Open access • Journal Article • DOI:10.1200/JCO.2009.26.6650

Phase I/IIa Study of Cilengitide and Temozolomide With Concomitant Radiotherapy Followed by Cilengitide and Temozolomide Maintenance Therapy in Patients With Newly Diagnosed Glioblastoma — Source link

Roger Stupp, Monika E. Hegi, Bart Neyns, Roland Goldbrunner ...+15 more authors

Institutions: University Hospital of Lausanne, Institut Jules Bordet

Published on: 01 Jun 2010 - Journal of Clinical Oncology (American Society of Clinical Oncology)

Topics: Cilengitide, Temozolomide and Chemoradiotherapy

Year: 2010

Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma

Stupp, R; Hegi, M E; Neyns, B; Goldbrunner, R; Schlegel, U; Clement, P M ; Grabenbauer, G G; Ochsenbein, A F; Simon, M; Dietrich, P Y; Pietsch, T; Hicking, C; Tonn, J C; Diserens, A C; Pica, A; Hermisson, M; Krueger, S; Picard, M; Weller, M

Phase I/IIa Study of Cilengitide and Temozolomide With Concomitant Radiotherapy Followed by Cilengitide and Temozolomide Maintenance Therapy in Patients With Newly Diagnosed Glioblastoma

Roger Stupp, Monika E. Hegi, Bart Neyns, Roland Goldbrunner, Uwe Schlegel, Paul M.J. Clement, Gerhard G. Grabenbauer, Adrian F. Ochsenbein, Matthias Simon, Pierre-Yves Dietrich, Torsten Pietsch, Christine Hicking, Joerg-Christian Tonn, Annie-Claire Diserens, Alessia Pica, Mirjam Hermisson, Stefan Krueger, Martin Picard, and Michael Weller

From the Centre Pluridisciplinaire d'Oncologie (Stupp), Department of Neurosurgery (Stupp, Diserens, Hegi), Service de Radio-Oncologie (Pica), Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne; Institute of Medical Oncology, University of Bern, Bern (Ochsenbein); Division d'Oncologie, Hôpitaux Universitaires de Genève, Geneva (Dietrich); Department of Neurology, University Hospital, Zurich, Switzerland (Weller); Department of Medical Oncology, UZ Brussel, Brussels (Neyns); Department of Clinical Oncology, Katholieke Universiteit Leuven, Leuven, Belgium (Clement); Department of Neurosurgery, University Hospital of Cologne, Cologne (Goldbrunner), Department of Neurosurgery, University of Munich, Munich (Tonn); Neurologische Universitätsklinik des Knappschaftskrankenhauses Bochum-Langendreer, Ruhr University, Bochum (Schlegel); Friedrich Alexander University, Erlangen (Grabenbauer); Department of Neurosurgery (Simon), Institute of

Neuropathology (Pietsch), University of Bonn, Bonn ; Merck Serono, Darmstadt (Hicking, Krueger, Picard); and Department of General Neurology, University of Tübingen, Germany (Hermisson, Weller).

Corresponding author: Roger Stupp, MD, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Rue du Bugnon 46, Lausanne, Switzerland CH-1011; Telephone: +41 21 314 0156; Fax: +41 21 314 0737; e-mail: roger.stupp@chuv.ch.

Supported by Merck Serono.

Running head: Cilengitide and temozolomide plus radiotherapy in glioblastoma

Presented at the 43rd American Society for Clinical Oncology Annual Meeting, Chicago, IL, June 1–5, 2007.

ABSTRACT

Purpose

Invasion and migration are key processes in the malignant phenotype of glioblastoma and are tightly linked to tumor recurrence. Integrin inhibition using cilengitide has shown synergy with chemo- and radiotherapy in vitro and promising activity in recurrent glioblastoma. This multicenter, open-label, phase I/IIa study investigated the efficacy and safety of cilengitide in combination with standard chemoradiotherapy in newly diagnosed glioblastoma.

Patients and Methods

Patients (≥18 to ≤70 years) were treated with cilengitide (500 mg) administered twice weekly i.v. in addition to standard radiotherapy with comcomitant and adjuvant temozolomide. Treatment was continued until disease progression or for a maximum of 35 weeks. The primary endpoint was progression-free survival (PFS) at 6 months.

Results

Fifty-two patients (median age 57 years; 62% male) were included. Six- and 12-month PFS rates were 69% (95% confidence interval [CI], 54-80%) and 33% (95% CI, 21-46%). Median PFS was 8 months (95% CI, 6.0-10.7). Twelve- and 24-month overall survival (OS) rates were 68% (95% CI, 53-79%) and 35% (95% CI, 22-48%). Median OS was 16.1 months (95% CI, 13.1-23.2). PFS and OS were longer in patients with tumors with O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation (13.4 and 23.2 months) *versus* those without *MGMT* promoter methylation (3.4 and 13.1 months). The combination of cilengitide with temozolomide and radiotherapy was well

tolerated, with no additional hematologic or nonhematologic toxicity. No pharmacokinetic interactions between temozolomide and cilengitide were identified.

Conclusion

Compared with historical controls, the addition of concomitant and adjuvant cilengitide to standard chemoradiation demonstrated promising activity in patients with glioblastoma with *MGMT* promoter methylation.

INTRODUCTION

Glioblastoma, the most aggressive and most common primary brain tumor in adults[1-3], is an intensely vascularized, invasive tumor with a high propensity to induce angiogenesis.[4] The standard of care for newly diagnosed glioblastoma includes surgical resection where feasible, involved-field radiotherapy (RT) and concomitant temozolomide (TMZ) chemotherapy followed by up to 6 months of adjuvant TMZ.[5] Methylation of the O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter is a putative predictive marker for benefit from TMZ plus RT.[6-8] Despite these diagnostic and therapeutic advances, most patients with glioblastoma still die within 2 years.

Cilengitide (EMD 121974) is the first agent in a novel class of integrin-directed anticancer therapies reaching the clinic.[9] Integrins are heterodimeric transmembrane receptors that play a key role in cellular interactions, adhesion to the extracellular matrix, and cell migration.[10] Cilengitide is a cyclized arginine–glycine–aspartic acid (RGD)-containing pentapeptide that selectively binds the cell surface receptors $\alpha_V\beta_3$ and $\alpha_V\beta_5$, which are expressed on activated endothelial cells during angiogenesis.[11-12] Moreover, cilengitide may act directly on $\alpha_V\beta_3$- and $\alpha_V\beta_5$-expressing tumor cells, depriving them of important signals involved in survival and growth, and indirectly by inhibiting angiogenesis and thereby tumor growth.[13-14] Glioblastomas express the target integrins of cilengitide on activated endothelial cells and on tumor cells.[15]

Single-agent cilengitide has been investigated in patients with recurrent glioblastoma in phase I and II studies and has shown an excellent safety profile with little toxicity and promising antitumor activity.[16-18] Preclinical models have indicated a synergistic effect when cilengitide is combined with RT[19-20] or with an alkylating agent (data on file, Merck

KGaA, Darmstadt). There is thus a strong scientific rationale for combining cilengitide with chemoradiotherapy to improve tumor control and inhibit tumor cell adhesion and migration.

This study investigated the efficacy and safety of concomitant and adjuvant cilengitide added to standard chemoradiation in patients with newly diagnosed glioblastoma.

PATIENTS AND METHODS

Study Design

This multicenter, open-label, single-arm, phase I/IIa trial investigated the efficacy and safety of cilengitide added to RT with concomitant and adjuvant TMZ in patients with newly diagnosed glioblastoma. It was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and International Conference on Harmonisation recommendations. The study protocol and informed-consent form were approved by Ethics Committees at each investigational site in accordance with local legislation. Written informed consent was obtained from each patient prior to study entry. Primary endpoint was the PFS rate at 6 months. Secondary endpoints were OS, safety, and pharmacokinetics. The influence of *MGMT* promoter methylation status on efficacy was investigated in a prospectively planned subgroup analysis.

Patient Selection

Main inclusion criteria were age ≥18 to ≤70 years; newly diagnosed, histologically proven supratentorial glioblastoma (World Health Organization [WHO] grade IV); Eastern Cooperative Oncology Group (ECOG) performance status ≤2; adequate renal (creatinine ≤1.5 × upper limit of normal [ULN] or creatine clearance rate ≥60 mL/min), hepatic (total bilirubin ≤1.5 × ULN and serum transaminases ≤2.5 × ULN), and hematologic (absolute neutrophil count ≥1,500/mm^3, platelets ≥100,000/mm^3, and hemoglobin ≥10 g/dL) function. Tumor tissue was required for central pathology review

and *MGMT* promoter methylation analysis. An interval of ≥3 weeks but ≤5 weeks between surgery or biopsy and enrollment was required.

Exclusion criteria were: prior chemotherapy or RT or a history of malignancy in the previous 5 years; previous anti-angiogenic therapy; and recent/concurrent treatment with other experimental therapies. Patients with planned surgery for other diseases, a history of peptic ulcer within the last 6 months, or a history of coagulation disorder associated with bleeding or recurrent thrombotic events were also excluded. A negative pregnancy test and adequate contraception were mandatory.

Treatment

RT and TMZ were administered as previously described.[5] Fractionated 3-dimensional conformal radiotherapy was delivered with a linear accelerator at 2 Gy per fraction for a total of 60 Gy in 30 fractions (5 days per week). The target volume was planned from postoperative magnetic resonance imaging (MRI) studies and included all gross disease plus a margin of 2-3 cm. TMZ 75 mg/m^2/day was administered orally 7 days each week from the first to the last day of RT. After a 4-week break, adjuvant TMZ 150–200 mg/m^2/day was given for 5 consecutive days every 4 weeks for a total of 6 cycles.

Cilengitide was administered at a flat dose of 500 mg as a 1-hour intravenous infusion twice weekly on days 1 and 4 (or occasionally on days 2 and 5), starting 1 week before (week -1) chemoradiation. Cilengitide was continued until progression or until the end of adjuvant TMZ, i.e., for up to 35 weeks, or until the occurrence of unacceptable adverse

events (AEs). It was administered approximately 2 h before TMZ, RT was delivered approximately 60 min after TMZ.

Pharmacokinetics (PK)

Pharmacokinetic analyses were performed in a subset of 13 patients (4 patients receiving enzyme-inducing antiepileptic drugs (EIAED), 4 on non-EIAED, and 5 patients with no anticonvulsants) to identify potential interactions between cilengitide and TMZ when administered concomitantly. Blood samples (4 mL) were taken for determination of cilengitide plasma concentrations before dosing and 1, 1.5, 2, 3, 4, 8, and 24 h after starting the infusion on day 1 of weeks 1, 2, 8, and 12; TMZ plasma concentrations were determined before dosing and 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after administration on days 3 and 4 of week 2. Cilengitide and TMZ plasma concentrations were determined using validated high-performance liquid chromatography and liquid chromatography tandem mass spectrometry [21-22]. The PK parameters of cilengitide and TMZ were calculated from plasma concentration data according to non-compartmental standard methods using the PK software program KINETICA™.

***MGMT* status**

MGMT gene promoter methylation status was determined by methylation-specific polymerase chain reaction (PCR), as previously described.[7,23] Genomic DNA was isolated from macrodissected paraffin sections or cryosections of glioblastoma tissue (Ex-Wax DNA Extraction Kit S4530, Chemicon, or Qiagen genomic kit, respectively) and

subjected to bisulfite modification using the EpiTect kit (Qiagen). Unmethylated cytosine, but not its methylated counterpart, is modified into uracil by bisulfite treatment. Methylation-specific PCR was performed in a nested two-step approach. The results were confirmed in an independent experiment, starting with reisolation of DNA from the tumor. PCR products were separated on 4% agarose gels.

Efficacy and Safety Assessments

A gadolinium-enhanced MRI scan was performed within 1 week before the first dose of cilengitide for patients who had undergone a tumor biopsy or <48 h after surgery for patients who had undergone open tumor resection. MRI was repeated 3-4 weeks after the end of RT and every 8 weeks thereafter for 6 months, subsequently every 3 months. Progression was based on investigators' clinical and radiological assessment according to Macdonald criteria.[24]

Safety was evaluated by descriptively summarizing AEs, laboratory assessments, and physical examinations, including ECOG performance status, vital signs, neurological examinations, hematology and coagulation parameters, weekly blood counts, serum chemistry, urinalysis, chest x-ray, and electrocardiogram. All treatment-emergent AEs were recorded. AEs possibly related to treatment with chemoradiotherapy or cilengitide and events that could not be attributed to another cause were reported as treatment-related AEs.

Statistical Methods

All analyses were performed on the intent-to-treat population defined as all patients who received at least one dose of cilengitide. Patient demographics and AEs are descriptive. The primary endpoint was the PFS rate at 6 months. PFS was defined as the time from start of cilengitide treatment until progression or death from any cause. In a sample of 50 patients, the target was to obtain a 6-month PFS rate of ≥65%. OS was defined as the time from the start of cilengitide treatment until death from any cause. Kaplan-Meier survival curves (product-limit estimates) were produced with a summary of associated statistics (median survival time, 6-monthly survival rate estimates) including the corresponding two-sided 95% confidence intervals (CIs).

RESULTS

Patient Characteristics

Of 59 patients screened, 52 met the inclusion criteria and were treated at 15 sites in Switzerland, Germany, and Belgium. Reasons for non-inclusion of screened patients were: failure to meet inclusion/exclusion criteria (n = 5), AEs (n = 1), or withdrawal of consent (n = 1). Patient characteristics are summarized in Table 1. Median age was 57 years, and most patients had a performance status of 0 or 1. Twenty-three patients had a gross total resection, 20 patients had a partial resection, 9 had only a diagnostic stereotactic biopsy. Thirty-five patients received AED at baseline, including 19 patients receiving EIAED. Histology was centrally confirmed as glioblastoma in 92% of patients. Central review reclassified two patients as anaplastic astrocytoma (WHO grade III). A confirmation of the diagnosis was not possible in two patients due to insufficient or missing samples. *MGMT* promoter methylation status could be determined in 45 patients (87%) and approximately half (23) had *MGMT* promoter methylation, a distribution comparable to our previous trials.[6-8] . The frequency observed in this study is similar to the randomized phase III EORTC-NCIC study (46%).[7]

Treatment Delivery

Fifty patients (96%) completed combination treatment with cilengitide plus TMZ and RT; 23 patients (44%) completed the planned study period of 35 weeks, including all 6

cycles of maintenance TMZ. Reasons for early discontinuation were disease progression (n = 19, 37%), AEs (n = 9, 17%), or withdrawal of consent (n = 1, 2%).

Efficacy

At the time of this analysis after a median follow-up of 34 months (minimum follow-up 24 months), 43 patients (83%) have progressed and 34 patients (65%) have died (Table 2). The 6-month PFS rate (primary endpoint) was 69% (95% CI, 54-80%). The median PFS was 8.0 months (95% CI, 6.0-10.7). The median OS was 16.1 months (95% CI, 13.1-23.2); 1- and 2-year OS rates were 68% (95% CI, 53-79%) and 35% (95% CI, 22-48%).

Subgroup analyses for known clinical prognostic factors demonstrated prolonged PFS and OS in patients younger than 50 years and in patients who had debulking surgery. PFS was significantly longer in patients with tumors with *MGMT* promoter methylation than those without (Fig. 1; Table 2). Six-months, 12-months, and median PFS were 91% (95% CI, 70-98%), 52% (95% CI, 30-70%), and 13.4 months (95% CI, 8.6-22.8) in patients with *MGMT* promoter methylation *v* 41% (95% CI, 21-60%), 9% (95% CI, 2-25%), and 3.4 months (95% CI, 2.3-7.1) in patients without. OS was also prolonged in patients with *MGMT* promoter methylation (Fig. 2, Table 2). Twelve-, 18-, and 24-month OS were 91% (95% CI, 69-98%), 68% (95% CI, 45-84%) and 46% (95% CI, 25-64%) in patients with *MGMT* promoter methylation *versus* 51% (95% CI, 28-70%), 25% (95% CI, 9-45%), and 20% (95% CI, 6-40%) in patients without.

Pharmacokinetics (PK)

Mean plasma time concentration curves and PK parameters for cilengitide and TMZ are summarized in Supplementary Tables 1 and 2 and in Fig. 3. Mean cilengitide plasma concentration time profiles and mean PK parameters (Cmax, tmax, AUC, t1/2, CL and Vz) were similar across the weeks of cilengitide treatment, indicating that the PK of cilengitide is independent from treatment time and neither influenced by co-treatment with TMZ alone nor with TMZ chemoradiation. Maximum plasma concentrations of cilengitide were reached at 1 h post-dose, i.e., at the end of infusion. Mean systemic CL of cilengitide ranged from 6.72 to 7.51 L/h, the mean terminal half-life (t1/2) from 2.02 to 2.16 h, and the mean volume of distribution (Vz) from 19.6 to 21.9 L, nominally equivalent to the extracellular fluid volume. There was no apparent accumulation of cilengitide upon repeated administration.

Also for TMZ plasma concentration time profiles and mean PK parameters were similar across treatment periods observed indicating that the PK of TMZ is not influenced by concomitant treatment with cilengitide. No influence of the concomitant administration of EIAED was evident. Maximum plasma concentrations of TMZ were reached within 1 h after oral administration. The mean systemic CL of TMZ was 7.42 L/h/m² on day 3 and 7.56 L/h/m² on day 4, the mean t1/2 was 1.84 h on both days and the Vz was 19.7 L/m² on day 3 and 20.1 L/m² on day 4. Overall, the PK parameters of TMZ were in good agreement with published data[25] and did not appear to be influenced by concomitant treatment with cilengitide and RT.

Kommentar [RS1]: To be confirmed by Merck.

Safety

The combination of cilengitide with standard chemoradiation was safe and well tolerated. Treatment-related AEs were usually mild to moderate and in the range previously observed with radiotherapy plus TMZ alone (Tables 3 and 4). Constitutive symptoms (nausea, headache, fatigue, vomiting, and anorexia) were the most common nonhematologic AEs. Seven patients (14%) had treatment-related AEs leading to withdrawal of further treatment including thrombocytopenia, asthenia, hepatitis, raised liver enzymes, aphasia related to cerebral hemorrhage, cerebral hemorrhage, memory impairment, lower limb paresthesias, pulmonary embolism, and deep vein thrombosis. Treatment-emergent AEs unlikely to be related to treatment included thrombosis and pulmonary emboli in two patients, further complicated in one patient by a fatal intracranial bleed 18 days after the last cilengitide administration. A minor intracranial hemorrhage was detected on MRI in a patient after a seizure. One patient with a history of diverticulosis required surgery for intestinal perforation; cilengitide therapy was subsequently resumed without further complications. Grade 1/2 hypertension was observed in 5 patients(9.6%) and mild proteinuria in 1 patient. Other toxicities were one case each of reversible peripheral grade 2 neuropathy not otherwise explained and idiosyncratic liver toxicity likely related to valproic acid. One patient died due to tumor progression within 28 days of the last dose of cilengitide. No safety differences were observed between patients with versus without *MGMT* promoter methylation (data not shown).

DISCUSSION

Various strategies are pursued to improve the outcome for newly diagnosed glioblastoma patients by adding new agents to the standard of radiotherapy plus concomitant and adjuvant TMZ. Here we report for the first time the use of an integrin inhibitor in newly diagnosed glioblastoma. The addition of cilengitide to the combined chemoradiation regimen showed clinical activity and was well tolerated. A total of 14% of patients discontinued treatment due to possibly treatment related adverse events, slightly higher than in the EORTC-NCIC experience [5]; this may in part be explained by the increased monitoring due to the twice weekly administration schedule. Overall, the toxicity was comparable to other reports with cilengitide, and no single AE could be specifically attributed to the study drug..[16-18, 29] + ref. NABTT 9911. Similarly, no increase in thromboembolic events and hemorrhagic complications was evident with the addition of this anti-angiogenic agent.

Of note, no severe hemorrhagic complications or wound-healing problems were observed in this population of patients treated shortly after brain surgery. Our safety findings are similar to those of another phase II study in which two doses of cilengitide (500 mg or 2000 mg) were administered before second surgery at recurrence.[16] Even though the last cilengitide dose was given 24 h before surgery, no relevant hemorrhagic or wound-healing AEs were observed. The pharmacokinetics of cilengitide and of TMZ in combination with RT were comparable to published data as single agents,[21-22,25] and no pharmacokinetic interaction or accumulation after repeated dosing was observed (Supplementary Tables 1 and 2, Figure 3).

The primary endpoint was the 6-months PFS rate, which was 69% (95% CI, 54-80%) – an improvement of about 15% over RT plus TMZ alone[5] – and thus met the predefined target of success of >65%. After a minimum follow-up of 2 years, six patients have not progressed. OS also compares favorably with previous reports, with a median of 16.1 months (95% CI, 13.1-23.2) and a 2-year survival rate of 35% (95% CI, 22-48%). A better outcome was seen in patients who had undergone debulking surgery. In the context of drugs like cilengitide, it is reasonable to assume that the inhibition of attachment and migration may become clinically relevant mainly after elimination of the tumor bulk.

The benefit of adding cilengitide, at least at the dose and schedule chosen here, to the standard regimen of TMZ and RT followed by TMZ alone appears to be limited to patients with glioblastoma with *MGMT* promoter methylation. The reasons for this non-intuitive interrelation remain unclear at present. No synergistic effects were identified for the combination of cilengitide and TMZ *in vitro*, and the *MGMT* status *per se* has no effect on the biological activity of cilengitide.[13] We speculate that cilengitide-induced vascular normalization improves the delivery of TMZ to tumor tissue, with a preferential benefit for patients with *MGMT* promoter methylation. This is in accordance with the observation of a better prognosis associated with increased expression of a signature for tumor endothelium markers in the treatment arm of the EORTC-NCIC study which may be linked to a better perfusion of the tumor with TMZ.[26]

Could the favorable results be related to an increased incidence of pseudoprogression in patients with MGMT-methylated tumors[27] which was successfully treated by cilengitide? We believe that this is not the case. In fact, it remains to be confirmed that

patients with *MGMT* promoter methylation are more likely to experience pseudoprogression, and unlike the VEGF antibody bevacizumab,[28] there is no data to suggest that cilengitide will ameliorate treatment-induced imaging changes in glioblastoma.

On the other hand, orthotopic *in vivo* glioblastoma models demonstrated both sensitivity to cilengitide[20]and striking synergy between cilengitide and RT.[19] One may speculate that patients without *MGMT* promoter methylation may require higher doses than 500 mg to experience such a radiosensitizing effect. We used a 500 mg dose based on a prior phase I dose-escalation study, in which dose-limiting toxicity was not reached at doses up to 2400 mg/m^2 and responses were observed at both lower and higher doses.[18] However, there is now increasing evidence that cilengitide was underdosed in our trial: A randomized trial evaluated tissue penetration of of cilengitide in patients treated prior to surgery for recurrent disease demonstrating higher drug concentrations in the tumor tissue at 2000 mg of cilengitide compared to 500 mg only.[17] Two randomized phase II studies evaluated a lower (500 mg) and higher (2000 mg) cilengitide dose in recurrent and newly diagnosed glioblastoma, both trials showed consistently a better outcome for patients treated with at the 2000 mg dose level. [16, 29] In newly diagnosed GBM patients cilengitide in addition to TMZ/RT demonstrated a median survival of 17 months and 21 months for the 500 mg and 2000 mg doses, respectively.[29]

Despite encouraging clinical activity throughout all phase I and II trials, a number of preclinical observations have recently provoked controversy about potential counter-productive effects of integrin antagonism in cancer treatment, including the paradoxical

promotion of angiogenesis and metastasis at extremely low doses.[30] A detailed review of these studies is beyond the scope of this discussion. Nevertheless, at the dose used in our regimen, sufficiently high tissue concentrations of cilengitide are achieved.[31]

Based on the results of the present trial and supportive data from complementary trials preclinical and clinical studies, a randomized phase III trial (CENTRIC, EORTC 26071-22072, NCT00689221) was launched at the end of 2008. The standard arm corresponds to standard TMZ/RT chemoradiation [5] whereas the experimental arm investigates the addition of high-dose (2000 mg cilengitide to TMZ/RT. Eligibility is restricted to patients with a methylated MGMT promoter, speculating that this anti-angiogenic strategy is most effective in the presence of an active chemotherapy regimen. The excellent safety profile and the observation of some early relapses after cessation of cilengitide suggest that the discontinuation of cilengitide after completion of 6 months in the trial reported here may have been premature. Indeed, mechanistic considerations warrant prolonged integrin inhibition. Accordingly, cilengitide will be administered until progression or for up to 18 months in the CENTRIC trial.

Acknowledgments

We thank the patients for their participation; nurses, data managers, and pharmacists for support and care. The following investigators also enrolled patients: Lionel. D'Hondt, Eric Joosens, Guido Nikkhah, Johannes Schramm, and Dietmar Krex. We thank Marie-France Hamou for technical and Kevin De-Voy and Andreas Eilers for editorial assistance.

REFERENCES

1. Ohgaki H, Dessen P, Jourde B, et al: Genetic pathways to glioblastoma: a population-based study. Cancer Res 64:6892-6899, 2004

2. Barnholtz-Sloan JS, Sloan AE, Schwartz AG: Cancer of the brain and other central nervous system. In: Ries LAG, Young JL, Keel GE, et al. (eds). SEER Survival Monograph: Cancer survival among adults: US SEER Program, 1988–2001, Patient and tumor characteristics. National Cancer Institute, SEER Program, NIH Pub. No. 07-6215, Bethesda, MD, 2007. 203-216. http://seer.cancer.gov/publications/survival/surv_brain.pdf

3. Ohgaki H, Kleihues P: Epidemiology and etiology of gliomas. Acta Neuropathol 109:93-108, 2005

4. Kargiotis O, Rao JS, Kyritsis AP: Mechanisms of angiogenesis in gliomas. J Neurooncol 78:281-293, 2006

5. Stupp R, Mason WP, van den Bent MJ, et al: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-996, 2005

6. Hegi ME, Diserens AC, Godard S, et al: Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clin Cancer Res 10:1871-1874, 2004

7. Hegi ME, Diserens AC, Gorlia T, et al: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997-1003, 2005

8. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME: *MGMT* promoter methylation in malignant gliomas: ready for personalized medicine? Nature Rev Neurol 2010, in press

9. Stupp R, Ruegg C. Integrin inhibitors reaching the clinic. J Clin Oncol 25:1637-1638, 2007

10. Tucker GC: Integrins: molecular targets in cancer therapy. Curr Oncol Rep 8:96-103, 2006

11. Dechantsreiter MA, Planker E, Mathä B, et al: *N*-Methylated cyclic RGD peptides as highly active and selective $\alpha_v\beta_3$ integrin antagonists. J Med Chem 42:3033-3040, 1999

12. Goodman SL, Hölzemann G, Sulyok GA, et al: Nanomolar small molecule inhibitors for $\alpha_v\beta_6$, $\alpha_v\beta_5$, and $\alpha_v\beta_3$ integrins. J Med Chem 45:1045-1051, 2002

13. Maurer GD, Tritschler I, Adams B, et al: Cilengitide modulates attachment and viability of human glioma cells, but not sensitivity to irradiation or temozolomide in vitro. Neuro Oncol 2009 Feb 16 [Epub ahead of print]

14. Nisato RE, Tille JC, Jonczyk A, et al: $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonists inhibit angiogenesis in vitro. Angiogenesis 6:105-119, 2003

15. Schnell O, Krebs B, Wagner E, et al: Expression of integrin $\alpha_v\beta_3$ in gliomas correlates with tumor grade and is not restricted to tumor vasculature. Brain Pathol 18:378-386, 2008

16. Reardon DA, Fink KL, Mikkelsen T, et al: Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme. J Clin Oncol 26:5610-5617, 2008

17. Gilbert M, Lamborn K, Lassman A, et al: Tumor tissue delivery of cilengitide after intravenous administration to patients with recurrent glioblastoma (GBM): Preliminary data from NABTC protocol 03-02. Presented at the 12th Annual Meeting of the Society for Neuro-Oncology, Dallas, TX, November 15-18, 2007, abstr MA-39

18. Nabors LB, Mikkelsen T, Rosenfeld SS, et al: Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. J Clin Oncol 25:1651-1657, 2007

19. Mikkelsen T, Brodie C, Finniss S, et al: Radiation sensitization of glioblastoma by cilengitide has unanticipated schedule-dependency. Int J Cancer 124:2719-2727, 2009

20. MacDonald TJ, Taga T, Shimada H, et al: Preferential susceptibility of brain tumors to the antiangiogenic effects of an alpha(v) integrin antagonist. Neurosurgery 48:151-157, 2001

21. Eskens FA, Dumez H, Hoekstra R, et al: Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in patients with advanced solid tumours. Eur J Cancer 39:917-926, 2003

22. Shen F, Decosterd LA, Gander M: Determination of temozolomide in human plasma and urine by high-performance liquid chromatography after solid-phase extraction. J Chromatogr 667:291-300, 1995

23. Vlassenbroeck I, Califice S, Diserens AC et al: Validation of real-time methylation-specific PCR to determine O^6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. J Mol Diagn 10:332-337, 2008

24. Macdonald DR, Cascino TL, Schold SC Jr, et al: Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8:1277-1280, 1990

25. Friedman HS, Kerby T, Calvert H: Temozolomide and treatment of malignant glioma. Clin Cancer Res 6:2585-2597, 2000

26. Murat A, Migliavacca E, Gorlia T, et al: Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol 26:3015-24, 2008

27. Brandes AA. Franceschi E, Tosoni A, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol 26:2192–2197, 2008

28. Torcuator R, Zuniga R, Mohan YS, et al. Initial experience with bevacizumab treatment for biopsy confirmed cerebral radiation necrosis. J Neurooncol 94: 63-68, 2009

29. Nabors LB, Mikkelsen T, Batchelor T, et al. NABTT 0306: A randomized phase II trial of EMD 121974 in conjunction with concomitant and adjuvant temozolomide with radiation therapy in patients with newly diagnosed glioblastoma multiforme (GBM). J Clin Oncol 2009;27(suppl.):abstract 2001

30. Reynolds AR, Hart IR, Watson AR, et al: Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. Nat Med 15:392-400, 2009

31. Weller M, Reardon D, Nabors B, et al: Will integrin inhibitors have proangiogenic effects in the clinic? Nat Med 15:726, 2009

Table 1. Baseline Patient Characteristics (n = 52)

Characteristic	Number of patients (%)
Median age, years (range)	57 (32-68)
Sex	
Male	32 (62)
Female	20 (38)
ECOG performance status	
0 or 1	48 (92)
2	4 (8)

Prior treatment	
Corticosteroids	43 (83)
Debulking surgery	43 (83)
Complete resection	23 (44)
Partial resection	20 (39)
Biopsy	9 (17)
Independent histopathologic confirmation of glioblastoma	48 (92)
Other	
Anaplastic astrocytoma (WHO grade III)	2 (4)
Material insufficient or missing for diagnosis	2 (4)
MGMT promoter status	
Methylated	23 (44)
Unmethylated	22 (42)
Unknown	7 (13)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; WHO, World Health Organization.

Table 2. Survival in the ITT Population and by *MGMT* Status

Endpoint	ITT Population (n = 52)	Patients With *MGMT* Promoter Methylation (n = 23)	Patients Without *MGMT* Promoter Methylation (n = 22)

Progression-free survival			
12 months	33 (21-46)	52 (30-70)	9 (2-25)
18 months	19 (9-31)	33 (15-52)	5 (0-19)
24 months	15 (6-26)	28 (12-48)	5 (0-19)
Overall survival			

Median OS, months (95% CI)	16.1 (13.1-23.2)	23.2 (15.5-NR)	13.1 (9.7-17.6)

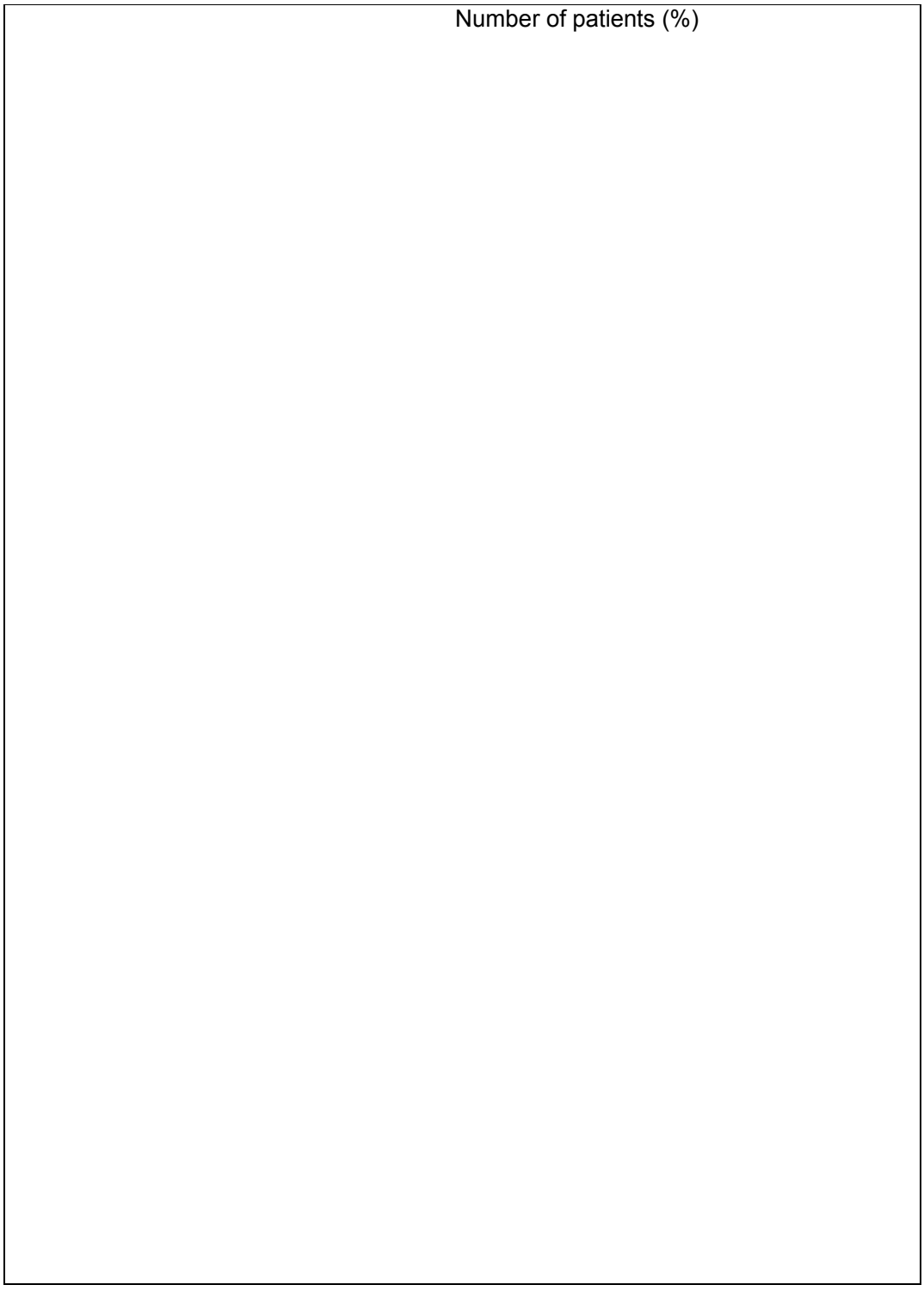
6 months	90 (78-96)	100 (NA)	81 (57-93)

Table 3. Hematologic Toxicity Reported by Laboratory Values, Irrespective of Relationship to Study Drug (n = 52)

	Number of patients (%)		
Lymphocytopenia	22 (42)	29 (56)	51 (98)

Thrombocytopenia	29 (56)	7 (14)	36 (69)
Neutropenia	9 (17)	5 (10)	14 (27)

Table 4. Treatment-Related Nonhematologic Adverse Events* (n = 52)


Number of patients (%)

Anorexia	15 (29)	1 (2)	16 (31)

Supplementary Table 1. Mean plasma PK parameters of cilengitide by week

Week	Statistic	PK Parameter

C_{max} [µg/mL] **t_{max} [h]**

λ_z [1/h]

$t_{1/2}$ [h]

MRT [h]

$AUC_{0-\infty}$ [µg/mL*h]

CL

1	**N**	**11**	**11**	**6**	**6**	**6**	**6**	**6**	**6**	**6**

Mean	25.6	1.14	72.1	0.350	2.02	2.75	7.51	21.9	21.0

%CV	27.1	9.2	27.1	16.6	15.5	17.5	35.1	40.1	48.6

Min	13.6	0.92	45.5	0.241	1.55	2.38	5.45	12.2	13.0

8	**N**	**9**	**9**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

%CV	21.8	6.8	38.8	28.8	34.7	28.7	28.2	10.3	13.5

Min	17.0	1.08	46.4	0.299	1.48	2.55	5.14	16.8	15.4

N: total number of subjects; Min: minimum; Max: maximum; %CV: percentage of coefficient variation.

Supplementary Table 2. Mean plasma PK parameters of temozolomide by day in week 2

Day	Statistic	PK Parameter

C_{max} [ng/mL]	t_{max} [h]	$AUC_{0-□}$ [ng/mL*h]	λ_z [1/h]	$t_{1/2}$ [h]	MRT [h]	CL/f [L/h/m²]	V_z/f [L/m²]	V_{ss}/f [L/m²]

Mean	3497	0.95	10269	0.380	1.84	3.10	7.42	19.7	23.0

4	**N**	**10**	**10**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Max	4982	2.00	12617	0.474	2.47	3.79	9.70	27.0	30.0

Figure legends

Fig 1a. Kaplan-Meier estimate for progression-free survival (all patients)

Fig 1b. Kaplan-Meier estimates for progression -free survival by *MGMT* promoter methylation status

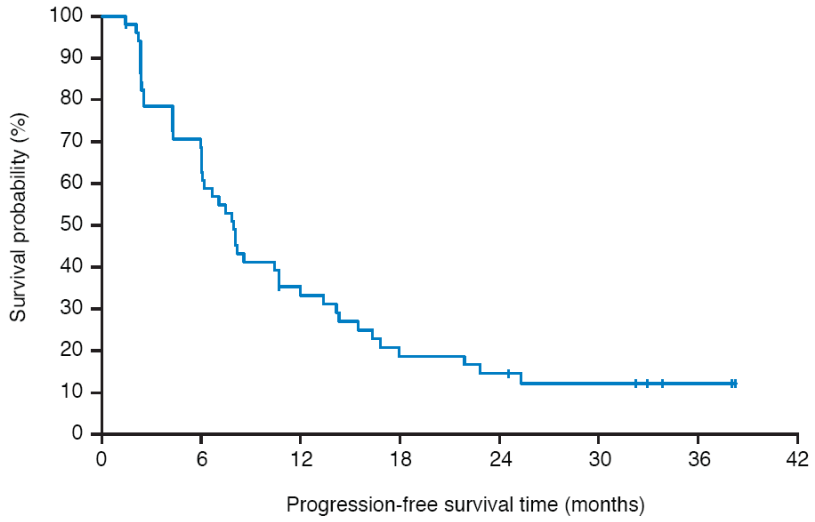
Fig 2a. Kaplan-Meier estimate for overall survival (all patients)

Fig 2b. Kaplan-Meier estimate for overall survival by *MGMT* promoter methylation status.

Fig 3a. Comparison of the mean (± s.d.) plasma cilengitide concentrations between weeks

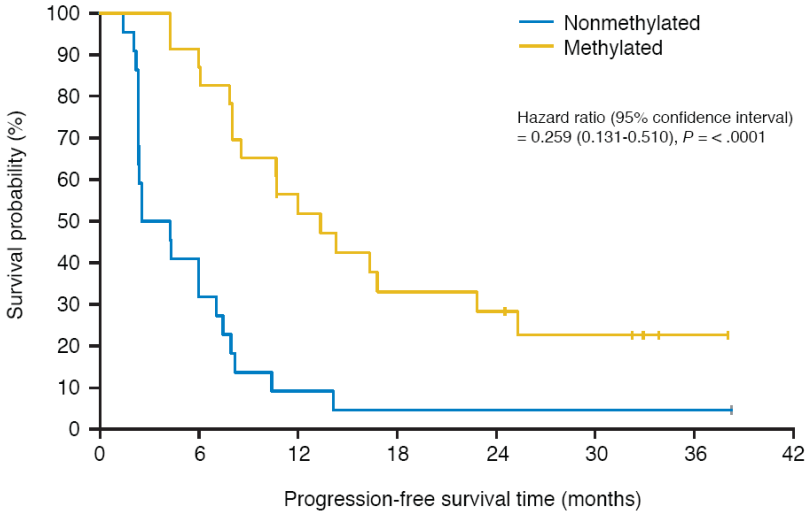
Fig 3b. Comparison of the mean (± s.d.) plasma TMZ concentrations between days in week 2

Fig 1a.



Number of patients at risk	52	35	16	9	7	5	2	0

Fig 1b.



Number of patients at risk								
Nonmethylated	22	9	2	1	1	1	1	0
Methylated	23	21	11	7	6	4	1	0

Fig 2a.

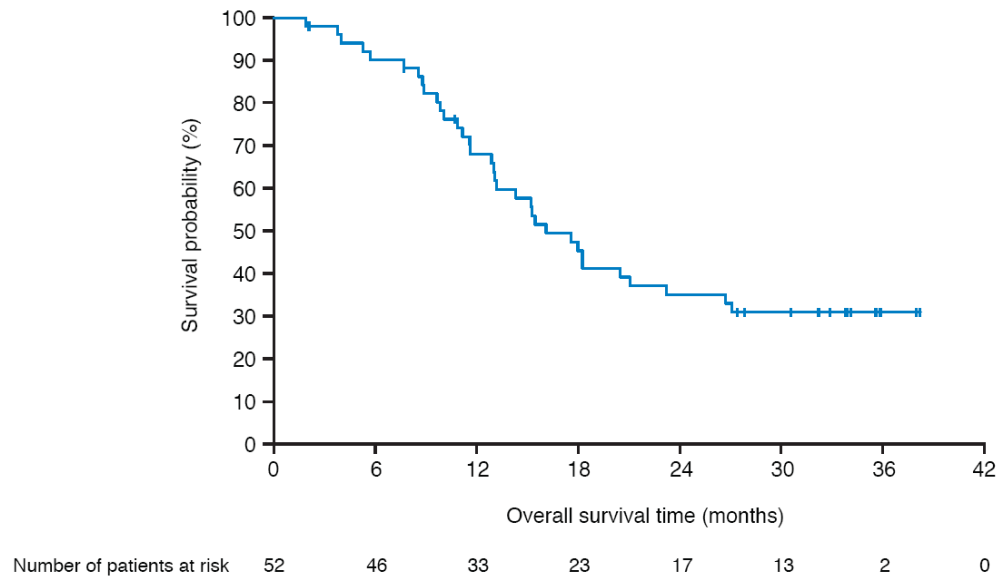
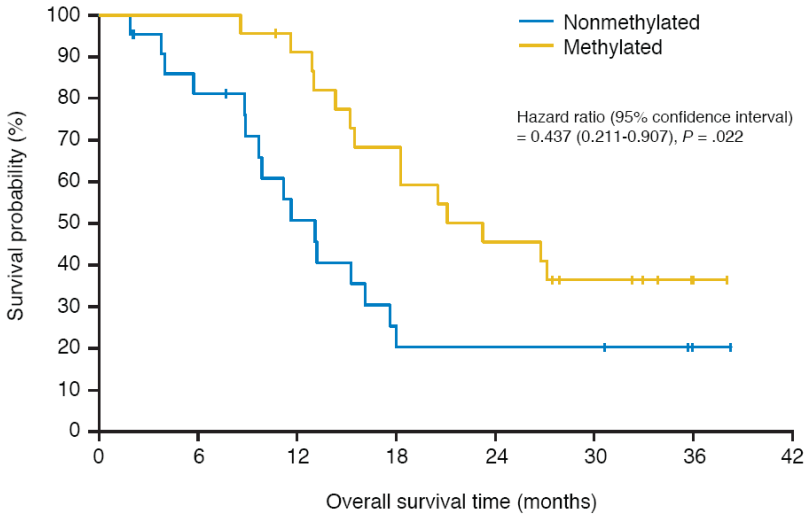


Fig 2b. Kaplan-Meier estimate for duration of overall survival according to methylation status.



Number of patients at risk								
Nonmethylated	22	17	10	5	4	4	1	0
Methylated	23	23	20	15	10	6	1	0

Fig 3a. Comparison of the mean (± s.d.) plasma cilengitide concentrations between Weeks

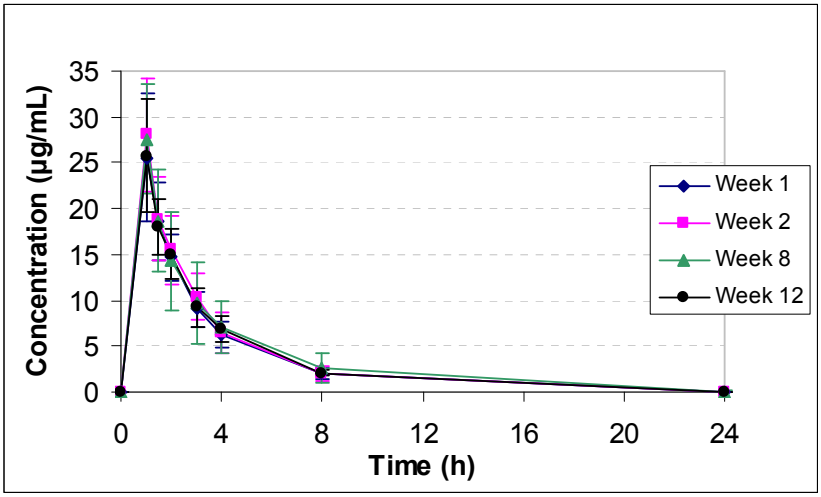


Fig 3b. Comparison of the mean (± s.d.) plasma TMZ concentrations between days in Week 2