Abstract

Purpose: Enzastaurin targets the protein kinase C and phosphatidylinositol 3-kinase/AKT pathways to reduce tumor angiogenesis and cell proliferation and to induce cell death. A phase I trial was conducted to evaluate the feasibility of combining enzastaurin with gemcitabine and cisplatin.

Experimental Design: Patients with advanced cancer received a 14-day lead-in treatment with oral enzastaurin followed by subsequent 21-day cycles of daily enzastaurin, gemcitabine on days 1 and 8, and cisplatin on day 1. Enzastaurin doses were escalated between 350 mg once daily to 500 mg twice daily, whereas gemcitabine doses were either 1,000 or 1,250 mg/m² and cisplatin doses were either 60 or 75 mg/m². Circulating endothelial cell numbers and CD146 and CD133 mRNA expression were evaluated as pharmacodynamic markers.

Results: Thirty-three patients (median age, 58 years) were enrolled in seven dose levels. The maximum tolerated dose was not identified. Two dose-limiting toxicities (grade 2 QT interval corrected for heart rate prolongation and grade 3 fatigue) were reported. Other toxicities included grade 3/4 neutropenia (3 of 6 patients), thrombocytopenia (1 of 6 patients), grade 3 leukopenia (2 patients), and fatigue (5 patients). Enzastaurin twice daily (≥250 mg) resulted in more discontinuations and low-grade toxicities. In the combination, enzastaurin exposures decreased slightly but remained above the target of 1,400 nmol/L, whereas gemcitabine/cisplatin exposures were unaltered. Three patients (9.1%) had partial responses and 13 (39.4%) had stable disease. Measurement of circulating endothelial cell numbers and CD146 and CD133 mRNA expression did not contribute to decision-making on dose escalation.

Conclusions: Recommended phase II dose is 500 mg enzastaurin once daily, 1,250 mg/m² gemcitabine, and 75 mg/m² cisplatin. This regimen is well tolerated with no significant alterations in the pharmacokinetic variables of any drug.
(Upstate kinase profiler data). In preclinical pharmacologic studies in rats and dogs, enzastaurin showed antitumor and antiangiogenic activity (13, 14) and was well tolerated. In some dogs given high daily doses (exposures higher than those expected to occur in most patients), QT and QT interval corrected for heart rate (QTc) prolongation was observed after 5 weeks, and cataracts were seen after 13 weeks.6 In a phase I study of cancer patients, oral enzastaurin was well tolerated at all doses tested [20-700 mg once daily (q.d); ref. 15]. A dose proportional increase in plasma enzastaurin exposures was observed at doses up to 340 mg. Dose escalation was stopped after 700 mg because enzastaurin exposures reached a plateau above 500 mg. Oral bioavailability and pharmacokinetic characteristics of enzastaurin observed in the study support a dose schedule that results in chronic continuous exposure, the most promising strategy for antiangiogenic drug use (16).

Based on preclinical studies showing significant antitumor effect when antiangiogenic drugs were added to cytotoxic chemotherapy (17, 18), and because the toxicity profiles of enzastaurin and cytotoxic agents are nonoverlapping, we initiated a phase I study of enzastaurin in combination with gemcitabine and cisplatin in patients with advanced or metastatic cancer for which no treatment of higher priority exists. The objectives were to determine the recommended phase II dose for the combination regimen and to evaluate safety, pharmacokinetics, and pharmacodynamic biomarkers.

In targeted therapies, biomarker development is important for evaluating treatment efficacy (19) because the relation between toxicity and antitumor effect is less defined (20–23). Quantification of circulating endothelial cells (CEC) and endothelial (progenitor)–specific mRNA expression (CD133 and CD146) in the peripheral blood mononuclear cells have been recognized as possible biomarkers of cancer activity (4, 24–29). Because enzastaurin inhibits vascular endothelial growth factor signaling, we hypothesized that these angiogenesis-related biomarkers may be useful to provide information of the investigated drug combination on cancer activity or to identify a putative nontoxic biologically active dose.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>No. patients</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>33</td>
</tr>
<tr>
<td>Male/female</td>
<td>22/11</td>
</tr>
<tr>
<td>Median age (range), y</td>
<td>58 (38-79)</td>
</tr>
</tbody>
</table>

**Tumor types**
- Esophagus: 2
- Pancreas: 10
- Ovary: 1
- Prostate: 1
- Kidney: 2
- Cholangiocarcinoma: 1
- Melanoma: 7
- Mesothelioma: 2
- Adenocarcinoma of unknown primary: 3
- Head and neck: 1
- Breast: 1
- Urothelial cancer: 1
- Carcinoma amp Vater: 1
- ECOG performance status:
  - 0: 19
  - 1: 13
  - 2: 1

**Prior therapy**
- Surgery: 17
- Chemotherapy: 11
- Radiotherapy: 9
- Hormonal therapy: 1

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

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6 N. Horton, personal communication.
phase II dose was defined as one dose level below that at which 33% of patients within a cohort experienced DLT. In the absence of maximum tolerated dose, the phase II dose would be identified as the dose that achieved the potential therapeutic plasma concentration of 1,400 nmol/L of enzastaurin and its metabolites based on IC90 of 70 nmol/L and plasma protein binding of 95%.

**Dose adjustments.** Enzastaurin dose was reduced by 50% if a patient had DLT and was escalated up to the original dose if the reduced dose was tolerated. If a gemcitabine dose was omitted during a 21-day cycle, no further gemcitabine was administered during that cycle. Absolute neutrophil count was to be no further gemcitabine was administered during that cycle. If a gemcitabine dose was omitted during a 21-day cycle, DLT and was escalated up to the original dose if the reduced dose had DLT and was escalated up to the original dose if the reduced dose was tolerated. If a gemcitabine dose had been omitted in the previous cycle because of hematologic toxicity, the next cycle would commence therapy. If a day 8 gemcitabine dose had been omitted in the previous cycle because of hematologic toxicity, the next cycle would commence with 75% of the full gemcitabine dose.

**Patient evaluation.** Before enrollment and during treatment, patients were assessed by physical examination, hematology, and serum chemistry, electrocardiogram, and a slit lamp ocular examination. Radiological scans of tumor measurements were done before enrollment, before every other combination cycle. Palpable or visible tumors were measured 2 weeks before enrollment and before each combination cycle. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (30). Toxicities were graded according to the common toxicity criteria version 2.0 (31). Poststudy follow-up included hematology, serum chemistry, electrocardiogram measurements, a slit lamp ocular examination, tumor measurements, and evaluation of performance status.

**Pharmacokinetic studies.** For enzastaurin analysis, heparinized blood samples were collected on day 14 of cycle 1; predose and 1, 2, 4, 6, and 8 h postdose and on day 1 of cycle 2; before gemcitabine infusion; and at 1, 2, 4, 5, 8, 10 to 12, and 23 to 25 h postinfusion. Analysis was done by Advion Biosciences using a validated liquid chromatography with tandem mass spectrometry analytic method. The lower limit of quantification was 0.5 ng/mL. Pharmacokinetic variables were determined using noncompartmental methods (WinNonlin version 4.1). For gemcitabine analysis, heparinized blood samples were collected on day 1 of cycle 2 at predose, 15, 30, and 35 min, and 1, 1.5, 2, 4.5, 8, 10 to 12, 23 to 25, and 47 to 49 h after start of the infusion. Analysis of gemcitabine and its metabolite 2′,2′-difluoro-2′-deoxy-uridine (dFdU) were done at the Slotervaart Hospital (Department of Pharmacy and Pharmacology), using high-performance liquid chromatography with UV detection as described previously. For cisplatin analysis, heparinized blood samples were collected at predose, 5 and 30 min, and 1, 1.5, 3, 4, 7.5, 9.5 to 11.5, 22.5 to 24.5, and 46.5 to 48.5 h after start of infusion. Sample pretreatment was done as described previously (32). A validated method using graphite furnace atomic absorption spectrometry was applied to determine the total and ultrafilterable platinum plasma concentrations (33). Cisplatin concentration-time data were analyzed for pharmacokinetic variables using noncompartmental methods with WinNonlin Pro 5.0.1 (Pharsight). Gemcitabine concentration-time data were analyzed using nonlinear mixed effect modeling.

**Quantification of surrogate angiogenesis markers from whole blood.** For the isolation and quantification of CEC biomarkers in peripheral blood mononuclear cells, blood was drawn on day 1, cycle 1 (predose), days 8 and 1 of cycles 2 and 3, before gemcitabine infusion, and at 2, 4, 8, 24, and 48 h after start of infusion (no 48-h sample in cycle 3). Viable CECs were isolated and quantified as described previously, using CD146 antibody-labeled immunomagnetic beads (34–40). Further- more, the relative CEC content and the endothelial and hematopoietic progenitor cell content in peripheral blood mononuclear cells were quantified by nucleic acid sequence-based amplification of CD146 and CD133 mRNA as described previously (26).

**Statistical analysis.** The (un)paired Student’s t test and ANOVA were applied. Bivariate correlations between CECs or copies of mRNA and patient variables were done by calculation of the Pearson correlation coefficient or Spearman’s ρ. Data are presented either as mean values ± SE or as mean or median values with range. P values of <0.05 were considered significant. All analyses were done using SPSS software version 12.0.1.

**Results**

**Patient characteristics.** Patient characteristics of the 33 patients enrolled in the study (May 2002-March 2005) are presented in Table 1. The most common diagnoses were adenocarcinoma of the pancreas and melanoma. All patients were Caucasian.

**Dose administration.** A total of 162 cycles were initiated, and the median number of cycles per patient was 5 (range, 1-12). Eleven patients were treated for seven or more cycles. Table 2 describes patient accrual and dosage escalation. Of the 48 dose adjustments in 22 patients (10 in 1 patient from dose level 6), 27 were due to adverse events. More patients in the bid cohorts than in the qd cohorts discontinued from the study in the early cycles. Overall, the reasons for discontinuation were disease progression (52%), lack of clinical benefit (18%),

<table>
<thead>
<tr>
<th>Table 2. Dose levels and DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose level</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

*One patient was added after a patient discontinued before DLT analysis.
†One patient was added to replace a patient who was unavailable for pharmacokinetic analysis.
‡DLT occurred during cycle 1 (enzastaurin monotherapy).
¶Three patients were added when DLT occurred. Another three patients were added to replace those in the cohort who were not evaluable for safety analysis due to discontinuations.

7 R. van Maanen, personal communication.
adverse events (27%), or other reasons (3%). Nineteen patients reported 45 gemcitabine dose adjustments, mostly due to scheduling conflicts. Eight dose omissions and five dose delays were due to adverse events. There were no cisplatin dose omissions. Dose adjustments for gemcitabine and cisplatin were required at all dose levels and were similar in the qd and bid cohorts.

**Maximum tolerated dose.** Two DLTs were recorded, both for patients in the bid cohorts. A common toxicity criteria grade 2 QTc interval prolongation (possibly related to enzastaurin and leading to discontinuation) was recorded for a patient in dose level 6. This patient had a fluctuating QTc interval at study entry, but the QTc interval was within the protocol limits. The other DLT (grade 3 fatigue) was reported by a patient with melanoma in dose level 7 who discontinued from the study during cycle 1. The fatigue ameliorated within days after discontinuation of study drug. Because there was not a dose level in which two or more patients experienced a DLT, the maximum tolerated dose was not identified. Therefore, the recommended phase II dose was based on tolerability and the ability to achieve therapeutic plasma concentrations.

**Toxicity.** Generally, no direct enzastaurin-related side effects were noted. Grade 3/4 laboratory toxicities (Table 3) were noted across all dose levels and were considered to be related to gemcitabine/cisplatin, resulting in a week’s delay of the second gemcitabine/cisplatin cycle. These toxicities were reversible. Four patients had study-related, grade 4 hematologic toxicity (Table 3). Grade 3 maximum toxicity was recorded in anemia, creatinine, and hyperglycemia for 1 patient (3%) each. Grade 4 nonhematologic toxicities were not observed (Table 4). Other toxicities were nonfrequent and of little discomfort. More grade 1 and 2 toxicities were reported by patients in the bid cohorts than in the qd cohorts. These low-grade toxicities led to several discontinuations.

### Table 3. Main laboratory toxicities observed during treatment with enzastaurin in combination with gemcitabine and cisplatin chemotherapy

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grades 1</th>
<th>Grades 2</th>
<th>Grades 3</th>
<th>Grades 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1 (3)</td>
<td>7 (21)</td>
<td>1 (3)</td>
<td>0</td>
<td>9 (27)</td>
</tr>
<tr>
<td>Leukocytopenia</td>
<td>0</td>
<td>2 (6)</td>
<td>2 (6)</td>
<td>0</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
<td>1 (3)</td>
<td>6 (18)</td>
<td>3 (9)</td>
<td>10 (30)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0</td>
<td>1 (3)</td>
<td>6 (18)</td>
<td>1 (3)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>0</td>
<td>2 (6)</td>
<td>0</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Increased ALT</td>
<td>11 (33)</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>12 (36)</td>
</tr>
<tr>
<td>Increased AST</td>
<td>8 (24)</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>9 (27)</td>
</tr>
</tbody>
</table>

**NOTE:** Enzastaurin monotherapy did not result in any hematologic toxicity. Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

### Table 4. Main nonhematologic toxicities observed during enzastaurin treatment with or without gemcitabine and cisplatin chemotherapy

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grades 1</th>
<th>Grades 2</th>
<th>Grades 3</th>
<th>Grades 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Gastrointestinal toxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>17 (52)</td>
<td>10 (30)</td>
<td>1 (3)</td>
<td>0</td>
<td>28 (85)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>12 (36)</td>
<td>8 (24)</td>
<td>1 (3)</td>
<td>0</td>
<td>21 (64)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (15)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>0</td>
<td>2 (6)</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (15)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Neurologic toxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensory neuropathy</td>
<td>1 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Ototoxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinnitus</td>
<td>0</td>
<td>8 (24)</td>
<td>0</td>
<td>0</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Other toxicities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>3 (9)</td>
<td>3 (9)</td>
<td>0</td>
<td>0</td>
<td>6 (18)</td>
</tr>
<tr>
<td>Left ventricular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 (18)</td>
<td>7 (21)</td>
<td>5 (15)</td>
<td>0</td>
<td>18 (55)</td>
</tr>
<tr>
<td>Urine color change</td>
<td>5 (15)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (15)</td>
</tr>
</tbody>
</table>
Pharmacokinetics. Data from 30 patients were available for pharmacokinetic evaluations of enzastaurin across all the dose levels. Exposures in each dose group were compared between cycles 1 and 2 (Table 5). Data indicated that the pharmacokinetics of enzastaurin were not altered when administered in combination for 350 mg qd, 500 mg qd, and 350 mg bid. Compared with cycle 1, there was a lower mean exposure for 250 mg bid and 500 mg bid in cycle 2.

Plasma concentrations of gemcitabine decreased quickly after termination of infusion and were generally below the minimum quantification limit of the assay within 2 h (data not shown). Plasma dFdU concentrations generally reached a maximum at termination of the gemcitabine infusion at 30 min after start of infusion, decreased slowly, and remained quantifiable at 48 h after start of infusion.

When platinum was analyzed, the median terminal half-life was 83 h (range, 43-261) for total platinum (data not shown). The maximum concentration occurred at or near the end of the 3-h i.v. infusion of cisplatin. The geometric mean (%CV) for the area under the curve was 54 (±50) μg h/mL for total platinum. The geometric mean (%CV) of total platinum plasma clearance and volume of distribution at steady state was 12.0 (±40.1) mL/min and 79.3 (±26.8) L, respectively. As shown in Fig. 1, the pharmacokinetic results for gemcitabine and cisplatin in combination with enzastaurin are comparable with historical, single-agent, pharmacokinetic data of gemcitabine.

Table 5. Summary of enzastaurin and total analyte exposure following enzastaurin dosing in cycles 1 and 2 in all dose cohorts

<table>
<thead>
<tr>
<th></th>
<th>Geometric mean (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qd dosing</td>
</tr>
<tr>
<td></td>
<td>350 mg</td>
</tr>
<tr>
<td>Enzastaurin</td>
<td>C_{ss,av} (nmol/L) cycle 1</td>
</tr>
<tr>
<td></td>
<td>C_{ss,av} (nmol/L) cycle 2</td>
</tr>
<tr>
<td>Total analyte (enzastaurin and its metabolites)</td>
<td>C_{ss,av} (nmol/L) cycle 1</td>
</tr>
<tr>
<td></td>
<td>C_{ss,av} (nmol/L) cycle 2</td>
</tr>
</tbody>
</table>

NOTE: n is the number of subjects for pharmacokinetic analysis. Abbreviation: C_{ss,av}, average steady-state concentration.

Fig. 1. Observed plasma concentrations versus time from start of infusion for gemcitabine (A), normalized to 1,000 mg/m², and total platinum (B), normalized to a cisplatin dose of 75 mg/m².
and cisplatim (39), suggesting that enzastaurin does not influence either platinum elimination or gemcitabine pharmacokinetics.

Based on the safety and pharmacokinetic data, the recommended phase II dose is 500 mg qd enzastaurin, 1,250 mg/m² gemcitabine, and 75 mg/m² cisplatim.

**Treatment response.** Data from nine patients were unavailable due to discontinuation (because of adverse events) before tumor assessment; thus, the remaining 24 patients were evaluable for response. Three patients from dose levels 3 (pancreatic cancer), 5 (head and neck cancer), and 6 (ovarian cancer) had partial responses. The duration of response was 3.5, 5.6, and 5.8 months, respectively. Thirteen patients reported a best response of stable disease, 6 of whom were stable for ≥5 months. Progressive disease was the best response for eight patients, and there were no complete remissions.

**Angiogenesis-related biomarkers.** Data were available from 28 patients for CEC quantification and 21 patients for CD133 mRNA quantification. For the analysis of CECs, the nonparametric Mann-Whitney test and Wilcoxon signed-rank test were used. CEC numbers and CD146 and CD133 mRNA expression did not change significantly during enzastaurin monotherapy (Fig. 1A-C). Furthermore, enzastaurin did not influence the change in CECs and CD146 mRNA expression after treatment with gemcitabine and cisplatim. CEC numbers and CD146 mRNA expression in peripheral blood mononuclear cells were increased to maximum levels over baseline after a median of 4 h after gemcitabine/cisplatim infusion in the first and second cycles of the combination treatment. CEC numbers increased significantly 2 h after gemcitabine infusion in the first cycle ($P = 0.003$) and with borderline significance ($P = 0.05$) in the second cycle of gemcitabine/cisplatim treatment. CD146 mRNA expression was significantly increased 2 h after gemcitabine infusion during the first ($P = 0.04$) and second cycle of gemcitabine/cisplatim treatment ($P = 0.04$). CD133 mRNA expression in peripheral blood mononuclear cells decreased directly after infusion of chemotherapy and was significantly decreased at 8, 24, and 48 h after the gemcitabine/cisplatim treatment ($P = 0.03, 0.002, and 0.0003$, respectively). The second cycle of gemcitabine/cisplatim also led to a significant CD133 mRNA decrease, 24 h after infusion ($P = 0.008$). A 10-fold median increase in CD133 mRNA expression between the first and second gemcitabine/cisplatim cycles ($P = 0.0003$) was observed (Fig. 2).

**Discussion**

In this clinical study, we assessed toxicity and safety of oral enzastaurin (qd or bid) combined with gemcitabine/cisplatim and evaluated the pharmacokinetic profile of the three drugs. Both monotherapy and the combination were generally well tolerated at the tested doses. Adding enzastaurin to gemcitabine/cisplatim chemotherapy did not seem to influence the known hematologic toxicity profile of gemcitabine/cisplatim.

No grade 3/4 bone marrow, hepatic, or ocular toxicity was reported. Pharmacokinetics revealed that cisplatim and gemcitabine exposures were not altered when given in combination with enzastaurin. Exposures of enzastaurin did not change with altered dosing or when combined with gemcitabine/cisplatim. Safety and pharmacokinetic data enabled dose escalation to continue. Due to less than dose proportional increase in enzastaurin exposures in a previous study, enzastaurin qd was changed to bid. The bid regimen resulted in slightly greater plasma exposures but was also associated with more low-grade toxicities, suggesting that bid dosing was less tolerable than the qd dosing. In light of the chronic daily dosing of enzastaurin, qd dosing is preferred for further studies.

Enzastaurin inhibits the protein kinase C family (8, 40, 41) and the phosphatidylinositol 3-kinase/AKT pathway (12) to inhibit tumor cell growth and tumor-induced angiogenesis. Blocking the protein kinase Cβ pathway results in abrogation of vascular endothelial growth factor–induced angiogenesis, vascular function, and vascular permeability (42, 43). Preclinical studies show significant antitumor effect of adding antiangiogenic drugs to cytotoxic chemotherapy (28, 44). An antiangiogenic drug may normalize tumor vessel perfusion, which enables better delivery of chemotherapy to the tumor cells (29). Furthermore, because both treatment modalities have a different target, the toxicity profiles are not expected to overlap.

Pharmacodynamic markers that identify biological efficacy are highly needed to select an optimal dose of angiogenesis inhibitor (45). CECs are increased in cancer patients during disease progression and in the hours following cytotoxic chemotherapy (23). In vitro toxicity data show that IC$_{50}$ of

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**Fig. 2.** Absolute CEC numbers (A) and CD146 (B) and CD133 (C) mRNA expression during 14-d enzastaurin lead-in followed by enzastaurin + gemcitabine/cisplatim chemotherapy (two cycles). Patients are grouped according to enzastaurin dose. Note truncations on X-axis at day 15; between days 17 and 36.
enzastaurin for endothelial precursor cell outgrowth is ~12- fold lower than IC50 for stationary or exponentially growing human umbilical vein endothelial cells (6 μmol/L; ref. 14). Because ablation of CECs affects tumor growth and because inhibitors of angiogenesis partly seem to elicit their antitumor effect through inhibition of vascular endothelial growth factor–mobilized CEC incorporation into tumor vasculature (46–49), the kinetics of CECs during antiangiogenic treatment may be a promising and easily accessible marker to assess efficacy of this class of drugs. However, the methodology to measure CEC and progenitor cells vary and their quantification is not a validated assay. Furthermore, very little is known about changes in these CECs during conventional chemotherapy. Our data should therefore be considered exploratory.

In this study, biomarkers were incorporated to possibly aid in the identification of a nontoxic biologically effective dose. The baseline amounts of CECs reflect the heterogeneity of the patient group that was studied. Single-agent enzastaurin had no effect on any of the angiogenesis biomarkers analyzed. However, a semiacute increase in the biomarkers was seen in the hours following gemcitabine/cisplatin infusion. Our assays did not allow an assessment of the source of the observed increase, which may be detachment of CECs from the (tumor) vessel wall or recruitment from the bone marrow (38, 49–51). The increased CD133 mRNA levels after chemotherapy may point to a rapid mobilization of progenitor cells from the bone marrow. It is tempting to speculate that these progenitor cells have an effect on treatment outcome and this deserves further study. In spite of interesting observations, in this exploratory analysis, CEC or CD133 and CD146 mRNA levels did not prove to be a useful pharmacodynamic marker that influenced decision-making on dose escalation or recommended phase II dose for this drug combination.

In conclusion, combination therapy of enzastaurin with gemcitabine/cisplatin chemotherapy is feasible and safe. Its toxicity profile seems comparable with that of gemcitabine/cisplatin chemotherapy alone. Enzastaurin addition did not increase the toxicity of gemcitabine/cisplatin chemotherapy. The combination regimen did not alter the exposures of individual drugs. The recommended phase II dose is 500 mg qd enzastaurin, 1,250 mg/m² gemcitabine, and 75 mg/m² cisplatin.

For pharmacodynamic purposes, repeated quantification of CECs and amplification of endothelial (progenitor) mRNAs as part of a clinical trial is feasible. Exploration of the full potential of CEC biomarkers as a possible surrogate marker of treatment effect of enzastaurin warrants further study.

Acknowledgments

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References


L.V. Beerepoot, unpublished data.


Phase I Pharmacokinetic and Pharmacodynamic Study of the Oral Protein Kinase C \( \beta \)-Inhibitor Enzastaurin in Combination with Gemcitabine and Cisplatin in Patients with Advanced Cancer

Jeany M. Rademaker-Lakhai, Laurens V. Beerepoot, Niven Mehra, et al.


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