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A phase I study of LY317615 (enzastaurin) and temozolomide in patients with gliomas (EORTC trial 26054)

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We report a phase 1 study to examine the safety and recommended dose of the oral protein kinase C-beta inhibitor (anti-angiogenic) enzastaurin in combination with single-agent temozolomide. The study was conducted in patients with recurrent glioblastoma or newly diagnosed disease that was not treatable with standard (chemo)radiotherapy. Patients were treated with standard dose temozolomide (200 mg/m^2 for 5 days every 4 weeks) together with daily oral enzastaurin. Three dose levels of enzastaurin were investigated: 250 mg daily (OD), 500 mg OD, and 250 mg twice daily (BID). Dose-limiting toxicity was determined in the first 2 cycles, but treatment continued until limiting toxicity or disease progression was identified. Twenty-eight patients were enrolled. No dose-limiting toxicity was noted at 250 mg OD or 500 mg OD. However, at 250 mg BID, 2 dose-limiting episodes of thrombocytopenia were noted. The recommended dose for enzastaurin in combination with standard 4-weekly temozolomide is therefore 500 mg OD. The pharmacokinetics of enzastaurin in combination with temozolomide was evaluated. Temozolomide did not appear to effect enzastaurin exposures at the 250 mg or 500 mg OD dose levels.

Keywords: Enzastaurin (Ly317615), phase 1, temozolomide, thrombocytopenia, toxicity.

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The standard of care for newly diagnosed glioblastoma (GBM) following surgery is the combination of radiation therapy and daily temozolomide followed by monthly 5-day temozolomide.[1] This results in a median survival of 14.6 months and a 2-year survival of 26%. Clearly, there is a need for improvement. Many tumors, including GBM, depend on angiogenesis. Angiogenesis is a complex process requiring the serial activation of receptors by a sequence of ligands.[2] A rate-limiting ligand in this process is vascular endothelial growth factor–A (VEGF).[3] VEGF is also important in pathological angiogenesis, as occurs in tumors such as GBM,[4] in which up-regulation leads to over-expression of the VEGF receptor and hyper-vascularity. Angiogenesis is thought to be essential for the rapid proliferation of tumors, and any agent-inhibiting angiogenesis might then also inhibit tumor growth.

Enzastaurin is an acyclic bisindolylmaleimide that acts as a serine/threonine kinase inhibitor. It is a potent selective inhibitor of classic and novel protein kinase C (PKC) isoforms and, in particular, targets PKC-beta.[5] Phase I studies of enzastaurin failed to reach a maximum tolerated dose (MTD) at doses up to 700 mg daily.[6] It is thought that biologically active plasma enzastaurin concentrations are achieved with daily doses of 500 mg and that steady state plasma concentrations occur within 14 days with doses around this level.[7] An enzastaurin dose of 500 mg daily has become the recommended dose for further study as a single agent. A loading dose is also recommended to more rapidly achieve therapeutic concentrations. PKC-β forms part of the signaling chain for VEGF, a signaling pathway of pivotal importance in glioblastomas.[8] In addition, PKC activates Akt through

phosphorylation and, thus, modulates the PTEN/PI3K/Akt pathway, which is also of central importance to gliomagenesis.[9] Therefore, PKC-β is a potential target for treatment of glioblastoma. Indeed, an early single agent study on recurrent glioblastoma reported a high objective response rate, although 6-month progression-free survival (PFS) rates were not provided at the time of the first report.[10,11] As a result of this report, further studies were initiated, including a randomized controlled evaluation of the activity of enzastaurin in recurrent GBM. In addition, studies were designed to investigate the addition of enzastaurin to standard of care in newly diagnosed glioblastomas. Tabatabai et al demonstrated that enzastaurin enhanced the activity of radiation in mice bearing gliomas, resulting in improved survival.[12] Combining cerebral irradiation with enzastaurin led to diminished tumor volume and irradiation-induced tumor satellite formation, up-regulation of VEGF expression, and enhanced microvessel density in vivo. Thus, if enzastaurin could safely be combined with the standard chemoradiation protocol, the possibility of enhanced efficacy could be explored. As part of a larger phase I program exploring combinations of enzastaurin with standard of care radiotherapy plus temozolomide, we conducted a phase I study, including pharmacokinetics, of the combination of enzastaurin with day 1–5, 150–200 mg/m^2 temozolomide in patients with relapsed high-grade glioma. This study was initiated before the results of other largely negative studies of enzastaurin in GBM were known.

Materials and Methods

Study Design

This was a classical 3 + 3 design, phase I, single-arm study of the combination of enzastaurin and temozolomide in patients with recurrent high-grade gliomas. Eligible patients were aged ≥18 years, with Eastern Oncology Cooperative Group (ECOG)/World Health Organization (WHO) PS 0-2, with any histologically proven supra-tentorial glioma (WHO grade 3 or 4) amenable to treatment with standard 5-day temozolomide. Patients could have first- or second-relapse disease after previous surgery and/or radiotherapy or have newly diagnosed disease considered to be not amenable to radiotherapy. These latter patients might have disease involving multiple lobes of the brain where the radiation fields are considered too large to allow safe but worthwhile dosing, and primary chemotherapy was a justifiable option. When radiotherapy had been previously given, at least 12 weeks must have elapsed prior to enrollment on study. Only 1 prior chemotherapy exposure was allowed (either adjuvant or for first recurrence) and must have been completed at least 4 weeks prior to study enrollment (6 weeks for nitrosoureas). Patient exposure to temozolomide was allowed except those progressing during temozolomide or within 6 weeks of temozolomide treatment completion. Patients with high-grade tumors transformed from low-grade glioma were also eligible.

Patients were required to have adequate bone marrow, renal, hepatic, and cardiac function. Those receiving corticosteroid treatment had to be receiving a stable or decreasing dose for at least 1 week. Patients requiring anticonvulsants had to be exclusively receiving non–enzyme-inducing antiepileptic drugs (EIADs) for at least 2 weeks. Anticoagulant treatment at study entry was not allowed. All patients with reproductive potential must have been using effective contraception. Exclusion criteria included previous or current malignancies at other sites (except nonmelanoma skin cancer or carcinoma in situ of the cervix), unstable systemic diseases, uncontrolled hypertension, pregnancy or lactation in women, or any condition potentially hampering compliance with the study protocol and follow-up schedule.

The study design was approved by the institutional review boards of all participating institutions according to local and national guidelines. All patients gave written informed consent prior to study entry. The study was conducted at 3 European institutions.

Dosing

Initially, 2 dose levels of enzastaurin were planned: regime 1 (loading dose of 500 mg followed by a single daily dose of 250 mg) and regime 2 (loading dose of 1125 mg followed by a single daily dose of 500 mg). For both enzastaurin dose levels, the daily dose of temozolomide was 150 mg/m^2 in cycle 1, increasing to 200 mg/m^2 from cycle 2 onward in the absence of toxicity. Provided no limiting toxicity was encountered, the highest dose level would be expanded to include 12 patients as an extended safety phase. Patients were entered regardless of their prior treatment status. However, it was ensured that at least 6 patients who had been pretreated with chemotherapy were entered in the extended safety phase. For patients completing 6 courses of the combined treatment, therapy could continue with enzastaurin alone at the same dose level. During the course of the study, pharmacokinetic data became available, showing that the same total daily dose of enzastaurin administered as a 250-mg twice daily regimen resulted in modest increases in exposure. To examine this phenomenon clinically, an amendment was added that allowed recruitment of an additional 12 patients (6 pretreated and 6 nonpretreated patients) into a third dose regime: enzastaurin regime 3 (loading dose of 1125 mg followed by a twice daily dose of 250 mg; the temozolomide dose was kept unchanged). Enzastaurin was taken orally daily without a break; temozolomide was taken on days 1–5 of each cycle. One cycle of treatment was defined as 28 days. Patients were advised to take temozolomide with water prior to eating and to take enzastaurin after a meal. Treatment continued until there was evidence of progressive disease or excessive toxicity, the patient

refused to continue, or the treating physician felt termination to be in the patient's best interest.

Dose Escalation

The study used a classical 3 + 3 dose escalation design. A minimum of 3 patients were to be included at each dose level and/or administration schedule. If no patient experienced dose-limiting toxicity (DLT) the next dose level was opened. If 1 of 3 patients experienced DLT, 3 additional patients to a maximum of 6 could be included at that level. If a second patient experienced DLT, no additional patients could be entered at that level, and MTD was defined. Otherwise, escalation to the next dose level was allowed. If $\geq$2 patients (of a maximum of 6) experienced DLT, dose escalation would cease and an additional 12 patients would be included at the next-lower dose (safety cohort). If well tolerated, this dose level would be documented as being the MTD or recommended dose. Patients at any particular dose level could be entered simultaneously. The next-highest dose level could be started when all patients at the prior dose level had completed cycle 1.

Assessment

Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. The DLT for this study was defined as any of the following adverse events related to the combination treatment occurring during cycles 1–2: any nonhematological grade 3/4 toxicity, with the exclusion of alopecia, nausea, vomiting, and fever, which can be rapidly controlled with appropriate measures; an absolute neutrophil count $<$500 neutrophils/mm^3 lasting for 7 days; febrile neutropenia, defined as an absolute neutrophil count $<1.0 \times 10^9$ neutrophils/L and temperature of at least 38.5°C; thrombocytopenia grade 3/4; and any toxicity that did not allow administering at least 80% of the intended dose intensity.

Response to Treatment

A full clinical review and laboratory examination were performed within a week after starting the treatment. A baseline MRI was done within 3 weeks after the first treatment. A full clinical review and laboratory review were done prior to each cycle, with additional hematological assessment at 14 and 21 days. A further gadolinium-enhanced MRI was performed after 3 completed cycles or when there was suspicion of disease progression. Disease assessment was based on Macdonald criteria,[13] and responses were centrally reviewed. For patients continuing to receive enzastaurin alone, review was undertaken on a monthly basis, with scans every 3 months. All patients were followed up for survival.

Dose Modification

If a patient experienced DLT level toxicity, enzastaurin was omitted until the event resolved; if not resolved by 2 weeks, the patient discontinued the study. Otherwise, they could restart enzastaurin at 50% dose, escalating back to 100% in the absence of toxicity for 2 weeks. If the patient experienced nausea and/or vomiting CTCAE grade 3 or 4, enzastaurin was omitted until the event resolved to CTCAE grade 1 or baseline; then, the enzastaurin could be restarted at full dose. If any toxicity could be clearly attributed to temozolomide, patients could continue therapy with enzastaurin alone. Three possible dose levels were allowed for temozolomide (200 mg/m^2, 150 mg/m^2, and 100 mg/m^2 days 1–5). A dose reduction of 1 level would be made in the event of nadir hematological toxicity CTCAE grade 3 or 4 or nonhematological toxicity CTCAE grade 3. If nonhematological toxicity CTCAE grade 4 occurred, the patient discontinued study. If toxicity requiring dose reductions of temozolomide occurred in patients treated at the 100 mg/m^2 daily level, the patient discontinued study.

PK Methods

Steady state plasma samples were collected for the 250 mg daily and 500 mg daily doses and evaluated for enzastaurin and its major active metabolite LY326020. No samples were collected for the 250 mg twice daily dose. Enzastaurin was evaluated alone on cycle 1 day 22 and with temozolomide on cycle 2 day 5. Samples were collected at predose and 2, 4, 6, and 24 h postdose for both cycles. Samples were assayed using a validated LC/MS/MS method by Advion BioServices. Pharmacokinetic parameters were estimated using noncompartmental methods from the plasma concentration–time profiles of enzastaurin and its metabolite with WinNonLin Professional Edition, version 5.3. Total analyte (enzastaurin + LY326020), the area under the concentration versus time curve during 1 dosing interval at steady state (AUCt,ss), and the average drug concentration under steady state conditions during multiple dosing (Cav,ss) were calculated by summing the enzastaurin and LY326020 values for these parameters.

Results

From August 2007 through November 2008, 28 patients were entered into the study from 3 centers (Chu Pitie-Salpetriere, France; U.Z. Rotterdam, Netherlands; and Beatson Cancer Centre, Scotland). Their dose level distribution and characteristics, including eligibility status, are given in Table 1. One patient at level 1 failed to start treatment and was replaced. All other patients completed the study and were evaluable. None was lost to follow-up. Four patients discontinued because of toxicity (level 2 thrombocytopenia); otherwise, discontinuation was attributable to progressive

Table 1. Eligibility status and characteristics of all patients in the study

Eligibility status or characteristic	Dose level, No. (%)		
	250 mg *N* = 4	500 mg *N* = 12	250 mg BID *N* = 12
Histologically proven primary supra-tentorial glioma amenable to Temozolomide treatment	4 (100)	12 (100)	12 (100)
WHO Histologic Grade			
Grade 3	2 (50.0)	3 (25.0)	4 (33.3)
Grade 4	2 (50.0)	6 (50.0)	6 (50)
Grade 3 transformed to Grade 4		3 (25.0)	2 (16.7)
Status of disease			
First recurrence	3 (75.0)	9 (75.0)	10 (83.3)
Second recurrence	0 (0)	2 (16.7)	1 (8.3)
Newly diagnosed not amenable to radiotherapy	1 (25.0)	1 (8.3)	1 (8.3)
Prior Surgery			
No	3 (75.0)	2 (16.7)	0 (0.0)
Yes for primary brain tumor	1 (25.0)	9 (75.0)	9 (75.0)
Yes for recurrence	0 (0.0)	0 (0.0)	1 (8.3)
Yes for both	0 (0.0)	1 (8.3)	2 (16.7)
Prior radiotherapy			
No	1 (25.0)	1 (8.3)	3 (25.0)
Yes	3 (75.0)	11 (91.7)	9 (75.0)
Prior chemotherapy			
No	2 (50.0)	6 (50.0)	4 (33.3)
Yes, adjuvant	2 (50.0)	6 (50.0)	7 (58.3)
Yes for first recurrence	0 (0.0)	0 (0.0)	1 (8.3)
Sex			
Male	3 (75.0)	10 (83.3)	7 (58.3)
Female	1 (25.0)	2 (16.7)	5 (41.7)
Age			
Median	65.8	52.3	51.4
Range	33.7–70.8	22.5–65.8	27.7–66.3
Performance status (ECOG)			
0	1 (25.0)	7 (58.3)	7 (58.3)
1	3 (75.0)	4 (33.3)	4 (33.3)
2	0 (0.0)	1 (8.3)	1 (8.3)

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

disease. All chemotherapy pretreated patients had received temozolomide, except one, who received prior nitrosourea. Following completion of study treatment, ~61% of patients received further chemotherapy, and 14% received radiotherapy.

A description of treatment delivery is given in Table 2. Only 3 cycles (1 at each dose level) were delivered with a reduction in temozolomide dose. These were all attributable to hematotoxicity. Four cycles (3 at dose level 3 and 1 at dose level 2) were given with toxicity-related enzastaurin reductions. No DLT was observed in either the 250 mg or 500 mg once daily dose levels (Table 3). No DLT was seen in the first 3 patients treated at 250 mg twice daily. This dose level was then expanded to include 12 patients. In this cohort, 2 DLTs were observed. Both were grade 3 thrombocytopenia: 1 in a pretreated and 1 in a nonpretreated patient. During the whole treatment period (153 cycles), 12 patients experienced hematological toxicity grade ≥3. Four patients experienced thrombocytopenia grade 3 and 1 grade 4; 8 had lymphopenia grade 3, and 2 had neutropenia grade 3.

Table 2. A summary of the experimental treatment delivered during the study

Enzastaurin dose level	No. of cycles delivered total, median (range)	No. (%) of Cycles delayed due to hematotoxicity	No. (%) of patients who discontinued due to toxicity
250 mg (*N* = 3)	17, 7.0 (2–8)	5 (29.4)	0 (0.0)
500 mg (*N* = 12)	73, 4.5 (2–16)	5 (6.9)	1 (8.3)
250 mg BID (*N* = 12)	63, 4.0 (2–12)	11 (17.5)	3 (25.0)

Abbreviations: BID, twice daily.

Table 3. Primary analysis of dose-limiting toxicity (DLT) incidence according to dose level

Enzastaurin Dose Level	DLT	Cause	Pretreated	Non pretreated
250 mg OD	0	-		
500 mg OD	0	-		
250 mg BID	2	Thrombocytopenia G3 (2)	1	1

Abbreviations: BID, twice daily; G, grade; OD, once daily.

The only nonhematological grade 3/4 related adverse event recorded was grade 3 limb edema in 1 patient. Serious adverse events occurred in 6 patients. These were attributable to nausea and vomiting in 1, pulmonary embolus in 2, raised intracranial pressure in 1, and seizure in 2 (1 also with aphasia). The pulmonary emboli were considered to be not related to treatment, but it was not thought possible to assess the relationship of the remaining events to treatment. In particular, there was no excess of thrombotic/embolic events, no hemorrhagic events grade ≥2, and no cardiac or hypertensive adverse events.

Although the study was not designed specifically to look at response rates, these data were collected (Table 4). There were 3 partial responses, and 15 patients recorded no change between their entry scan and the first assessment 2 months later. In addition, the median PFS was 5.5 months (95% confidence interval [CI], 4.5–8.3 months), and median overall survival was 11.7 months (95% CI, 10.4–14.3 months).

Pharmacokinetics

Plasma concentration-time data and dosing information for pharmacokinetic evaluation were available from 11 patients (Tables 5 and 6). The Cav,ss and AUC for total analyte (enzastaurin + LY326020) were similar at both the 250 mg and 500 mg once daily dose levels when enzastaurin was given alone or with temozolomide. Enzastaurin variability was high across all pharmacokinetic parameters but was similar to that seen in other studies.

Discussion

When initial results of early phase II trials of enzastaurin in recurrent high-grade glioma were reported, the high level of radiological response seen generated considerable excitement.[10,11] However, subsequent evaluation based on survival in a randomized phase III trial failed to confirm benefit over conventional cytotoxic treatment (lomustine).[14,15] This study was stopped after the recruitment of 293 patients when a planned interim analysis showed no improvement of enzastaurin over lomustine. Moreover, when the final results of the early phase I/II studies were published with a 6 months PFS of 7%, the high initial response rates (25%) observed were not reflected in a significant response durability.[14]

The present study was intended just to establish DLT in 2 enzastaurin dose levels (250 mg and 500 mg daily dose) in combination with standard dose temozolomide. Treatment in both these cohorts was well tolerated, with no DLT seen in the first 2 cycles in any patient. However, because of new data showing higher exposure with twice daily dosage (250 mg twice daily), an amendment was raised to examine this dose also. This led to the identification of 2 DLT's in this cohort, both thrombocytopenia, giving a DLT rate of 17% (2 of 12). This rate is borderline for acceptance, and our feeling is that the slightly lower exposure resulting from the once daily dose of 500 mg and the resulting lack of DLT makes this the preferred MTD. There were no other significant toxicities at any dose level. It is concluded that enzastaurin (500 mg once daily) in combination with temozolomide (200 mg/m^2 daily) for 5 days represents the recommended dose. Although only the first 2 cycles of treatment were used to define MTD in our study, treatment and evaluation were continued thereafter. At 250 mg and 500 mg once daily, there did not seem to be a greater level of toxicity than that expected with temozolomide alone. However, at 250 mg twice daily, the number of cycles delayed for reasons of hematological toxicity was more than doubled (5–11) and the number of patients discontinuing treatment because of toxicity also increased (1–3). Because of this, it seems

Table 4. Patient best response following treatment with enzastaurin and temozolomide

Best overall response	Enz 250 mg (*N* = 4)	Enz 500 mg (*N* = 12)	Enz 250 mg BID (*N* = 12)
PR	1 (25.0)	2 (16.7)	0 (0.0)
No Change	1 (25.0)	5 (41.7)	9 (75.0)
PD	1 (25.0)	4 (33.3)	3 (25.0)
Not assessable	0 (0.0)	1 (8.3)	0 (0.0)
Treatment not started	1 (25.0)	0 (0.0)	0 (0.0)

Abbreviations: BID, twice daily; Enz, enzastaurin; PD, progressive disease; PR, partial response.

Table 5. Summary of steady-state plasma pharmacokinetic parameter estimates of enzastaurin, LY326020, and total analyte (enzastaurin + LY326020) in glioma patients following 250-mg once-daily doses of enzastaurin alone and with temozolomide

Geometric mean (CV%)						
	Enzastaurin 250 mg QD			Enzastaurin 250 mg QD + Temozolomide		
	Enz	LY326020	Total analyte	Enz	LY326020	Total analyte
N	3	3	3	3	3	3
$C_{max,ss}$ (nmol/L)	1340 (66)	758 (84)	2070 (74)	1120 (47)	715 (68)	1820 (54)
$t^{a}_{max,ss}$ (h)	3.97 (2.00–4.08)	4.03 (3.97–4.08)	4.03 (3.97–4.08)	4.00 (2.08–4.08)	4.08 (4.00–22.75)	4.00 (2.08–4.08)
$AUC^{b}_{\tau,ss}$ (nmol · h/L)	17 500 (99)	15 000 (90)	32 600 (95)	15 600 (92)	14 300[c] (70)	29 900[c] (82)
$C_{av,ss}$ nmol/L	730 (99)	625 (90)	1360 (95)	650 (92)	604[c] (74)	1260[c] (84)
CL_{ss}/F L/h	27.7 (99)	NC	NC	31.1 (92)	NC	NC
MR	NC	0.855 (8)	NC	NC	0.914[c] (16)	NC

Abbreviations: $AUC_{\tau,ss}$, area under the concentration versus time curve during one dosing interval at steady state; $C_{av,ss}$, average drug concentration under steady state conditions during multiple dosing; CL_{ss}/F, apparent clearance at steady-state; $C_{max,ss}$, maximum observed drug concentration during a dosing interval at steady state; CV, coefficient of variation; h, hour; Enz, enzastaurin; MR, metabolic ratio; *N*, number of subjects; NC, not calculable; QD, once daily; $t_{max,ss}$, time of maximum observed drug concentration during a dosing interval at steady state.
[a]Median (range).
[b]τ equals 24 h.
[c]The $AUC_{(0\text{-tlast})}$ for Patient 3 was used in the calculation of this estimate's geometric mean and CV because the LY326020 $AUC_{\tau,ss}$ could not be calculated. See text for details. The t_{last} for this profile was 22.75 h, which differs only 5% from the τ of 24 h. The error introduced by this imputation was considered inconsequential.

Table 6. Summary of steady-state plasma pharmacokinetic parameter estimates of enzastaurin, LY326020, and total analyte (enzastaurin + LY326020) in glioma patients following 500-mg once-daily doses of enzastaurin alone and with temozolomide

Geometric mean (CV%)						
	Enzastaurin 500 mg QD			Enzastaurin 500 mg QD + Temozolomide		
	Enz	LY326020	Total analyte	Enzastaurin	LY326020	Total analyte
N	7	7	7	7	7	7
$C_{max,ss}$ (nmol/L)	739 (43)	590 (43)	1330 (40)	985 (49)	621 (35)	1620 (41)
$t^{a}_{max,ss}$ (h)	4.00 (1.92–6.17)	2.08 (2.00–6.17)	4.00 (1.92–6.17)	4.00 (2.00–6.25)	6.00 (2.00–6.25)	4.00 (2.00–6.25)
$AUC^{b}_{\tau,ss}$ (nmol · h/L)	9190 (48)	11 500 (46)	21 000 (43)	12 100 (68)	12 800 (38)	25 500 (49)
$C_{av,ss}$ nmol/L	383 (48)	481 (46)	875 (43)	504 (68)	534 (38)	1060 (49)
CL_{ss}/F L/h	106 (48)	NC	NC	80.1 (68)	NC	NC
MR	NC	1.26 (36)	NC	NC	1.06 (48)	NC

Abbreviations: $AUC_{\tau,ss}$, area under the concentration versus time curve during one dosing interval at steady state; QD, once daily; $C_{av,ss}$, average drug concentration under steady state conditions during multiple dosing; CL_{ss}/F, apparent clearance at steady-state; $C_{max,ss}$, maximum observed drug concentration during a dosing interval at steady state; CV, coefficient of variation; Enz, Enzastaurin MR, metabolic ratio; *N*, number of subjects; NC, not calculable; $t_{max,ss}$, time of maximum observed drug concentration during a dosing interval at steady state.
[a]Median (range).
[b]τ equals 24 h.

that the split-dose regime is prohibitively toxic, and 500 mg once daily is the recommended dose.

Simultaneously, Butowski et al. conducted a phase I/II study of enzastaurin in patients with newly diagnosed GBM with the aim of establishing the MTD of enzastaurin administered concomitantly with radiation therapy and daily temozolomide (75 mg/m^2), followed by adjuvant enzastaurin and temozolomide.[16] They explored 2 dose levels of enzastaurin (250 mg and 500 mg) in combination with both the concomitant and adjuvant phases of the standard protocol. In their report, they identified excess toxicity at 500 mg and recommended 250 mg as the appropriate dose. Two DLTs (both thrombocytopenia) were identified in 6 patients treated at the 500 mg level, and they concluded that the recommended dose should be 250 mg. Both DLTs occurred during the last week of combined chemoradiotherapy; thus, the combination of 5-day temozolomide and 500 mg enzastaurin daily has not been addressed in this study. In both our study and that of Butowski et al., the DLT was attributable to thrombocytopenia. Low platelet counts can occur with temozolomide alone or in combination with radiation.[17,18] Clearly, enzastaurin can enhance this toxicity, although the reason behind this is currently obscure.

With respect to the pharmacokinetics, although the number of patients in this study is low, these results suggest that temozolomide does not alter enzastaurin exposure, which is in agreement with the results reported by Butowski et al.[16]

In this study, primarily intended to establish safety, 3 partial responses and 15 patients with stable disease as best response were documented, which we consider to be within the expectation for temozolomide alone. Although the majority of patients had prior exposure to temozolomide, it must be remembered that this was a mixed cohort, including some patients with newly diagnosed disease and patients with (malignant) transformed tumors who had not had previous exposure to temozolomide and among whom higher response rates are to be expected. When the survivals were examined, the outcome was much as expected for this cohort of patients treated with temozolomide alone, and there is nothing to lead us to suspect that enzastaurin was adding to the outcome. This conclusion fits with other efficacy reports.[11,15] The reason for the poor performance of enzastaurin, even with regard to reduction of contrast enhancement in the MRI, is unclear. Galanis and Buckner argue that intratumoral concentrations may be too low or that complementary angiogenesis pathways that bypass PKC inhibition and negate its antitumor effect develop in patients.[19] In any event, it is clear that further single agent development of this drug is not justified in this population. Should further rationally designed combination studies become justified in the future, this phase I trial provides toxicity data to facilitate the approach.

Conclusion

The combination of enzastaurin (500 mg daily dose) with temozolomide (20 mg/m^2 daily) for 5 days each month is feasible and well tolerated. Split dose (250 mg twice daily) with the same chemotherapy leads to prohibitive myelosuppression. The total analyte (enzastaurin + LY326020) exposure in this study, along with the data from Butowski et al., suggest that temozolomide does not affect enzastaurin exposure when given as 250 or 500 mg once daily doses.

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References

1. Stupp R, Mason WP, Van Den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987–996.
2. Yancopoulos G, Davis S, Gale N, Rudge J, Wiegand S, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature*. 2000;407:242–248.
3. Ferrara N, Gerber H, LeCouter J. The Biology of VEGF and its receptors. *Nat Med*. 2003;9:669–676.
4. Zhou Y-H, Tan F, Hess KR, Yung WKA. The Expression of PAX6, PTEN, Vascular Endothelial Growth Factor, and Epidermal Growth Factor Receptor in Gliomas. *Clin Cancer Res*. 2003;9:3369–3375.
5. Kuo W-L, Liu J, Mauceri H, et al. Efficacy of the multi-kinase inhibitor enzastaurin is dependent on cellular signaling context. *Molecular Cancer Therapeutics*. 2010;9:2814–2824.
6. Carducci MA, Musib L, Kies MS, et al. Phase I dose escalation and pharmacokinetic study of enzastaurin, an oral protein kinase C beta inhibitor, in patients with advanced cancer. *J Clin Oncol*. 2006;24:4092–4099.
7. Welch PA, Sinha VP, Cleverly AL, Darstein C, Flanagan SD, Musib LC. Safety, tolerability, QTc evaluation, and pharmacokinetics of single and multiple doses of enzastaurin HCl (LY317615), a protein kinase C-beta inhibitor, in healthy subjects. *J Clin Pharmacol*. 2007;47:1138–1151.
8. Keyes KA, Mann L, Sherman M, et al. LY317615 decreases plasma VEGF levels in human tumor xenograft-bearing mice. *Cancer Chemother Pharmacol*. 2004;53:133–140.
9. Graff JR, McNulty AM, Hanna KR, et al. The protein kinase Cbeta-selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. *Cancer Res*. 2005;65:7462–7469.
10. Fine HA, Kim L, Royce C, et al. Results from phase II trial of enzastaurin (LY317615) in patients with recurrent high grade gliomas. *J Clin Oncol*. 2005;15s (suppl; abstr 1504).
11. Fine HA, Kim L, Royce C, et al. A phase II trial of LY317615 in patients with recurrent high grade gliomas. *J Clin Oncol*. 2004;22:14s (suppl: abstr 1511).
12. Tabatabai G, Frank B, Wick A, et al. Synergistic antiglioma activity of radiotherapy and enzastaurin. *Ann Neurol*. 2007;61:153–161.
13. Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG, et al. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8:1277–1280.
14. Kreisl TN, Kotliarova S, Butman JA, et al. A phase I/II trial of enzastaurin in patients with recurrent high-grade gliomas. *Neuro-Oncology*. 2010;12:181–189.
15. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J Clin Oncol*. 2010;28:1168–1174.
16. Butowski N, Chang SM, Lamborn KR, et al. Enzastaurin plus temozolomide with radiation therapy in glioblastoma multiforme: a phase I study. *Neuro-Oncol*. 2010;12:608–613.
17. Nagane M, Nozue K, Shimizu S, et al. Prolonged and severe thrombocytopenia with pancytopenia induced by radiation-combined temozolomide therapy in a patient with newly diagnosed glioblastoma - Analysis of O6-methylguanine-DNA methyltransferase status. *J Neuro Oncol*. 2009;92:227–232.
18. Sure D, Dunn I, Norden A, Anderson WS. Intracerebral hemorrhage secondary to thrombocytopenia in a patient treated with temozolomide. *Clin Neurol Neurosurg*. 2010;112:741–742.
19. Galanis E, Buckner JC. Enzastaurin in the treatment of recurrent glioblastoma: a promise that did not materialize. *J Clin Oncol*. 2010;28:1097–1098.