Reynolds *et al.* reply:

Outcome in glioblastoma multiforme (GBM) is extremely poor, with median progression-free survival and overall survival of only 6.9 and 14.6 months, respectively[1]. It is therefore vital that new treatment options for this disease are explored. In particular, because these tumors are highly vascularized, the use of antiangiogenic therapy in affected individuals is logical and should be prioritized. Therefore, we strongly support ongoing clinical trials that are designed to elucidate the best way to integrate antiangiogenic therapy into the management of this disease. However, we also believe that a better understanding of antiangiogenic agents and their effects in preclinical models will assist in the successful design of these trials.

We agree with Weller *et al.*[2] that cilengitide may have multiple actions in glioma. Evidence suggests that integrin inhibitors can inhibit angiogenesis[3,4], exert cytotoxic effects on tumor cells[5], increase endothelial cell permeability[6] and act syngergistically with radiation therapy[7]. However, although high micromolar concentrations of integrin inhibitors may inhibit angiogenesis and tumor growth, lower concentrations of integrin inhibitors may actually enhance angiogenesis[8] and promote tumor invasion[9]. These data suggest that, whereas higher concentrations of integrin inhibitors such as cilengitide could be therapeutically active, lower concentrations could be detrimental. In the phase 3 clinical trial of cilengitide (European Organisation for Research and Treatment of Cancer (EORTC) 26071–22072), subjects will receive a very high dose of cilengitide (2,000 mg biweekly). This schedule results in plasma and intratumoral concentrations of cilengitide in the high micromolar range. Encouragingly, these concentrations far exceed the concentrations that promote angiogenesis and tumor invasion[8,9]. Therefore, the selection of this very high cilengitide dose should, in theory, maximize the clinical benefit of cilengitide while also minimizing the potential for any tumor growth–promoting side effects.

Weller *et al.*[2] suggest that the clinical importance of our findings[8] may be questionable because we did not use *in vivo* glioma models. A key study published in 2002 showed that cilengitide monotherapy prolongs the survival of nude mice injected orthotopically with DAOY or U87 human brain tumor cell lines[4]. However, in a more recent study, the survival of nude rats implanted orthotopically with U251 glioma cells was not prolonged by cilengitide monotherapy[7]. These contrasting observations suggest that, even when an orthotopic murine model of glioma is used, the efficacy of cilengitide as an antitumor agent may be dependent on the specific glioma cell lines studied. We would also like to stress that the purpose of our study was not to assess the action of cilengitide in glioma *per se*. Instead, we provide proof of principle that low concentrations of integrin inhibitors can promote angiogenesis and tumor growth[8]. We feel that our recently reported data may explain, at least in part, the mixed results seen with cilengitide in preclinical and clinical studies. That said, because glioma is now the main indication for which cilengitide is being pursued, it will be informative to examine the effects of low and high doses of cilengitide in the glioma setting.

Agonistic effects of integrin inhibitors on tumor growth[8,9] may at first seem paradoxical. However, recent work shows that vascular endothelial growth factor (VEGF) inhibitors may also produce paradoxical effects in cancer, that is, they may lead to increased tumor invasion and metastasis[10,11]. This may explain, at least in part, why a recent adjuvant trial of bevacizumab in colorectal cancer (National Surgical Adjuvant Breast and Bowel Project C-08) did not reach its primary end point of improved disease-free survival[12]. Importantly, these findings do not preclude the use of antiangiogenic agents in patients, but they do have implications for *how* they are used in patients. Perhaps these drugs should only be used in select patient subgroups or used only in combination with appropriate co-administered therapies. Notably, the EORTC 26071–22072 trial will enroll only subjects with glioma who have O^6-methylguanine methyltransferase (*MGMT*) promoter methylation and will randomize them into two arms: temozolimide plus radiation therapy plus cilengitide versus temozolimide plus radiation therapy. In a recent phase 2 trial, this subgroup of subjects with *MGMT* promoter methylation responded better to the temozolimide, radiation therapy and cilengitide combination[13]. Treatment of this molecularly defined patient subgroup is innovative and should increase the chance that the EORTC 26071–22072 trial is successful.

Weller *et al.*[2] suggest that integrin inhibitors may act as chemosensitizing or radiosensitizing agents. A recently published preclinical study provides encouraging evidence that cilengitide may indeed work synergistically with radiation therapy[7]. However, in the rodent glioma model used, the synergistic benefit of cilengitide in sensitizing tumors to radiation therapy was maximal when systemic concentrations of cilengitide were in the low nanomolar range. Intriguingly, these data suggest that low nanomolar concentrations of cilengitide (that is, concentrations similar to those that promoted angiogenesis in our study[8]) may be more effective at synergizing with radiation therapy than high micromolar doses. However, these preclinical data seem to have been overlooked in the proposed EORTC 26071–22072 trial, in which cilengitide is administered at high doses that result in plasma and intratumoral concentrations of cilengitide in the high micromolar range. We find this to be a puzzling disconnect between the preclinical data and the EORTC 26071–22072 trial protocol.

Weller *et al.*[2] compare the radiological response to cilengitide with the radiological response seen with VEGF-antagonizing agents. Recent work shows that the clinical benefit of antiangiogenic therapy in glioma may be due to suppression of brain edema rather than due to inhibitory effects on tumor growth[14]. In this paper, the edema-suppressive effect led to improvements in overall survival, despite radiological progression of disease[14]. Therefore, radiological responses may not always be predictive of overall survival when using antiangiogenic agents in the clinic.

In conclusion, as Weller *et al.*[2] correctly point out, it is vital that appropriately designed clinical trials are employed to investigate the use of antiangiogenic agents in glioma. However, as we have explained here, many conundrums remain regarding how to use these agents appropriately in glioma. We believe that complementary pre-clinical studies should therefore continue, because they may help to design better trials that eventually lead to regulatory agency approval of cilengitide for glioma. It is our sincerest hope that ongoing work in this area will provide new therapeutic options for individuals diagnosed with this devastating condition.

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