Cilengitide Treatment for Malignant Glioma: Current Status and Future Direction

Kazuhiko KUROZUMI,1 Tomotsugu ICHIKAWA,1 Manabu ONISHI,1 Kentaro FUJII,1 and Isao DATE1

1Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama

Abstract
Malignant glioma is the most common primary brain tumor and accounts for the majority of diagnoses. Treatment has involved a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of the patient to more than one year from diagnosis. Integrins are expressed in tumor cells and tumor endothelial cells, and are important in angiogenesis and invasion in glioma. αvβ3 and αvβ5 integrins regulate cell adhesion, and inhibitors of these integrins suppress tumor growth in certain pre-clinical models. Several integrin-targeted drugs are in clinical trials as potential compounds for the treatment of cancer. Among them, cilengitide is a novel integrin antagonist for the treatment of glioblastoma. The multimodal anti-glioma effects are based on its cytotoxic, anti-angiogenic, anti-invasive, and synergetic effects. Preclinical studies showed a promising synergy between cilengitide and radiochemotherapy in order to normalize tumor vasculature and attenuate tumor invasion. Cilengitide is currently being assessed in phase III trials for patients with glioblastoma multiforme and in phase II trials for other types of cancers, demonstrating promising therapeutic outcomes to date. The results of these and other clinical studies are expected with great hope and interest. A more clear understanding of the benefits and pitfalls of each approach can then lead to the design of strategies to derive maximal benefit from these therapies.

Key words: malignant glioma, integrin, cilengitide, angiogenesis, invasion

Introduction
Malignant gliomas are the most common type of primary brain tumor. Their treatment has involved a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of the patient to more than one year from diagnosis. Several modalities have been and continue to be tested for the treatment of these tumors. Malignant gliomas remain a challenging tumor to treat, and a variety of experimental therapies have failed to show effectiveness in clinical trials.54 The pathophysiological processes of angiogenesis and tumor cell invasion play pivotal roles in glioma development and growth from the earliest stages.55 The formation of abnormal tumor vasculature and glioma cell invasion along white matter tracts are believed to be the major reasons for the resistance of these tumors to treatment. This angiogenesis or invasion causes the production of many different angiogenic or invasive factors, respectively, such as vascular endothelial growth factor receptor (VEGFR) platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), hepatocytes growth factor, integrins, etc. Among these factors, the overexpression of integrins is well documented.59,60 Emerging evidence indicates that integrins promote the adhesion, migration, and angiogenesis of glioblastoma.61
Integrins

Integrins are a superfamily of cell adhesion receptors that bind to extracellular matrix (ECM) ligands, cell-surface ligands, and soluble ligands. Integrins are heterodimeric transmembrane cell surface receptors that play a key role in the crosstalk between the cell and its surrounding stroma. Twenty-four different integrin heterodimers are currently recognized, and are formed by the combination of at least 18 α-subunits and 8 β-subunits. Upon ligation to extracellular ligands such as fibronectin, vitronectin, collagen, and fibrinogen, the integrin dimers activate downstream signaling pathways in concert with growth factor receptors, including PDGFR, EGFR, and VEGFR. The physical interaction of integrins with ECM proteins promotes signal transduction, gene expression, proliferation, apoptosis regulation, angiogenesis, invasion, and metastasis.

αvβ3 and αvβ5 integrins are usually expressed at low levels in most adult epithelia but can be highly upregulated in some tumors. Human gliomas also overexpress αvβ3 and αvβ5 integrins. These integrins are expressed on certain tumor cells during their growth and on activated endothelial cells during tumor angiogenesis and invasion of the surrounding tissue.

Cilengitide

A vast number of integrin antagonists have been reported such as monoclonal antibodies, peptide and peptidomimetic antagonists, and small molecules. αvβ3 and αvβ5 integrins regulate cell adhesion and inhibitors of these integrins suppress tumor growth in certain pre-clinical models. Currently, several compounds targeting integrins are in clinical trials as potential drugs for the treatment of numerous diseases including cancer. Among them, cilengitide is the first integrin antagonist in clinical phase III for the treatment of glioblastoma and in phase II trials for several other tumors.

Cilengitide is a vast number of integrin antagonists have been reported such as monoclonal antibodies, peptide and peptidomimetic antagonists, and small molecules. αvβ3 and αvβ5 integrins regulate cell adhesion and inhibitors of these integrins suppress tumor growth in certain pre-clinical models. Currently, several compounds targeting integrins are in clinical trials as potential drugs for the treatment of numerous diseases including cancer. Among them, cilengitide is the first integrin antagonist in clinical phase III for the treatment of glioblastoma and in phase II trials for several other tumors.
tumors. This drug is the only anti-angiogenic small molecule showing subnanomolar antagonistic activity for αvβ3 and affinities in the low nanomolar range for αvβ5 and α5β1. Cilengitide was shown to influence cellular adhesion to αvβ3 ligands, to induce increased apoptosis after the detachment of αvβ3- and αvβ3-expressing cells in vitro, and to block the growth of human xenografts in nude mice. Moreover, cilengitide demonstrated anti-angiogenic and anti-tumor activity in different animal models. Cilengitide has multimodal anti-glioma effects such as cytotoxic, anti-angiogenic, anti-invasive, and synergetic effects (Fig. 1).

I. Cytotoxic effects of cilengitide
Recent studies reported that cilengitide exerts direct cytotoxic effects on glioma cells via an as yet unknown mechanism. Several studies have shown that various cells are dependent on integrin-mediated adhesion to specific ECM proteins for their growth and survival and that detachment induces apoptotic cell death. Cilengitide reportedly induces apoptosis in αv-integrin-expressing tumor cell lines by detaching tumor cells from vitronectin and tenasin, which are matrix proteins that are essential for tumor growth and invasion. Apoptosis in response to lack of adhesion or inappropriate adhesion has been termed anoikis. Cilengitide induces anoikis in brain tumor cells by inhibiting the phosphorylation of focal adhesion kinase (FAK), Src, and Akt. In addition to apoptosis, detachment has also been associated with the induction of autophagy. Autophagy may contribute to the cytotoxic effects of cilengitide. We showed that cilengitide treatment of αvβ3-expressing glioma cells induced changes in cell morphology, cell detachment, and decreased cell viability in vitro. Anoikis of glioma cells was induced by intraperitoneal cilengitide injection.

II. Anti-angiogenic effects of cilengitide
Angiogenesis is the formation of new blood vessels by the rerouting or remodeling of existing vessels, and is believed to be the primary method of vessel formation in gliomas. Cilengitide was highly effective in suppressing blood vessel growth, thereby inhibiting the orthotopic growth of human glioblastoma cells in animals. Reduction in the size of tumors increased survival in mice with orthotopic brain tumors treated with cilengitide compared to mice treated with an inactive peptide. Therefore, brain tumors, which are highly angiogenic, may be more susceptible to growth inhibition by integrin antagonists. Angiogenesis requires three distinct steps: 1) blood vessel breakdown; 2) degradation of the vessel basement membrane and surrounding ECM; and 3) migration of endothelial cells and the formation of new blood vessels. During the third step, endothelial cells proliferate and begin to migrate toward tumor cells expressing pro-angiogenic compounds. Endothelial cell activation upregulates the expression of cell surface adhesion/migration molecules. Specifically, αvβ3 integrin is upregulated in endothelial cells during angiogenesis, enhancing endothelial cell adhesion and migration. Cilengitide might prevent the third step of angiogenesis and reduce the size of tumor vessels.

III. Anti-invasive effects of cilengitide
Glioma cell invasion requires four distinct steps: 1) detachment of the invading cells from the primary tumor mass; 2) adhesion to the ECM; 3) degradation of the ECM; and 4) cell motility and contractility. During the second step, the molecules allowing glioma cells to adhere to the ECM are integrins, αvβ3 integrin in particular, which binds to fibronectin, vitronectin, and tenasin-C in the ECM. Integrin αvβ3 plays a central role in glioma invasion. Inhibition of integrin αvβ3 decreased glioma cell motility in vitro. Cilengitide might inhibit the second step, thereby suppressing the invasion of glioma. Although most of the animal models with glioma xenografts have tumors with borders that are easily distinguished and dissected from normal brain tissue, we recently established two novel invasive animal glioma models (J3T-1 and J3T-2) that reflect the invasive phenotype of human malignant gliomas. These models were particularly beneficial to investigate the anti-invasive effects of cilengitide. Cilengitide suppressed the invasiveness of these animal glioma models. The borders of cilengitide-treated J3T-2 tumors (angiogenesis-independent invasive tumors) were more easily distinguished than the borders of control J3T-2 tumors.

IV. Other anti-tumor effects of cilengitide
Treatment of glioblastoma cells with cilengitide led to a significant and dose-dependent decrease in the intracellular levels of hypoxia-inducible factor 1 (HIF-1) under hypoxic conditions. Hypoxia stimulates the αvβ3 and αvβ5 integrin pathways through FAK and that hypoxia activates FAK in glioblastoma cell lines. This study suggests that αvβ3 and αvβ5 are activated by hypoxia and are key regulators of the response of glioma to hypoxic conditions by controlling HIF-1 degradation. Cysteine-rich protein 61 (CYR61), a member of the CCN (CYR61/CTGF/NOV) family of matricellular proteins that regulate cell growth, differentiation, survival, angiogenesis, and migration in development, tissue remodeling, and development.
repair, and \( \alpha \beta 5 \) integrin (a receptor for CYR61) are expressed by tumor cells as critical molecules that cooperate to promote local invasion and distant metastases. Importantly, a function-blocking anti-\( \alpha \) \( \beta 3 \) monoclonal antibody (17E6) and cilengitide inhibits CYR61-mediated angiogenesis, invasion, and metastasis.

\[ \text{V. Synergistic effects of combination with radiation, chemotherapy, or other new therapeutic strategies} \]

An increased understanding of the molecular mechanisms in the tumorigenesis of glioblastomas has led to the evaluation of targeted agents as potential radiosensitizers. Preclinical studies showed a promising synergy between cilengitide and radiochemotherapy (RCT) in order to normalize tumor vasculature and attenuate tumor invasion. The combination of an integrin antagonist and radiotherapy (RT) showed a significant delay of tumor growth in glioblastoma xenografts compared with either treatment individually. Irradiation of tumors reduces the local tumor growth, but at the same time upregulates \( \alpha \beta 3 \) expression and enhances local invasion. Hence, cilengitide conceivably may normalize the tumor vasculature, lower tumor interstitial fluid pressure, and improve vascular function and tumor oxygenation. Such activity of cilengitide may promote enhanced responsiveness to RT. Most recently, dramatically enhanced antitumor activity of RT was induced by cilengitide in 2-week-old intracranial U251 gliomas in nude rats, but only when cilengitide was given at 4–8 hours before radiation.

Cilengitide acts primarily to block survival pathways, and its enhanced antitumor activity may occur in combination with conventional cytotoxic or pro-apoptotic therapies. As a new therapeutic approach, we demonstrated that anti-angiogenic cilengitide treatment before oncolytic virus (OV) treatment enhanced the antitumor efficacy of the virus (Fig. 2). This study showed that pretreatment of gliomas with cilengitide reduced inflammation, vascular hyperpermeability, and leukocyte infiltration of tumor tissue upon treatment with the OV. The reduction of host immune responses by cilengitide treatment enhanced the anticancer efficacy of OV treatment by increasing the propagation of this virus in tumors. We also reported that oncolytic herpes simplex virus 1 infection of tumors induced angiogenesis and upregulated the expression of CYR61. In order to test the role of CYR61-mediated integrin activation in OV-induced angiogenesis, we showed the impact of cilengitide on OV treatment-induced angiogenesis.

\[ \text{Clinical Trials} \]

\[ \text{I. Completed clinical trials} \]

The first clinical trial using cilengitide was reported in a phase I trial in patients with recurrent glioblastoma. This multi-institutional phase I trial was designed to determine the maximum-tolerated dose of cilengitide (EMD 121974) and to evaluate the use of perfusion magnetic resonance imaging in patients with glioblastoma. In this study, cilengitide demonstrated an unexpected single agent activity for these tumors with limited toxicity for doses up to 2,400 mg/m². A multicenter, open-label, phase II study was conducted to evaluate the activity and safety of cilengitide in glioblastoma patients at their first recurrence. As previous clinical studies showed responses at both the lower and higher dose levels, reported follow-up (>4 years) data recently showed that the long-term survival rates were consistently greater with 2000 mg (10.0% after 54 months) versus 500 mg (2.4% after 54 months). Another phase II trial to evaluate the efficacy and tumor delivery of cilengitide in patients with recurrent glioblastoma detected in all tumor specimens with higher levels in the group receiving 2000 mg dosing while corresponding plasma concentrations were low. This study provides evidence that with established dosing, cilengitide is adequately delivered to the tumor.

Preclinical studies revealed that cilengitide in combination with RT and chemotherapy could have enhanced anti-tumor activity. A phase II pilot trial added cilengitide (500 mg) to standard chemoradiotherapy with temozolomide (TMZ). In a multicenter pilot study, the progression-free survival (PFS) rates at 6 months in primary endpoint were greater than historical controls (69% versus 54%). Median survival was 16.1 months, with a 2-year survival rate of 35%. These results suggested cilengitide acted as a chemosensitizer but not as a toxic substance. Interestingly, the authors of this study also showed that this treatment was more effective in patients whose tumors had O\( \text{6-} \)methylguanine-deoxyribonucleic acid methyltransferase (MGMT) promoter methylation, exhibiting longer PFS and overall survival (OS). On the basis of these results, international, randomized, controlled phase III (CENTRIC) and phase II (CORE) trials were launched in 2008.

\[ \text{II. Clinical trials of cilengitide currently in progress} \]

**CENTRIC:** The CENTRIC study was designed to test the efficacy and tolerability of cilengitide in patients with newly diagnosed glioblastoma with a
methylated MGMT gene promoter. In the investigational arm, patients receive cilengitide at a dose of 2000 mg intravenously twice weekly in combination with standard RCT (concomitant RT/TMZ for 6 weeks, followed by 6 cycles of TMZ maintenance therapy). Patients in the control arm receive only standard RCT. The treatment duration is 18 months for patients in the cilengitide group and 8 months for those in the control group. Patients in the cilengitide group are allowed to continue with cilengitide after completion of the 18 months of the study treatment until the occurrence of progression disease or unacceptable toxicity, or withdrawal for any other reason. The study design is shown in Fig. 3.

**CORE:** The CORE study is investigating the efficacy and safety of 2 regimens of cilengitide in glioblastoma patients with an unmethylated MGMT promoter. CORE is a multicenter, open-label, phase II study. Cilengitide (2000 mg intravenously over 60 min) is administered at 4 hours before RT and TMZ is given orally for 7 days a week after the completion of cilengitide infusion at least 1 hour before RT. The primary objective of this study is to investigate the OS time in subjects receiving 2 different regimens of 2000 mg of cilengitide in combination with RT and TMZ standard therapy. Secondary objectives of this study are 1) to evaluate PFS time, 2) to evaluate the safety and tolerability of the combination of cilengitide with standard RT and TMZ therapy, and 3) to evaluate the pharmacokinetic profile of cilengitide (Fig. 4).

**III. Other on-going trials (Table 1)**

Several preclinical studies have shown an enhanced antitumor effect of cilengitide when administered in combinatorial therapeutic regimens. RCT with cilengitide or cetuximab is being investigated in a randomized, non-comparative trial in patients with newly diagnosed MGMT-promoter unmethylated glioblastoma (CeCil). Chemoresistance was examined in the MGMT unmethylated population, building on preclinical data, prior experience with cilengitide, and the combination of low dose TMZ and procarbazine (ExCentric). Cediranib maleate and cilengitide may stop the growth of tumor cells by blocking blood flow to the tumor; therefore, the co-administration of cediranib maleate and cilengitide may kill more tumor cells. This phase I trial is studying the side effects and best dose

---

**Fig. 3** Design of the CENTRIC study. An estimated 504 patients (approximately 250 patients in each treatment arm) from ~200 centers worldwide will be randomized. *Shaded columns:* focal radiotherapy (RT) 5×/week for 6 weeks (30×2 Gy, total dose 60 Gy); *open columns:* cilengitide 2000 mg intravenously 2×/week until week 34, for 18 months (until week 77) optional thereafter; *closed columns:* temozolomide (TMZ) 75 mg/m2 per oral daily for 6 weeks (during RT), followed by 150–200 mg/m2 per oral on days 1–5 every 28 days for 6 cycles. MGMT: O6-methylguanine-deoxyribonucleic acid methyltransferase gene promoter, R: randomization. Modified from Stupp et al.: Cilengitide in newly diagnosed glioblastoma with MGMT promoter methylation: protocol of a multicenter, randomized, openlabel, controlled phase III trial (CENTRIC) [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS152).

**Fig. 4** Design of the randomized part of the CORE study. An estimated 252 patients from up to 85 centers in the US, Canada, Europe, and Asia will be randomized in the second part of the study. *Shaded columns:* focal radiotherapy (RT) 5×/week for 6 weeks (30×2 Gy, total dose 60 Gy); *open columns:* cilengitide 2000 mg intravenously 2×/week until week 34; *hatched column:* cilengitide 2000 mg intravenously 5×/week during weeks 1–6; *closed columns:* temozolomide (TMZ) 75 mg/m2 per oral daily for 6 weeks (during RT), followed by 150–200 mg/m2 per oral on days 1–5 every 28 days for 6 cycles. MGMT: O6-methylguanine-deoxyribonucleic acid methyltransferase gene promoter, R: randomization. Modified from Nabors et al.: Cilengitide in patients with newly diagnosed glioblastoma multiforme and unmethylated MGMT gene promoter: Protocol of a multicenter, randomized, open-label, controlled phase II study [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS151).
Table 1 Clinical trials of cilengitide currently in progress*

<table>
<thead>
<tr>
<th>Trial</th>
<th>Estimated no. of patients</th>
<th>Disease setting</th>
<th>Purpose/Treatment</th>
<th>Start date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II ExCentric</td>
<td>48</td>
<td>newly diagnosed GBM (unmethylated gene promoter status)</td>
<td>evaluate safety and efficacy/ cilengitide + RT + TMZ + PCB</td>
<td>November 2009</td>
</tr>
<tr>
<td>Phase II Cecil</td>
<td>108</td>
<td>newly diagnosed GBM (unmethylated gene promoter status)</td>
<td>evaluate safety and efficacy/ cilengitide or cetuximab + RT + TMZ</td>
<td>September 2009</td>
</tr>
<tr>
<td>Phase I</td>
<td>28</td>
<td>newly diagnosed GBM</td>
<td>evaluate safety and dosage/ cilengitide + sunitinib maleate</td>
<td>January 2010</td>
</tr>
<tr>
<td>Phase I</td>
<td>52</td>
<td>progressive/recurrent GBM</td>
<td>evaluate safety and dosage/ cilengitide + cediranib maleate</td>
<td>March 2010</td>
</tr>
</tbody>
</table>


Conclusions

The management of glioblastoma remains a challenging area in oncology. Angiogenesis and invasion are undoubtedly critical in the development and survival of glioblastoma. Cilengitide is the first integrin antagonist for the treatment of glioblastoma. Integrins are expressed in tumor cells and tumor endothelial cells, and are important in angiogenesis and invasion in glioma. Cilengitide is currently being assessed in phase III trials for glioblastoma patients and phase II trials for other types of cancers, with promising therapeutic outcomes to date. The CENTRIC controlled phase III study was launched in 2008, with primary outcome measures due in September 2012. The results of this and other clinical studies are expected with great hope and interest. A more clear understanding of the benefits and pitfalls of each approach can then lead to the design of strategies to derive maximal benefit from these therapies.

Funding

Cilengitide was generously provided by Merck KGaA (Darmstadt, Germany) and Cancer Therapy Evaluation Program, the National Cancer Institute at the National Institutes of Health (Bethesda, Maryland, USA).

Conflict of Interest

No author has any conflict of interest to declare.

References

8) Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Chereb DA: Integrin alpha v beta 3 antagonists promote tumor regression by inducing...
34) Meyer A, Auernheimer J, Modlinger A, Kessler H:


49) Schaller MD: Biochemical signals and biological responses elicited by the focal adhesion kinase. Biochim Biophys Acta 1540: 1–21, 2001


Neurol Med Chir (Tokyo) 52, August, 2012


*Address reprint requests to: Kazuhiko Kurozumi, MD, PhD, Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2–5–1 Shikata–cho, Kita–ku, Okayama 700–8558, Japan.
e-mail: kkuro@md.okayama-u.ac.jp*