Original article

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Phase I study of PKC412 (*N*-benzoyl-staurosporine), a novel oral protein kinase C inhibitor, combined with gemcitabine and cisplatin in patients with non-small-cell lung cancer

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Background: PKC412 (*N*-benzoyl-staurosporine), an oral inhibitor of protein kinase C, is capable of cell cycle inhibition and is endowed with anti-angiogenic properties. This dose-finding phase I study was designed to establish the maximum tolerated dose (MTD) of PKC412 when combined with cisplatin–gemcitabine.

Patients and methods: Escalating doses of PKC412 were given every day of a 4 week cycle with cisplatin 100 mg/m^2 on day 2 and gemcitabine 1000 mg/m^2 on days 1, 8 and 15 in patients with non-small-cell lung cancer. Dose escalation was based on a modified continuous reassessment method.

Results: Twenty-three patients, assigned to four cohorts receiving PKC412 at a dose ranging from 25 to 150 mg/day were evaluable. Grade 3 diarrhea occurring in 3/4 patients at cycle 1 led us to define 150 mg/day as the MTD. The MTD based on multiple cycles was redefined as 100 mg/day, since prolonged grade 2–3 nausea/vomiting leading to treatment discontinuation occurred in 3/7 patients after repeated cycles. The next lower dose tested of 50 mg/day was therefore considered as the recommended dose for phase II trials. Among 33 cycles in eight patients, toxicity consisted of grade 1–2 diarrhea (12.5%) and asthenia (50%) with only one patient experiencing grade 3 headache at this dose level. A partial response was observed in three patients.

Conclusions: The results of the present study indicate that PKC412 at a dose of 50 mg/day can be safely added to cisplatin and gemcitabine in patients with advanced non-small-cell lung cancer.

Key words: anti-angiogenesis, cell cycle inhibitor, protein kinase C

Introduction

Protein kinase C (PKC) belongs to a family of serine-threonine kinases involved in several signaling pathways [1]. The PKC enzyme family comprises more than 12 isoenzymes which can be classified into the following three main groups: (a) conventional calcium- and diacylglycerol (DAG)-dependent PKCs-α, -β and -γ; (b) calcium-independent but DAG-dependent PKCs-δ, -ε, -η and -θ; and (c) calcium-independent and DAG-unresponsive PKCs-ζ and -ι [1, 2]. PKC isoenzymes play key roles in the up- and down-regulation of the G_1–S and G_2–M cell cycle checkpoints, but also in apoptosis, angiogenesis, differentiation, invasion, senescence and drug efflux [3]. PKCs also act as secondary messengers in the mitogenic signaling pathway of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) [4, 5]. Most non-small-cell lung cancer (NSCLC) cell lines express high PDGF and PDGF-R (PDGF-receptor) mRNA levels [6], while PDGF-Rs are highly expressed in endothelial cells in and around tumors [7]. VEGF overexpression also carries a poor prognosis in resected NSCLC [8] and targeting VEGF pathways downstream might also be a way of preventing tumor growth [9]. PKC inhibitors have been investigated for the treatment of cancer due to their capacity to modulate the cell cycle and angiogenesis [1]. Two indolocarbazole staurosporine analogues, UCN-01 (7-hydroxy-staurosporine) and PKC412 (*N*-benzoyl-staurosporine) (Figure 1), which compete for binding to the ATP site on PKCs, as well as ISIS-3521, an antisense oligodeoxynucleotide that inhibits PKC-α mRNA, have been included in clinical trials [10–12].

PKC412 interacts strongly with ATP binding sites of the conventional PKCs-α, -β and -γ, PDGF-Rβ, VEGF-R2, VEGF-R1 and the cyclin-dependant kinase 1–cyclin B complex. At the cellular level, inhibition of the conventional PKCs-α, -β and -γ by PKC412 [1] leads to inactivation of the mitogen-activated protein kinase pathway induced by tumor promoting phobol esters and ultimately affects c-fos expression [13]. In human T98 glioblastoma cells, exposure to PKC412 decreases cdk1/cdk2 kinase activity [14], blocks the cell cycle at the G_2–M boundary and induces apoptosis [15]. In addition, PKC412 has been demonstrated to elicit anti-angiogenic effects by inhibiting PDGF-R and VEGF-R expression in mice implanted with a porous teflon chamber containing VEGF [13, 16]. The antitumor activity of PKC412 in nude

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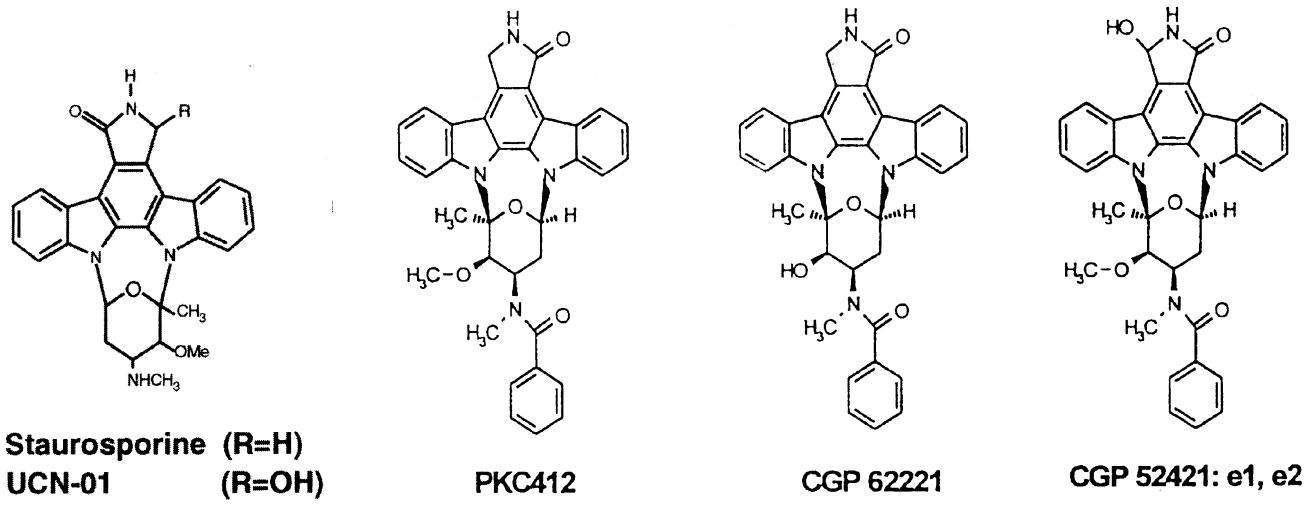

Figure 1. Structure of staurosporine, UCN-01 (7-hydroxystaurosporine), PKC412 (N-benzoyl-staurosporine), CGP62221 and CGP52421-epimer. Adapted from Fabbro et al. [15] and Senderowicz and Sausville [30].

mice bearing human H460 NSCLC tumor xenografts was found to be significantly higher than that observed with a number of conventional cytotoxic agents, including doxorubicin, cyclophosphamide, cisplatin and gemcitabine [15]. No sequence dependent toxicity was reported from animal experiments. In a phase I study in patients with advanced cancer, oral PKC412 was given daily at doses ranging from 12.5 to 300 mg [11]. No hematological toxicities were reported. Dose-limiting toxicities (DLT) consisted of nausea/vomiting, diarrhea, headache and lethargy/fatigue. In that trial, no formal maximum tolerated dose (MTD) could be established even though grade 2–3 non-hematological toxicities in more than half of the patients prohibited higher doses (225 and 300 mg/day) for chronic oral administration. The recommended dose (RD) of single agent PKC412 was 150 mg/day. The median elimination half-life of PKC412 was 1.6 days. The steady state plasma PKC412 levels at the RD were in the range of concentrations associated with cytotoxicity in cancer cell lines (114–399 ng/ml). Human α1-acidic glycoprotein (AAG) binds strongly (99%) to PKC412 and still more firmly (99.9%) to its two major active metabolites (CGP62221 and CGP52421) [15]. A partial response and one case of disease stabilization lasting 4.5 months have been reported.

Based on single agent activity of PKC412 in NSCLC and the additive effects evidenced *in vitro* with cisplatin [15], a phase I study was designed using a cisplatin–gemcitabine regimen with escalating doses of PKC412. The primary objective was to determine the MTD of PKC412 combined with cisplatin and gemcitabine. Secondary objectives were as follows: (a) to define the DLT of PKC412 when combined with cisplatin–gemcitabine; (b) to establish the RD of PKC412 when combined with chemotherapy containing cisplatin–gemcitabine; (c) to characterize the pharmacokinetic (PK) profile of PKC412 at the RD; and (d) to report evidence for anti-tumor activity in patients with NSCLC.

Patients and methods

Eligibility criteria

Patients with histologically-proven stage IIIB–IV NSCLC who were suitable for cisplatin and gemcitabine chemotherapy were eligible for entry onto the study. Other eligibility criteria were as follows: age ≥18 years; World Health Organization (WHO) performance status <2; life expectancy ≥3 months; no chemotherapy or investigational agent administered during the previous 28 days (40 days for mitomycin C, nitrosoureas or immunotherapy); no major surgery or radiotherapy during the previous 28 days; no evidence of brain metastases; no concurrent malignancy (except basal cell carcinoma and *in situ* cervical cancer); no active infection and no other severe concomitant illness. Women who were pregnant, breast feeding, or adults of reproductive potential without an effective birth control method at least 1 week prior to treatment were excluded; an effective contraception method was recommended for the overall duration of treatment and for at least 6 months post-treatment.

Eligible patients fulfilled the following criteria for study entry: neutrophil count, $\geq 1.5 \times 10^9$/l; hemoglobin ≥10 g/dl; platelet count $\geq 100 \times 10^9$/l; serum creatinine <1.5 × upper limit of normal (ULN); and total bilirubin <1.5 × ULN with serum aspartate aminotransferase and alanine aminotransferase levels <2.0 × ULN.

All patients gave their written informed consent before treatment according to institutional and national guidelines. The study was performed according to French and Swedish drug regulations and complied with the principles of the Helsinki Declaration. The protocol was approved by the University of Kremlin-Bicêtre (France) and the University of Umea (Sweden) Ethics Committees.

The present analysis is based on the examination of the source case report forms by the participating investigators who had access to all study data.

Study design

Before entry onto the study, all patients were required to have a clinical history, physical examination, chest and abdominal computed tomography (CT) scan, complete blood work-up comprising complete blood count and serum chemistries (sodium, potassium, calcium, creatinine, urea, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and total protein) and an electrocardiogram. During

treatment, a medical history, toxicity assessment and complete blood work-up were performed weekly. Toxicity grading was performed in accordance with the National Cancer Institute–common toxicity criteria (NCI–CTC), version 2.0 [17]. Patients were evaluated for tumor response every two cycles. Standard Southwest Oncology Group (SWOG) criteria were used to determine response [18]. Criteria for withdrawing patients from the study included tumor progression, dose-limiting or unacceptable toxicity as determined by the treating physician and patient refusal.

Drug administration

PKC412 was supplied by Novartis (Basel, Switzerland) in soft gelatin capsules containing 25 mg of the active drug substance. Capsules were taken with water during or following meals. The first dose of PKC412 was taken before administering chemotherapy. PKC412 was given orally daily during a 28-day cycle without interruption during and between cycles. Scheduled dose levels were 25 mg (25 mg taken o.d.), 50 mg (25 mg taken b.i.d.), 75 mg (25 mg taken t.i.d.), 100 mg (50 mg taken b.i.d.), 150 mg (50 mg taken t.i.d.) and 225 mg (75 mg taken t.i.d.). Fractionation of the daily doses was empirically chosen to improve the digestive tolerability and to reduce the number of pills that needed to be swallowed at one time. Dosage of PKC412 was reduced by 50% at cycle two in case of grade 3–4 PKC412-related toxicities.

On days 1, 8 and 15, gemcitabine 1000 mg/m^2 diluted in 100 ml of 0.9% NaCl was given as a 30-min intravenous infusion 1 h after the administration of PKC412. Antiemetic treatment before gemcitabine consisted of ondansetron 8 mg and methylprednisolone 125 mg. On day 2, cisplatin 100 mg/m^2 diluted in 250 ml of 0.9% NaCl was given intravenously over 60 min and hydration with 3 l 0.9% NaCl, 1 h after the administration of PKC412. Antiemetic treatment during cisplatin therapy consisted of ondansetron 8 mg per os twice to three times daily, that was continued, in case of prolonged nausea, with dexamethasone 20 mg with or without bromazepam 1 mg four times daily for 2 days. In case of prolonged nausea 2 days after cisplatin therapy, the antiemetic regimen was intensified with prednisone 100 mg and metoclopramide 40 mg [19].

Gemcitabine dose was adjusted in case of hematological toxicity. If grade 2 neutropenia and/or thrombocytopenia occurred at day 8 and/or 15, 75% of the initial gemcitabine dose was given. If grade 3 or 4 neutropenia and/or thrombocytopenia occurred at days 8 and/or 15, gemcitabine was omitted and a decrease of 25% was made at the subsequent cycle. The next cycle was delayed until hematological recovery. Dose adjustments for non-hematological toxicities were as follows: a 50% decrease in the initial gemcitabine dose was recommended in case of grade 3–4 transaminase elevation at day 8 and/or 15. A 50% decrease in the cisplatin dose was recommended in case of grade 1 nephrotoxicity and no cisplatin was given until recovery if nephrotoxicity exceeded grade >1. Cisplatin was discontinued when grade 3 neurotoxicity and grade 2 ototoxicity occurred.

Dose escalation procedure

The starting dose of PKC412 was 25 mg daily, i.e. 1/6 of the recommended dose of single agent PKC412 established in the phase I study [11]. A modified continual reassessment method (MCRM) was used for dose escalation [20]. For dose-finding purposes, four patients were assigned sequentially to each cohort. The daily PKC412 dose increments foreseen, namely 25, 50, 75, 100, 150 and 225 mg, were based on a single agent phase I study [11].

DLTs were defined as grade 4 neutropenia lasting at least 7 days, grade 4 febrile neutropenia, grade 4 thrombocytopenia, grade ≥2 nephrotoxicity and any other grade 3–4 non-hematological toxicity (excepted alopecia and acute nausea/vomiting). Nausea/vomiting occurring during the first week of treatment were considered as acute and cisplatin related, while nausea/vomiting lasting >1 week and disappearing after discontinuation of PKC412 were considered as prolonged and PKC related. Prolonged nausea/vomiting that persisted for >1 week, despite an adapted anti-emetic regimen, and any other toxicity that required dose reduction or treatment discontinuation were considered as DLT.

Dose escalation was primarily designed to define the MTD based on acute DLT of PKC412 when combined with cisplatin and gemcitabine during cycle 1 of treatment. The MTD was defined as the highest dose of PKC412 at which >35% of the patient population would experience DLT during cycle 1. The DLT observed after treatment of each cohort of patients was noted and the dose-toxicity model defined by the MCRM was updated accordingly. If no DLT was observed in a group of four patients, then the dose escalation proceeded to a further dose level. If one of four patients experienced DLT at cycle 1, then four new patients had to be entered at this dose level. In that situation, dose escalation was allowed to resume to a further dose level if three or less patients of eight experienced DLT at cycle 1. The probability of DLT at each of the PKC412 dose levels under study was estimated. The next cohort of patients was administered the dose level at which the estimated probability of DLT was closest to the 35% threshold DLT rate, provided that the new dose level did not exceed double the previous dose. The final RD was based on the MTD based on multiple cycles, since a prolonged administration of PKC412 should be possible at that dose level.

Pharmacokinetics study

In humans, PKC412 has two main metabolites, the *O*-demethylation product of PKC412, CGP62221 (*O*-desmethyl-*N*-benzoyl-staurosporine) and 7-hydroxy-PKC412, referred to as CGP52421 (*N*-benzoyl-7-hydroxy-staurosporine) (Figure 1). CGP52421 is a mixture of two epimers (epimer 1, 62.5% of CGP52421; epimer 2, 37.5% of CGP52421) and has structural homology with UCN-01 [15]. CGP52421-epimer1 has a short half-life and cannot be measured in plasma. CGP52421-epimer2 has a long half-life and accumulates over time [11]. A 24-h PK profile was obtained on day 28 of the first cycle and day 1 of the second cycle, before administering the second cycle of chemotherapy. Blood samples were collected prior to the first dose and at 1, 2, 4 (prior to the midday dose), 6, 10 (prior to the evening dose) and 24 h after the first intake. Blood samples were processed rapidly and protected from light. Samples of venous blood (3 ml) were collected in heparinized tubes and immediately centrifuged at 4000 r.p.m. Plasma samples were stored at –20°C until analysis.

Determination of PKC412 and its two main metabolites CGP62221 and CGP52421-epimer2, in plasma was performed by HPLC with fluorescence detection. The plasma PKC412 levels were analyzed by Novartis Pharma AG (Basel, Switzerland) according to published procedures [21]. An Agilent HP 1090 and an Agilent HP1100 HPLC system with a LiChrospher 100 RP18ec, 250 × 4 mm, 5 μm column, protected by the same type of pre-column, were used. The lowest quantification limit was 75 ng/ml for PKC412, 100 ng/ml for CGP62221 and 38 ng/ml for CGP52421-epimer2. Non-compartmental pharmacokinetic parameters were calculated for PKC412 and its metabolites in order to define the maximum concentration (C_{max}) and the area under the plasma concentration time curve over 24 h (AUC_{24h}) for each dose level, using the software Turbochrom, versions 6.1.0 and 6.1.2 (Perkin-Elmer, Norwalk, CT, USA) and Microsoft Excel, version 97 SR-2 (Microsoft, Redmond, WA, USA).

Results

General

Twenty-four patients with advanced stage IIIB/IV NSCLC entered the study, including 17 chemotherapy- and radiotherapy-naïve patients. One patient withdrew his consent before the first PKC412 dose and was not considered evaluable for this study. Characteristics of the 23 evaluable patients are listed in Table 1.

Table 1. Patient characteristics

Characteristics	No. of patients
Total number of patients[a]	23
Gender (male/female)	17/6
Median age, years (range)	56 (39–73)
WHO performance status	
0	6
1	11
2	6
Histology of NSCLC	
Epidermoid	5
Adenocarcinoma	9
Large cell	2
Others[b]	7
Stage of NSCLC	
IIIB	5
IV	18
Prior treatment	
Chemotherapy only[c]	3
Radiotherapy only	2
Chemotherapy[c] and radiotherapy	1
No prior therapy	17

[a]Twenty-four patients were enrolled, but one patient withdrew his consent before administration of PKC412 and was no longer further analyzed.
[b]One bronchiolo-alveolar carcinoma, one undifferentiated carcinoma, five NSCLC not specified further (diagnosis was performed by a fine needle biopsy in three patients).
[c]For metastatic disease: one chemotherapy line in three patients; two chemotherapy lines in one patient.
NSCLC, non-small-cell lung cancer; WHO, World Health Organization.

None of the four patients included at dose level I experienced DLT during cycle 1 and consequently dose escalation was authorized. At dose level II, one of the first four patients included experienced a grade 3 headache during cycle 1 that disappeared after discontinuation of PKC412. Four additional patients were included at dose level II and no other DLT was observed during the first cycle. Based on the MCRM, the 75 mg/day dose level was skipped and the 100 mg/day dose was tested as dose level III. At dose level III, none of the four patients experienced DLT during cycle 1. At dose level IV, three of four patients had grade 3 diarrhea during cycle 1. For these patients, diarrhea occurred during the first week of treatment and persisted despite therapy with loperamide 2 mg given after each stool up to a maximum of 16 mg/day. In each patient, the diarrhea disappeared in <12 h after discontinuation of PKC412. Two patients were re-challenged with PKC412 at the same dose and grade 3 diarrhea reappeared 12 h after the first intake. The 150 mg/day PKC412 dose was therefore considered MTD based on acute toxicity.

At dose level 100 mg/day, three more patients were added to the four initially included to evaluate toxicities beyond the first cycle. The last three included patients who experienced prolonged grade 2 or 3 toxicities at cycle two. One patient experienced grade 3 fatigue beginning at day 2 of cycle 2 with grade 3 nausea/vomiting beginning at day 6 and lasting until discontinuation of PKC412 at day 10. The second patient experienced grade 2 nausea/vomiting during cycles 1 and 2; despite intensive anti-emetic regimens, PKC412 dose reduction (to 50 mg/day) was required at cycle two without symptom improvement and treatment was interrupted. The third patient also had long-lasting grade 2 vomiting during cycles 1 and 2 that disappeared after discontinuation of PKC412 at day 21 of cycle 2. At dose level 3, the last three patients included did not differ from the first four patients in terms of PS, metastatic sites, prior irradiation or concomitant medications. Since 3/7 patients required treatment discontinuation due to unacceptable toxicity after repeated cycles, we decided not to include additional patients at dose level III and this dose level was not considered as meeting criteria for the RD for phase II studies.

Thus, 50 mg/day, the next dose level tested below 100 mg/day was considered. In addition to DLT at cycle 1 at 50 mg/day, one patient had grade 3 nausea/vomiting during the first week of cycle two that lasted 2 weeks until discontinuation of PKC412. No dose reduction was performed. As a total of 2/8 patients experienced PKC412-related DLT at dose level II, the 50 mg/day dose level was considered as the RD for chronic administration of PKC412 combined with cisplatin and gemcitabine (see Table 2). At this dose level, PKC412 plasma concentrations were in the range of the IC_{50} for preclinical studies. This dose of 50 mg/day was near

Table 2. Number of patients, cycles and DLTs at cycle 1 by PKC412 dose levels

Dose level	Dose (mg/day)	No. of patients	Patients with PS<1	No. of cycles			DLTs at cycle 1	
				Median	Range	Total	No. per patient	Types of DLTs
I	25	4	3	5	2–6	18	0/4	
II	50	8	6	5	1–6	33	1/8	1 grade 3 headache
III	100	7	5	2	1–4	14	0/7	
IV	150	4	3	1	1–1	4	3/4	3 grade 3 diarrhea
	Total	23	17			69	4	

DLT, dose-limiting toxicity; PS, performance status.

Table 3. Cisplatin–gemcitabine-related hematological toxicity per patient and per cycle

	Daily doses of PKC412 (mg/m^2)							
	25 (n = 4; cycles = 18)		50 (n = 8; cycles = 33)		100 (n = 7; cycles = 14)		150 (n = 4; cycles = 4)	
	G1–2	G3–4	G1–2	G3–4	G1–2	G3–4	G1–2	G3–4
Neutropenia								
Per patient	2	0	1	2	1	2	1	1
Per cycle (%)	2 (11)	0 (0)	9 (27)	5 (15)	1 (7)	3 (21)	1 (25)	1 (25)
Thrombocytopenia								
Per patient	1	3	3	5	5	1	2	0
Per cycle (%)	5 (27)	9 (50)	19 (57)	11 (33)	11 (79)	1 (7)	2 (50)	0 (0)
Anemia								
Per patient	4	0	8	0	7	0	1	1
Per cycle (%)	17 (94)	0 (0)	31 (94)	0 (0)	11 (79)	0	1 (25)	1 (25)

Worst NCI-CTC grades for all courses of PKC412 [17].

G, grade; NCI-CTC, National Cancer Institute-common toxicity criteria.

the MTD. For several kinase inhibitors the dose recommendation for phase I trials has been selected based on a compromise between toxicity, plasma concentrations and surrogate biological end points of antitumor activity, if any. For PKC412, there was no clear evidence to support an increase in dose above 50 mg/day in order to achieve higher activity against PKC enzymes in tumors. Therefore we decided not to investigate further doses >50 mg/day and consequently the dose level of 75 mg/day was not explored.

Hematological toxicities

As expected for the cisplatin–gemcitabine regimen, the toxicities commonly observed were hematological. Cisplatin–gemcitabine-related hematological toxicities stratified according to PKC412 dose levels are listed in Table 3. Of the 207 scheduled gemcitabine administrations, dose reduction and omission were necessary in 20% and 29% of cycles, respectively.

At dose level II, among a total of 33 cycles, grade 3–4 neutropenia occurred in 2/8 patients. The median ANC nadir was 2.400×10^6/l (range 600–9700×10^6/l). No grade 3–4 neutropenia lasting >7 days occurred nor was there febrile neutropenia. Grade 3–4 thrombocytopenia occurred in 5/8 patients. The median platelet nadir was 60×10^9/l (range 9–491×10^9/l). Only two patients required platelet transfusions because of grade 4 thrombocytopenia. Thrombocytopenia frequently occurred in patients who received more than two cycles compared with recipients of only one or two cycle(s), indicating that the cisplatin–gemcitabine combination may induce cumulative platelet toxicity. Anemia was usually mild and red blood cell transfusion was not required.

Non-hematological toxicities

Table 4 shows non-hematological toxicities. At dose level II, the above described DLT was a grade 3 headache at cycle 1. Other PKC412-related toxicities at the RD were mild to moderate grade 1–2 diarrhea, asthenia/fatigue, headache and prolonged nausea/vomiting. As mentioned above, one patient had grade 3 nausea/vomiting that was considered related to PKC412. Other toxicities were either considered related to cisplatin and/or gemcitabine. They included grade 2 hearing loss in one patient at level I, grade 1 peripheral neuropathy in two patients at level II, grade 2 anorexia in two patients at level I and one patient at level II, grade 2 infections without neutropenia in two patients at level II and one patient at level IV. Five patients presented cisplatin-related grade 1 elevation of serum creatinine (three patients at dose level II, one patient at dose level I and one patient at dose level IV).

Activity

Among the 23 patients, 17 (14 chemotherapy naïve) were evaluable for tumor response. Three chemotherapy-naïve patients achieved a partial response, one at dose level I and two at dose level II. Nine patients had tumor stabilization, which lasted for 4, 5, 7 and 10 months, respectively, in four cases and two of these four had previously been treated.

Pharmacokinetics

The PKC412 pharmacokinetic profile was assessed in four patients at the 50 mg dose level. Figure 2 shows the mean plasma concentration–time curves for PKC412, CGP62221 and CGP52421-epimer2 at dose level II (50 mg/day). At this dose level, the mean AUC_{24} of PKC412, CGP62221 and CGP52421-epimer2 was 11389 (range 6447–16739), 17923 (range 10948–25049) and 1513 ng·h/ml (range 1075–1927), respectively. The mean C_{max} of PKC412, CGP62221 and CGP52421-epimer2 was 799 (range 501–1070), 861 (range 569–1189) and 69 (range 47–87) ng/ml, respectively.

Discussion

Among the PKC inhibitors under clinical development, antisense oligodeoxynucleotide ISIS-3521 and staurosporine analogues UCN-01 and PKC412 have been investigated as single agents in

Table 4. PKC412-related non-hematological toxicity per patient and per cycle

	Daily doses of PKC412 (mg/m^2)							
	25 (n = 4; cycles = 18)		50 (n = 8; cycles = 33)		100 (n = 7; cycles = 14)		150 (n = 4; cycles = 4)	
	G1–2	G3	G1–2	G3	G1–2	G3	G1–2	G3
Headache								
Per patient, n	0	0	2	1	0	0	0	0
Per cycle, n (%)	0 (0)	0 (0)	2 (6)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea								
Per patient, n	1	0	1	0	2	0	0	3
Per cycle, n (%)	2 (11)	0 (0)	1 (3)	0 (0)	2 (14)	0 (0)	0 (0)	3 (75)
Asthenia/fatigue								
Per patient, n	2	0	4	0	1	1	2	0
Per cycle, n (%)	4 (22)	0 (0)	8 (24)	(0)	3 (21)	1 (7)	2 (50)	0 (0)
Prolonged nausea/vomiting[a]								
Per patient, n	0	0	0	1	2	1	0	0
Per cycle, n (%)	0 (0)	0 (0)	0 (0)	1 (3)	4 (29)	1 (7)	0(0)	0 (0)

Worst NCI-CTC grades for all courses of PKC412 are reported per patient (no. of patients) and per cycle (no. and percentage of cycles). No grade 4 non-hematological toxicities occurred during any course.

[a]Only prolonged nausea/vomiting are reported in the table.

G, grade; NCI-CTC, National Cancer Institute-common toxicity criteria.

patients with advanced malignancies in several phase I/II trials [10–12]. Schedules, based on laboratory data and selected for application in clinical studies, have led to prolonged exposure to PKC inhibitors. ISIS-3521 was given in a 2-h continuous intravenous infusion at doses ranging from 0.1 to 6 mg/m^2/day three-times-a-week for 3 consecutive weeks every 4 weeks [1, 12, 22]. Although no DLT was identified, common toxicities such as nausea/vomiting, fever and chills were reported [12]. UCN-01 was the first staurosporine derivative to be introduced in clinical trials at a RD of 42.5 mg/m^2 given in a 72-h continuous intravenous infusion every 4 weeks. DLTs were nausea/vomiting, symptomatic insulin-resistant hyperglycemia and pulmonary toxicity leading to hypoxemia [10].

PKC412 can be administered orally and it provided an opportunity to explore the effects of prolonged inhibition of PKC during a clinical trial in patients with advanced cancer. In a previously published phase I trial, the RD of single agent PKC412 was 150 mg daily with toxicities consisting of nausea/vomiting, diarrhea and lethargy/fatigue [11]. In our study, toxicities were either related to PKC412 or cisplatin–gemcitabine chemotherapy. Diarrhea was the main DLT of PKC412 when combined with chemotherapy and prohibited dose escalation >150 mg/day. As gemcitabine and cisplatin do not induce diarrhea, this toxicity was considered solely attributable to PKC412. Attempts to control diarrhea with loperamide were not satisfactory and treatment discontinuation was required in all patients experiencing diarrhea at a dose level of 150 mg/day. When re-challenged with PKC412, patients re-experienced grade 3 diarrhea and this strongly pointed to the role of PKC412 in this toxicity.

Nausea and vomiting were also frequently documented in our study and greatly influenced our recommendation for the definition of MTD and RD. Since PKC412 is given orally for a prolonged period of time, discomfort caused by persistent nausea/vomiting could have an impact on schedule compliance and the quality of life of patients in future phase II clinical trials. As cisplatin is also known to induce nausea and vomiting, we proposed considering grade 2 nausea and vomiting lasting >1 week as a DLT in our study, in addition to the classic grade 3–4 threshold for drug-induced DLT. At the dose of 100 mg/day, grade 2–3 nausea/vomiting lasting >1 week was observed in 3/7 patients who required treatment discontinuation or dose reduction. The frequency and severity of nausea/vomiting were also increased in other phase I studies when PKC412 was combined with paclitaxel and carboplatin [23] or protracted infusional 5-fluorouracil [24]. We had no alternative but to propose the daily dose of 50 mg as the RD in combination with cisplatin and gemcitabine in our study because of nausea and vomiting.

From the PKC412 single agent phase I study, we know that side-effects are dose related and usually occur within the first week of treatment. PKC isoenzymes are found in every tissue and the pattern and frequency of toxicities are identical when PKC412 is given as a single agent or in combination [11]. Toxicities, however, did occur at lower doses of PKC412 and a drug interaction at the PK level is suspected. However, as PK samples were scheduled only at day 28, we cannot confirm this hypothesis, as DLTs supervene earlier. However, at the pharmacodynamic level, we should be reminded that PKC412 caused nausea and vomiting as a single agent and it is not surprising to observe that the addition of an highly emetic drug such as cisplatin was probably responsible for the prolonged nausea and vomiting occurring at a much lower PKC412 dose level.

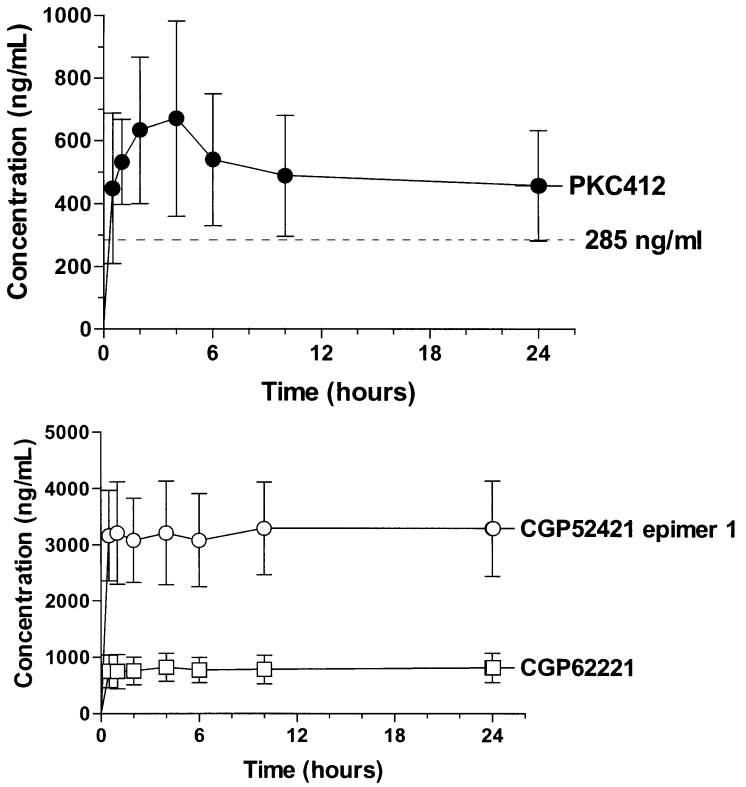

Figure 2. Mean plasma concentration–time profiles of PKC412, CGP62221 and CGP52421-epimer2 at the recommended dose of 50 mg/day.

In our study, the adjunction of PKC412 had no influence on cisplatin–gemcitabine-induced hematological toxicity. At a daily dose of PKC412 50 mg, hematological toxicity was similar in intensity and frequency to that previously reported with cisplatin–gemcitabine chemotherapy. With the same schedule of administration for the gemcitabine-cisplatin regimen as in our study, Crinò et al. reported percentages of gemcitabine dose reduction or omission of 27% and 29%, respectively, compared with 20% and 29% in our phase I trial [25]. Only a larger randomized phase II trial comparing chemotherapy with and without PKC412 would allow a better assessment of the toxicities.

The three partial responses and the four long-lasting (>4 months) tumor stabilizations all occurred at the RD and below. At the RD, the mean PKC412 C_{max} at day 28 was 799 ng/ml (range 501–1070). The mean plasma PKC412 concentration–time profiles (Figure 2) at the RD were consistently above the minimal target concentration required for cytotoxicity in all cell lines, i.e. an inhibitory concentration (IC_{50}) of 285 ng/ml (range 114–399). This target concentration level was reported to exert anti-proliferative and anti-tumor activity in human tumor models without the presence of human AAG and it is difficult to be confident of the potential value of this clinically achieved plasma concentration [26]. However, previously published data have shown that PKC412 pharmacokinetics are very complex due to the high-affinity protein binding of PKC412 and its metabolites. In the single-agent PKC412 study, the linear relationship between the dosage and plasma PKC412 level seen during the first week of treatment disappeared with prolonged drug intake. At day 28, no increase was observed in the plasma PKC412 level with increasing doses ranging from 150 to 300 mg/day. There was accumulation of CGP52421-epimer2, a 10-fold less active metabolite known for its very long elimination time (median half-life of 36 days; range 27–164 days). CGP52421 has the highest affinity for human AAG (>99.9% binding) due to its structural homology with UNC-01 [10, 15, 27]. It is possible that CGP52421-epimer2 displaces PKC412 and CGP62221 from AAG and that further interaction with cisplatin protein adducts reinforces this mechanism [28]. Our trial was not designed to test further these drug interactions. In other studies, attempts were made to identify biological end points that would correlate with the biological anti-tumor activity of PKC412. Blood plasma TNF-α and IL-6 levels and the level of extracellular signal-regulated kinase 2 using western blotting in peripheral blood mononuclear cells have been proposed as candidate surrogate end points for PKC412 [29], but have still not been validated for routine clinical trials.

In summary, the results of this study indicate that PKC412 at a dose of 50 mg/day can be safely added to cisplatin–gemcitabine chemotherapy in patients with advanced NSCLC. The plasma PKC412 levels achieved at the RD are in the IC_{50} range of preclinical models without the presence of human AAG. Further trials are warranted to evaluate the anti-tumor activity of PKC412 in the treatment of advanced NSCLC, either combined with cisplatin–gemcitabine or with other chemotherapies.

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