Phase I and Pharmacokinetic Study of Brostallicin (PNU-166196), a New DNA Minor-Groove Binder, Administered Intravenously Every 3 Weeks to Adult Patients with Metastatic Cancer[1]

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ABSTRACT

***Purpose*: Brostallicin (PNU-166196) is a cytotoxic agent that binds to the minor groove of DNA with significant antitumor activity in preclinical studies. This trial was designed to determine the maximum tolerated dose, the toxicity profile, and the pharmacokinetics of Brostallicin in cancer patients.**

***Experimental Design*: Patients were treated with escalating doses of Brostallicin ranging from 0.85 to 15 mg/m^2 administered as a 10-min i.v. infusion every 3 weeks. Blood samples for pharmacokinetic analysis were collected during the first and second course, and analyzed by liquid-chromatography with tandem-mass spectrometric detection.**

***Results*: Twenty-seven evaluable patients received a total of 73 courses. Grade 4 neutropenia was the only dose-limiting toxicity at 12.5 mg/m^2, whereas grade 4 thrombocytopenia (1 patient) and grade 4 neutropenia (2 patients) were the dose-limiting toxicities at 15 mg/m^2. Other side effects, including thrombocytopenia and nausea, were generally mild. The maximum tolerated dose was defined at 10 mg/m^2. The clearance and terminal half-life of Brostallicin were dose-independent, with mean (±SD) values of 9.33 ± 2.38 liters/h/m^2 and 4.69 ± 1.88 h, respectively. There was no significant accumulation of Brostallicin with repeated administration. Significant relationships were observed between systemic exposure to Brostallicin and neutrophil counts at nadir. One partial response was observed in a patient with a gastrointestinal stromal tumor.**

***Conclusion*: Brostallicin was found to be well tolerated, with neutropenia being the principal toxicity. The recommended dose for additional evaluation in this schedule is 10 mg/m^2.**

INTRODUCTION

Brostallicin (PNU-166196) is a new synthetic α-bromoacrylic derivative of distamycin A-like structures, characterized by a four-unit pyrrolcarbamoyl frame ending with a guanidine moiety (Fig. 1). It belongs to the class of DNA MGBs,[4] which comprises synthetic and semisynthetic compounds of very different molecular structure, acting through different mechanisms. Although MGB alkylating compounds (*e.g., tallimustine, carselezin,* and *adozelesin*) have shown excellent antitumor activity in preclinical studies, they have failed to demonstrate relevant antitumor activity in clinical trials because of severe myelotoxicity preventing the administration of potentially therapeutic doses (1–7).

Brostallicin has been selected for clinical development because of its reduced myelotoxicity in comparison with other MGBs in preclinical models translating into an 80-fold increase of therapeutic index in comparison with tallimustine (8).

In preclinical experiments, Brostallicin exerts antitumor activity against several murine and human tumor xenografts (8) Brostallicin is also highly proapoptotic (more than camptothecin) and, unlike alkylating agents and other MGBs, is fully active against DNA mismatch repair-deficient tumor cells (9–12). Brostallicin is more effective against cell sublines selected for resistance to alkylating agents and expressing high levels of GSH. Interestingly, it was found that Brostallicin reacts *in vitro* with GSH, but instead of causing inactivation of the drug, as one might expect, this reaction increases its cytotoxicity and the antitumor effect. The reaction between Brostallicin and GSH is catalyzed by GST with the π and μ isoenzymes being more effective than the α isoenzyme. Isogenic cell systems differing only for the expression of GST-π isoenzyme, allowed the ver-

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[4] The abbreviations used are: MGB, minor groove binder; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; GSH, glutathione; GST, glutathione-S-transferase; RD, recommended dose; ANC, absolute neutrophil count; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine-aminotransferase; C_{max}, peak concentration; CTC, common toxicity criteria; CL, clearance; AUC, area under the plasma concentration *versus* time curve; $T_{1/2}$, terminal disposition half-life; V, volume of distribution; BSA, body surface area.

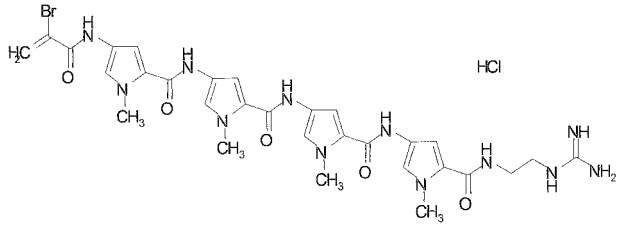

Fig. 1 Chemical structure of Brostallicin (N-[5-[[[5-[[[2-[(amino-iminomethyl)amino]ethyl]amino] carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H.pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl]carbonyl]amino]-1-methyl-1H-pyrrole-2-carbo-xamide hydrochloride).

ification that the greater sensitivity to Brostallicin occurs not only in *in vitro* cultured cells but also in tumors transplanted in nude mice (13, 14). This might be important clinically, as GSH and GST overexpression in comparison with normal tissues occurs *de novo* or as a consequence of cytotoxic treatment in a number of cancers, and GST-π is the most prevalent GST isoenzyme in tumors (15, 16).

Preclinical toxicology studies in mice and monkeys identified the hematopoietic system as the principal target of Brostallicin toxicity, mainly affecting the white cell lineage and much less frequently PLTs. The LD_{10} of a single administration of Brostallicin in mice were 3.54 and 2.86 mg/kg in males and females, respectively (10.6 and 8.6 mg/m^2).

We performed a dose-finding and pharmacological study to evaluate the toxicity of 3-weekly i.v. administration of Brostallicin, to determine the MTD, to describe the pharmacokinetics of Brostallicin, to document any antitumor effects, and to establish a dose suitable for additional Phase II evaluation of activity of the compound.

PATIENTS AND METHODS

Eligibility Criteria. Patients with a cytologically or histologically confirmed diagnosis of a solid tumor refractory to standard treatment or for whom no standard treatment was available were eligible for this study. Patients with primary central nervous system neoplasm, brain or leptomeningeal metastases, or known bone marrow involvement were excluded. Additional eligibility criteria included the following: age ≥18 years; Eastern Cooperative Oncology Group performance status ≤2; life expectancy of ≥12 weeks; no anticancer therapy in the previous 4 weeks; adequate bone marrow function (ANC ≥1.5 × 10^9/liter and PLT ≥100 × 10^9/liter); adequate liver function (total bilirubin ≤20 μmol/liter, AST and ALT within the normal limits or up to 2.5 upper normal limit in case of liver metastasis); adequate renal function (serum creatinine <133 μmol/liter); no more than two prior heavily myelosuppressive chemotherapeutic regimens; no prior high dose chemotherapy requiring bone marrow rescue; and no radiotherapy involving >25% of bone marrow. Concomitant use of growth factors was not allowed. The institutional medical ethics committee approved the protocol. All of the patients gave written informed consent at study entry.

Treatment Assessment. Before therapy, a complete medical history was taken, and a physical examination was performed. A complete blood count, including WBC differential, and serum chemistry, including sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, AST, ALT, and γ-glutamyl transferase were performed, as were urine analysis, electrocardiogram, and chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the National Cancer Institute Common Toxicity Criteria (version date January 1998), serum chemistries, urine analysis, and electrocardiogram. Complete blood counts and liver function tests were taken twice weekly in the first three cycles and weekly thereafter. Tumor measurements were performed before treatment, after the second course or earlier in case of early progression, and every two cycles thereafter, and were evaluated according to the WHO criteria for response (17). In case of progressive disease patients were taken off study.

Drug and Drug Administration. Pharmacia Corporation supplied Brostallicin in vials, as freeze-dried powder for injection containing 1 and 10 mg of active drug. The vials were stored at +5°C protected from light. The content of the vials was reconstituted with 2 ml and 10 ml of dextrose 5%, respectively, using a plastic syringe. The calculated dose to be administered was put in a Baxter infusion bag containing 100 ml of dextrose 5%. The solution was kept at 2–8°C protected from light until administration. Brostallicin was administered within 4 h from drug preparation. With the exception of the first and second course, in which patients were hospitalized for pharmacokinetic sampling, patients were treated on an outpatient basis. Prophylactic antiemetics were not allowed during the first cycle of treatment.

Dosage and Dose Escalation. Brostallicin was administered as a 10-min i.v. infusion every 3 weeks. On the basis of animal data the starting dose was 0.85 mg/m^2, corresponding approximately to one-tenth of the LD_{10} in mice. Dose escalation proceeded by an accelerated phase consisting of 100% increments over the previous dose level in cohorts of 1 patient each. The accelerated scheme was chosen because of the linear pharmacokinetic behavior of the drug in animals. During the accelerated phase intrapatient dose escalation was allowed if the patient experienced only toxicities grade 0–1, and provided that 1 patient had completed one cycle at the escalated dose level, and no side effects of grade 3 and 4 were observed at the higher dose level. This accelerated phase was terminated at the first occurrence of DLT or the second instance of grade 2 toxicity in the first course. Additional dose escalations were based on the prior dose level toxicity allowing a dose escalation of 15–50% in cohorts of at least 3 patients. DLT was defined as any more than or equal to grade 3 nonhematological toxicity attributable to Brostallicin, with the exception of nausea and vomiting responding to antiemetic treatment, and a transient grade 3 increase in transaminases lasting ≤7 days. Treatment-related neurotoxicity of grade 2 or more was also defined as DLT. Grade 4 neutropenia for at least 7 days or complicated with infection of grade >2, febrile neutropenia, or thrombocytopenia with PLTs ≥10 < 25 × 10^9/liter for ≥7 days, or PLTs <10 × 10^9/liter of any duration determined the hematological DLT. The MTD (or recommended Phase II dose) was defined as the highest dose to be administered at which 0 of 6 or 1 of 6 patients experienced DLT, with the next higher dose having at least 2 of

3 or 2 of 6 patients encountering DLT. Once the MTD was reached, additional patients were treated at the same dose level, to characterize the safety profile of Brostallicin at the expected Phase II dose. If a patient encountered DLT, the dose of Brostallicin was to be decreased to the next previous level at retreatment. The treatment was resumed when ANC had recovered to $>1.5 \times 10^9$/liter, and the PLT count to $>100 \times 10^9$/liter, and nonhematological toxicity had recovered to less than grade 2. In case the toxicity had not recovered within 2 weeks of the planned retreatment time, the patient went off study.

Pharmacokinetic Analysis. Eleven blood samples of 6 ml were taken on the first day of cycles 1 and 2 from the arm opposite of the infusion site via an i.v. canula before administration (predose); at the end of infusion; at 5, 15, and 30 min; and 1, 2, 4, 8, 10, and 24 h after the end of infusion. The blood was collected in tubes containing sodium heparin and was immediately centrifuged at $1200 \times g$ at 4°C for 10 min. Plasma was aliquoted and stored at −80°C in the dark until analysis. Plasma samples were assayed using a specific and sensitive method based on liquid chromatography with tandem-mass spectrometric detection using a turbo-ionspray interface. The sample pretreatment procedure involved a solid-phase extraction of 400-μl sample aliquots using 96-well-solid-phase extraction plates [Isolute C2(EC); 50 mg for each well]. The deuterated version of Brostallicin ([$^2H_4$]PNU-166196A) was used as an internal standard. The lower limit of quantitation of the assay was 0.1 ng/ml, and the overall chromatographic run time was 5 min. The assay showed an acceptable interday and intra-assay precision and accuracy (coefficient of variation <10%).

For each patient the pharmacokinetic parameters were calculated by standard noncompartmental methods. Although limited sampling time points were available up to 24 h, pyruvate kinase parameters for all of the patients, including half-lives, were calculated. The C_{max} was put on par with the observed concentration directly at the end of infusion as read directly from the raw data. For each patient, the AUC was calculated up to the last detectable concentration ($AUC_{0\text{-}tz}$), with the linear trapezoidal rule and extrapolated to infinity ($AUC_{0\text{-}inf}$) using the terminal rate constant (*k*), estimated by linear-regression analysis of the final concentration-time data. The $T_{1/2}$ was calculated as *ln2/k*, the total plasma CL as dose divided by $AUC_{0\text{-}inf}$, and the V as CL divided by *k*. $T_{1/2}$ was evaluated using at least three concentration time points of the terminal phase. V at steady state was calculated as: mean residence time × CL.

Hematological pharmacodynamics in the first cycle were evaluated using nadir values of ANCs, PLT counts, and WBC counts, determined on a twice weekly basis in all of the patients. Relationships between various exposure measures and drug-induced myelosuppression were performed using both absolute nadir values during the first treatment course or by using the percentage decrease in blood cell count at nadir. This latter variable was calculated as: % decrease = [(pretreatment count − nadir count)/pretreatment count] × 100.

Statistical Analysis. All of the pharmacokinetic data are presented as mean values ± SD, unless stated otherwise. The relationship between peak concentration or AUC and administered absolute dose was evaluated by a least-squares linear regression analysis. The effect of drug dose on CL, V, and $T_{1/2}$ was analyzed using a Kruskal-Wallis multiple comparison test, followed by Dunn's test to detect significantly different groups. The effect of repeated drug administration on drug CL was tested by a paired (two-tailed) Student's *t* test at a hypothesized mean difference of 0. Interindividual variability in kinetic parameters was evaluated by the coefficient of variation, expressed as a percentage of the ratio of the SD and the observed mean value. For this purpose, CL was calculated using the absolute dose in mg (CL in liters/h) or the BSA-corrected dose in mg/m^2 (CL in liters/h/m^2). The level of significance was set at $P = 0.05$. Statistical correlations between exposure measures and toxic side effects or patient characteristics were evaluated using Spearman's correlation coefficient and least-squares linear regression analysis. All of the statistical evaluations were performed on the NCSS v5.X software package (J. L. Hintze, East Kaysville, UT) except the correlation analysis performed on SAS System V6.12.

Table 1 Patient characteristics

Characteristic	No. of patients
Number entered	28
Number evaluated	27
Male/female	16/11 (12)
Age (years)	
Median	55
Range	36–72
Eastern Cooperative Oncology Group PS[c]	
Median	1 (range 0–2)
Tumor type (treated patients)	
Colorectal	6
Stomach	1
Ovarian	1
ACUP[a]	5
Sarcoma (including GIST)	5
Cervix	2[b]
Non-small cell lung	3
Adeno cystic carcinoma	1
Esophageal	2
Urothelial	1
Pretreatment	
Chemotherapy	19
Chemotherapy and radiation	5
None	3

[a] Adenocarcinoma with unknown primary.
[b] One patient with a double tumor (cervix/non-small cell lung cancer).
[c] PS, performance score.

RESULTS

Twenty-eight patients (16 male and 12 female), with a median age of 55 years were enrolled into the study between October 1999 and June 2001. Patient characteristics are listed in Table 1. All but 3 of the patients received prior chemotherapy. Because 1 patient did not start treatment for personal reasons, 27 patients were actually treated and evaluable for toxicity. The total and median number of courses was 73 and 3 (range, 1–12). Dose levels studied were 0.85 ($n = 1$), 1.7 ($n = 1$), 3.4 ($n = 3$), 5.1 ($n = 3$), 7.5 ($n = 3$), 10 ($n = 6$), 12.5 ($n = 8$), and 15 mg/m^2 ($n = 2$). In 1 patient treated at 15 mg/m^2 the dose was reduced in the second cycle after experiencing DLT in the first cycle.

Table 2 Hematological toxicity in first cycle[a]

Dose (mg/m^2)	No. of patients	ANC[b]		PLT		DLT in first cycle	DLT in subsequent cycles
		CTC grade: 1–2	3–4	1–2	3–4		
0.85	1	—	—	—	—	—	—
1.7	1	—	—	—	—	—	—
3.4	3	—	—	—	—	—	—
5.1	3	3	—	1	—	—	—
7.5	3	1	1	—	—	—	—
10	6	1	4	1	—	—	—
12.5	8	1	7	4	4	3	—
15	2	—	2	1	1	2	NA

[a] Expressed in number of patients scored to the worst CTC grade experienced during the first treatment cycle.
[b] ANC, neutropenia; PLT, thrombocytopenia; NA, not applicable.

Table 3 Summary of worst treatment emergent nonhematologic toxicity by cycle (all cycles)[a]

Dose level (mg/m^2)	No. of patients	No. of cycles	Nausea/Vomiting		Fatigue		Diarrhea	
			½	¾	½	¾	½	¾
0.85	1	2	2	—	—	—	—	—
1.7	1	2	—	—	1	—	1−	—
3.4[b]	3	7	2	2	5	1	3	—
5.1	3	15	6	—	13	0	3	—
7.5	3	8	6	—	7	1	1	—
10	6	9	4	1	2	1	—	—
12.5	8	28	10	—	10	2	4	—
15[c]	2	2	4	—	7	—	1	—

[a] Expressed in number of cycles worsening by ≥1 CTC grade over baseline.
[b] After this dose levels, emesis prophylaxis with 5HT3 inhibitors was started.
[c] One patient received one course with 15 mg/m^2 and thereafter 12.5 mg/m^2.

DLT. At the first dose levels (0.85 and 1.7 mg/m^2) no toxicity above CTC level 1 was noted. At dose level 3.4 mg/m^2 the first 2 patients experienced nausea grade 2 and vomiting grade 3 shortly after the drug infusion. Therefore, 5HT3 inhibitors were added to the regimen to prevent this observed early onset emesis. At this dose the accelerated phase was terminated, and 2 more patients were studied at this level. No DLT was observed until 12.5 mg/m^2. The dose was raised to 15 mg/m^2. At this dose both patients experienced DLT consisting of grade 4 neutropenia lasting >7 days, and 1 patient also experienced grade 4 thrombocytopenia. This patient developed intestinal bleeding of a colon tumor, and bacteriaemia during ANC grade 4 and PLTs grade 4 (neutropenic sepsis). Five additional patients were then treated at the next lower dose level of 12.5 mg/m^2. Although no DLTs had been observed in the first 3 patients treated at this dose level, of the additional 5 patients, 3 developed DLT consisting of uncomplicated neutropenia grade 4 for 8 days. Thus, in accordance with the protocol the dose level of 12.5 mg/m^2 could not be considered the MTD. However, the patients treated at 12.5 mg/m^2 who experienced prolonged uncomplicated neutropenia were retreated for, respectively, 5, 1, and 3 cycles at the same dose under carefully controlled conditions with biweekly blood sampling because of the uncomplicated nature of the neutropenia. In these subsequent cycles no DLT was observed. Three additional patients were treated at the next lower dose level of 10 mg/m^2. At this level no DLTs were observed. So according to protocol definitions the RD of Brostallicin for Phase II trials was set at 10 mg/m^2 once every 3 weeks. However, because at 12.5 mg/m^2 only 3 of 28 courses yielded DLT and retreatment at the same dose did not lead to recurrence of DLT, in case of carefully controlled conditions a dose of 12.5 mg/m^2 once every 3 weeks could be considered.

Hematological Toxicity. Hematological toxicities observed are listed in Table 2. Leukopenia, more specific absolute neutropenia, was dose-dependent. The neutrophil nadir was recorded in the second week with recovery during the third week in the majority of patients (recovery: in cycle 1 median 6 days, range, 2–14; all cycles median 7 days, range, 2–18). Grade 4 neutropenia was first observed at 10 mg/m^2. At 12.5 mg/m^2, 6 of 8 patients developed grade 4 neutropenia in cycle 1, lasting 8 days in 3 of these patients. The degree of leukopenia did not correspond to the degree of neutropenia, suggesting selective neutrophil damage. Neutropenia did not seem to be cumulative. Thrombocytopenia grade 1–2 was observed in 6 of 27 patients and in 21% of cycles. Six patients in total developed grade 3 thrombocytopenia over all cycles. Thrombocytopenia grade 4 occurred in only 1 patient receiving the highest dose of 15 mg/m^2. Thrombocytopenia did not appear to be cumulative, because 4 of 9 patients of cohort 12.5 mg/m^2 developed thrombocytopenia grade 3 at their first course and not in latter courses. However, the median number of courses administered was only 3. Anemia emerging during treatment grade 1–3 was observed in 21 of 27 patients (56% of cycles). Anemia grade 4 was not noted.

Table 4 Summary of Brostallicin pharmacokinetics as a function of dose (first course)[a]

Dose (mg/m^2)	*n*	C_{max} (ng/ml)	$T_{1/2}$ (h)	AUC (ng/h/ml)	CL (liter/h/m^2)	V at steady state (liter/m^2)
0.85	1	114	0.85	53	13.0	4.5
1.7	1	196	2.12	103	15.2	9.20
3.4	3	504 (352–625)	3.60 (2.1–6.0)	280.2 (228–346)	11.7 (9.4–14.6)	10.0 (6.6–16.1)
5.1	3	998.7 (936–1090)	5.80 (2.2–9.1)	567.7 (408–830)	9.3 (5.7–11.9)	8.30 (4.3–11.4)
7.5	3	1369.8 (997–1681)	4.84 (4.4–5.5)	808.5 (713–908)	9.0 (7.7–10.0)	7.2 (5.8–8.0)
10	6	1689.9 (920–2006)	5.70 (3.4–8.6)	1096.6 (997–1311)	8.60 (7.1–11.2)	8.5 (5.8–11.9)
12.5	8	2550.8 (1776–318)	4.70 (4.2–6.5)	1424.1 (1167–1617)	8.3 (7.6–10311)	6.90 (5.1–10.4)
15	2	2784 (2569–2999)	4.48 (4.1–4.9)	2011.4 (1779–2244)	7.0 (6.2–7.8)	5.70 (5.2–6.2)
Overall mean[b]			4.69 ± 1.88		9.33 ± 2.38	7.72 ± 2.62

[a] Mean values with range in parenthesis.
[b] Mean values with SD.

Nonhematologic Toxicity. Major nonhematologic side effects observed are listed in Table 3. Toxicity was mild and consisted of nausea/vomiting, fatigue, and a transient rise of transaminases (maximum grade 2). Nausea and vomiting grade 1–2 was observed in 17 patients (63%) within 24 h after treatment start (early onset emesis), and only 1 patient (3.5%) experienced grade 3 vomiting. No delayed emesis was observed. At the dose levels of 3.4 mg/m^2 prophylactic antiemetics were introduced (5HT3 blocking agents). Thereafter, nausea could be limited to grade 1–2. Treatment emergent fatigue grade 1–2 was noted in 45 of 73 cycles (17 of 27 patients), and 5 patients developed fatigue grade 3–4 without any correlation with anemia. Fatigue was observed in all of the marginally pretreated patients, including the 3 chemonaive patients. A transient rise of transaminases, not attributable to the study drug, was noted in 4 patients but not during all of the courses. In 3 patients a hypersensitivity reaction was noted. The first patient developed a transient skin rash at dose level 10 mg/m^2 only in her first course. The second patient was treated at 12.5 mg/m^2 and became hypotensive with tachycardia at the fifth cycle. At the sixth cycle premedication (dexamethasone 10 mg i.v.) could not prevent the reoccurrence of these symptoms, and the patient went off study. The third patient also treated at 12.5 mg/m^2 developed short-lasting grade 2 fever and thoracic pain, shortly after the seventh administration, and went off study.

Pharmacokinetics and Dynamics. Pharmacokinetic studies were performed in all 27 of the evaluable patients during the first course and 20 patients in the second course of Brostallicin. A summary of pharmacokinetic data are given in Table 4. The plasma concentration-time profiles of Brostallicin were very similar between the patients. These profiles were characterized by peak concentrations occurring immediately after cessation of the infusion. This was followed by a rapid decline in the plasma concentrations in an apparent multiexponential fashion, with a short terminal half-life of 4.69 ± 1.88 h. Overall, the total plasma CL was relatively slow (mean, 9.61 ± 2.81 and 10.22 ± 3.42 liters/h/m^2) in the patients that underwent the first and second cycle, respectively. No statistical difference was evident between the two cycle of treatment for the CL value (paired *t* test) with a small V of only 7.72 ± 2.62 liters/m^2, suggesting limited distribution toward the extravascular compartment.

The relationships between peak concentration or AUC achieved and the absolute dose of Brostallicin administered, is shown in Fig. 2, and indicate linear behavior. The values for total plasma CL of Brostallicin observed at the three lowest dose levels were slightly higher compared with the other dose levels. This likely relates to underestimation of the total systemic exposure resulting from constraints in assay sensitivity, precluding accurate

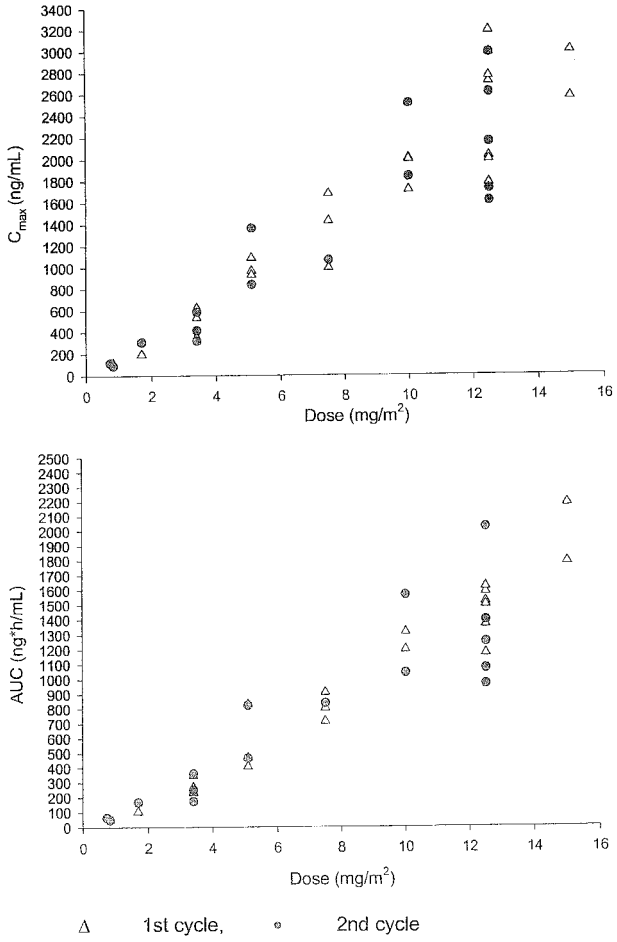

Fig. 2 Relationship between C_{max} and dose (*top*), and AUC and dose (*bottom*).

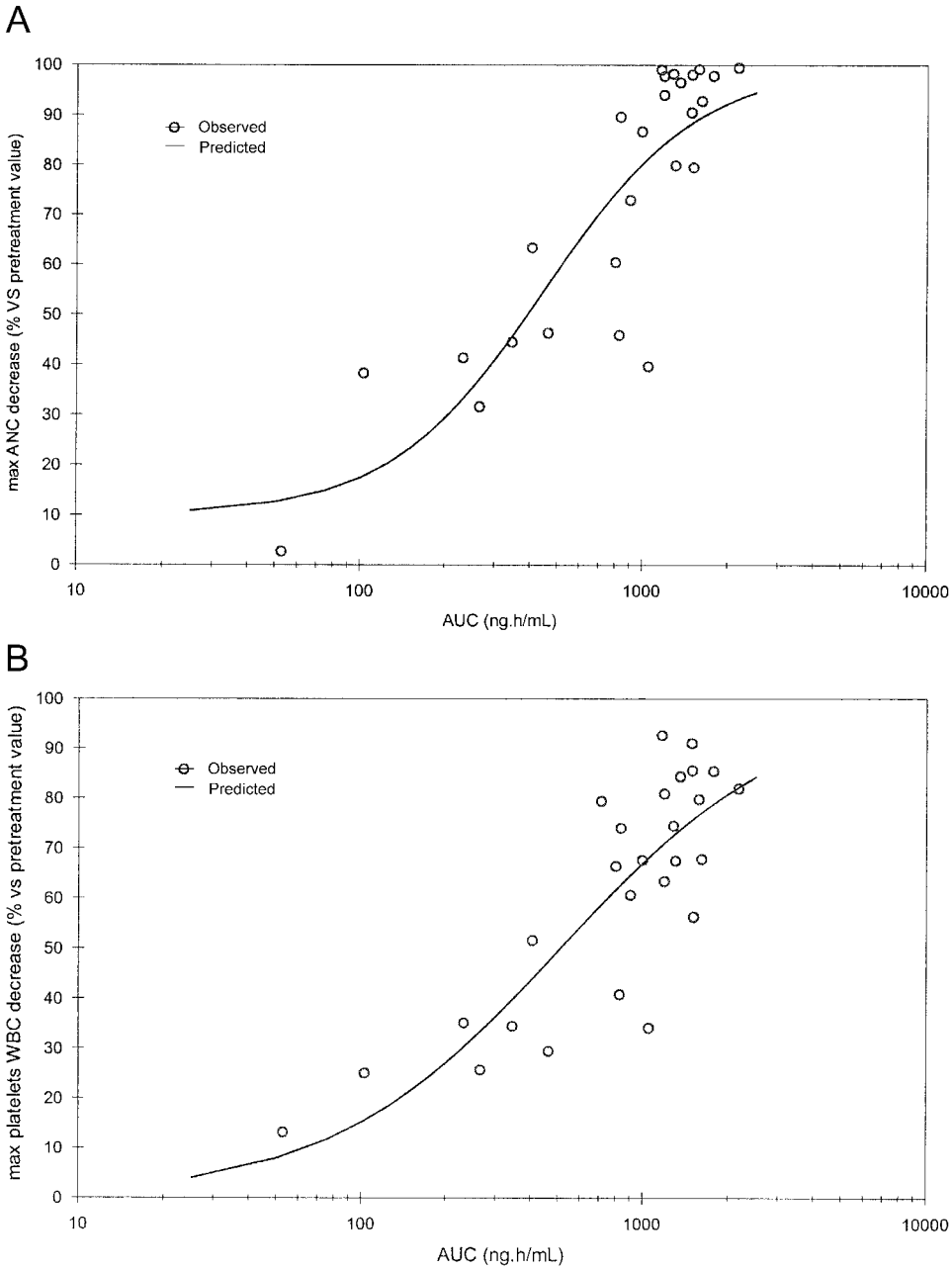

Fig. 3 Scatterplots depicting the relationship between ANC decrements during the first time course and AUC or C_{max} of Brostallicin (*A*, AUC; *B*, C_{max}).

determination of the terminal disposition phase at the lower dose levels. However, the total plasma CL of Brostallicin was not significantly different between the various dose levels ($P = 0.123$, Kruskal-Wallis test), suggesting a linear and dose-independent pharmacokinetic behavior. Likewise, the estimated $T_{1/2}$ ($P = 0.264$) and the V ($P = 0.428$) were relatively constant in all of the subjects and independent of the administered dose. The AUC extrapolated from the last time point (24 h) up to infinitive time was $<1\%$ in all of the subjects ($0.57\% \pm 0.31$). The mean ($\pm$SD) ratio of total plasma CL obtained during cycles 1 and 2 was 0.99 ± 0.28 suggesting that repeated administration of Brostallicin had no influence on total body CL and was not associated with drug accumulation (data not shown).

Sigmoidal maximum effect modeling of pharmacokinetic and hematological toxicity data revealed that both the AUC and C_{max} of Brostallicin were significantly correlated with hematological toxicity parameters, including the lowest observed value during the treatment interval (*i.e.*, nadir), and the maximum percentage decrease in ANC, PLT count, and WBC count (Fig. 3).

To explain the observed interpatient variability in Brostallicin pharmacokinetics, relationships were evaluated between individual kinetic parameters and several other patient characteristics, including BSA, age, serum albumin, renal function (creatinine), and hepatic function (AST and ALT; Table 5). No clinically significant correlation was observed between C_{max} or AUC and BSA, serum albumin, creatinine, or age.

Table 5 Correlation between Brostallicin pharmacokinetics and patient characteristics[a]

Variable	*n*	C_{max}		AUC	
		r[b]	p	r	p
Age	27	−0.01	NS	−0.03	NS
BSA	27	−0.08	NS	0.03	NS
Baseline albumin	27	−0.21	NS	−0.21	NS
Baseline creatinine	27	−0.58	0.002	−0.50	0.008
Baseline AST	27	0.39	0.047	0.44	0.022
Baseline ALT	27	0.41	0.036	0.37	NS
Peak creatinine	27	−0.66	0.0002	−0.59	0.001
Peak AST	27	0.43	0.025	0.45	0.019
Peak ALT	27	0.59	0.001	0.59	0.001

[a] Only data from the first treatment course were taken into account.
[b] r, Spearman's correlation coefficient; p, probability value; NS, not statistically significant.

Antitumor Activity. No complete responses were seen. One patient with extensive liver metastases of a gastrointestinal stromal tumor had a partial response at a dose level of 5.1 mg/m^2 lasting 16 months. Two other patients, 1 with a synovial sarcoma and 1 with a cervix cancer/non-small cell lung cancer, had disease stabilization of 13 and 16 weeks at the dose level 12.5 mg/m^2.

DISCUSSION

The current study was performed to explore the safety, tolerability, and pharmacokinetics of Brostallicin, a novel DNA MGB. The compound shows broad antitumor activity in preclinical models and a level of *in vitro* myelotoxicity on human hematopoietic progenitor cells dramatically reduced in comparison with that of other MGBs. It is conceivable that Brostallicin exhibits different biological properties because it may have a different ability to alkylate DNA in the minor groove with a different sequence specificity. Characterizing the pharmacological properties of Brostallicin, mechanistic studies have suggested that its antitumor activity is increased in cells with a high GSH/GST content. This finding has potential value in cancer treatment, because GSH and GST play a role in cellular resistance to different cytotoxic drugs, and the majority of human tumors display increased levels of GSH and/or GST with respect to normal tissues.

We determined (according to the protocol definitions) the MTD of Brostallicin administered by 10-min i.v. infusion given once every 3 weeks at 10 mg/m^2. However, because at 12.5 mg/m^2 only 3 of 28 courses yielded DLT (consisting of prolonged uncomplicated neutropenia grade 4), and retreatment at the same dose did not lead to recurrence of DLT, in case of carefully controlled conditions a dose of 12.5 mg/m^2 once every 3 weeks could also be considered. The toxicity profile of this agent is mainly determined by reversible myelotoxicity, particularly neutropenia. At 12.5 mg/m^2 grade 3 and 4 neutropenia was recorded in 7 of 26 cycles (27%). At the RD (10 mg/m^2) grade 3 or 4 neutropenia occurred in 4 of 8 cycles (all of them in the first cycle). The nadir value occurred approximately at day 11; the median duration of grade 3 and 4 was 6.5 days (range, 3–8 days). No double WBC nadir occurred, at variance with the MGB carzelesin (18). Nausea and vomiting were also recorded, but were well manageable with antiemetics. Special care was taken for hepatotoxicity, because in preclinical studies the liver was identified as another target of Brostallicin. In contrast to ecteinascidin-743, a currently investigated MGB, no major transaminitis was noted (19). In any other aspect Brostallicin was well tolerated.

After the administration of Brostallicin to various animal species, plasma CL was slow (11.7, 8.0, and 12.6 ml/min/kg in mice, dogs and monkeys, respectively), and represented <25% of the hepatic blood flow (20). The V of the central compartment exceeded the plasma volume by 5–6-fold, and the V at steady state was 310–439 ml/kg, suggesting a moderate distribution into tissues. This is in agreement with the high hydrophilic nature of this compound. In our study the CL of Brostallicin in cancer patients was similarly slow, with a mean value of <10 liters/h/m^2 albeit with a relatively fast $T_{1/2}$ of ~5 h. As predicted from the animal pharmacokinetic data, the V at steady state was small, ~10 liters/m^2. Thus, the pharmacokinetic results in our study in humans are in line with the results of studies in different animal species, which had clear predictive value for pharmacokinetic studies of Brostallicin and are very similar to those of tallimustine (4, 21, 22).

In the present study, Brostallicin demonstrated linear and dose-independent pharmacokinetics over the total dose range studied, without any major time dependency. Repeated administrations of Brostallicin to the same cancer patient had no measurable effect on either C_{max} and AUC or CL, which indicates a lack of accumulation and/or autoinduction.

A moderate degree of interpatient variability in kinetic parameters was apparent, with ~2-fold variation in AUC values. To explain the observed interpatient variability in Brostallicin pharmacokinetics, relationships were evaluated between individual kinetic parameters and several other patient characteristics. No clinically significant correlation was observed between C_{max} or AUC and BSA, serum albumin, creatinine, or age, suggesting that reduced starting doses are not required in elderly patients. Although significant *P*s were obtained with the baseline and peak transaminase evaluations when correlated to pyruvate kinase parameters of C_{max} and AUC, low Spearman coefficients less than the absolute value of 0.7 indicate there is a minimal association between these parameters, and as such it can be concluded that these relationships are not of clinical significance. Additional analysis is clearly required, *e.g.*, using

a multiple regression approach to construct a clinically relevant model that will include patients with altered liver and/or renal function.

Relative to the absolute CL of Brostallicin (expressed in liters/h), the interpatient variability in CL remained in a similar order of magnitude after correction for the BSA of individual patients (expressed in liters/h/m^2), with coefficients of variation of 30.8% and 28.5%, respectively, indicating that BSA contributes to only 7.5% of the total kinetic variability between patients. In addition, a linear-regression analysis of absolute CL of Brostallicin *versus* BSA did not result in a significant relationship ($r = 0.23$; $P = 0.30$). This suggests that BSA is not a significant predictor of Brostallicin CL and that flat-fixed dosing regimens might be applied in future studies without compromising overall safety profiles.

The pharmacodynamic analysis was focused on hematological toxicity, particularly the dose-limiting neutropenia. The modeling of the pharmacokinetic and pharmacodynamic data in the present study revealed a significant relation between the hematological toxicity, and both AUC and C_{max} of Brostallicin.

One patient with extensive liver metastases of a gastrointestinal stromal tumor, a known chemotherapy-resistant tumor type, clearly responded to the treatment with an objective partial response lasting 16 months. Two patients, 1 with synovial sarcoma and 1 with cervix cancer/non-small-cell lung cancer demonstrated disease stabilization for, respectively, 16 and 13 weeks. Phase 2 studies on Brostallicin are currently ongoing using the 3-weekly schedule.

In conclusion, the RD for Brostallicin is 10 mg/m^2 given once every 3 weeks. At this dose the plasma systemic exposure (AUC) is twice as high as the exposure needed in mice models to achieve antitumor effects. Alternatively, a flat/fixed dosing can be considered. The current clinical findings and the novel mechanism of action of Brostallicin suggest that additional clinical research on this agent is clearly warranted.

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