009). Unfortunately, even with the best available standard of care, patients with this disease still face a poor clinical outcome (Stupp et al., 2005). Based on the discovery of molecular targets that are involved in tumorigenesis and maintenance of the malignant cellular phenotype, new therapeutic strategies were developed. In about half of all glioblastomas, the epidermal growth factor receptor (HER1/EGFR) was shown to be amplified and overexpressed, rendering it an outstanding target in this disease (Libermann et al., 1985; Ekstrand et al., 1991). Thus, great interest was generated in the creation of HER1/EGFR-targeted agents. The clinically most advanced compounds that were developed to target HER1/EGFR for the treatment of GBM are small-molecule tyrosine kinase (TK) inhibitors such as erlotinib (Tarceva®, Genentech Inc., San Francisco, CA, U.S.A.). TK inhibitors reversibly bind to the intracellular catalytic TK domain of HER1/EGFR followed by the inhibition of autophosphorylation of the receptor as well as further downstream signaling involving phosphatidylinositol 3-kinase/murine thymoma viral oncogene homolog (PI3-K/AKT) and mitogen-activated protein kinase (MAPK) pathways (Arteaga, 2001; Busse et al., 2000; Scaglotti et al., 2004). Erlotinib does not only inhibit HER1/EGFR but also EGFRvIII, the most frequent mutant form of HER1/EGFR which is characterized by ligand-independent activation (Chu et al., 1997). In experimental studies, erlotinib was shown to inhibit the expression of genes encoding pro-invasive proteins and to significantly diminish EGFRvIII expression in transfected glioblastoma cells (Lal et al., 2002). Moreover, the extent of erlotinib-mediated inhibition of anchorage-independent growth of glioblastoma-derived cell lines was shown to correlate inversely with the cellular capability to induce HER1/EGFR mRNA (Halatsch et al., 2004). However, clinical studies examining the therapeutic efficacy of erlotinib in the setting of GBM have so far failed to prove a therapeutic benefit (Raizer et al., 2010; van den Bent et al., 2009). In a randomized, controlled phase II trial, only 11.4% of the patients with recurrent glioblastoma treated with erlotinib were free of progression after 6 months compared to 24.1% of the patients treated with temozolomide or carmustine (van den Bent et al., 2009). In addition, overall survival of the two treatment groups was found to be similar (7.7 months for the erlotinib group versus 7.3 months for the temozolomide/carmustine group).
In addition, several studies examined the therapeutic efficacy of erlotinib when combined with standard radiochemotherapy (Brown et al., 2008; Peereboom et al., 2010; Prados et al., 2009). Overall, the results of these studies appear unfavorable and discourage the use of erlotinib in combination with temozolomide and radiotherapy.

Combined inhibition of HER1/EGFR and downstream key regulators such as mammalian target of rapamycin (mTOR) and PI3-K represents another approach that has been evaluated. In an experimental study, combined treatment with erlotinib and rapamycin, an mTOR inhibitor, resulted in significantly increased anti-proliferative effects on phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-deficient U87 and SF295 glioblastoma cells when compared to cells receiving erlotinib alone (Wang et al., 2006). Moreover, additional inhibition of PI3-K using a dual mTOR/PI3-K inhibitor (PI-103) was shown to result in even more pronounced antineoplastic effects when combined with erlotinib in comparison to erlotinib combined with either mTOR or PI3-K inhibition (Fan et al., 2007). In the clinical setting, in a pilot study, a 6-month progression-free survival of 25% was reported for 22 recurrent glioblastoma patients who were treated with erlotinib or gefitinib in combination with sirolimus (rapamycin, Rapamune®, Wyeth Pharmaceuticals Inc., Ayerst, PA, U.S.A.) (Doherty et al., 2006). In a phase II clinical trial, no complete or partial responses were observed in 32 patients with recurrent glioblastoma treated with erlotinib and sirolimus in combination (Reardon et al., 2010). Median progression-free survival and median overall survival were shown to be 6.9 weeks and 33.8 weeks, respectively.

The therapeutic efficacy of a combined treatment with erlotinib and bevacizumab, a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody, on patients with recurrent high-grade glioma was recently evaluated by a phase II clinical trial (Sathornsumetee et al., 2010). For glioblastoma patients, median 6-month progression-free survival and overall survival were reported as 28% and 42 weeks, respectively. In addition, for 48% of the glioblastoma patients radiographic response was reported. However, progression-free survival and radiographic response were similar to historical data of patients treated with bevacizumab alone.

In conclusion, current data suggest that the targeted therapeutic approach against HER1/EGFR may require a synergistic drug combination strategy involving other targeted agents in addition to HER1/EGFR-targeted TK inhibitors. This chapter focuses on innovative therapeutic strategies combining HER1/EGFR-targeted TK inhibitors with novel agents aiming to enhance the antineoplastic effect exerted by erlotinib. Most of the agents discussed in this chapter have not been evaluated for the treatment of GBM yet but constitute worthy candidates for further evaluation in this setting.

2. Promising candidates for enhancing the antineoplastic activity of erlotinib

2.1 Inhibitors of Kit

Kit (CD117) is a receptor tyrosine kinase which is related to the macrophage colony-stimulating factor receptor (c-fms) and to the platelet-derived growth factor receptor (PDGFR) (Heinrich et al., 2002; Yarden et al., 1987). Its physiologic ligand is stem cell factor, also known as mast cell factor or steel factor (Nocka et al., 1990). Ligand-binding is followed by receptor dimerization, autophosphorylation and activation of downward signaling
pathways such as MAPK, JAK/STAT and PI3K/AKT pathways (Duensing et al., 2004; Mol et al., 2003). Kit was found to be expressed by a variety of cell types including the interstitial cells of Cajal, mast cells, haemopoietic progenitor cells or melanocytes (Natali et al., 1992; Nocka et al., 1989; Turner et al., 1992; Ishikawa et al., 1997), and its dysregulation has been associated with the pathogenesis of various different human malignancies (Duensing & Duensing, 2010; Heinrich et al., 2002; Woodman & Davies, 2010).

In glioma, about 75% of the tumors were reported to express Kit (Cetin et al., 2005). Interestingly, amplification and expression of Kit were shown to be significantly higher in high-grade gliomas when compared to low-grade gliomas (Joensuu et al., 2005; Puputti et al., 2006). These findings suggest that Kit may be involved in the tumorigenesis and malignant transformation of gliomas.

Different mutational changes of Kit have been described, such as the D816V mutation conferring an enhanced catalytic activity and an increased affinity for adenosine triphosphate or small in-frame deletions or insertions in the inhibitory juxtamembrane region causing ligand-independent activation of the receptor (Heinrich et al., 2002). Such genetic alterations of Kit have not been reported for gliomas yet. In other human malignancies including gastrointestinal stromal tumors (GIST) or mast cell leukemia, these mutations are quite frequently encountered (Duensing & Duensing, 2010). As a consequence, Kit-targeted agents such as imatinib mesylate (Gleevec®, Novartis, East Hanover, NJ, U.S.A.), a small molecule tyrosine kinase inhibitor, were developed. Imatinib was shown to significantly increase median overall survival of patients with GIST from 19 months to more than 50 months (Blanke et al., 2008a, 2008b; Gold et al., 2007).

Imatinib was shown to inhibit the proliferation of certain glioblastoma cell lines in vitro (Hagerstrand et al., 2006). In another experimental study, imatinib significantly inhibited the proliferation of human U87 glioblastoma cells and significantly increased the radiosensitivity of this glioma cell line in vitro and in vivo (Oertel et al., 2006). However, in clinical phase I and II trials, imatinib was shown to exert only moderate antitumor activity (Razis et al., 2009; Wen et al., 2006). In a phase I/II study, 34 patients with glioblastoma were treated with imatinib monotherapy at a dose of 800 mg/d (Wen et al., 2006). Progression-free survival at 6 months was only 3%, no patient achieved complete response and only 6 patients reached stable disease while 2 patients showed partial response. In a different phase II study, 20 patients with glioblastoma were diagnosed by tumor biopsy and treated with 400 mg imatinib administered twice a day for a period of 7 days prior to re-biopsy or tumor resection. Molecular examination of the tumor specimens showed that treatment with imatinib did not significantly change Ki67 expression, suggesting that treatment with imatinib did not affect tumor proliferation (Razis et al., 2009).

The fact that inhibition of Kit and co-targeted tyrosine kinases such as the platelet-derived growth factor (PDGFR), alone, does not sufficiently suppress tumor growth in glioblastoma might be explained by co-activation of other growth factor receptors such as HER1/EGFR. Cellular signaling derived from activated HER1/EGFR might interfere with the inhibitory effects of imatinib on Kit and preserve the cancerous cellular phenotype. In this regard, additional inhibition of HER1/EGFR by erlotinib might prove beneficial in terms of a more pronounced therapeutic efficacy. To date, no experimental or clinical data exist with respect to a combined therapeutic approach with erlotinib and an inhibitor of Kit in this disease.
However, in the setting of recurrent glioblastoma, encouraging results were reported by a phase II study evaluating the therapeutic efficacy of a combination therapy with imatinib and hydroxyurea, a ribonucleotide reductase inhibitor (Reardon et al., 2005). Median overall survival, progression-free survival at 6 months and median progression-free survival were 48.9 weeks, 27% and 14.4 weeks, respectively. Nine percent of the patients achieved radiographic response and 42% had stable disease within a median follow-up of 58 weeks.

In conclusion, despite rather discouraging results of Kit inhibitors used as single agent therapies in clinical trials, Kit inhibitors may prove as valuable partners for the treatment of glioblastoma when combined with other agents such as erlotinib.

2.2 Histone deacetylase (HDAC) inhibitors

In humans, 18 HDACs with different tissue distributions and functions have been identified. Class I, IIA and IV HDACs are found in the brain (Marsoni et al., 2008). HDACs induce an increased packaging of chromatin and subsequent suppression of transcription (Lane & Chabner, 2009; Svechnikova et al., 2008). Modulation of the chromatin state through enzymatic histone modification may alter the transcriptional activity of genes involved in cell cycle control which is considered to be an important factor in tumorigenesis (Yoo & Jones, 2006). HDACs were shown to be overexpressed in a variety of human cancers including breast cancer, hematologic malignancies, colorectal cancer or pancreatic carcinoma (Lane & Chabner, 2009; Nakagawa et al., 2007). Moreover, inhibition of HDAC was shown to induce apoptosis by different mechanisms (Insinga et al., 2005; Nebbioso et al., 2005; Zhang et al., 2006; Zhao et al., 2005). In addition, inhibition of HDAC was shown to disrupt the function of the heat shock protein 90 which promotes the degradation of oncogenic proteins such as HER1/EGFR, AKT or BCR-ABL (Bolden et al., 2006; Kovacs et al., 2005; Whitesell & Lindquist, 2005). Thus, HDAC inhibition may constitute a promising approach in cancer therapy.

Romidepsin is a bicyclic peptide that was shown to have anti-microbial, immunosuppressive and antineoplastic activities (Ritchie et al., 2009; Ueda et al., 1994). It was shown to selectively inhibit deacetylases such as HDAC or tubulin deacetylase and represents one of the best studied HDAC inhibitors in the clinical setting (Yoo & Jones, 2006). The clinical experience with HDAC inhibitors is most advanced for the treatment of cutaneous T-cell lymphoma (CTCL) and hematologic malignancies (Lane & Chabner, 2009; Prince et al., 2009). In an early phase I trial, 10 patients with chronic lymphocytic leukemia (CLL) and 10 patients with acute myeloid leukemia (AML) were treated with romidepsin at a dose of 13 mg/m² on day 1, 8, and 15 of a 4-week cycle (Byrd et al., 2005). Despite absence of formal complete or partial responses, all seven CLL patients who had elevated leukocyte counts at the beginning of the therapy showed an improvement in peripheral leukocyte counts, while in the AML group one patient developed a tumor lysis syndrome. Moreover, in a phase II clinical trial, treatment with romidepsin resulted in a decrease of bone marrow blasts in 5 of 7 patients with AML (Odenike et al., 2008). However, within a month after achieving their best response towards romidepsin, these 5 patients developed disease progression. In the clinical setting of refractory CTCL, two phase II clinical trials examining the therapeutic efficacy of romidepsin were recently published (Piekarz et al., 2009; Whittaker et al., 2010). In 71 patients with treatment-refractory or advanced CTCL treated with a starting dose of 14 mg/m² romidepsin administered as a 4-h
intravenous infusion on days 1, 8, and 15 of a 28-day cycle, an overall response rate of 34% was found (Piekarz et al., 2009). Partial response, complete response and stable disease were reported as 26%, 7% and 38%, respectively. Similar findings were reported by a different group (Whittaker et al., 2010). Overall, the safety profile of romidepsin has been favorable, and serious adverse events were shown to be rare (Byrd et al., 2005; Odenike et al., 2008; Piekarz et al., 2009; Prince et al., 2009; Whittaker et al., 2010).

There is no clinical data on romidepsin in glioblastoma and only little data on other HDAC inhibitors in this setting. However, in experimental studies, a radiosensitizing effect was observed in glioblastoma cells treated with HDAC inhibitors. The fraction of surviving SF539 and U251 glioblastoma cells that were treated with valproic acid (VA), an anticonvulsive drug known to also inhibit HDACs, and radiation was significantly lower in comparison to cells that were treated with radiation only (Camphausen et al., 2005). Moreover, in a murine heterotopic U251 xenograft model, treatment with VA and irradiation was shown to result in a significantly greater delay of tumor growth when compared to animals treated with either VA or irradiation alone. These findings were confirmed by other groups using different HDAC inhibitors (Entin-Meer et al., 2007; Lucio-Eterovic et al., 2008). In another experimental study, treatment with the HDAC inhibitor trichostatin A or 4-phenyl-butyrate was shown to induce cellular differentiation of different human glioblastoma cell lines (Svechnikova et al., 2008). In addition, both HDAC inhibitors were shown to inhibit cellular proliferation and to promote apoptosis in glioblastoma cell lines.

In the setting of glioblastoma, so far only one experimental study was published examining the effects of romidepsin. In that study, treatment with romidepsin at a concentration of 1 ng/ml was shown to significantly reduce proliferation of T98G, U251MG and U87MG glioblastoma cells (Sawa et al., 2004). In addition, U251MG cells treated with romidepsin were shown to be significantly less invasive when compared to controls. Moreover, in a heterotopic xenograft model, mice treated with romidepsin were shown to have significantly reduced tumor growth of subcutaneously inoculated EGFRvIII-bearing U87MG glioblastoma cells.

Both erlotinib and romidepsin are promising anticancer agents fitting a reasonable safety profile. However, further studies are needed to elucidate if combining the antineoplastic effects of erlotinib and HDAC inhibitors such as romidepsin may result in a significant improvement of the current clinical course of glioblastoma.

### 2.3 Vascular disrupting agents

Tumor angiogenesis stands for cancers’ development of their own blood supply. This process was found to be crucial for the growth and metastasis of solid tumors and can be achieved by different mechanisms such as sprouting angiogenesis, recruitment of bone marrow-derived endothelial progenitor cells or the longitudinal splitting of existing blood vessels called intussusception (reviewed in Heath & Bicknell, 2009).

Different anti-angiogenic agents were developed for the treatment of human malignancies including high-grade glioma. One such agent is bevacizumab (Avastin®, Genentech Inc., San Francisco, CA, U.S.A.), a humanized monoclonal antibody targeted to VEGF. Numerous clinical studies were conducted evaluating the therapeutic efficacy of bevacizumab in
glioblastoma. In a phase II study, 20 of 35 patients (57%) with recurrent glioblastoma who were treated with bevacizumab in combination with irinotecan showed at least partial response. The 6-month progression-free survival and 6-month overall survival rates were 46% and 77%, respectively (Vredenburgh et al., 2007). Similar findings were reported for patients with recurrent World Health Organization (WHO) grade III gliomas (Desjardins et al., 2008). More recently, Friedman et al. reported the results of a phase II multicenter clinical trial (BRAIN) studying a larger patient population (Friedman et al., 2009). In this study, 167 patients with recurrent glioblastoma were randomly assigned to either treatment with bevacizumab alone (n=85) or in combination with irinotecan (n=82). Median overall survival was 9.2 months and 8.7 months, respectively, 6-month progression-free survival rates were 42.6% and 50.3%, and objective response rates were 28.2% and 37.8%, respectively.

The tumor blood supply may not only be therapeutically attacked by anti-angiogenic means inhibiting the formation of new tumor-supplying blood vessels, but also by destroying already existing tumor blood vessels. The combretastatins are small molecule microtubule-depolymerising agents which cause selective disruption of the tumor-supplying vasculature. The best studied member of this group of agents is represented by CA4P (Zybrestat™, Oxigene Inc., Lund, Sweden).

The blood supply of spontaneous and ortho- and heterotopically transplanted rodent tumors as well as human xenografted tumors was shown to be significantly reduced within 10-20 min after application of CA4P, an effect lasting for up to 24 hrs in some tumors (Kanthou & Tozer, 2007; Tozer et al., 2001). However, despite the fact that a single-dose application of CA4P was shown to induce abundant tumor necrosis within a short period of time, cells in the outer rim of the tumor survived (Dark et al., 1997; Tozer et al., 2001). The cells in this niche may continue or restart to grow causing tumor recurrence. In a heterotopic rat glioma model, blood flow in subcutaneous tumors dropped to about half of the initial tumor blood flow during the first 110 min after administration of CA4P (Eikesdal et al., 2000). However, treatment with CA4P at a dose of 50 mg/kg did not significantly affect tumor growth in comparison to controls. Remarkably, when the treatment with CA4P preceded a hyperthermic treatment by 3 hrs, tumor growth was significantly more delayed when compared to animals receiving CA4P immediately before hyperthermia or animals subjected to hyperthermic treatment alone. In conclusion, if applied at the right time, treatment with CA4P may increase thermally induced antitumor activity.

To date, there are no clinical studies examining the effects of CA4P in glioblastoma. However, CA4P was shown to diminish perfusion and blood flow in different advanced solid tumors (Dowlati et al., 2002; Rustin et al., 2003; Stevenson et al., 2003). In addition, some patients were reported to have experienced a notable clinical benefit from the treatment with CA4P. Complete response was reported for a patient with anaplastic thyroid cancer. This patient was free of disease for more than 5 years. Another patient suffering from fibrosarcoma achieved partial response.

Aiming at the elimination of viable tumor cells remaining at the periphery of the tumor despite treatment with VDAs, a therapeutic approach was attempted combining VDAs with radiotherapy or conventional chemotherapy. Eight patients with advanced non-small cell lung cancer (NSCLC) were treated with radiotherapy (27 Gy) and CA4P at a dose of 50 mg/m² starting after the second fraction of radiotherapy (Ng et al., 2007). The tumor blood
volume was shown to be reduced by 22.9% at 4 hrs after application of CA4P and by 29.4% after 72 hrs. Moreover, the decrease in blood volume was shown to be more pronounced at the outer rim of the tumor than at its center (51.4% vs 22.8%). These findings suggest that the antivascular effect exerted by CA4P can be enhanced by radiotherapy in the setting of NSCLC. In another study, CA4P was applied for the treatment of patients with different advanced cancers refractory to standard therapy 18-22 hrs prior to a single-agent treatment with paclitaxel or carboplatin or combination therapy with paclitaxel and carboplatin in sequential order (Rustin et al., 2010). A formal response was noted in 7 of 18 patients with ovarian cancer, primary peritoneal carcinoma, or cancer of the fallopian tube. Partial remission was achieved in another 3 out of 30 patients with non-ovarian cancer. Thus, this combinatorial regimen displays antitumor activity in patients with difficult-to-treat cancers.

Overall, VDAs are promising anticancer agents and might provide an additional benefit when combined with other antineoplastic drugs. Other therapeutics administered in addition to VDAs might be trapped in the tumor tissue due to the shut-down of tumor blood flow. Thereby, tumor cells might not only die secondary to ischemia, but surviving cells in the outer rim of the tumor may also be eliminated. This way, tumor regrowth might be retarded or prevented. At this point, there is no data on the therapeutic efficacy of a combined treatment with erlotinib and VDAs for the treatment of glioblastoma. Further studies are warranted to examine the overall antineoplastic effect of a combined treatment with erlotinib and a VDA in glioblastoma.

3. Conclusion

Unfortunately, in glioblastoma, HER1/EGFR-targeted small-molecule TK inhibitors such as erlotinib did not fulfill the enthusiastic expectations derived from the promising results obtained by preclinical studies (Brown et al., 2008; van den Bent et al., 2009). Thus, the fate of patients diagnosed with glioblastoma remains dismal despite employing the currently best standard of care. New therapeutic strategies are undoubtedly needed to overcome this frustrating situation.

One such new therapeutic approach which aims at enhancing the therapeutic efficacy against glioblastoma involves the combination of erlotinib with other targeted agents in order to inhibit key regulators that are located further downstream of the signaling cascade or with agents inhibiting other signaling pathways. Several clinical studies are ongoing to evaluate this therapeutic option. In patients with recurrent glioblastoma or gliosarcoma, a phase I/II clinical trial currently evaluates the therapeutic effects of a combined treatment with erlotinib, sorafenib (BAY 54-9085, Bayer HealthCare Pharmaceuticals, Montville, NJ, U.S.A.), an inhibitor of murine leukemia viral oncogene homolog (RAF)/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and VEGFR-2/PDGFR-β signaling pathways, and temsirolimus (CCI-779, Wyeth Pharmaceuticals, Madison, NJ, U.S.A.), an inhibitor of mTOR. The results are awaited. A different clinical trial investigated the effects of dual therapy with erlotinib and sorafenib in patients with progressive or recurrent glioblastoma. This study has been completed, and the results are pending.

In this chapter, we emphasize the need for a continuous search for new agents replenishing our armory for the fight against glioblastoma. Some of the novel agents presented herein may allow to enhance overall antitumor activity when applied together with other
compounds such as erlotinib. In addition, several candidate erlotinib resistance genes have been proposed from genetic analysis of glioblastoma cell lines (Halatsch et al., 2009) and further validation is under way.

4. References


Future Perspectives of Enhancing the Therapeutic Efficacy of Epidermal Growth Factor Receptor Inhibition in Malignant Gliomas


receptor messenger RNA induction and suppression of anchorage-independent growth by OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in glioblastoma multiforme cell lines. *J Neurosurg*, 100, pp. 523-533.


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