Cancer Chemother Pharmacol (2012) 69:281–287
DOI 10.1007/s00280-011-1754-1

SHORT COMMUNICATION

Pharmacokinetic drug interaction between AEE788 and RAD001 causing thrombocytopenia in patients with glioblastoma

David A. Reardon · Timothy Cloughesy · Jeremy Rich · W. K. Alfred Yung · Lotus Yung · Clifford DiLea · Jerry Huang · Margaret Dugan · William Mietlowski · Andrea Maes · Charles Conrad

Received: 10 August 2011 / Accepted: 22 September 2011 / Published online: 9 October 2011

Abstract
Purpose Treating glioblastoma through the simultaneous inhibition of multiple transduction pathways may prove more effective than single-pathway inhibition. We evaluated the safety, biologic activity, and pharmacokinetic profile of oral AEE788, a selective inhibitor of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF), plus oral RAD001, a mammalian target of rapamycin inhibitor, in glioblastoma patients.
Methods This phase IB/II, open-label, multicenter, 2-arm, dose-escalation study enrolled adult glioblastoma patients at first or second recurrence/relapse. Primary objective was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of AEE788 combined with RAD001. Secondary objectives included determining the safety/tolerability, pharmacokinetics, pharmacodynamics, and antitumor activity of the combination.
Results Sixteen patients were enrolled (AEE788 200 mg/day + RAD001 5 mg/day, 2 patients; AEE788 150 mg/day + RAD001 5 mg every other day [qod], 14); all patients discontinued the study most commonly because of disease progression. Four patients experienced DLT (AEE788 200 mg/day + RAD001 5 mg/day, 1 patient; AEE788 150 mg/day + RAD001 5 mg qod, 3). Both patients receiving AEE788 (200 mg/day) plus RAD001 (5 mg/day) experienced clinically significant thrombocytopenia requiring a dose reduction/interruption. AEE788 appeared to inhibit the metabolism of RAD001. The study was terminated prematurely before an MTD was determined because of safety findings in other studies evaluating AEE788 monotherapy.
Conclusions The coadministration of AEE788 and RAD001 in glioblastoma patients caused a clinically significant thrombocytopenia and a higher-than-expected RAD001 area under the curve concentration when dosed at 200 and 5 mg/day, respectively. After a dose reduction to AEE788 (150 mg/day) and RAD001 (5 mg qod), the combination appeared to be better tolerated.

Keywords Glioblastoma · AEE788 · Tyrosine kinase inhibitor · RAD001 · mTOR

D. A. Reardon (✉)
Center for Neuro-Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, SW-460F, Boston, MA 02215, USA
e-mail: David_Reardon@DFCI.harvard.edu

T. Cloughesy
University of California, Los Angeles, Los Angeles, CA, USA

J. Rich
Cleveland Clinic Lerner Research Institute, Cleveland, OH, USA

W. K. Alfred Yung · C. Conrad
University of Texas MD Anderson Cancer Center, Houston, TX, USA

L. Yung
Celgene Corporation, Summit, NJ, USA

C. DiLea · J. Huang · M. Dugan · W. Mietlowski · A. Maes
Novartis Pharmaceutical Corporation, East Hanover, NJ, USA

Introduction

The role of various signal transduction pathways, in particular the ErbB/epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and phosphatidylinositol-3-kinase (PI3K)/Akt pathways, in the pathophysiology of glioblastoma has been well described [1–4]. The activities of some of these pathways have, therefore, become promising targets for glioblastoma treatment.

Results of preclinical data have shown that inhibition of EGFR with simultaneous blockade of either PI3K or Akt inhibits tumor cell proliferation [5, 6]. Additionally, the absence of the tumor suppressor gene–phosphatase and tensin homolog deleted on chromosome ten (PTEN) is associated with constitutive activation of the PI3 K/Akt signaling pathway, resulting in resistance to EGFR tyrosine kinase inhibition [6, 7]. This inhibition can be reversed, however, with PTEN expression or pharmacologic suppression of PI3 k/Akt pathway activity. Clinically effective PI3 K/Akt inhibitors with acceptable toxicity profiles have been difficult to develop, but inhibitors of mammalian target of rapamycin (mTOR) kinase activity, which upregulate the Akt/mTOR signaling pathway, have shown efficacy in preclinical tumor cell lines lacking PTEN expression [3, 8]. Therefore, Goudar et al. [9] evaluated the simultaneous administration of AEE788, an oral receptor tyrosine kinase inhibitor (TKI) that selectively inhibits EGFR/ErbB-2 and VEGF, and RAD001, an mTOR inhibitor, in a glioblastoma model. This combination, compared with controls, was well tolerated and effectively inhibited tumor growth and extended survival times. Similar results of significant inhibition of cell growth were observed in an EGFR TKI-resistant non-small cell lung cancer cell line treated with the combination of AEE788 and RAD001 [10].

AEE788 and RAD001, both oral agents, are absorbed through the gastrointestinal tract and are metabolic substrates of cytochrome P-450 enzyme 3A4 (CYP3A4) [11, 12]. In human liver microsomes incubated with selective probe substrates of the cytochrome P-450 isoforms, AEE788 moderately inhibits CYP3A4/5 (half maximal inhibitory concentration [IC_{50}] $\approx$ 2–7.5 μM) (data on file). Therefore, drug–drug interactions may result from the concomitant administration of AEE788 and drugs eliminated primarily by CYP3A4/5-dependent metabolism, including RAD001. Indeed, in vitro study results have estimated that at pharmacologically active concentrations (i.e., mean steady-state plasma concentration 0.25 μM [110 ng/mL]), AEE788 inhibits metabolism of RAD001 by 20% (at mean steady-state concentration 0.08 μM [100 ng/mL]), increasing RAD001 blood concentrations by 1.3 times concentrations observed with RAD001 monotherapy (data on file). Furthermore, data from a dose-escalation trial (AEE788 2101) in cancer patients with solid tumors indicate that exposure of AEE788's pharmacologically active metabolite, AQM674, is similar to that of AEE788 (data on file). Drug interactions between RAD001 and AQM674, therefore, are also likely, further increasing RAD001 blood concentrations. RAD001 dose reductions may, therefore, be necessary to obtain the desired exposure of RAD001 coadministered with AEE788. Additionally, other drugs known to interact with CYP3A4, such as enzyme-inducing anticonvulsant drugs (EIACDs) (e.g., phenytoin, carbamazepine, phenobarbital, oxcarbazepine), may also affect RAD001 exposure.

Based on these preclinical and pharmacokinetic (PK) data, we designed a study evaluating the safety, tolerability, biologic activity, and PK profile of AEE788 coadministered daily with RAD001 in adult patients experiencing a first or second recurrence or relapse of glioblastoma who were receiving non-EIACDs or no anticonvulsant drugs. Because of the possible drug–drug interactions between AEE788 and RAD001, RAD001 was administered at a fixed dose determined by RAD001 phase I studies; AEE788 doses were escalated according to a model that assessed the drug's toxicity during the study (data on file). This article reviews the PK findings of this trial, in particular, the PK drug interaction observed between AEE788 and RAD001.

Patients and methods

Study population

This phase IB/II, open-label, multicenter, 2-arm dose-escalation study enrolled patients (≥18 years) with histologically confirmed glioblastoma at first or second recurrence or relapse who were not receiving an EIACD and had (1) a Karnofsky Performance Status (KPS) ≥70, (2) acceptable laboratory values, and (3) a life expectancy ≥12 weeks. The study excluded patients with Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) grade-2 or greater peripheral neuropathy or unresolved diarrhea or any concurrent severe and/or uncontrolled medical conditions that could compromise study participation.

Study objectives

The primary objective of this study was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of escalated doses of oral AEE788 (initial dose, 200 mg/day) combined with fixed doses of oral RAD001 (5 or 10 mg/day), administered once daily (qd) in 28-day cycles, in adult patients who had a first or second recurrence or relapse of glioblastoma and not receiving an EIACD. Secondary objectives included characterizing the safety, PKs, pharmacodynamics, and antitumor activity of this drug combination.

Dose-escalation methods

Dose escalation was guided using a 6-parameter Bayesian logistic regression model for the probabilities of DLT incorporating the escalation with overdose control (EWOC) principle described by Babb and colleagues [13, 14]. The DLT rates were grouped into four intervals: under-dosing

(<20%), targeted toxicity (20–35%), excessive toxicity (35–60%), and unacceptable toxicity (≥60%). The EWOC principle dictated that a dose combination was potentially unsafe if it had ≥25% chance of excessive and/or unacceptable toxicity and/or ≥5% chance of unacceptable toxicity when the model was updated with DLT data from a new cohort of patients enrolled in the study or from glioblastoma patients enrolled in another AEE788 monotherapy trial. A dose-escalation teleconference among investigators was held to discuss the next dose combination considering available adverse event (AE) (non-DLT), PK, and pharmacodynamic data, as well as the "safe" dose combinations from the Bayesian logistic regression model and meeting the EWOC criteria. Patient cohorts consisted of newly enrolled patients for each dose level (i.e., intrapatient dose escalation was not permitted) who received escalating doses of AEE788 until an MTD was established.

DLTs included the following AEs that occurred during the first 28 days after the initiation of study therapy: CTCAE grade 1 or greater neurotoxicity; grade 2 or greater renal or cardiac toxicities; grade 3 or greater hematologic or hepatic toxicity or nausea/vomiting despite antiemetic administration. Any grade 3 or greater toxicity resulting in a 7-day or greater delay in AEE788 or RAD001 administration was also considered a DLT.

The DLT rate was determined according to dose cohorts using the MTD-determining population; patients who experienced a DLT were reported individually by dose cohort and specific DLT.

Pharmacokinetics

PK parameters were computed for AEE788 and its primary metabolite, AQM674, and RAD001 (parent compound only). Maximum matrix (serum/blood) concentrations (C_{max}) and time at which maximum concentration was reached (T_{max}) were determined using compartment model independent methods. The area under the curve (AUC) from pre-administration to each quantifiable data point (C_{last}) (i.e., $AUC_{0–Clast}$) and to 24 h post-administration ($AUC_{0–24}$) was computed using the linear trapezoidal rule. An accumulation index (RA) ($AUC_{0–24}$ day X/$AUC_{0–24}$ day 1) was used to determine drug accumulation.

Safety

Safety assessments consisted of monitoring and recording all AEs and serious AEs (SAEs); regularly monitoring hematology, blood chemistry, and urine values, and vital signs; performing physical examinations, including weight and KPS; and assessing cardiac function. Chest radiography was repeated as clinically indicated. AE grading was based on CTCAE version 3.0.

Efficacy

Objective changes in tumor size were assessed by Gd-MRI using a method adapted from Macdonald et al. [15] at end of cycle 1 and all subsequent even-number cycles. Tumor responses were defined as complete response (CR), partial response (PR), progressive disease (PD), stable disease (SD), or unknown and summarized according to dose cohort. The response assessment also included neurologic examinations performed within 1 week of Gd-MRI.

Statistical methods

Summary statistics were used to report AUC and C_{max} on days 1, 15, and 28 of cycle 1 ($AUC_{0–\infty}$ was used for day 1 and $AUC_{0–24}$ for days 15 and 28). An analysis of variance (ANOVA) was performed on log-transformed AUC and C_{max} (days 1, 15, 28) using a linear mixed-effects model to assess day effect. Median values and ranges were reported for T_{max}.

To determine the relative exposure (i.e., AUC) of patients receiving combination therapy compared with patients receiving the RAD001 monotherapy, a parametric tolerance interval approach was used [16]. A 95% limit that controlled the upper tail of the AUC distribution to less than or equal to 5% was calculated for patients receiving RAD001 5 mg monotherapy. The AUC data were assumed to follow a lognormal distribution with an assumed mean (standard deviation) of 5.52 (0.34) ln ng h/mL. The individual observed AUC values under combination therapy were then compared relative to the upper 95% limit. Data from 40 RAD001-treated patients were used in this analysis (data on file).

Results

A total of 16 patients (AEE788 200 mg/day + RAD001 5 mg/day: 2 patients; AEE788 150 mg/day + RAD001 5 mg qod: 14 patients) were enrolled between November 2004 and November 2005 (Table 1).

Four patients experienced DLT (1 patient at AEE788 [200 mg/day] + RAD001 [5 mg/day]; 3 patients at AEE788 [150 mg/day] + RAD001 [5 mg qod]). Because 1 of 2 patients (50%) receiving the initial doses (AEE788 [200 mg/day] and RAD001 [5 mg/day]) developed grade 3 thrombocytopenia that was reported as a DLT, subsequent patients received the provisional dose level of AEE788 (150 mg/day) and RAD001 (5 mg qod). Among 14 patients treated with AEE788 (150 mg/day) plus RAD001 (5 mg qod), 13 patients met the study protocol-defined criteria for inclusion in the MTD-determining population. Of these patients, 1 patient experienced grade 3 thrombocytopenia

Table 1 Patient demographics

Variable	AEE788 200 mg qd + RAD001 5 mg qd $n = 2$	AEE788 150 mg qd + RAD001 5 mg qod $n = 14$	All patients $n = 16$
Study populations, *n* (%)			
ITT	2 (100)	14 (100)	16 (100)
Safety	2 (100)	14 (100)	16 (100)
MTD evaluation	2 (100)	13 (92.9)	15 (93.8)
Gender, *n* (%)			
Male	2 (100)	9 (64.3)	11 (68.8)
Female	0 (0)	5 (35.7)	5 (31.3)
Age, year	45.5 (40–51)	53 (28–71)	52 (28–71)
Median (range)			
Race, *n* (%)	2 (100)	14 (100)	16 (100)
White			
KPS (%), *n* (%)			
100	0 (0)	2 (14.3)	2 (12.5)
90	0 (0)	7 (50.0)	7 (43.8)
80	2 (100)	4 (28.6)	6 (37.5)
70	0 (0)	1 (7.1)	1 (6.3)
Prior relapse, *n* (%)			
1st	1 (50.0)	6 (42.9)	7 (43.8)
2nd	1 (50.0)	8 (57.1)	9 (56.3)
Prior treatment, *n* (%)			
Surgery			
Yes	2 (100)	14 (100)	16 (100)
No	0 (0)	0 (0)	0 (0)
Radiation therapy			
Yes	2 (100)	14 (100)	16 (100)
No	0 (0)	0 (0)	0 (0)
Chemotherapy			
Yes	2 (100)	13 (92.9)	15 (93.8)
No	0 (0)	1 (7.1)	1 (6.2)

ITT intent-to-treat, *KPS* Karnofsky performance status, *MTD* maximum tolerated dose

that was reported as a DLT; 2 other DLTs were reported including grade 3 diarrhea and grade 4 creatine phosphokinase (CPK) elevation. Both cases of grade 3 thrombocytopenia were reversible.

AEs were reported for all patients and most commonly included diarrhea, fatigue, rash, and alanine aminotransferase (ALT) increases (Table 2). Additionally, 4 patients (28.6%) each experienced grades 2–3 aspartate aminotransferase (AST) or ALT abnormalities, grade 3 thrombocytopenia, and grades 2–3 mucositis. One patient (6.3%) died within 28 days of receiving the last dose; the cause of death was a pulmonary embolism and reported as unrelated to the study drug.

Mean AUCs for AEE788, AQM674, and RAD001 are outlined in Tables 3 and 4. Because patients receiving the AEE788-RAD001 combination did not also receive RAD001 monotherapy, no direct measure of increased RAD001 exposure indicative of a drug–drug interaction was possible. An indirect estimate of increased RAD001 exposure, however, was obtained by calculating an upper normal limit for the AUC_{0-24} (h ng/mL) in patients receiving RAD001 (5 mg/day). The upper normal limit was based on a 95% tolerance limit for the upper 95th percentile of historical PK data observed in patients treated with 5 mg of daily RAD001 monotherapy (data on file). The AUC in both patients in this study treated with the combination of AEE788 (200 mg/day) plus RAD001 (5 mg/day) exceeded the calculated upper normal limit (536 ng h/mL) of the RAD001 AUC (117.4 and 147.6% of upper normal limit, respectively). This supports the claim of a drug–drug interaction increasing the RAD001 exposure (Table 4) (data on file). The resulting mean RAD001 exposure in patients treated with RAD001 (5 mg/day) and AEE788 (200 mg/day) was approximately equivalent to RAD001 (10 mg/day) monotherapy (mean AUC_{0-24} = 514 h ng/mL) (data on file). Mean RAD001 exposure after

Table 2 AEs occurring in >15% of patients

Adverse event	No. of patients (%)		
	AEE788 200 mg qd + RAD001 5 mg qd $n = 2$	AEE788 150 mg qd + RAD001 5 mg qod $n = 14$	All patients $n = 16$
Diarrhea	1 (50.0)	8 (57.1)	9 (56.3)
Fatigue	1 (50.0)	7 (50.0)	8 (50.0)
Rash	1 (50.0)	7 (50.0)	8 (50.0)
ALT increase	0 (0)	5 (35.7)	5 (31.3)
Thrombocytopenia	2 (100)	3 (21.4)	5 (31.3)
Hyperglycemia	0 (0)	4 (28.6)	4 (25.0)
Muscular weakness	1 (50.0)	3 (21.4)	4 (25.0)
Stomatitis	0 (0)	4 (28.6)	4 (25.0)
AST increase	0 (0)	3 (21.4)	3 (18.8)
Blood CK increase	1 (50.0)	2 (14.3)	3 (18.8)
Convulsion	2 (100)	1 (7.1)	3 (18.8)
Nausea	0 (0)	3 (21.4)	3 (18.8)
Peripheral edema	1 (50.0)	2 (14.3)	3 (18.8)
Urinary tract infection	1 (50.0)	2 (14.3)	3 (18.8)

AE adverse event, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CP* creatinine phosphokinase

Table 3 Mean $AUC_{0\text{-}24}$ of AEE788 and AQM674 during cycle 1

AEE788 dose (mg/day)	RAD001 dose (mg)	RAD001 dose interval (h)	Study day	$AUC_{0\text{-}24}$ h ng/mL Value (range) (No. of patients)	
				AEE788	AQM674
200	5	24	1	268 (235–301) ($n = 2$)	57 (55–59) ($n = 2$)
			15	1,344 (981–1,707) ($n = 2$)	187 (167–207) ($n = 2$)
150	5	48	1	178 (49–514) ($n = 13$)	73 (27–139) ($n = 13$)
			15	679 (96–2,314) ($n = 10$)	238 (51–559) ($n = 10$)
			28	1,083 (244–5,744) ($n = 9$)	206 (49–539) ($n = 8$)

AUC area under the curve

dose reductions of both RAD001 to 5 mg qod and AEE788 to 150 mg qd was similar to mean $AUC_{0–24}$ in patients treated with (RAD001 5 mg/day) monotherapy. Relative to the initial dose cohort of AEE788 (200 mg/day) plus RAD001 (5 mg/day), the second dose cohort of AEE788 (150 mg/day) plus RAD001 (5 mg qod) appeared to be better tolerated in the patients studied. After multiple doses, RAD001 increased the exposure of AEE788, but did not affect AQM674 exposure. The exposure of AEE788 (200 mg/day) on day 1 ($AUC_{0–24}$ = 268 h ng/mL) appears similar to the mean values that have been observed with AEE788 (150 mg) monotherapy ($AUC_{0–24}$ = 221 h ng/mL). AEE788 (200 mg/day) exposure on days 15 ($AUC_{0–24}$ = 1,089 h ng/mL) and 28 ($AUC_{0–24}$ = 1,603 h ng/mL), however, was similar to that observed with AEE788 (250 mg/day) monotherapy (day 15: $AUC_{0–24}$ = 2,038 h ng/mL; day 28: $AUC_{0–24}$ = 1,574 h ng/mL). The exposure of AEE788 in the AEE788 (150 mg/day) plus RAD (5 mg qod) group is similar to that of AEE788 monotherapy at 150 mg qd in patients with advanced tumors. The increased AEE788 exposure did not appear to be clinically relevant.

Table 4 Mean AUC_{0-24} of RAD001 during cycle 1

AEE788 dose (mg/day)	RAD001 dose (mg)	RAD001 dose interval (h)	Study day	Value (range) (No. of patients)	
				$AUC_{0–24}$ (h ng/mL)	$AUC_{0–48}$ (h ng/mL)
200	5	24	15	710 (629–791) (n = 2)	NA
150	5	48	1	NA	361 (233–589) (n = 8)
			15	NA	563 (199–1,057) (n = 8)
			28	NA	482 (80–1,050) (n = 9)

AUC area under the curve, *NA* not applicable

Overall, no CRs or PRs were observed. SD at end of cycle 2 occurred in 7 patients (AEE788 [200 mg/day] + RAD001 [5 mg/day]: 1 patient; AEE788 [150 mg/day] + RAD [5 mg qod]: 6 patients). Median number of days on treatment was 46.5 days (range 15–78 days) for those receiving AEE788 (200 mg/day) + RAD001 (5 mg/day) and 59.7 days (range 9–241) for the AEE788 (150 mg/day) + RAD (5 mg qod) group.

All patients discontinued the study (10, disease progression; 3, AE; 2, consent withdrawal; 1, abnormal laboratory value). Meaningful comparisons between the 2 dose-escalation groups were not possible because of the small number of patients. Liver function abnormalities observed in other studies evaluating AEE788 prompted premature termination of this study before an MTD was determined; therefore, the dose-expansion phase was not initiated.

Discussion

In this study, the most commonly reported AEs included diarrhea, fatigue, rash, thrombocytopenia, and ALT elevations. DLTs included thrombocytopenia, diarrhea, and CPK elevations. Most of the AEs were similar to AEs observed in studies evaluating single-agent AEE788 or single-agent RAD001. However, both patients who received AEE788 (200 mg) plus RAD001 (5 mg/day) experienced a clinically significant thrombocytopenia requiring a dose reduction/interruption in both patients; 1 of these cases was reported as a DLT. Thrombocytopenia has been observed in clinical trials evaluating daily, single-agent RAD001 (up to 10 mg/day) in cancer patients; the severity, however, was not considered dose limiting [12]. Indeed, grade 3 thrombocytopenia was reported in only 2 of 33 (6%) patients receiving single-agent RAD001 10 mg/day [12]. Likewise, the rate of grade 3 thrombocytopenia ranges from 8% to 12% in patients receiving single-agent RAD001 (10 mg/day) for other oncology indications (e.g., renal cell carcinoma) [17, 18]. Whether the higher rate of grade 3 thrombocytopenia (25%) in the current study is specific to this drug combination is unknown and could not be determined since patient numbers were relatively small. Furthermore, because a direct comparison of the pharmacodynamics and/or PKs between patients receiving the combination and patients receiving monotherapy with either AEE788 or RAD001 was not within the scope of this study, it was not possible to evaluate the causes of AEs. The observed thrombocytopenia may not be due only to a PK interaction as the AUCs of RAD001 5 mg qd and 5 mg qod in combination with AEE788 were similar to that observed with RAD001 10 mg qd monotherapy, a safe and well-tolerated dose used in clinical practice. Interestingly, these safety findings are similar to those from other studies evaluating the mTOR inhibitor temsirolimus combined with other targeted small molecules (i.e., sorafenib, erlotinib, sunitinib) where higher than expected toxicity occurred, often resulting in dose reductions of at least 1 agent within the combination or discontinuation of therapy [19–21].

Because AEE788 and RAD001 share a similar metabolic pathway (i.e., CYP3A4 substrates), the study was designed to incorporate a drug interaction between these agents. Based on the results of both an in vitro study of human liver microsomes and a single-agent AEE788 dose-escalation study, AEE788 and its active metabolite, AQM674, were expected to increase RAD001 concentrations by 11% compared with RAD001 monotherapy levels in the first dose-combination cohort (AEE788 [200 mg/day] and RAD001 [5 mg/day]) of this study (data on file). Using historical RAD001 PK data, it appeared that AEE788 increased (80%) RAD001 exposure considerably more than predicted (data on file).

The study was terminated early before enrollment was completed or all objectives could be achieved because of safety findings (i.e., liver function abnormalities) from other studies evaluating AEE788 monotherapy. Whether

we would have been able to use PK data and a dose-escalation study design to obtain an effective MTD is unknown. Because of unacceptable toxicity, further investigation of AEE788, alone or in combinations with agents such as RAD001, has been abated. However, based on the results of this study, future phase 1 studies evaluating agents with known PK drug interactions should incorporate study designs that determine dose estimates based on known single-agent data as well as tolerability of and PK data from the combination therapy observed during first and subsequent cycles. Likewise, direct comparisons of monotherapy and combination therapy pharmacodynamic and PK data would also prove beneficial in evaluating unexpected drug–drug interactions.

Acknowledgments We are indebted to the patients who participated in this study and study coordinators. We thank Syntaxx Communications, Inc. for assistance with manuscript development and editing. Research support by Novartis Pharmaceuticals Corporation, East Hanover, NJ.

References

1. Humphrey PA, Wong AJ, Vogelstein B et al (1998) Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts. Cancer Res 48:2231–2238
2. Frederick L, Wang XY, Eley G et al (2000) Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res 60:1383–1387
3. Neshat MS, Mellinghoff IK, Tran C et al (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci USA 98:10314–10319
4. Stockhammer G, Obwegeser A, Kostron H et al (2000) Vascular endothelial growth factor (VEGF) is elevated in brain tumor cysts and correlates with tumor progression. Acta Neuropathol 100:101–105
5. Fan QW, Specht KM, Zhang C et al (2003) Combinatorial efficacy achieved through two-point blockade within a signaling pathway-A chemical genetic approach. Cancer Res 63:8930–8938
6. She QB, Solit D, Basso A et al (2003) Resistance to gefitinib in PTEN-null HER overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3′-kinase/Akt pathway signaling. Clin Cancer Res 9:4340–4346
7. Bianco R, Shin I, Ritter CA et al (2003) Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. Oncogene 22:2812–2822
8. Podsypanina K, Lee RT, Politis C et al (2001) An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten ± mice. Proc Natl Acad Sci USA 98:10320–10325
9. Goudar RK, Shi Q, Hjelmeland MD et al (2005) Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma multiforme tumor growth inhibition. Mol Cancer Ther 4:101–112
10. Nakachi I, Naoki K, Soejima K et al (2010) The combination of multiple receptor tyrosine kinase inhibitor and mammalian target of rapamycin inhibitor overcomes erlotinib-resistant lung cancer cell line through c-Met inhibition. Mol Cancer Res 8:1142–1151
11. Nashan B (2002) Review of the proliferation inhibitor everolimus. Exp Opin Investig Drugs 11:1845–1857
12. O'Donnell A, Faivre S, Burris HA III et al (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. J Clin Oncol 26:1588–1595
13. Thall PF, Millikan RE, Mueller P et al (2003) Dose-finding with two agents in phase I oncology trials. Biometrics 59:487–496
14. Babb J, Rogatko A, Zacks S (1998) Cancer phase I clinical trials: efficient dose escalation with overdose control. Stat Med 17:1103–1120
15. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG (1990) Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8:1277–1280
16. Hahn GJ, Meeker WQ (1991) Statistical intervals: a guide for practioners. Wiley, New York
17. Amato RJ, Jac J, Giessinger S et al (2009) A phase 2 study with a daily regimen of the oral mTOR inhibitor RAD001 (everolimus) in patients with metastatic clear cell renal cell cancer. Cancer 115:2438–2446
18. Wolpin BM, Hezel AF, Abrams T et al (2008) Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. J Clin Oncol 27:193–198
19. Patnaik A, Ricart A, Cooper J et al. (2007) A phase I, pharmacokinetic and pharmacodynamic study of sorafenib (S), a multitargeted kinase inhibitor in combination with temsirolimus (T), an mTOR inhibitor in patients with advanced solid malignancies. J Clin Oncol 25(suppl 18):141 s. abstract 3512
20. Chang SM, Kuhn J, Lamborn T et al. (2009) Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas (MG)(NABTC 04–02). J Clin Oncol 27(suppl 15):88 s. abstract 2004
21. Patel PH, Senico PL, Curiel RE, Motzer RJ (2009) Phase I study combining treatment with temsirolimus and sunitinib malate in patients with advanced renal cell carcinoma. Clin Genitourin Cancer 7:24–27