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Phase I and pharmacokinetic studies of PNU-159548, a novel alkycycline, administered intravenously to patients with advanced solid tumours

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Abstract

PNU-159548 (4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-daunorubicin) is the lead compound of a novel class of cytotoxic agents (alkycyclines) with a unique mechanism of action combining DNA intercalation with alkylation of guanines in the DNA major groove. The objectives of two phase I studies were to assess the dose-limiting toxicities (DLTs), to determine the maximum tolerated dose (MTD) and to study the pharmacokinetics (PKs) of PNU-159548 and its active metabolite PNU-169884 when administered intravenously (i.v.) over 10 or 60 min to patients with advanced solid tumours. Patients were treated with escalating doses of PNU-159548, courses repeated every 21 days at doses ranging from 1.0 to 16 mg/m^2. For pharmacokinetic analysis, plasma sampling was performed during the first course and assayed using a validated high-performance liquid chromatographic assay with mass spectrometric detection. 69 patients received a total of 161 courses. The MTD was reached at 14 and 16 mg/m^2 in heavily (HP) and minimally pretreated/non-pretreated (MP) patients, respectively, with thrombocytopenia as the DLT. A hypersensitivity reaction was observed in 8 patients across all dose levels, characterised by fever with chills, erythema, facial oedema and dyspnoea. The PKs of PNU-159548 and PNU-169884 were linear over the dose range studied. A significant correlation was observed between the percentage decrease in platelet count and the AUC of PNU-159548. In these studies, the DLT of PNU-159548 was thrombocytopenia. The recommended dose for phase II studies of PNU-159548 is 12 and 14 mg/m^2 administered i.v. over 10 min, once every 21 days, in HP and MP patients, respectively.

Keywords: Cytotoxic; Accelerated titration design; Thrombocytopenia

1. Introduction

PNU-159548 (4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-daunorubicin) is the lead compound of a novel class of cytotoxic agents (termed alkycyclines) (Fig. 1), whose interactions with DNA appear to be different from those of previous identified DNA interacting anticancer agents [1]. PNU-159548 was designed using the knowledge that modification of the structure and stereochemistry of the amino sugar (daunosamine) of the anthracyclines plays a major role in the determination of the chemical and/or pharmacological characteristics of the compounds [2]. In order to modify the mode of action of idarubicin, an alkylating substituent was introduced on position C-3′ of the amino sugar, resulting in activity against tumour cells expressing multidrug resistance (MDR) [3,4] and a methylsulphonyl group was introduced on position C-4′ increasing

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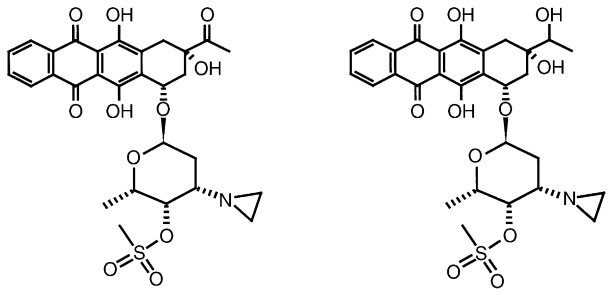

Fig. 1. Chemical structures of PNU-159548 and its metabolite PNU-169884.

the lipophilicity of the drug and improving the stability of the compound [5].

The mechanism of action of PNU-159548 is unique, combining DNA intercalation with alkylation of guanines in the DNA major groove. Unlike other intercalating agents, PNU-159548 does not interfere with DNA topoisomerase II (topo II) function [6].

In vitro, PNU-159548 exerted an antitumour activity against a variety of murine and human tumour cell lines including rapidly proliferating murine leukaemias and human colon, ovarian and prostatic carcinoma cell lines. *In vivo*, PNU-159548 was highly active (>95% tumour growth inhibition and cured mice) against human ovarian, breast and small cell lung cancer xenografts, and active (>70% tumour growth inhibition) against human pancreatic, colon, epidermoid carcinoma and glioblastoma xenografts when administered as a single agent. A synergistic antitumour effect was observed combining PNU-159548 with another antitumour compound, such as CPT-11, paclitaxel, docetaxel, doxorubicin, etoposide and gemcitabine [7,8].

PNU-159548 was found to be cytotoxic both against tumours expressing P-glycoprotein (Pgp)-MDR and altered topo II associated-MDR and it lacked cross resistance with alkylating agents, paclitaxel and topo-I and II inhibitors [9]. In animals, PNU-159548 induced bone marrow and gastrointestinal toxicity, but no notable renal or hepatic toxicity. At equimyelotoxic doses, the compound was markedly less cardiotoxic than doxorubicin in rats [6,10]. The LD_{10} (dose causing 10% of deaths) of a single administration of PNU-159548 to mice was 3.0 mg/kg (equivalent to 9 mg/m^2). A dose of one tenth of the mouse equivalent LD_{10} (1.0 mg/m^2) was recommended as a starting dose for phase I studies in humans.

The pharmacokinetics (PKs) of PNU-159548 in animal species were characterised by high volumes of distribution in agreement with the high lipophilicity of the drug, high plasma protein binding and a rapid elimination. The major route of metabolism constituted the reduction of the 13-keto group of PNU-159548 to the 13-dihydro derivative, PNU-169884. The conversion of the parent compound to its 13-dihydro metabolite was observed to take place both in the blood and in liver microsomes; it is likely to involve aldoketoreductase activity which probably accounts for most of the extrahepatic metabolism of PNU-159548. The main route of elimination of PNU-159548 was faecal. The 13-dihydro metabolite mantained *in vivo* antitumour activity against doxorubicin-resistant tumours, unlike 13-dihydro metabolites of anthracyclines [11], and showed in rodents a toxicological profile comparable to that of the parent drug.

The purposes of the present phase I studies, which were performed in parallel, were to determine the maximum tolerated dose (MTD) of PNU-159548 administered intravenously (i.v.) once every 21 days, to establish the dose-limiting and other toxic effects, to describe the PKs of PNU-159548 and its metabolite PNU-169884 with respect to interpatient and intrapatient variation, to document any antitumour effects and to establish a dose suitable for further phase II evaluation of activity of the compound for both heavily pretreated (HP) and minimally pretreated/non-pretreated (MP) patients.

The results of the present phase I studies were also used to validate a haematotoxicity model predicting human MTD [12]. The validation of this model is detailed in a separate paper.

2. Patients and methods

2.1. Patient selection

Patients with a cytologically- or histologically-confirmed diagnosis of a malignant solid tumour refractory to standard forms of therapy were eligible for these studies provided that they met the following criteria: age 18–75 years; Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant 2$; estimated life expectancy $\geqslant 12$ weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas, carboplatin or mitomycin C); no previous intensive ablative regimens; no more than two prior chemotherapeutic regimens for metastatic disease; and adequate haematopoietic (haemoglobin $\geqslant 6.2$ mmol/l, absolute peripheral granulocyte count $\geqslant 2.0\times10^9$/l and platelet count $\geqslant 100\times10^9$/l), hepatic (bilirubin within normal limits, and serum aspartate aminotransferase and alanine aminotransferase $\leqslant 2.5$ times the upper normal limit) and renal (serum creatinine concentration <133 μmol/l or creatinine clearance >1 ml/s) functions. The left ventricle ejection fraction (LVEF), measured by either echo or multigated acquisition (MUGA) scan, should be within the limits of normal. All patients gave written informed consent before study entry. The studies were approved by the Institutional Medical Ethics Committees.

2.2. Treatment and dose escalation

PNU-159548 was supplied by Pharmacia Corporation (Nerviano, Italy) as freeze-dried powder for injection in glass vials, containing 10 mg of active drug and dimyristoylphosphatidylcholine, dimyristoylphosphatidylglycerol (sodium salt), cholesterol, mannitol, sodium phosphate monobasic and sodium phosphate dibasic as excipients. The vials were stored at −20 °C. The content of the vial was reconstituted with 10 ml of water for injection and added to saline to a total volume of 100 ml in a polyvinyl chloride-free bag or glass bottle. The solution was kept at 2–4 °C protected from light until administration. PNU-159548 was administered i.v. over 10 min within 4 h from drug preparation. During the study, the infusion duration was amended to 1 hour to evaluate the influence of the infusion duration of PNU-159548 on toxicity. With the exception of the first course, during which patients were hospitalised for pharmacokinetic sampling, patients were treated on an outpatient basis.

The starting dose of PNU-159548 was 1 mg/m^2, corresponding to approximately 1/10 of the LD_{10} in mice. Courses were to be repeated every 21 days. Initially, an accelerated escalation scheme was applied with two 100% increments and one 50% increment over the previous dose level with only 1 patient treated at each dose level [13,14]. During the accelerated phase, intrapatient dose escalation was allowed if the patient experienced only grade 0 or 1 toxicities (excluding alopecia) and grade 0 for neurotoxicity and bilirubin and provided that a new patient had completed one cycle at the escalated dose level. At the occurrence of DLT or the second instance of grade 2 toxicity of any type in any course, the accelerated phase was abandoned. Further dose escalations were based on the prior dose level toxicity allowing a dose escalation of 15–50% (which was determined by the worst significant toxicity). From that moment on, at least 3 patients were entered at each dose level. The MTD was defined as the dose level that induced dose-limiting toxicity (DLT) during course 1 in $\geqslant 2/3$ or $\geqslant 2/6$ patients. DLTs were defined as grade 4 granulocytopenia for at least 5 days or of any duration if associated with infection of severity grade $\geqslant 3$, febrile neutropenia, platelets $\geqslant 25.0$ and $< 50.0\times10^9$/l for 5 days or more, platelets $< 25.0\times10^9$/l, haemoglobin < 4 mmol/l. Non-haematological toxicity $\geqslant$ grade 3 (grade 2 for neurotoxicity and bilirubin), and/or the occurrence of congestive heart failure or a decline in LVEF of either $\geqslant 20\%$ if the drop is within normal limits, or $\geqslant 10\%$ if the value attained is below the lower limit of normal were also considered as DLT. Nausea and vomiting subsequently responding to antiemetic therapy were excluded. If a patient encountered DLT, the dose of PNU-159548 was decreased by one dose level at retreatment. The treatment was resumed when the absolute neutrophil count (ANC) had recovered to $\geqslant 2.0\times10^9$/l and the platelet count to $\geqslant 100\times10^9$/l and non-haematological toxicity had recovered to $\leqslant$ grade 1 (bilirubin grade 0). In case the toxicity had not recovered within 2 weeks of the planned re-treatment time, the patient would go off-study. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria, version 2.0 [15].

After definition of the MTD in HP patients, further dose escalation was pursued in MP patients in order to determine the MTD in this patient subgroup.

Patients were considered to be HP or MP if they had undergone two or more prior chemotherapeutic regimens and one or no prior chemotherapy, respectively.

2.3. Treatment assessment

Before treatment, a complete medical history was recorded and a physical examination performed. A complete blood cell count (CBC) including white blood cell differential, and serum biochemistry, which involved sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, glucose and uric acid, were performed, as were urinalysis, relevant tumour markers, electrocardiogram, LVEF and a chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the Common Toxicity Criteria (CTC), and serum chemistries. CBC was determined twice weekly. Evaluation of the LVEF with a MUGA scan or echo was performed every two cycles from the first instance a DLT was observed and also in patients pretreated with anthracyclines. Tumour evaluation was performed after every two courses according to the modified World Health Organization criteria for response [16]. Patients were taken off protocol at the onset of disease progression.

2.4. Sample collection and drug analysis

For pharmacokinetic analysis, 11–13 blood samples (approximately 1 ml each) were obtained from an indwelling i.v. canula and collected in vials containing lithium heparin as the anticoagulant. The samples were taken immediately before dosing, at the end of the infusion and at 5, 15 and 30 min, and 1, 2, 4, 8, 10 and 24 h after administration of the drug on day 1 of the first course. At higher dose levels also samples at 34, 48 and 96 h after drug infusion were also obtained. In some patients, additional samples were drawn in the second course, to determine intrapatient variability in PKs of PNU-159548 and its metabolite, at the following times: immediately before dosing, at the end of the infusion and at 30 min and 1, 4, 10 and 24 h after the end of the infusion.

During the study, the infusion duration of PNU-159548 was amended from 10 min to 1 h. This also resulted in an adaptation of the sampling time-points for the pharmacokinetic analysis to: immediately before dosing, 30 min after the start of the infusion, at the end of the infusion, at 10 and 30 min, and 1 and 6 h after the end of the infusion.

All samples were centrifuged immediately after sampling at 1200*g* for 10 min at 4 °C and the plasma was stored at −80 °C in polypropylene tubes in the dark until analysis. Concentrations of PNU-159548 and its active 13-hydroxy metabolite PNU-169884 in plasma were determined according to a validated method based on a liquid chromatography system with mass spectrometric detection, described in detail elsewhere [17]. The lower limits of quantitation were 0.1 ng/ml for both compounds using 0.05 ml volumes for sample clean-up and analysis.

Pharmacokinetic and pharmacodynamic data analysis

The terminal disposition half-life ($T_{1/2}(z)$) of PNU-159548 and PNU-169884 was calculated as $\ln 2/k$, where k is the terminal elimination rate constant (expressed in h^{-1}). The peak plasma concentrations (C_{max}) and the time to peak plasma concentration (T_{max}) were determined graphically from the experimental values. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) of PNU-159548 and PNU-169884 were estimated using the experimental values (trapezoidal rule) with extrapolation to infinity ($AUC_{0-\infty}$) using the terminal elimination rate constant, defined as the slope of the final 3–4 data points of the log-linear concentration–time plot. The total body clearance (CL) was calculated as the ratio between the administered dose and the $AUC_{0-\infty}$. PK data analysis was carried out using a non-compartmental analysis approach with the aid of the WinNonlin package (Scientific Consulting, Inc).

Pharmacokinetic/pharmacodynamic relationships between PNU-159548 kinetic parameters and haematological toxicity associated with drug administration were explored using a non-linear regression model. Within individual patients, myelosuppression was described as the continuous variable, consisting of the percentage decrease in white blood cell, ANC and platelet count. The relative haematological toxicity was defined as: % decrease = (pretreatment value − nadir value)/(pretreatment value) × 100. Only the first course of each patient was taken into consideration to avoid potentially confounding bias due to cumulative toxicity. All data were fitted to a sigmoidal maximum effect (E_{max}) model based on the modified Hill equation, as follows: $E = E_{max} \times [(KP^{\gamma})/(KP^{\gamma} + KP_{50}{}^{\gamma})]$. In this equation, E_{max} is the maximum response, KP is the pharmacokinetic parameter of interest, KP_{50} the value of the pharmacokinetic parameter predicted to result in half of the maximum response, and γ is the Hill constant describing the sigmoidicity of the curve. Models were evaluated for goodness of fit by minimisation of sums of the squared residuals and by reduction of the estimated coefficient of variation for fitted parameters. Significance of the relationships was assessed by construction of contingency tables with subsequent χ^2 analysis.

Statistical analysis: all pharmacokinetic data are presented as mean values standard deviation. The effect of drug dose on clearance, volume of distribution and terminal disposition half-life was analysed using a Kruskal–Wallis multiple comparison test. The level of significance was set at $P = 0.05$.

3. Results

The data presented in this article derive from two phase I studies with the same study design, in- and exclusion criteria and treatment plan. When the two trials were implemented in early 1998 the accelerated titration scheme was a new approach for phase I studies in oncology [13]. The lack of experience with such dose escalation scheme justified the conduction of two separate studies to gain adequate tolerability and pharmacokinetic data also at the lower dose levels. Despite the implementation of two parallel trials, the number of patients treated in the accelerated escalation phase was lower than in a conventional 3-patient cohort phase I study and a well characterised toxicity profile was obtained at the recommended doses for phase II studies.

A total of 70 patients, whose main characteristics are listed in Table 1, were enrolled onto the studies at three

Table 1
Patients' characteristics

Characteristic	No. of patients
No. entered	70
No. assessable	69
Age (years)	
Median	58
Range	22–74
Sex	
Female	46
Male	23
Performance status	
ECOG 0	34
ECOG 1	31
ECOG 2	4
Tumour type	
NSCLC	12
Colorectal	11
Gastric	8
Renal	7
Head and neck	6
Miscellaneous	25
Previous treatment	
Chemotherapy and/or immunotherapy	41
Chemotherapy and radiation	20
Radiation	3
None	5

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer.

Institutions. 69 patients were eligible and assessable for toxicity and response. One patient developed progressive liver dysfunction after consenting to participate in the study and did not receive any treatment. The majority of the patients were either asymptomatic (49%) or had only mild symptoms (45%) at study entry. The most common tumour types were non-small cell lung cancer (NSCLC) and colorectal cancer. The total number of assessable courses was 161. The median number of courses per patient was 2 (range 1–8). Dose levels studied were 1, 2, 4, 6, 9, 12, 14 and 16 mg/m^2 administered i.v. over 10 min and 14 mg/m^2 administered in 1 h, with courses repeated once every 21 days. In 3 patients treated at 1 and 2 mg/m^2, the dose of PNU-159548 was escalated from the third and fourth cycle onwards to 2 (2 patients) and 4 mg/m^2 (1 patient), respectively, as allowed by protocol. 8 out of 31 patients treated at 14 and 16 mg/m^2 required dose reductions in the second cycle for toxicity. Treatment was discontinued in 1 patient at 14 mg/m^2 due to the experienced side-effects.

In absence of drug-related effects in the 2 patients treated at the 1 mg/m^2 dose level, the dose of PNU-159548 was doubled twice to 2 and 4 mg/m^2 followed by a 50% increment of the dose to 6 mg/m^2 as defined per protocol. At this dose level, 1 patient experienced dose-limiting thrombocytopenia in the first course, thereby ending the accelerated phase of the study. Haematological DLT was observed in 1 out of 10 and in 2 out of 16 patients at 9 and 12 mg/m^2, respectively. In addition, 1 patient at 9 mg/m^2 experienced non-haematological DLT, consisting of a hypersensitivity reaction. At the 14 mg/m^2 dose level, dose-limiting thrombocytopenia occurred in 4 of 9 HP patients, making 14 mg/m^2 the MTD in this patient subgroup. However, at the same dose level DLT was only observed in 2 out of 11 MP patients who were subsequently entered. In this patient subset, the dose of PNU-159548 was escalated to 16 mg/m^2, resulting in DLT in 2 out of 3 patients, therefore defining 16 mg/m^2 as MTD. On the basis of these results, the recommended PNU-159548 dose for further studies was 12 and 14 mg/m^2 in HP and MP patients, respectively, administered i.v. over 10 min. To evaluate the influence of the infusion duration of PNU-159548 on the toxicity, the compound was administered at 14 mg/m^2 over 1 h in an additional cohort of HP patients. In addition, in this case, 4 out of 8 patients experienced haematological DLT, making the prolonged infusion not effective in the reduction of haematological toxicity.

3.1. Tolerability

Thrombocytopenia was the principal DLT and was observed from the 6 mg/m^2 dose level onwards (Table 2). The platelet nadir occurred between days 13 and 16, generally followed by a spontaneous recovery to platelet values $\geqslant 100\times10^9$/l allowing patients to be re-treated on time. However, platelet transfusions were required in 7 patients, 4 of which experienced thrombocytopenia-related bleeding. In the 5 patients, who received six or more cycles, there was no evidence of a cumulative effect of PNU-159548 on the thrombocytopenia. Leucocytopenia and neutropenia were never dose-limiting and were observed in eight cycles involving 6 patients at the 14 and 16 mg/m^2 dose levels. Even if grade 3 or 4 neutropenia was encountered, it was not complicated by fever. The median time to ANC nadir was 16 days (range 11–21 days) with a recovery to ANC $\geqslant 2.0\times10^9$/l in a median of 5 days (range 1–11 days). Grade 3 anaemia, possibly

Table 2
Worst toxicity per cycle per dose level[a]

Dose (mg/m^2)	Patients/cycles	Haematological				Non-haematological			
		ANC		Platelets		Nausea/vomiting		Hypersensitivity reaction	
Grade:		1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4
1	2/8	–	–	–	–	–	–	–	–
2	2/9	–	–	–	–	1	–	1	–
4	2/4	–	–	–	–	2	–	–	–
6	6/10	3	–	1	1	7	–	2	–
9	10/25	7	–	9	4	12	1	1	1
12	16/39	9	–	16	7	19	3	2	–
14[b]	9/20	12	–	4	9	5	–	3	3
14[c]	11/22	4	4	13	7	12	2	1	–
14[d]	8/19	11	2	7	10	2	–	–	–
16[c]	3/5	2	2	1	4	4	–	–	–

[a] Numbers in table for toxicity are numbers of cycles.
[b] Heavily pretreated patients.
[c] Minimally pretreated/non-pretreated patients.
[d] Infusion duration 1 h.

related to the study treatment, was observed in two patients treated at the 6 and 9 mg/m^2 levels, respectively.

The most common non-haematological effects of PNU-159548 in both HP and MP patients were nausea and vomiting (Table 2). The episodes had an early onset (median day 2) with a median duration of 3 days and seemed to be dose-related. Patients treated at the lower dose levels did not routinely receive antiemetic premedication with their first dose. Subsequently, considering the frequency and intensity of nausea and vomiting, the use of antiemetic prophylaxis with the administration of a 5-hydroxytryptamine-3 receptor antagonist prior to drug administration was introduced in both studies, starting from the 9 and 12 mg/m^2 dose levels, respectively. The antiemetic premedication was effective in 87% of the patients. However, grade 3 nausea and/or vomiting was observed in 13% of patients, treated at 9 (1 patient), 12 (3 patients) and 14 mg/m^2 (2 patients).

A hypersensitivity reaction was observed in 8 patients (14/161 cycles) across all dose levels. Usually it developed during or just after the end of the infusion and was characterised by fever, chills, erythema, facial oedema and dyspnoea or a feeling of pressure on the chest. The recovery was mostly spontaneous after interruption of the infusion or following therapy with antihistamines. At re-challenge in 2 out of 5 patients, the hypersensitivity reaction occurred despite antihistamine prophylaxis and/or prolongation of the infusion duration. Other mild to moderate effects that were possibly related to PNU-159548 administration included fatigue (17 patients grade 1/2, 2 patients grade 3/4), headache and anorexia.

Cardiac function was assessed in all patients prior to the start of treatment. In 30% of the patients, the LVEF was evaluated during treatment, mainly at the end of the second cycle. No decline in LVEF $\geqslant$ 20% or below the lower normal limit was noted. Furthermore, no clinical signs of impaired cardiac function were observed in any of the treated patients.

3.2. Antitumor activity

No objective responses were recorded. Disease stabilisation was observed in four patients. 2 patients with NSCLC, both pretreated, had stabilisation of their disease for 30 and 28 weeks (at dose levels 9 and 12 mg/m^2, respectively). One patient with renal cancer treated at 12 mg/m^2 showed disease stabilisation for 23 weeks. One patient with thyroid cancer treated at 14 mg/m^2 administered over 1 h experienced disease stabilisation for 34 weeks.

3.3. Pharmacokinetics and dynamics

Complete plasma sampling was performed in 57 patients for pharmacokinetic analysis of PNU-159548 and its metabolite PNU-169884. After i.v. administration, the plasma concentration-time profiles of PNU-159548 were similar for all patients studied and showed a polyexponential decline. Representative plasma concentration time profiles of a patient treated at 14 mg/m^2 are shown in Fig. 2. Inspection of the scatterplots of dose versus either $AUC_{0-\infty}$ (Fig. 3a) or C_{max} (Fig. 3b) for PNU-159548 and its metabolite PNU-169884 revealed an increase of both parameters with the dose.

The total plasma clearance, estimated terminal half-life and volume of distribution of PNU-159548 were not different between the various dose levels suggesting a linear pharmacokinetic behaviour. The mean pharmacokinetic parameters determined using a non-compartmental analysis are listed in Table 3. The systemic exposure to the metabolite was similar or slightly higher than that to the parent drug. The ratio of the AUC of PNU-159548 and PNU-169884 was approximately 1.0 and independent of the dose administered. Maximum plasma levels of PNU-169884 were reached approximately 1 h after the start of the administration of PNU-159548. The estimated terminal half-life of the metabolite was relatively constant in all subjects, and was not dependent on the dose of PNU-159548. In 4 patients, the PKs of both the parent compound and the metabolite were also determined in the second cycle using a limited sample scheme. There were no significant differences between pharmacokinetic parameters derived from paired data sets (data not shown), suggesting a time-independent pharmacokinetic behaviour when PNU-159548 is administered every 21 days. In 15 patients, the free fraction of the parent drug and its active metabolite were evaluated by ultrafiltration at the dose levels 6, 12 and 14 mg/m^2. The free fraction of PNU-159548 was on average 0.6$\pm$0.4% and 1.1$\pm$0.7% for PNU-169884 (data not shown), indicating an extensive binding to the plasma proteins.

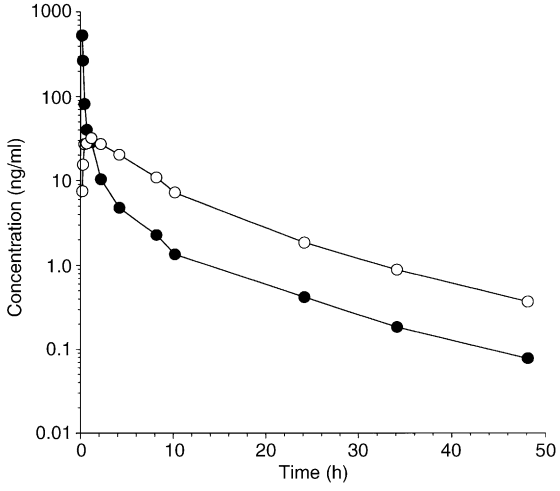

Fig. 2. Representative plasma concentration–time profile of PNU-159548 (●) and its metabolite PNU-169884 (○) in 1 patient treated at 14 mg/m^2.

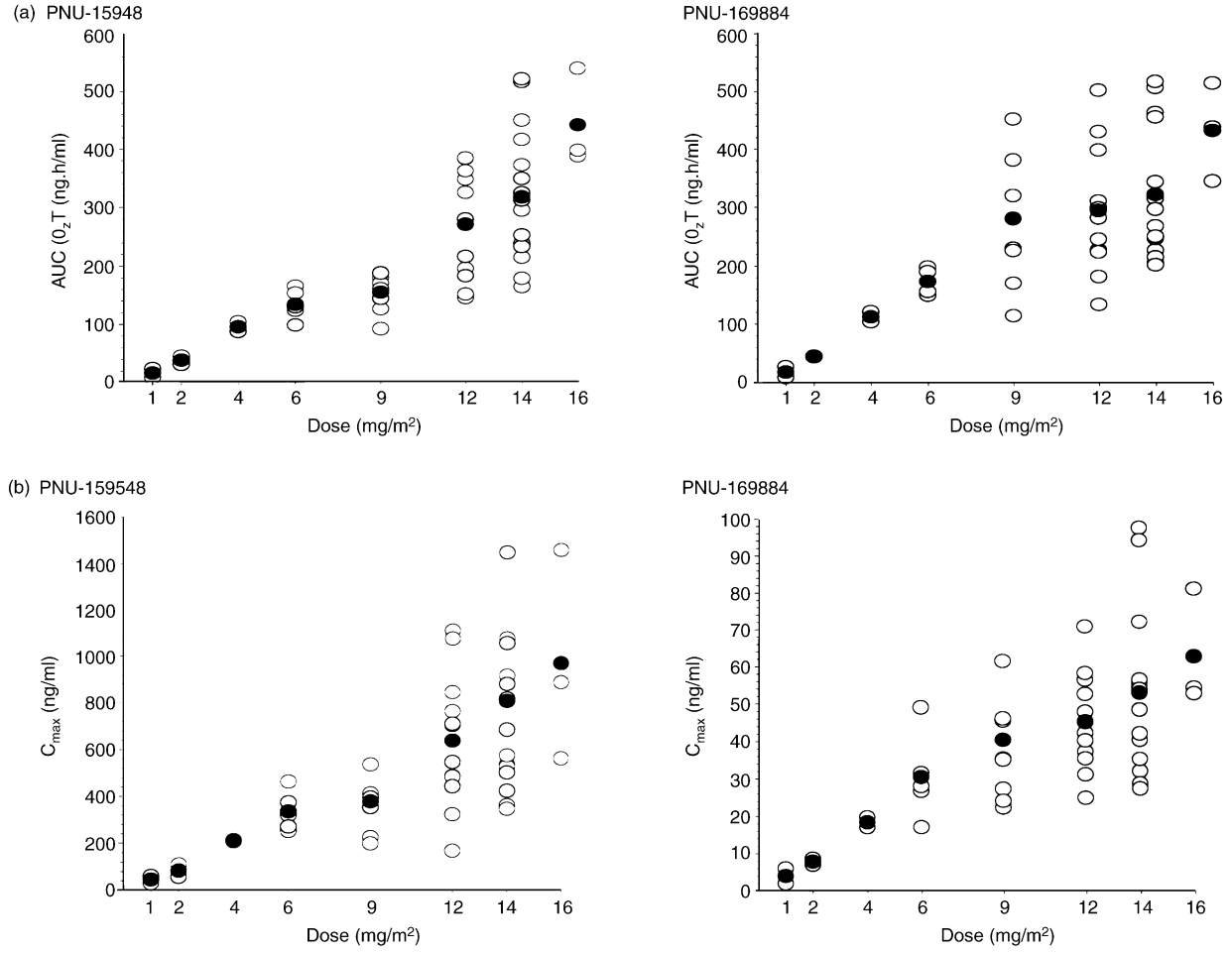

Fig. 3. Relationship between the area under the plasma concentration-time curve AUC (3a) and C_{max} (3b) of PNU-159548 and PNU-169884 as a function of PNU-159548 dose administered. The AUC values are indicated: ○, single patient AUC value; ●, mean AUC value at each dose level.

Table 3
Summary of the pharmacokinetics of PNU-159548 and its metabolite PNU-169884 and percentage decrements in platelets during the first treatment course

Dose (mg/m^2)	N	PNU-159548					PNU-169884				% platelets decrease
		C_{max} (ng/ml)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng.h/ml)	CL (ml/h/kg)	V_{ss} (ml/kg)	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng.h/ml)	(pret.–nadir)/(pret.)×100
1	2	44±22	2.9	17±10	2105±1177	2883±2029	4±2.5	1.2	5.2±2.7	18±13	13±11
2	2	82±37	6.4±1.4	38±11	1463±511	3763±2184	8±1.0	1.2	6.4±1.3	44±0.7	10±5
4	2	208±3	6.7±0.5	96±13	1094±221	1993±30	18±1.0	0.7	6.9±1.5	113±12	24±11
6	5	334±86	6.3±3.3	134±27	1230±365	2383±508	30±12	0.8±0.5	6.4±2.0	173±21	43±29
9	8	378±122	6.3±1.0	154±40	1697±398	3172±967	40±14	1.1±0.2	5.7±1.1	280±134	69±14
12	12	523±192	6.9±2.5	236±104	1544±619	2579±706	52±9	1.0±0.3	9.2±1.3	351±108	74±15
14	15	822±439	10.5±7.9	324±116	1257±447	3599±4823	53±21	1.1±0.2	7.5±3.3	321±111	77±13
14[a]	8	309±162	1.3±0.1[b]	356±188	1240±428		75±25	1.7±0.3	3.2±0.5[b]	395±85	74±18
16	3	969±454	7.4±0.9	441±85	926±143	1645±375	62±16	0.9±0.3	7.2±1.9	430±85	85±11

N, number of patients; C_{max}, peak plasma level; $AUC_{0-\infty}$, area under the plasma concentration–time curve; CL, total body clearance; V_{ss}, volume of distribution at steady state; $T_{1/2}$, terminal elimination half-life; T_{max}, time to maximal concentration; pret., pretreatment value.

[a] Infusion duration 1 h.

[b] Being 6 h the last sampling time point, the reported $T_{1/2}$ is an underestimation of the 'true' half life of the compounds. Kinetic values, as well as percentage platelets decrements, are mean values±standard deviation.

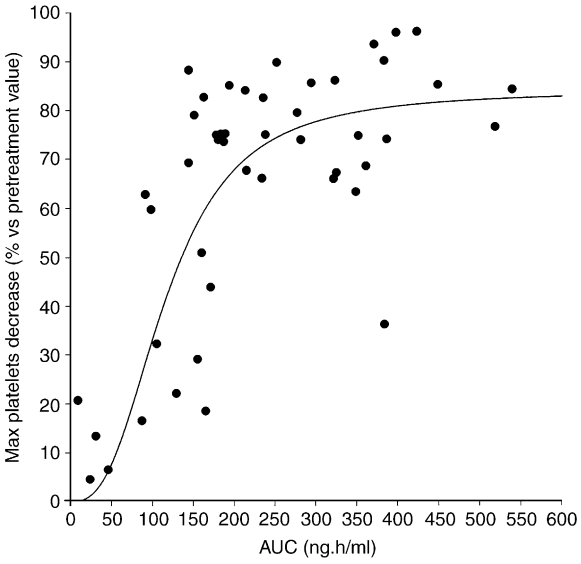

Fig. 4. Scatterplots depicting the individual relationships between percentage decrements in platelets during the first treatment course and AUC of PNU-159548. The infusion duration is indicated with ●, 10 min. The solid lines represent fits of sigmoidal E_{max} models to the data.

Sigmoidal maximum effect modelling of pharmacokinetic and haematological toxicity data (maximum percentage decrements in platelets during the first treatment course versus pretreatment value) revealed that both the AUC (Fig. 4) and C_{max} (data not shown) of PNU-159548 were significantly correlated with the percentage decrease in platelets count. To understand if the haematological toxicity depended on AUC and/or C_{max}, an alternative schedule was explored. To this purpose, the duration of the infusion of PNU-159548 was amended from 10 min to 1 h, resulting in a decrease in C_{max}, without any significant change in the AUC values of PNU-159548 (Table 3). In addition, also the AUC of PNU-169884, as well as the C_{max}, were independent of the infusion time. Despite the significant reduction in the plasma peak level of the parent compound, no improvement in the haematological safety profile was observed (Tables 2 and 3).

4. Discussion

PNU-159548 is a novel agent with an unique mechanism of action combining DNA intercalation with alkylation of guanines in the DNA major groove. Modification of idarubicin resulted in this new compound with a different mode of action in comparison with anthracyclines, increased lipophilicity and improved stability. In preclinical studies, PNU-159548 demonstrated activity against several human tumour cell lines and xenografts. These trials were designed to test the feasibility and describe the clinical toxicity and pharmacokinetics of PNU-159548 when administered i.v. over a 10 min infusion.

The DLT of PNU-159548 was thrombocytopenia. Other haematological toxicity was mild. Grade 3 or 4 neutropenia was only encountered in 5% of the courses and was not complicated by fever. The most prominent non-haematological side-effects of PNU-159548 were nausea and vomiting. However, grade 3 nausea/vomiting were rare with the prophylactic use of 5-hydroxytryptamine-3 receptor antagonists. A less frequent, but notable, side-effect of treatment with PNU-159548 was a complex of symptoms consisting of fever with chills, facial erythema and oedema, and dyspnoea, which was interpreted as a hypersensitivity reaction. Usually, it developed during or shortly after the infusion of PNU-159548 and ceased either spontaneously at interruption of the drug administration or following antihistamine therapy. Prolongation of the infusion duration could not prevent the occurrence of the hypersensitivity reaction, indicating that the event was not related to the peak plasma concentration. Furthermore, a relationship with the dose of PNU-159548 administered cannot be established. In view of the rare frequency of the hypersensitivity reaction and the limited value of prophylactic therapy, no routine premedication is presently recommended. However, this phenomenon will require further evaluation during the following phase 2 studies.

Since PNU-159548 is a derivative of idarubicin, cardiac function was evaluated during treatment. Although the cumulative dose administered in a phase I study is limited, no cardiac toxicity could be discerned.

In the present study, PNU-159548 and its 13-dihydro metabolite PNU-169884 revealed a dose-independent pharmacokinetics over the dose range studied with the AUC of PNU-159548 increasing from 17.0±10 to 441 85 ng h/ml. The systemic exposure to PNU-169884 was similar or slightly higher than that to the parent drug indicating that PNU-169884 is also the main metabolite in humans. Interpatient variability in the concentrations of PNU-159548 at each of the sample-time points, as well as in the AUC were substantial, with values for the coefficient of variation ranging from 14 to 59%. The volume of distribution of PNU-159548 was large which is in agreement with the drug lipophilicity enabling diffusion into tissues.

The sigmoidal maximum effect modelling of the pharmacokinetic and pharmacodynamic data of the present study revealed a relationship between the main haematological toxicity—percentage decrease in platelets—and both the C_{max} and AUC of PNU-159548. In order to evaluate the importance of both parameters in the induction of thrombocytopenia, the duration of treatment administration was prolonged from 10 minutes to 1 h, reducing the C_{max} of PNU-159548. The significantly lower plasma peak level of PNU-159548 after

prolonged infusion did not result in any improvement of the toxicity profile, indicating that the thrombocytopenic effect of the compound is related to the drug exposure.

The MTDs identified in this study were also compared with the human MTDs predicted from mice and dogs data using the haematotoxicity model proposed by Parchment [12]. The application and validation of the model, as well as its usefulness in the qualitative identification of the haematological DLTs, are critically described in a separate paper.

In conclusion, in this study with PNU-159548 administered i.v. over 10 min every 21 days, the DLT was thrombocytopenia. The recommended dose for phase II studies was 12 and 14 mg/m^2 in HP and MP patients, respectively. Phase II studies in ovary, breast, colorectal cancer, NSCLC and malignant melanoma are ongoing.

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