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Phase I study and pharmacokinetic of CHS-828, a guanidino-containing compound, administered orally as a single dose every 3 weeks in solid tumours: An ECSG/EORTC study

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Abstract

CHS 828 is a new guanidino-containing drug. The aim of this study was to determine the maximum tolerated dose (MTD), the recommended dose and the toxicity of CHS 828. CHS 828 was given orally once every 3 weeks. The starting dose was 50 mg, which was escalated to 500 mg. A total of 107 courses was administered to 37 patients. At the 500-mg dose level, two of three patients experienced dose-limiting toxicities (DLT) (grade 3 mucositis and grade 4 thrombocytopenia), establishing this as the MTD. One of seven patients treated at 420 mg dose experienced DLT (grade 4 leucopenia, grade 4 mucositis and grade 4 diarrhoea), and this was considered the recommended dose for phase II studies. Vomiting, haematuria, leucopenia and thrombocytopenia were other significant toxicities. The pharmacokinetics of CHS 828 showed large variations both between and within patients. No objective responses were seen. A dose of 420 mg of CHS 828 administered every 3 weeks is the recommended dose, while 500 mg is the MTD.

Keywords: Phase I; Solid tumours; CHS 828; Chemotherapy; EORTC

1. Introduction

CHS 828, *N*-(6-(4-chlorophenoxy) hexyl)-*N′*-cyano-*N″*-4-pyridylguanidine is a new guanidino-containing drug with antitumoral activity *in vitro* and *in vivo* [1–4]. The *in vitro* pattern of activity of CHS 828 showed a low to moderate correlation with other antineoplastic agents suggesting a unique mechanism of action [1,2]. Moreover, CHS 828 is not affected by some of the known mechanisms of drug resistance [2].

The greatest cytotoxic effects *in vitro* have been obtained in OC-NYH small cell lung, PC3 prostatic and in U373MG glioma cell lines, with IC_{50} values of 0.0003, 0.2, 0.8 nM, respectively [2]. In most cell lines tested, CHS 828 was generally more potent than doxorubicin [2]. CHS 828 inhibits DNA synthesis in human cell lines (NYH small cell lung cancer cells, MCF-7 breast cancer cells) with a potency equivalent to that

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of daunorubicin or paclitaxel [2]. Regarding the mode of cytotoxicity, CHS 828 induces an abruptly shut-off DNA synthesis, protein synthesis and cellular proliferation 24–30 h after exposure, followed by the first signs of cell death. At 72 h, all cells have lost their membrane integrity. CHS 828 induces late programmed cell death leading to non-classic apoptosis [3,4].

The objectives of this phase I study were to determine the maximum-tolerated dose (MTD), to assess the toxicity profile, to propose a safety dose for phase II evaluation, to study the pharmacokinetics and to document any antitumoral effects of CHS 828 given orally as a single dose every 3 weeks to patients with advanced solid tumours.

2. Patients and methods

2.1. Patients

This study was designed to comply with the ethical principles of Good Clinical Practice in accordance with the Declaration of Helsinki. The local ethics board of St. Gallen (Switzerland) and the CCPPRB (Comité Consultatif de Protection des Personnes en Recherche Biomédicale) of Bordeaux approved the protocol and informed consent brochures. All patients gave written informed consent at study entry.

Patients with a cytologically or histologically confirmed diagnosis of a solid tumour that was refractory to standard treatment and not amenable to established forms of treatment were eligible. Patients were adult, had an Eastern Cooperative Oncology Group (ECOG) status of $\leqslant 2$ and a life-expectancy of $\geqslant 3$ months. They were not to have received chemotherapy, immunotherapy or radiotherapy in the previous 4 weeks. Laboratory eligibility criteria included the following: white blood cell counts $\geqslant 4 \times 10^9$ cells/L, platelet count $\geqslant 100 \times 10^9$ cells/L, bilirubin <25 μmol/L, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) within twice the normal upper limit, serum creatinine $\leqslant 120$ μmol/L. Patients with infection or clinical signs of brain involvement or leptomeningeal disease were excluded, as were pregnant or lactating women.

2.2. Pretreatment evaluation

Before treatment, a clinical history and complete physical examination were recorded for all patients. A complete blood count (including white blood cell (WBC) differential), and serum chemistry (including sodium, potassium, calcium, magnesium, phosphorus, urea, uric acid, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, ASAT, ALAT, γ-glutamyl transferase, and lactate dehydrogenase) were performed as were urine analysis, electrocardiogram (ECG), and chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to National Cancer Institute Common Toxicity Criteria (NCI-CTC version 2.0), complete blood count, serum chemistry and urine analysis. Tumour measurements were performed before treatment and after every two courses according to the World Health Organisation (WHO) Criteria for response.

2.3. Definition of dose-limiting toxicity and maximum tolerated dose

Definition of dose-limiting toxicity (DLT) was defined as grade 4 haematological toxicity, lasting more than 4 days, and/or complicated by fever, infection or bleeding episodes, or any $\geqslant$ grade 3 non–haematological toxicity, except inadequately treated grade 3 nausea or vomiting.

MTD was defined as the dose producing DLT in at least two patients of a 3–6-patient cohort.

2.4. Drug administration

LEO Pharma, Ballerup, Denmark supplied the CHS 828 as 10-, 30-, 100- and 300-mg gelatine capsules for oral administration. All patients fasted overnight before they received the study drug once, 1 h before breakfast. Prophylactic antiemetics were not prescribed. Once nausea and vomiting were identified as toxicities, prophylactive antiemetics were given in the subsequent courses for those patients.

The starting dose of CHS 828, given orally, once every 3 weeks, was planned to be 150 mg, based on 1/10 of the lethal dose in mice (853 mg/m^2). However, preliminary results from another phase I study with CHS 828 [5] showed that a dose of 150 mg might already be close to the MTD. In this study [5], the schedule tested was a single oral dose, on days 1–5, with an increasing planning total dose per cycle from 30 to 200 mg. At 130 mg, four of seven patients experienced DLT including thrombocytopenia, thrombosis, oesophagitis, diarrhoea and constipation. Accordingly, the starting dose was amended before the start of the study to 50 mg.

CHS 828 was administered once every 3 weeks or delayed until full recovery from the previous treatment cycle. Initially, one patient per dose level was treated. Doses were escalated using a modified Fibonacci scheme. At least three patients were entered at each dose level. If no or minimal (grade 1) toxicity was observed, the dose was escalated by 50–100%. If significant grade 2 toxicity was observed, excluding alopecia, anaemia, inadequately treated nausea or vomiting, a 20–33% dosage increment was allowed. Once significant grade 2 toxicity or a DLT was observed in one out of three patients, up to three additional patients were treated at that dose

level. DLT was considered only during the first course of treatment. Treatment continued in patients who responded or had stable disease if they wished. Adverse events occurring over the full treatment period were included in the final evaluation of CHS 828 toxicity. Intrapatient dose escalation was not allowed. The dose escalation scheme could be amended during the study based on pharmacokinetic findings.

2.5. Pharmacokinetics

For pharmacokinetic analysis of CHS 828, 10 mL blood samples were drawn before drug administration and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration in the first cycle. Patients with aberrant pharmacokinetic results could be sampled again during the next cycle pre-dose, 1, 2, 4 and 8 h after dosing. Samples were analysed using an high performance liquid chromatography (HPLC) method with ultraviolet (UV) detection provided by Leo Pharma [6]. Briefly, an internal standard was added to human serum and transferred to a *tert*-butyl methyl ether layer before partitioning CHS 828 and the internal standard. Then, the ether phase was mixed with an aqueous solution of phosphoric acid and the two compounds were re-extracted to the aqueous phase. The acidic aqueous phase was neutralised by adding aqueous ammonia and was injected into the column. The HPLC system was a Waters Alliance 2690 Separation Module (Waters, Milford, MA, USA). UV detection was performed at 277 nm and with a lower limit of quantification of 2.5 ng/ml.

AUC_{inf} (area under the curve from time 0 h to infinity), AUC_t (area under the curve from time 0 h to time t), and $T_{1/2}$ (half-life) were obtained using WinNonlin Standard version 2.0 or 2.1 (Parsight Corporation, El Camino Real, Mountain View, CA, USA), while T_{max} (time at which the highest drug concentration occurs) and C_{max} (maximum plasma concentration) were determined by direct observation of the serum concentrations.

3. Results

3.1. Patient characteristics

Thirty-eight patients were included in this study, but one patient was not treated having developed symptomatic cerebral metastases shortly after registration. The characteristics of the 37 patients teated are listed in Table 1.

Table 1
Characteristics of patients

Characteristics	No.	(%)
Number of patients	38	
Assessable patients		
For toxicity	37	(97)
For response	37	(97)
Men/women	24/14	(63)/(37)
Age (years) median (range)	56 (30–70)	
Performance status (ECOG)		
0	6	(16)
1	19	(50)
2	13	(34)
Primary tumour		
Renal cell carcinoma	15	(39)
Lung cancer	4	(11)
Colorectal cancer	3	(8)
Melanoma	3	(8)
Breast carcinoma	3	(8)
Hepatocarcinoma	2	(5)
Urothelium cancer	2	(5)
Others	6	
Prior treatment	37	(97)
Surgery	1	(3)
Surgery and radiotherapy	1	(3)
Surgery and systemic therapy	15	(39)
Surgery and systemic therapy and radiotherapy	20	(53)
Number of prior chemo- and immunotherapy regimens		
1 regimen	6	(16)
2 regimens	12	(32)
$\geqslant$3 regimens	17	(45)

ECOG, Eastern Cooperative Oncology Group.

3.2. Dose escalation

The starting dose was 50 mg every 3 weeks. The dose was escalated to 100, 150, 200, 250, 300, 350, 420 and 500 mg every 3 weeks. A total of 107 courses were administered to 37 patients. Twenty-seven patients received at least two cycles of treatment.

No DLTs were seen at the 50, 100, 150, 250 and 350 mg every 3 weeks dose levels. DLT occurred in one of six patients at the 200 mg every 3 weeks dose level consisting of a grade 3 thrombocytopenia. Another one of six patients experienced DLT (grade 3 haematuria) at the 300 mg every 3 weeks dose level. None of the initial three patients treated with the 420 mg every 3 weeks dose level experienced DLT so the 500 mg every 3 weeks dose level was opened. This cohort consisted of three patients of whom two experienced DLT: grade 3 mucositis and grade 4 thrombocytopenia, indicating that the MTD was exceeded. So far, an additional four patients have received 420 mg of whom 1 experienced DLT: grade 4 leucopenia for at least 4 days, grade 4 mucositis and grade 4 blood-stained diarrhoea. Another patient experienced a deterioration of a pre-existing muscle weakness to grade 3, from previous neurotoxicity induced by chemotherapy, but this was not considered as a DLT.

Consequently, oral CHS 828 at a dose of 420 mg once every 3 weeks was established as a safe dose for phase II studies.

Table 2
Main worst toxicities per course ($n = 107$) related to CHS 828

	Grade			
	1	2	3	4
Anaemia	28	21	6	1
Abdominal pain	1	4	1	
Anorexia	3	6	1	
Arthralgia	7	4		
Asthenia	6	10	7	
Balanoposthiis			1	
Diarrhoea	9	3	2	1
Fever		1	1	
Gait abnormal			1	
Haematuria			1	
Hypoaesthesia			1	
Leuco/neutropenia	9/–	4/–	1/1	3/–
Malaise		1	1	
Mucositis	1	1	2	
Muscle weakness			1	
Myalgia	2	1		
Nausea	19	8	2	
Paresthesia		1	1	
Pulmonary oedema			1	
Stomatitis	1	1	1	
Sweating increased	2	1		
Thrombocytopenia	19	7	8	1
Vaginitis			1	
Vomiting	13	11	2	1

3.3. Haematological toxicity

Myelosuppression occurred with a total of 35 drug-related thrombocytopenic events noted. Eight episodes of grade 3 thrombocytopenia occurred, that were judged drug-related, all at dose levels $\geqslant$200 mg (Table 2); one episode of grade 4 thrombocytopenia occurred at the 500 mg dose. In seven patients, the thrombocytopenia lasted more than one course. Leucopenia was observed in 17 out of 107 cycles and only occurred at dose levels $\geqslant$250 mg. Only one drug-related episode of grade 3 neutropenia occurred at the 500-mg dose level. Anaemia occurred in 56 out of 107 cycles; eight episodes were of grade 3–4 severity, all occurring at dose levels $\geqslant$250 mg, of which seven were considered drug-related.

3.4. Non–haematological toxicity

Most of the non-haematological adverse events attributed to CHS 828 were grade 1 and 2. Mucositis, diarrhoea and haematuria were the non-haematological DLTs. Two patients had dose-limiting mucositis. At the 420 and 500 mg dose level, one patient each had a grade 3 mucositis. Two patients had diarrhoea grade 3, one each at the 420 and 500 mg dose level, while one had grade 4 diarrhoea at the 420-mg dose level. Grade 3 haematuria was seen in one patient at the 300-mg dose level; this patient had a renal cell carcinoma.

3.5. Tumour response

Patients were evaluated after the first two cycles and every two cycles thereafter. There were no objective responses observed, but 11 patients (30%) had a stabilisation of the disease. Eighteen patients showed early progressive disease.

3.6. Pharmacokinetics

All patients were assessable for pharmacokinetic analyses after the first oral treatment with CHS 828.

Mean serum concentrations of CHS 828 following a single administration at the nine dose levels are shown in Table 3. Over the dose range studied, mean serum concentrations of CHS 828 were attained between 1.5 (150 mg) and 5.3 h (500 mg) after dosing (Table 3). Thereafter, serum concentrations declined with an average terminal half-life at each dose level of 2.2–7.9 h.

The linearity of CHS 828 pharmacokinetics with regard to C_{max} and AUC_{inf} was examined. The extent of the deviation from dose proportionality, as expressed by the exponent of the power function, was 1.1 (Table 4, Fig. 1). Over the dose range studied, there was a greater than dose-proportional increase in AUC_{inf} (Table 4). The extent of the deviation from dose proportionality was 1.6 (Table 4, Fig. 1). By calculating the mean pharmacokinetic parameters at each dose level,

Table 3
Pharmacokinetic parameters

Dose level (mg/3 weeks)	No. of patients	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{inf} (h × ng/ml)
50	3	224 ± 169	1.7 ± 0.6	4 ± 4.3	747 ± 586
100	3	761 ± 400	3.2 ± 1.4	2.2 ± 0.7	5014 ± 3701
150	3	558 ± 650	1.5 ± 0.5	2.5 ± 0.4	2517 ± 2951
200	6	881 ± 953	4 ± 1.7	4.2 ± 2.2	9253 ± 11636
250	3	1267 ± 1098	2.5 ± 1.3	2.9 ± 0.0	14503 ± 9536
300	6	2112 ± 2314	2.5 ± 1.8	3.5 ± 0.6	17337 ± 15946
350	3	2733 ± 1591	3.4 ± 2.5	3.3 ± 0.8	20247 ± 18975
420	7	1723 ± 1642	3.2 ± 0.9	6.1 ± 1.4	16063 ± 16110
500	3	3999 ± 2850	5.3 ± 1.2	7.9 ± 3.7	54529 ± 43499

C_{max}, maximum plasma concentration; AUC_{inf}, area under curve from time 0 h to infinity.

Table 4
Relationship between C_{max} and AUC_{inf} values and dose of CHS 828

Dose level (mg/3 weeks)	Fold-increase	C_{max} (ng/ml)	Fold-increase	AUC_{inf} (h × ng/ml)	Fold-increase
50	–	224	–	747	–
100	2.0	761	3.4	5014	6.7
150	1.5	558	0.7	2517	0.5
200	1.3	881	1.6	9253	3.7
250	1.3	1267	1.4	14503	1.6
300	1.2	2112	1.7	17337	1.2
350	1.2	2733	1.3	20247	1.2
420	1.2	1723	0.6	16063	0.8
500	1.2	3999	2.3	54529	3.4

C_{max}, maximum plasma concentration; AUC_{inf}, area under curve from time 0 h to infinity.

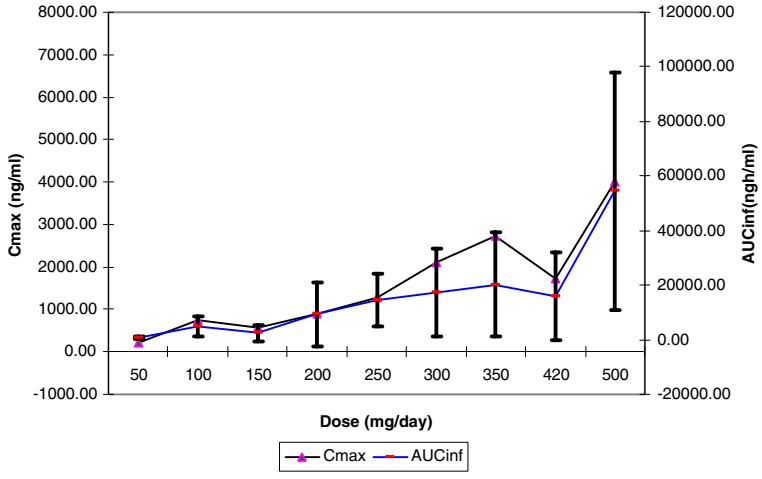

Fig. 1. CHS 828 pharmacokinetic. C_{max} and AUC_{inf}/dose.

Table 5
Serum levels of CHS 828 and DLT

Dose level (mg)	DLT	CTC grade	C_{max} (ng/ml)	AUC_{inf} (h × ng/ml)
300	Haematuria	3	1890	14144
420	Leucopenia	4		
420	Mucositis	4	568	6933
420	Diarrhoea	4		
500	Thrombocytopenia	4	804	8576
500	Mucositis	3	6280	95066

large variations in C_{max} and AUC_{inf} were found. The coefficient of variation ranged from 53% to 116% for C_{max} and 66–126% for AUC_{inf}. Moreover, up to a 50- and a 185-fold difference was observed with regard to the inter-individual C_{max} and AUC_{inf} values, respectively.

In addition, no conclusion can be drawn about the relationship between toxicity and serum levels of CHS 828. The patients who experienced DLT did not have values of C_{max} and AUC_{inf} (Table 5) outside the range of that dose level (Table 4).

4. Discussion

The recommended dose for phase II CHS 828 as a single oral administration once every 3 weeks in this study was 420 mg. DLTs at the 420-mg level occurred in patients who were heavily pretreated so this dose might not be appropriate for those patients. Since it was clear at this point that the current formulation of CHS 828 was not going to be used in further phase II studies, possible investigation of different recommended doses according to the extent of prior chemotherapy was

not carried out. Nevertheless, a lower starting dose should be considered in heavily pretreated patients. Of note, this study reached a recommended dose higher than the equivalent cumulative dose of 20 mg/day for 5 days every 28 days used in the previous phase I study [5] and in an ongoing phase II study.

DLTs were mucositis, diarrhoea, haematuria, leucopenia/neutropenia and thrombocytopenia. Mucositis, diarrhoea and thrombocytopenia were reported in a previous study using a different schedule with a daily dose for 5 days every 28 days [5].

Gastrointestinal side-effects were frequent, including abdominal pain, nausea, vomiting and diarrhoea, usually mild ($\leqslant$grade 2) for most patients (80–88%). Nevertheless, diarrhoea and vomiting induced DLTs. Diarrhoea was limited to grade 1 up to a dose of 350 mg CHS 828 but reached grade 3 and 4 at a dose $\geqslant$420 mg, which is considered as the recommended dose for phase II. Diarrhoea was reported as frequent (53% of cycles) in the other phase I study [5], with less severe diarrhoea (0 grade 3 out of 25 cycles for a total dose of 51–100 mg over 5 days) at the recommended dose.

Nausea and vomiting showed a similar toxicity profile, occurring already at a dose of 150 mg and reaching grade 3 at 200 mg. Despite the non-comparative total dose, this profile was similar to that of the daily dose over 5 days used previously [5].

Mucositis and genital mucositis seem to be associated with this drug. Stomatitis and mucositis were infrequent, but could reach a grade 3 level of toxicity. Moreover, one episode of grade 3 blanoptosthiis and one grade 3 vaginitis occurred in our study, while 24% of patients in the other phase I had genital mucositis $\leqslant$grade 2 [5]. It is thought that this atypical reaction could involve a high local concentration of active drug or metabolite from residual urine around the urethral orifice [5].

Haematological toxicities such as anaemia and thrombocytopenia were frequent. While anaemia usually remained asymptomatic (49/56 courses $\leqslant$grade 2), thrombocytopenia could induce clinical symptoms and frequently reached a grade 3. Grade 3 anaemia and thrombocytopenia were already observed at 200 mg, with no clear impact of the increase in dose.

The pharmacokinetic study of CHS 828 in this study showed both wide variations in the results between and within patients and no predictive data for toxicity. This is likely due to the involvement of cytochrome P450/CYP 3 A4 in the metabolism of CHS 828 and the impact of local factors in the absorption of an oral drug.

In conclusion, this phase I study with oral CHS 828 once a day every 3 weeks demonstrates an MTD of 500 mg and a recommended dose of 420 mg.

Conflict of interest statement

None declared.

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