A Phase I Study of CHS 828 in Patients with Solid Tumor Malignancy[1]

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ABSTRACT

CHS 828 is a cyanoguanidine, which has demonstrated potent antitumor activity in preclinical tumor models. The activity of CHS 828 *in vitro* showed only low to moderate correlation to other antineoplastic agents suggesting a unique mechanism of action. Ten females and 6 males (median age 58 years) with solid tumors refractory to standard therapy were included in this Phase I study. The study drug was administered to fasting patients as a single oral dose on days 1–5 of each treatment cycle. Patients received one to six cycles of treatment. The doses ranged from 30 mg to 200 mg (total dose within a cycle). Hematological toxicity was generally mild and dominated by transient thrombocytopenia and lymphocytopenia. Nonhematological toxicity most frequently consisted of nausea, vomiting, diarrhea, fatigue, and localized genital mucositis. The dose-limiting toxicities were thrombocytopenia, thrombosis, esophagitis, diarrhea, and constipation. The recommended Phase II dose of CHS 828 was 20 mg once daily for 5 days in cycles of 28 days duration. The extent of systemic exposure of CHS 828 across patients was approximately dose proportional. The time at which the highest drug concentration occurs was 2.2 ± 1.3 h and half-life was 2.1 ± 0.52 h (mean ± SD). Large intra- and interindividual variation in dose level-adjusted maximum plasma concentration and the area under the curve from time 0 h to infinity were observed. There was an apparent inverse relationship between systemic exposure of CHS 828, and thrombocyte and lymphocyte nadir levels. No objective tumor responses were observed, and 7 patients showed stable disease after two courses of therapy.

INTRODUCTION

CHS 828 is a cyanoguanidine, which has shown interesting properties as a potential anticancer agent (1–4). Early preclinical studies revealed a high *in vitro* activity of CHS 828 in human tumor cell lines, a low cross-reactivity with clinically used anticancer agents, and no significant sensitivity to some of the known mechanisms of resistance (2). In the subsequent pharmacodynamic evaluation, CHS 828 demonstrated significant antitumor activity in several *in vivo* tumor models, especially pronounced in a nude mouse model of small cell lung cancer (NYH; 2). Using a hollow fiber model in rats, CHS 828 exerted a high antitumor activity on eight tumor samples derived from patients with ovarian cancer and chronic lymphocytic leukemia (3).

The molecular events leading to CHS 828-induced cell death are not fully elucidated. CHS 828 exerts an immediate effect on extracellular acidification, possibly caused by an inhibition of mitochondrial respiration followed by an increase in glycolysis (5). *In vitro*, DNA and protein synthesis are unaffected during the first 24 h after CHS 828 exposure but are then abruptly inhibited (6).

For the vast majority of patients with nonresectable solid tumor malignancy the disease is incurable, prompting a need for new, efficient cytotoxic drugs.

The main objective of this trial was to establish a RPTD[3] for CHS 828 using once daily dosing for 5 consecutive days in cycles of 28 days duration. This was done safely and efficiently by combining an accelerated titration design (7) followed by a modified Fibonacci procedure.

PATIENTS AND METHODS

Patient Population. This study was approved by the Medical Product Agency in Sweden and by the Uppsala University ethics committee. The study was carried out according to the International Conference on Harmonisation guidelines for good clinical practice. Patients were required to have a histologically proven solid tumor malignancy for which no satisfactory therapy was available or had failed (for details on eligibility criteria, please refer to Table 1). On study entry, patients were

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[3] The abbreviations used are: RPTD, recommended phase two dose; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; ECOG, Eastern Cooperative Oncology Group; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; HPLC, high performance liquid chromatography; AUC, area under the curve; AUC_{inf}, the area under the curve from time 0 h to infinity; T_{max}, time at which the highest drug concentration occurs; $C_{max,}$ maximum plasma concentration; $T_{1/2,}$ half-life.

Table 1 Inclusion/exclusion criteria

Important inclusion criteria	Important exclusion criteria
Histologically proven solid tumor malignancy for which no satisfactory therapy was available or had failed	Treatment with chemotherapy, immunotherapy, or radiotherapy within 4 weeks before the baseline visit (6 weeks for nitrosoureas or mitomycin C)
Age between 18 and 75 years	Patients who had received investigational products within 30 days before the baseline visit
Life expectancy of at least 3 months	Female patients of childbearing potential who were not using adequate contraception, and patients who were pregnant or lactating
ECOG performance status of 0–2	Patients with a history of current unstable angina, congestive heart failure, or irreversible arrhythmias requiring permanent medication
WBC count $\geq 3.0 \times 10^9$/liter	Patients with psychotic or neurological disorders
Platelet count $\geq 100 \times 10^9$/liter	Patients with major gastrointestinal disease or dysfunction
Serum creatinine level $< 1.5 \times$ upper normal limit	Patients with infections requiring specific therapy
ASAT/ALAT and bilirubin level $< 2.0 \times$ upper normal limit (however, ASAT/ALAT $< 5.0 \times$ upper normal limit if judged as attributable to liver metastases)	Patients with current alcohol or substance abuse

Table 2 Study design

Accelerated phase	➡ Switch	➡ Standard design stage
Cohorts of 1 new subject per dose level Dose increments of 100% Intrapatient dose escalation	Second instance of grade 2 toxicity or first instance of DLT at any course	Cohorts of 3–6 subjects per dose level Dose increments of 30–40% No intrapatient dose escalation Two cycles per treatment

informed of the investigational nature of the treatment and potential side effects. All of the patients were required to give written informed consent to participate in the trial.

Drug Administration. CHS 828 was manufactured by Nova Laboratories Ltd. and was certified and supplied by LEO Pharma, Ballerup, Denmark. The dosage form was 10-mg gelatin capsules for oral administration. Personnel at the study site supervised all of the study drug administrations. All of the patients were fasting for 8 h before they received the study drug, and hospital breakfast was not allowed until 2 h after dosing. The starting dose was selected based on principles described by DeGeorge *et al.* (8), using one-sixth of the no adverse effect level for Beagle dogs receiving the proposed schedule of administration. This corresponded to 10-mg daily for 3 days in cycles of 28 days duration.

Definition of DLT, MTD, and RPTD. The following events were predefined as DLT: (*a*) any life-threatening event, which in opinion of the investigator was possibly, probably, or definitely related to the study drug; (*b*) a persistent thrombocytopenia, defined as failure to reach a platelet count of $\geq 100 \times 10^9$/liter 2 weeks after the end of the 28-day cycle; (*c*) persistent neutropenia, defined as failure to reach a neutrophile count of $\geq 1.5 \times 10^9$/liter 2 weeks after the end of the 28-day cycle; and (*d*) any common toxicity criteria grade 3 or 4 adverse event, which was considered to be possibly, probably, or definitely related to the study medication. The following exceptions were made: (*a*) transient hematological toxicity, *i.e.*, absolute neutrophile count of $< 0.5 \times 10^9$/liter for <7 days and/or platelets of $< 50 \times 10^9$/liter for <7 days; (*b*) grade 3 nausea or vomiting; (*c*) alopecia; and (*d*) grade 3 diarrhea not requiring parenteral rehydration. MTD was defined as the dose for which the incidence of DLT was >1 in 6 patients. Only DLT in the first two cycles were used to define the MTD.

The RPTD was defined as the dose just below the MTD.

Study Design/Treatment Plan. This study was an open-label, one-armed, Phase I safety study involving dose escalation until MTD was reached. To minimize the number of patients treated at inactive concentrations and increase the probability for the individual patient to receive bioactive concentrations, a design with an initial accelerated titration stage was used (Ref. 7; Table 2). Except for the first dose level where patients received 10 mg for 3 consecutive days, each patient was allocated a dose of CHS 828, which was given p.o. every 24 h for the first 5 days of each 28-day cycle. To proceed to the subsequent cycles patients had to have a WBC count of $\geq 3.0 \times 10^9$/liter and a platelet count of $\geq 100 \times 10^9$/liter.

During the accelerated stage of the study, patients were escalated one dose step for each treatment cycle as long as no more than CTC grade 2 toxicity occurred. The dose was to be escalated in 100% steps. One new patient was added at each dose level except on the first dose level where 3 patients were recruited. Treatment was discontinued when it was regarded to be in the best interest of the patient. When the first patient experienced DLT or when the second instance of CTC grade 2 toxicity was encountered during any course of treatment, the accelerated stage was stopped and the design switched to a modified Fibonacci procedure. All of the cycles from the accelerated stage were included in the final evaluation of the safety profile of CHS 828. During the standard stage of the study, each patient received two cycles of treatment on the allocated dose level. Dose escalation steps were 30–40%, and no intraindividual dose escalation was permitted. Cohorts of 3–6 patients were entered on each dose level until the MTD was reached. If there was no DLT after the first cycle, the next cohort of 3 patients was entered at the next higher dose. Dose escalation was to be continued until DLT occurred as follows:

Table 3 Patient characteristics, and study medication and important adverse events encountered in the study

				DOSE LEVEL (total dose in mg, divided into five daily doses)										
				Accelerated phase				Standard phase		Extended treatment				
Gender	Age	Diagnosis	ECOG	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Important adverse events
Male	32	Leiomyosarcoma	1	30	50	100	200							
Female	68	Renal carcinoma	1	30	50	100[a]								[a]Genital mucositis CTC2
Female	52	Renal carcinoma	2	30	50	100				100	100			
Male	66	Prostate cancer	0	50	100					100	100			
Female	74	Leiomyosarcoma	0	100[a]	50									[a]Diarrhoea CTC2
Female	48	Ovarian carcinoma	1	100[b]						100[b]				[b]Thrombosis CTC3
Female	73	Ovarian carcinoma	0					100	100	80	80	80	80	
Female	56	Leiomyosarcoma	1					100	100					
Male	51	Renal carcinoma	0					100	100	100	100	130	130	
Male	61	Malignant melanoma	0					130	130					
Female	58	Ovarian carcinoma	0					130	130	130	130[b]			[b]Vomiting CTC3
Female	49	Ovarian carcinoma	0					130[b]	130					[b]Diarrhoea CTC3
Female	58	Leiomyosarcoma	0					130	130[b]					[b]Esophagitis, constipation CTC3
Male	68	Soft tissue sarcoma	2					130						
Female	68	Ovarian carcinoma	1					130	130[b]					[b]Thrombocytopenia CTC3
Male	43	Soft tissue sarcoma	1					130	130 (80)[b,c]					[b]Diarrhoea CTC3

[a] 2 adverse events CTC grade 2 causing the switch from accelerated to standard phase of the study.
[b] DLTs experienced in the study.
[c] Intended dose level, medication stopped because of progressive disease. Actual dose given in parenthesis.

If 1 of 3 patients experienced DLT, then 3 more patients were to be treated at the same dose level. If 2 or 3 of 3 patients experienced DLT, then 3 more patients were to be treated at the next lower level unless 6 patients had already been treated at that dose. If the incidence of DLT was 1 in 6, then the next cohort was treated at the next higher dose. If 2 or more of 6 patients treated at the same dose level experienced DLT, the MTD was considered to be reached. Cycles from both accelerated and standard stage were included in the evaluation of the MTD of CHS 828.

Extended treatment cycles were offered to patients who responded or had stable disease. The degree of dose modification was at the discretion of the investigator. All of the adverse events during the extended treatment were included in the final evaluation of the toxicity profile of CHS 828 but were not considered for the identification of MTD.

Patient Evaluation. Pretherapy evaluation included a complete medical history and physical examination with ECOG, chest X-ray, and electrocardiogram. Hematological assessment consisted of hemoglobin, erythrocytes, platelets, WBC count and differential count, C-reactive protein, prothrombin complex, and activated partial thromboplastin time. Biochemical profile included sodium, potassium, calcium, uric acid, creatinine, glucose, albumin, bilirubin, ASAT, ALAT, alkaline phosphatase, and lactic dehydrogenase. Urine analysis included albumin, glucose, acetate, and erythrocytes (test strip). Regular assessments were required at frequent intervals during the cycle. A follow-up visit was performed 30 days after the end of the last treatment cycle. Laboratory measurements for hematological parameters were classified according to the CTC scale. Nadir values for each patient in every cycle were used for tabulating hematological toxicity. The study procedures adhered to European Organization for Research and Treatment of Cancer and the European Agency for the Evaluation of Medical Products guidelines (9).

Response Criteria. During the baseline procedure all of the patients had their tumor lesions assessed with different techniques including computed tomography scan, ultrasound scan, or X-rays. At the end of treatment cycle 2, these assessments were repeated using the same technique used for the baseline assessment. The tumor status was then compared, and a response designation could be made according to WHO criteria (10). If the patient continued treatment with the study drug, tumor assessment procedures were repeated at the end of every second additional cycle.

Pharmacokinetic Sampling. Blood samples for pharmacokinetic analysis were drawn on days 1 and 5 in the first two cycles per patient. Blood samples on day 1 were drawn before drug administration and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h after dose administration. On day 5 one sample was drawn before drug administration, and at 2 and 4 h after dosing. The samples were analyzed by LEO Pharma using an HPLC method with UV detection (11). Briefly, 1 ml of serum was extracted with 4-ml tert-butyl methyl ether after addition of internal standard and 0.1 ml 1 N ammonia. The ether phase was isolated, and CHS 828 and internal standard were re-extracted to the aqueous phase after addition of 0.1 ml 2 M phosphoric acid. The acidic aqueous phase was neutralized by addition of 0.1 ml 1 N ammonia before injection to the HPLC system. The reversed-phase HPLC system consisted of a Waters Alliance 2690 Separation Module (Waters, Milford, MA). The analytical column was a Symmetri C18 100 × 2.1 mm 3.5 μm (Waters) and kept at 40°C. The mobile phase was a gradient from 18 to 60% acetonitrile with a constant concentration of 50 mM phosphate buffer (pH 2.2) and 0.2% *N*,*N*-dimethyloctyl amine with a flow rate of 0.35 ml/min. The injection volume was 100 μl. The UV detection was performed at 277 nm and with a lower limit of quantification of 2.5 ng/ml.

From samples collected on day 1, AUC_{inf} and $T_{1/2}$ were determined using WinNonlin Standard version 2.0 or 2.1 (Sci-

Table 4 Hematological toxicity of CHS 828 (possible/probable relationship to CHS 828)[a]

CTC grade	≤50 mg (n = 8)	51–100 mg (n = 25)	101–150 mg (n = 15)	151–200 mg (n = 1)	Total (n = 49)	Percentage of all cycles
Thrombocytopenia						47%
1	1	1	2	0	4	
2	0	5	4	0	9	
3	0	4	5	1	10	
Lymphocytopenia						29%
1	0	1	0	0	1	
2	0	2	0	0	2	
3	0	6	5	0	11	
Anemia						18%
1	0	0	1	0	1	
2	0	2	5	0	7	
3	0	1	0	0	1	
Leukopenia (WBC)						10%
1	1	0	0	0	1	
2	1	0	2	0	3	
3	0	0	1	0	1	

[a] n = total number of evaluable cycles. If more than one occurrence per patient in the same cycle, AE is recorded only once with maximum severity.

entific Consulting Inc., Cary, NC). T_{max} and C_{max} were determined directly by observation of the serum concentration data. Presentation of pyruvate kinase parameters at each dose level was performed using the mean values from each patient. On day 5 serum concentrations were used for evaluation of intraindividual variation only.

To investigate a potential relationship between drug exposure and hematological toxicity, the total AUC for every cycle was estimated based on the AUC determined after the first dose, assuming linear pharmacokinetics. This AUC was related to the lowest value of WBCs and platelet counts measured during the cycle. For lymphocytes, because fewer measurements were available, the sample on day 8 was used. The potential relationship between estimated AUC and hematological parameters was investigated with linear and nonlinear regression using the GraphPad Prism software. No relationship, a linear model using log AUC, and an Emax model were tested, and compared using an F test. A simpler equation was used unless $P < 0.05$.

RESULTS

The study included 16 patients (6 males and 10 females). The baseline characteristics and tumor types of the 16 patients are shown in Table 3. Mean age was 58 years. All of the patients had received previous treatment with chemotherapy, and/or radiotherapy, and/or surgery, and/or hormonal therapy. The total numbers of evaluable cycles of CHS 828 were 49 (Table 3).

Six patients were entered in the accelerated stage, which included four different dose levels (30, 50, 100, and 200 mg total dose within cycle). Two CTC grade 2 toxicities in this stage resulted in the switch to the standard design stage. Ten patients were treated in the standard stage, which included two different dose levels (100 and 130 mg, total dose within cycle).

Six patients continued in the extended treatment with a total of 15 cycles, which also were included in the evaluation of CHS 828 toxicity. Two treatment cycles were discontinued after 3 treatment days, and these cycles were evaluated as planned in the study design but were included in the lower dose level in the summary of drug-related toxicities. In 13 patients, progressive disease was the reason for discontinuation of CHS 828. In the remaining 3 patients, treatment was discontinued because of toxicity and progressive disease.

Hematological Toxicity. Hematological toxicities were generally mild (Table 4).

Thrombocytopenia judged possibly or probably related to the study drug was reported in 47% of all of the cycles, and nadir generally occurred between days 10 and 15 of the cycle. The incidence of thrombocytopenia appeared to increase at the highest of the two dose levels of the standard design phase. In all but two of the cases the thrombocytopenia was transient and resolved within 1 week. In these two cases the thrombocytopenia normalized within 8 days. No CTC grade 4 thrombocytopenia was observed. In all of the treatment cycles in which the total dose administered was 100 mg or higher, a decrease in platelets followed by an overshoot was clearly evident from the time *versus* platelet count (Fig. 1*a*). When all of the treatment cycles (n = 35) for which pharmacokinetic data were available were analyzed with respect to thrombocytopenia, a possible relationship between platelet nadir and dose level could be discerned (Fig. 1*a*). Additional support for this relationship was seen when platelet nadir was related to the estimated systemic exposure (AUC, ng/ml × h) for the treatment cycle (Fig. 2*a*). This data were best fitted to an Emax model with the inhibitory Emax fixed to 100%, and the EC_{50} was estimated to 610 ng/ml*h. One patient developed CTC grade 3 thrombosis (lower extremity) requiring treatment during both of the treatment cycles (100 mg/week) administered (Table 3).

Lymphocytopenia judged possibly or probably related to the study drug was reported in 29% of all of the cycles. A decreased lymphocyte count was often present at baseline, and was probably because of underlying disease and heavy prestudy treatments. However, a tendency toward lower relative counts on day 8 compared with baseline was evident for several patients, especially at the higher CHS 828 doses (Fig. 1*c*). The lowest lymphocyte counts on day 8 were observed among

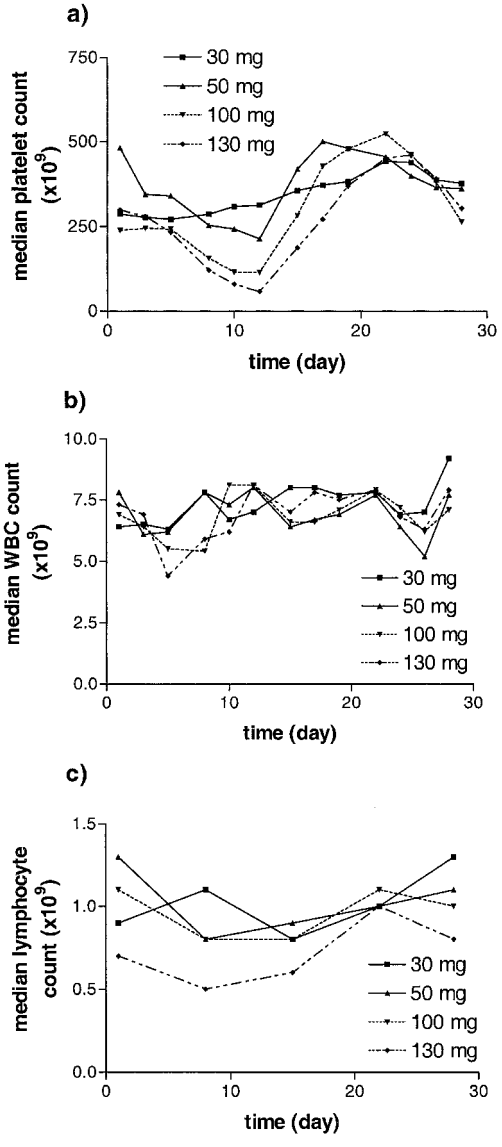

Fig. 1 Influence of CHS 828 on platelet count (*a*), WBC count (*b*), and lymphocyte count (*c*) during the first two treatment cycles in the study. Median values at every measurement time are displayed for every dose level (30 mg, $n = 3$; 50 mg, $n = 5$; 100 mg, $n = 10$; and 130 mg, $n = 13$).

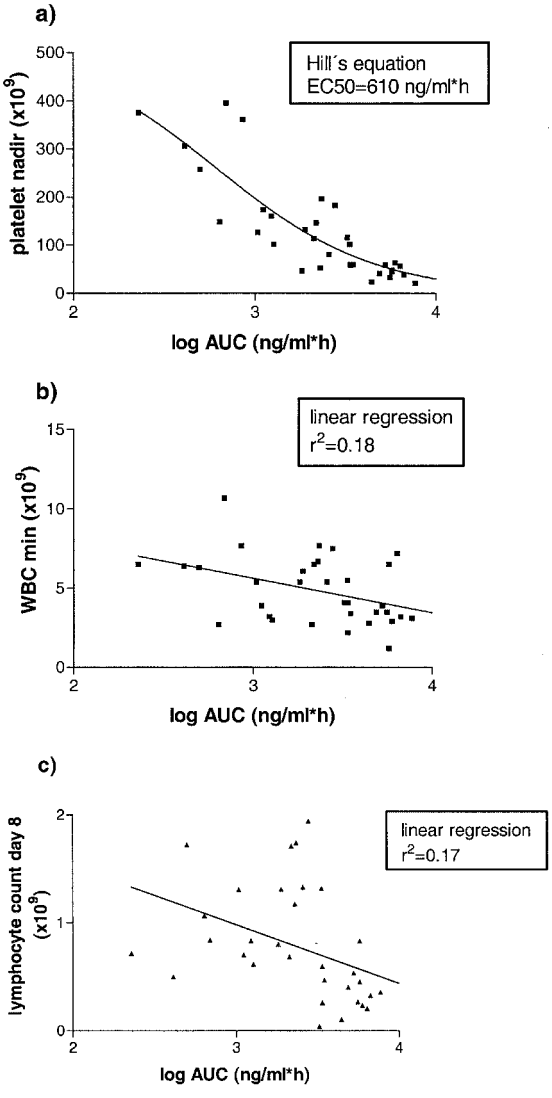

Fig. 2 Relationship between drug exposure and hematological toxicity. Drug exposure expressed as log AUC estimated from the samples taken during the first day of the treatment cycle. Hematological toxicity expressed as the lowest value of platelets (*a*) or WBCs (*b*) encountered during the cycle. For lymphocytes (*c*), which was measured less frequently, the value at day 8 after treatment start was used. The relationship between log AUC and the hematological toxicity was described with an E_{max}-model for platelets, and with linear regression for WBCs and lymphocytes.

patients with the highest estimated CHS 828 exposure (Fig. 2*c*). There was a weak ($r^2 = 0.18$) but statistically significant ($P < 0.05$; linear regression) correlation between estimated log AUC and lymphocyte count on day 8.

Anemia judged possibly or probably related to the study drug was reported in 18% of all of the cycles.

Leukopenia judged possibly or probably related to the study drug was reported in 10% of all of the cycles. CTC grade 2 was observed in three cycles. WBC CTC grade 3 was recorded once, in 1 patient during cycle 1. There was a weak ($r^2 = 0.17$) although statistically significant ($P < 0.05$; linear regression) relationship between log systemic exposure and total WBC (Fig. 2*b*). However, no apparent systematic pattern of treatment-induced changes could be discerned from the time *versus* WBC graphs (Fig. 1*b*).

In 2 of 3 of the patients (29 cycles) there were also apparent decreases of the monocyte fraction on day 8 compared with baseline (not shown).

There was no treatment delay because of hematological toxicities.

Nonhematological Toxicity. Nonhematological toxicity was diverse but most frequently consisted of nausea, diarrhea, vomiting, fatigue, and localized genital mucositis (Table 5). Nonhematological adverse events occurred at all of the dose

Table 5 Nonhematological toxicity of CHS 828 with a frequency of > 10% of all cycles (possible/probable relationship to CHS 828)[a]

Side effects (CTC grade)	Dose level (total dose within cycle) ≤50 mg (n = 8)	51–100 mg (n = 25)	101–150 mg (n = 15)	151–200 mg (n = 1)	Total (n = 49)	Percentage of all cycles
Nausea						57%
1	2	4	3	0	9	
2	2	9	6	1	18	
3	0	1	0	0	1	
Diarrohea						53%
1	1	8	2	0	11	
2	1	8	3	0	12	
3	0	0	3	0	3	
Vomiting						43%
1	2	3	3	1	9	
2	1	4	5	0	10	
3	0	1	1	0	2	
Fatigue						29%
1	1	1	3	0	5	
2	1	3	3	1	8	
3	0	1	0	0	1	
Mucositis genital (vulvitis/vaginitis/balanitis)						24%
1	0	1	2	0	3	
2	0	4	4	1	9	
Muscle cramps						16%
1	0	0	2	0	2	
2	0	2	4	0	6	
Dyspepsia/heartburn						14%
1	0	1	0	0	1	
2	1	4	1	0	6	
Infection without neutropenia						14%
1	0	1	3	0	4	
2	0	3	0	0	3	
Headache						14%
1	1	3	2	0	6	
2	0	0	1	0	1	

[a] n = total number of evaluable cycles. If more than one occurrence per patient in the same cycle, AE is recorded only once with maximum severity.

levels, although the incidence tended to increase with higher doses. The majority of these side effects occurred within the first 8 days of the cycle and were usually mild to moderate. No specific treatments were generally required. In 11 patients (21 cycles) conventional antiemetic and antidiarrhoic therapy were administered. One patient treated at 130 mg/cycle developed CTC grade 3 diarrhea requiring total parenteral nutrition. In another patient CTC grade 3 diarrhea associated with melena was recorded. One patient developed severe (CTC grade 3) esophagitis in combination with CTC grade 3 constipation, judged possibly related to the study drug.

Localized genital mucositis was observed in 5 females and in 2 male patients, and consisted of CTC grade 1–2 mucositis with red painful erosions of the mucosa around the orifice of urethra. This was observed on days 3–15. In 2 of these patients the genital mucositis was accompanied by rash in the axilla and on the chest, respectively. Among the other adverse events headache, dyspnea, muscle cramps, anorexia, rash, constipation, and infection without neutropenia were the ones occurring most frequently.

DLT. In a patient with a previous history of thrombotic episodes judged as being related to the underlying disease (ovarian carcinoma), CTC grade 3 thrombosis developed during the first and second cycle at 20 mg × 5 days and required anticoagulant therapy.

One patient, a 60-year-old woman with widespread metastatic deposits of an ovarian carcinoma in the abdominal cavity, was admitted to hospital on day 3 of cycle 4. She was dehydrated, and in the need of i.v. rehydration and hospitalization. This was judged as mainly attributable to vomiting, which consequently corresponded to grade 3 in the CTC grading system. Nausea and her malignancy probably contributed to the clinical picture.

On day 3 of the first treatment cycle, after treatment of 80 mg (total cycle dose 130 mg), 1 patient had profuse diarrhea, CTC grade 3, and subsequently required parenteral rehydration. The diarrhea spontaneously resolved after 3 days.

On day 10, cycle 2 (total cycle dose 130 mg) 1 patient presented with gastrointestinal symptoms including esophagitis, constipation, and abdominal pain. At day 15, cycle 2, an esophago-gastroscopy was performed, which revealed minimal erosions of the esophagus, changes compatible with esophagitis. When the esophago-gastroscopy was done, the patient had had omeprazol 40 mg i.v. for 5 days. The clinical picture of this event corresponded to esophagitis CTC grade 3. The relation to the study drug was deemed probable because the patient had

Table 6 Pharmacokinetic parameters

Average (SD) pharmacokinetic parameters after administration of different doses of CHS 828[a]

Dose (mg)	n^a	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{inf} (ng/ml × h)
10	5	75 (43)	1.7 (0.67)	2.2 (0.89)	257 (143)
20	9	153 (85)	2.3 (1.4)	2.1 (0.47)	639 (384)
30	7	312 (153)	2.3 (1.4)	2.2 (0.44)	1086 (519)
40	1	232	2.0	2.2	973

[a] The average values from each individual patient were used to calculate the present average values.
[b] n = number of patients.

similar but less severe symptoms (heartburn; CTC grade 2) during the first cycle. No evidence of bowel obstruction was discerned by diagnostic investigations, and the clinical picture of the constipation resembled paralytic ileus, as can be observed after treatment with *Vinca* alkaloids. The patient was treated with parenteral nutrition, and the bowel paresis had completely resolved after 42 days.

One patient, at a total cycle dose of 130 mg/cycle, showed transient CTC grade 3 thrombocytopenia during both cycles 1 and 2. In the second cycle the thrombocytes recovered after 8 days, and this episode was consequently classified as a DLT.

One patient developed CTC grade 3 diarrhea associated with melena and required parenteral support after receiving treatment of 80 mg of CHS 828 (planned total cycle dose of 130 mg).

RPTD of CHS 828. Four of 7 patients experienced DLT on 30 + 20 + 30 + 20 + 30 mg/cycle (total dose of 130 mg/cycle). Accordingly, the recommended dose for additional Phase II trials with the present schedule was set to 20 mg once daily for 5 days in cycles of 28 days duration.

Pharmacokinetics. The overall, average T_{max} value across all of the doses was found to be 2.2 ± 1.2 h. The overall terminal $T_{1/2}$ was 2.2 ± 0.54 h (mean ± SD). T_{max} and $T_{1/2}$ were similar between dose levels (Table 6). The ratio between the dose-level adjusted C_{max} and AUC_{inf} values were 1.0:0.85: 1.2:0.88 and 1.0:0.83:1.0:0.95, respectively, indicating that the extent of systemic exposure across patients was approximately dose proportional in the dose range investigated (Table 6).

Treating patients with 10–40 mg CHS 828 resulted in a large variation in both C_{max} and AUC_{inf} values. Up to 10-fold variation in dose-level adjusted AUC_{inf} values was seen between patients, whereas the variation in AUC_{inf} within the same patient was up to 4-fold. The variation in dose-level adjusted C_{max} between and within patients was 20-fold and 6-fold, respectively. When comparing the measured CHS 828 concentration 2 h after dosing in the same patient on days 1 and 5 of a cycle, the difference in dose-level adjusted serum concentration ranged from no difference to a 10-fold difference. A large within-patient variability was especially pronounced in 4 of the patients, where some of the doses given produced unexpectedly low plasma concentrations.

Response Evaluation. No partial or complete responses were observed in the present study. Stable disease was observed in 7 patients after two treatment cycles. Seven patients had progressive disease after two treatment cycles, and 2 patients were unavailable for response evaluation.

DISCUSSION

The RPTD of CHS 828 in this study was determined to be 20 mg/day for 5 days in cycles of 28 days duration. The main DLTs when using the present dosing schedule seemed to be of gastrointestinal origin. Diarrhea, nausea, and vomiting were observed frequently, and there was a tendency toward an increase in incidence with higher doses. Premedication with 5HT blockers and/or antidiarrhoeic agents was not systematically applied in the present study but may provide means for reducing GI toxicity in future studies. The spectrum of GI toxicity reported was diverse. Apart from nausea, vomiting, and diarrhea there were also patients for which constipation, esophagitis, and dyspepsia were reported to be possibly related to study drug.

Hematological toxicity was generally mild at the dose levels studied. The most frequently encountered toxicity was transient thrombocytopenia. The mechanism for this is not clear. In 1 patient thrombosis CTC grade 3 developed during the first cycle and reappeared during the second treatment cycle. However, the patient had had several episodes with thrombosis before entering the study judged to be associated with the underlying malignant disease (ovarian carcinoma). CHS 828 may have contributed to the triggering of these new events by inducing thrombocytosis in this predisposed patient. Moderate thrombocytosis was also observed in other patients with or without preceding thrombocytopenia; thus, the potential causality with this adverse event has to be determined in future clinical trials. In the present study the lymphocyte count decreased after CHS 828 administration in several patients without parallel decrease in total WBC. The decrease in lymphocyte count seemed inversely related to systemic exposure (AUC), which is in accordance with preclinical observations of CHS 828-induced lymphocytopenic effects in both mice and dogs.[4] Because malignant lymphocytes are significantly more sensitive to the cytotoxic effect of CHS 828 compared with the nonmalignant counterpart, acute and chronic lymphocytic leukemias appear to be well-suited target diagnoses (4) for additional Phase II evaluation of the present schedule of administration.

Localized mucositis of the genital tract, generally presented as a reddish erosion of the mucosa surrounding the urethral orifice, was a relatively frequent adverse reaction. The mechanism behind this atypical reaction is not clear but may involve high local concentrations of active drug or metabolite from residual urine around the orifice. This problem may be at least partly avoided by simply instructing the patients to carefully clean the urethral orifice after passing urine.

There was a large variation in pharmacokinetics of CHS 828 both between and within patients. The large interindividual variation may in part be explained by the fact that CHS 828 has been shown to be a substrate for CYP 3A4, a drug metabolizing enzyme known to produce large variation in systemic exposure of many drugs (12, 13). The intraindividual variation, on the

[4] Jonsson, *et al*., unpublished observations.

other hand, may reflect the impact of local factors in the gastrointestinal tract such as unpredictable fluctuations in gastric pH and/or inherent properties of the present drug formulation (14). There is an uncertainty to the attained RPTD in view of the large intraindividual variation in systemic exposure.

There was an apparent inverse relationship between AUC and platelet nadir. These results demonstrate that the attained RPTD in the present study was able to produce detectable systemic biological effects in all of the patients receiving this dose, which is encouraging, because this dosing schedule is applied in an ongoing Phase II trial.

CHS 828 has shown remarkable antitumor activity in several *in vivo* human tumor models, demonstrating complete tumor responses practically in the absence of toxicity (2, 3, 11). In the present study there was an apparent lack of objective tumor responses. However, this may be explained by the heavily pretreated patient population and advanced disease state. Furthermore, compared with the *in vivo* tumor models the attained doses in this study were much lower than those attainable in animals. The reason for this difference is not clear but does not seem to have a pharmacokinetic explanation, because the pharmacokinetic behavior of the drug appears to be rather similar between human and murine species (11).[4]

Preclinical studies have indicated that the cytotoxic effect of CHS 828 is dependent on exposure time or dosing schedule. Exposing cells *in vitro* to CHS 828 for more than ~30 h increases drug potency substantially, and *in vivo* studies show that changing the dosing schedule keeping the total dose constant strongly influences the cytotoxic effect (15). To determine whether the nature and intensity of the toxicity encountered in humans is schedule dependent will require additional clinical trials with different dosing strategies. One ongoing study administrating CHS 828 once every 3 weeks seems to reach a higher MTD than was reached in the present study (16).

The present study was performed using an accelerated design combined with a modified Fibonacci titration. The intention was to make the study less time- and resource-consuming, and to increase the chance for a clinical benefit for the first patients in the study. However, because of encountered toxicity at relative low dose levels, only 6 patients were included in the accelerated phase, and only 4 patients were individually dose-escalated. Thus, the advantage of the novel study design applied in this study was smaller than expected.

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