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ORIGINAL ARTICLE

Phase 1 study of N^1,N^{11}-diethylnorspermine (DENSPM) in patients with advanced hepatocellular carcinoma

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Abstract
Purpose N^1,N^{11}-diethylnorspermine (DENSPM), a synthetic analog of the naturally occurring polyamine spermine, can induce polyamine depletion and inhibit tumor cell growth. The objectives of this phase I study were to assess the safety, maximum-tolerated dose (MTD), pharmacokinetics, and preliminary antitumor activity of DENSPM in advanced HCC.
Methods Patients with measurable advanced HCC, Child-Pugh A or B cirrhosis, CLIP score ≤3, and Karnofsky score ≥60 % were eligible. DENSPM was given as a short intravenous infusion on days 1, 3, 5, 8, 10, and 12 of each 28-day cycle. The starting dose of 30 mg/m^2 was escalated at a fixed increment of 15 mg/m^2 until the MTD was identified. The plasma pharmacokinetics of DENSPM for the first and last doses given in cycle 1 was characterized.
Results Thirty-eight patients (male 79 %; median age 61 years; Child-Pugh A 84 %; ≥1 prior systemic therapy 45 %) were enrolled and treated. The most common adverse events (AEs) ≥grade 1 were fatigue (53 %), nausea (34 %), diarrhea (32 %), vomiting (32 %), anemia (29 %), and elevated AST (29 %). The most common grade 3–4 AEs were fatigue/asthenia (13 %), elevated AST (13 %), hyperbilirubinemia (11 %), renal failure (8 %), and hyperglycemia (8 %). The MTD was 75 mg/m^2. There were no objective responses, although 7/38 (18 %) patients achieved stable disease for ≥16 weeks. The overall mean (±SD) total body clearance for the initial dose, 66.3 ± 35.9 L/h/m^2 ($n = 16$), was comparable to the clearance in patients with normal to near normal hepatic function. Drug levels in plasma decayed rapidly immediately after the infusion but remained above 10 nM for several days after dosing at the MTD.
Conclusions N^1,N^{11}-diethylnorspermine treatment at the MTD of 75 mg/m^2, given intravenously every other weekday for two consecutive weeks of each 28-day cycle, was relatively well tolerated in patients with advanced HCC including those with mild-to-moderate liver dysfunction. This administration schedule provided prolonged systemic exposure to potentially effective

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concentrations of the drug. Stable disease was seen in 18 % of patients receiving DENSPM treatment. Further evaluation of DENSPM monotherapy for advanced HCC does not appear to be justified because of insufficient evidence of clinical benefit in the patients evaluated in this study.

Keywords Hepatocellular carcinoma · DENSPM · Pharmacokinetics · Phase I trial

Introduction

While liver transplantation, resection, and early detection strategies have improved survival outcomes in patients with hepatocellular carcinoma (HCC), HCC remains the third most lethal cancer worldwide [1]. Most patients develop incurable recurrent disease or present with advanced disease outside the scope of liver-directed therapies, and for these patients, systemic therapy has historically offered limited benefit. In 2007, the first systemic therapy for advanced HCC was approved by the Food and Drug Administration—sorafenib. A tyrosine kinase inhibitor, sorafenib, demonstrated a survival benefit over placebo (10.7 vs. 7.9 months, respectively) in a randomized controlled trial [2]. Although this represents modest progress, new agents are needed to improve outcomes for patients with advanced HCC.

The polyamine pathway has been identified as a novel target for antineoplastic therapy. Polyamines are ubiquitous intracellular molecules that play an essential, yet undefined role in cell growth and proliferation. They, and their biosynthetic enzymes, ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (SAMDC), are found at increased levels in malignant tissue compared to normal tissue. Inducing polyamine depletion via the cellular uptake of dysfunctional synthetic polyamine analogs has been proposed as an antitumor strategy [3]. The concept is akin to the one used by cytotoxic anticancer drugs that serve as dysfunctional analogs of endogenous pyrimidines and purines. Polyamine analogs enter cells via polyamine transporters, substitute for natural polyamines in their self-regulatory roles, but fail to function as natural polyamines in promoting cell growth. Consequently, a state of "pseudo-polyamine" excess is created in cells, thereby down-regulating the enzymes responsible for polyamine synthesis, ODC and SAMDC [4], and in some cases, inducing spermidine/spermine-*N*-acetyltransferase (SSAT), the key enzyme responsible for intracellular polyamine catabolism [5].

N^1,N^{11}-diethylnorspermine tetrahydrochloride (DENSPM) is a dysfunctional analog of the naturally occurring polyamine spermine. DENSPM inhibits cell growth by substituting for spermine and depleting intracellular pools of endogenous polyamines [6–9]. The rationale for exploring the potential of DENSPM in patients with advanced HCC is fivefold. First, DENSPM avidly concentrates in the liver as shown in primate models [10]. Second, polyamine dysregulation has been demonstrated in patients with HCC [11, 12]. Third, polyamine levels and ODC activity are increased in human hepatomas [12]. Fourth, in preclinical studies, DENSPM has shown antitumor activity against a number of human tumor cell lines [7, 13–16], including the Hep3B and HUH7 hepatocellular carcinoma cell lines [17]. Finally, DENSPM has been previously studied in phase I and phase II clinical trials in over 200 patients with various solid tumors including renal, lung, and breast cancer [18–22] and has demonstrated modest antitumor activity.

This phase I clinical trial is the first study to evaluate the administration of single-agent DENSPM to a selected population of patients with advanced HCC. It was initiated in 2004, before the US FDA approval of sorafenib for HCC. Accordingly, all patients enrolled were either previously untreated or had received other systemic therapies. DENSPM was administered as a short intravenous infusion given every other weekday for two consecutive weeks of each 28-day cycle with the intention of sustaining exposure of the tumor to drug and minimizing toxicity. The primary objectives of this phase I study were to characterize the toxicity profile and establish the maximum-tolerated dose (MTD) for this novel administration schedule of DENSPM in patients with advanced HCC. Secondary objectives were to obtain a preliminary assessment of antitumor activity and characterize the pharmacokinetic behavior of the drug in patients with mild-to-moderate hepatic dysfunction.

Patients and methods

Study population

Patients with histologically proven, measurable, locally advanced or metastatic HCC were eligible for inclusion. For the patients who did not have a biopsy, the following criteria were required: (1) history of cirrhosis or chronic HBV or chronic HCV, (2) focal liver lesion ≥3 cm on computed tomography (CT) or magnetic resonance imaging (MRI) with arterial hypervascularization as confirmed by a second modality (CT/MRI), and 3) AFP ≥1,000 ng/mL (≥4,000 ng/mL if hepatitis B surface antigen positive). Other eligibility criteria included age ≥18 years; Karnofsky performance status (KPS) ≥60 %; Cancer of the Liver Italian Program (CLIP) score ≤3; adequate bone marrow, renal, and hepatic function (while blood cells ≥1,500/μL, platelet count ≥75,000/μL; serum creatinine ≤1.2 mg/dL; total bilirubin <3.5 mg/dL, aspartate aminotransferase (AST)

and alanine aminotransferase (ALT) ≤5 times upper normal limit; albumin ≥2.0 g/dL); and sodium ≥130 mEq/L. Patients enrolled after the MTD was established could have a serum creatinine ≤1.4 mg/dL. Exclusion criteria included concurrent malignancies; significant medical comorbidities including active gastrointestinal bleeding, active inflammatory bowel disease, and seizure disorder; newly noted clinically significant ECG abnormality; Child-Pugh Class C cirrhosis (Child-Pugh Class B cirrhosis was added as an exclusion criterion in a protocol amendment); ascites refractory to diuretic therapy; localized therapy (e.g., radiotherapy, radiofrequency ablation, injection therapy, or chemoembolization) within 6 weeks of starting treatment; systemic therapy including investigational agents for HCC within 3 weeks of starting treatment; pregnancy or lactation; and brain metastases. All patients provided written informed consent before study participation. The protocol was approved by the Institutional Review Board at each participating institution.

Study design

This multicenter phase I study was designed to characterize the toxicity profile and establish the MTD of DENSPM given as a 15-min intravenous infusion on days 1, 3, 5, 8, 10, and 12 of 28-day cycle to patients with advanced HCC. In the original protocol, the starting dose was 60 mg/m^2, and the dose was escalated in 30 mg/m^2 increments. Due to unanticipated toxicities in the first patient treated at 60 mg/m^2, the protocol was amended to decrease the starting dose to 30 mg/m^2 and to escalate the dose in 15 mg/m^2 increments to 120 mg/m^2. Further escalation of the dose, if necessary, was to proceed in 20 % intervals until the MTD was determined, up to a maximum of 175 mg/m^2.

The MTD was established by the occurrence of dose-limiting toxicity (DLT) during the initial cycle of therapy. Cohorts of three patients were initially treated at each dose level. Escalation to the next dose level proceeded in the absence of DLTs in the patients evaluated at the current dose level. An additional three patients were enrolled if one of the initial patients experienced a DLT. Dose escalation then proceeded only if there were no DLTs in these additional patients. The occurrence of a DLT in two or more patients at any dose level resulted in declaring the previous dose as the MTD. Once the MTD was reached, additional patients were to be enrolled at that dose until a total of 15 patients were treated and assessed for toxicity and antitumor response. Patients who discontinued treatment in the first cycle for reasons other than an adverse event (AE) meeting DLT criteria were censored and replaced.

Dose-limiting toxicity T was defined as any of the following events: (1) ≥grade 3 treatment-related thrombocytopenia associated with bleeding requiring a blood or platelet transfusion; (2) grade 4 treatment-related platelet count, total WBC, ANC, or total lymphocyte count lasting >5 days; (3) grade 3 treatment-related ALT and AST elevations lasting >21 days after the start of a treatment cycle and all grade 4 ALT and AST elevations; (4) delayed recovery of creatinine as defined by creatinine elevations that did not resolve to ≤1.8 mg/dL within 21 days of the scheduled start of the next treatment cycle; and (5) any other ≥grade 3 treatment-related AE.

Safety and efficacy assessments

Patients were monitored for safety by assessing all AEs weekly according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 3.0. Other safety evaluations included vital signs, physical examination, performance status evaluation, complete blood count, chemistries, coagulation, urinalyses, and electrocardiogram. Safety data were monitored on an ongoing basis by an independent Data Monitoring Committee (DMC). The DMC reviewed the safety data after the first cohort of patients completed one cycle of therapy and quarterly thereafter.

Patients were evaluated for response by CT or MRI after every two cycles. Response and progression were determined by an independent radiologic review using RECIST criteria [23]. Long-term follow-up of patients to assess toxicity and survival occurred at 3-month intervals until death or until 2 years after the last study visit. Patients were permitted to receive up to 8 cycles of therapy as permitted by toxicity and disease progression.

Pharmacokinetic studies

Participation in the pharmacokinetic studies was optional for patients. Sampling was performed to define the time course of the DENSPM concentration in plasma for doses given on days 1 and 12 of cycle 1. Blood (5 mL) was drawn from a peripheral vein in the arm opposite to that used for infusing the drug into plastic tubes containing freeze-dried sodium heparin immediately before dosing, at the midpoint and end of the 15-min infusion, and at 13 additional time points ranging from 5 min to 3.75 h post-infusion. Sample tubes were mixed by inversion and placed over ice until centrifuged (1,300*g*, 10 min, 4 °C). Plasma was stored in polypropylene tubes at ≤−70 °C until assayed.

A LC–MS/MS assay facilitating determination of the drug in plasma with high sensitivity and specificity was developed for use in this study and validated according to current recommendations [24]. Plasma samples (250 μL) were prepared for analysis by spiking with internal standard working solution (2.5 μg/mL 1,8-diaminooctane in water, 10 μL,) and vigorously mixed with 10 % (w/v)

trichloroacetic acid (250 μL) to precipitate proteins. The supernatant (300 μL) afforded by centrifugation (12,000 rpm, 5 min) was extracted with *tert*-butyl methyl ether (1.0 mL) to remove excess trichloroacetic acid. The remaining aqueous phase was mixed with 1.0 M sodium hydroxide (20 μL) and acetonitrile (250 μL). Freshly distilled acetic anhydride (20 μL) was added to the solution, which was immediately mixed by vortexing, to effect the rapid acetylation of amine groups present in the drug and internal standard. The reaction mixture was allowed to stand for 15 min before evaporating the liquid phase using a centrifugal vacuum concentrator at 45 °C. The sample was reconstituted with *N,N*-dimethylformamide (60 μL) and water (90 μL). The final sample solution (25 μL) was loaded onto a Phenomenex (Torrance, CA) Luna C18(2) analytical column (4.6 mm × 150 mm) and eluted with a binary gradient composed of methanol and 25 mM ammonium formate buffer, pH 3.75 at 1.0 mL/min. The amount of methanol was increased from 20 to 50 % over 15 min. An Agilent Technologies (Santa Clara, CA) 1100 series XCT ion trap mass spectrometer with an atmospheric pressure ionization–electrospray interface was used for detection. Nitrogen was used as the nebulizing gas (50 psi) and drying gas (11 L/min, 350 °C). Positive ion tandem mass spectrometry was performed using helium as the fragmentation gas. Multiple reaction monitoring was used to measure the *m/z* 413.3 → 353.3 transition for the tetraacetate derivative of DENSPM and the *m/z* 229.2 → 170.1 transition for the internal standard diacetate derivative. Quantitation was based upon integrating the product ion chromatograms to provide peak areas.

Study samples were assayed together with a series of 8 calibration standards of DENSPM·4HCl in human donor plasma at concentrations ranging from 2.5 to 500 ng/mL (6.4–1,281 nM). The relationship between the drug/internal standard peak area ratio (y) and known concentration of DENSPM in each calibration standard (x) was best described by an exponential equation, $y = a + b \cdot x^c$. Nonlinear regression was performed with weighting in proportion to $1/x$ normalized to the number of calibration standards. Values of the y-intercept (a), coefficient (b), and exponent (c) of the best-fit curve were used to calculate the drug concentration in study samples. DENSPM was determined with an interday accuracy of 108 % and a precision of 11 % at the lower limit of quantitation (2.5 ng/mL, 6.4 nM). At all other concentrations in the calibration curves, DENSPM was determined with 95–100 % accuracy and precision <11 %.

N^1,N^{11}-diethylnorspermine plasma concentration–time curves were analyzed by standard non-compartmental methods using WinNonlin Professional 5.0 software (Pharsight Corp., Cary, NC) [25]. Pharmacokinetic parameters are reported as the geometric mean ± SD of values for individual patients at each dose level, with the exception of $t_{1/2,z}$, which was calculated as the harmonic mean [26–28]. The jackknife technique was used to estimate the standard deviation of geometric and harmonic means [29]. The paired two-tailed t test was used to compare mean pharmacokinetic parameters for the doses given on days 1 and 12 after logarithmic transformation of the data. $P < 0.05$ was the criterion for statistical significance.

Statistical methods

Descriptive statistical analysis was conducted, and all analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

Study population

The study was conducted between May 2004 and November 2007 at six sites in the United States. The baseline characteristics of the 38 patients who were enrolled in the study and treated with DENSPM are summarized in Table 1. The median age was 61 years (range, 25–81 years), and 2 (5 %) were Asian. Thirty-two (84 %) patients had Child-Pugh A liver cirrhosis, and 6 (16 %) had Child-Pugh B cirrhosis. The documented etiologies of their HCC were alcohol (9 patients, 24 %), hepatitis C (9, 24 %), hepatitis B (6, 16 %), and hemochromatosis (2, 5 %) with some patients having >1 etiology. Some patients had undergone previous treatments for HCC, including 6 (16 %) with radiofrequency ablation, 10 (26 %) with chemoembolization, 2 (5 %) with radiation to the liver, and 17 (45 %) with systemic chemotherapy. Metastatic disease was present in 22 (58 %) patients at study enrollment.

Drug delivery and toxicity

Thirty-eight patients received at least 1 dose of drug, and the median number of completed cycles was 2 (range, 0–8). Ten (26 %) patients received ≥4 cycles, and 2 (5 %) patients received 8 cycles. The median duration of treatment with DENSPM was 42 days (range, 8–207 days). The most common reason for not completing the study was disease progression (25 patients, 66 %). Patients also discontinued because of a medical issue (6 patients, 16 %), a DLT (3 patients, 8 %), and upon request (1 patient, 3 %).

The data for all 38 patients were included in the safety analysis, and every patient experienced at least one AE ≥grade 1. The most frequently reported AEs (>25 % of patients) were fatigue, nausea, diarrhea, vomiting, anemia, and increased AST. Thirty-one (82 %) patients experienced

Table 1 Patient baseline characteristics (MITT population: $N = 38$)

Characteristic	Value
Median age, year (range)	61 (25–81)
Age, *N* (%)	
≤49	5 (13 %)
50–69	24 (63 %)
≥70	9 (24 %)
Sex, *N* (%)	
Male	30 (79 %)
Female	8 (21 %)
Race, *N* (%)	
White	24 (63 %)
Black	9 (24 %)
Hispanic	2 (5 %)
Asian	2 (5 %)
Other	1 (3 %)
Karnofsky performance status, *N* (%)	
100	7 (18 %)
90	18 (47 %)
80	11 (29 %)
70	2 (5 %)
Hepatocellular carcinoma etiology, *N* (%)	
Alcohol	9 (24 %)
Hepatitis C	9 (24 %)
Hepatitis B	6 (16 %)
Hemochromatosis	2 (5 %)
CLIP score, *N* (%)	
0	2 (5 %)
1	10 (26 %)
2	14 (37 %)
3	12 (32 %)
Child-Turcotte-Pugh, *N* (%)	
A	32 (84 %)
B	6 (16 %)
AJCC stage, *N* (%)	
I	0
II	3 (8 %)
III	13 (34 %)
IV	22 (58 %)
Okuda stage, *N* (%)	
I	29 (76 %)
II	9 (24 %)
Prior treatment regimens, *N* (%)	
Radiofrequency ablation	6 (16 %)
Chemoembolization	10 (26 %)
Radiation to the liver	2 (5 %)
Systemic chemotherapy	17 (45 %)

MITT modified intent to treat

drug-related AEs, the most common (>10 % of patients) of which were nausea, vomiting, fatigue, increased AST, diarrhea, anemia, leukopenia, asthenia, hyperbilirubinemia, increased ALT, increased alkaline phosphatase (AP), increased serum creatinine, hyperglycemia, and hypokalemia (Table 2). Important drug-related AEs occurring with lower frequency included neutropenia and thrombocytopenia, which occurred in 8 % of patients each.

Twenty-four (63 %) patients experienced grade 3 or 4 AEs. The most common (≥5 % of patients) grade 3 and 4 toxicities were fatigue/asthenia (13 %), increased AST (13 %), hyperbilirubinemia (11 %), renal failure (8 %), hyperglycemia (8 %), and anemia, leukopenia, neutropenia, abdominal pain, vomiting, increased AP, hyperkalemia, and dyspnea (5 % each) (Table 3). It is uncertain whether changes from baseline in ALT, AST, AP, and total bilirubin were due to underlying cirrhosis, progression of HCC, or DENSPM treatment.

Fifteen (40 %) patients experienced a serious AE, which was defined as the occurrence of any of the following: death, a life-threatening experience, a persistent or significant disability or incapacity, or inpatient hospitalization or prolongation of an existing hospitalization. Thirteen of the serious AEs were assessed as being at least possibly related to DENSPM. Seven patients died while on the study. One of these deaths was due to acute renal failure and was considered to be definitely attributable to the study drug, and this occurred at the 105 mg/m^2 dose level. The remaining six deaths were due to encephalopathy (2), hepatic hemorrhage (1), respiratory failure (1), disease progression (1), and acute renal failure (1). The second death from acute renal failure was believed to be possibly related to the study drug, and the remainder of the deaths was determined to be unrelated or unlikely related to the study drug.

Ten patients experienced DLTs, with the majority of DLTs occurring in the 30 mg/m^2 (5 patients) and 45 mg/m^2 (3 patients) dose levels. Single patients in the 75 and 90 mg/m^2 dose levels experienced a DLT. There were no DLTs in patients in the 105 and 120 mg/m^2 dose levels. The DLTs included asthenia, dehydration, respiratory failure, leukopenia, neutropenia, hepatic failure, acute renal failure, endocarditis, hyperbilirubinemia, and encephalopathy. Three patients discontinued treatment due to a DLT, one each from the 30, 45, and 75 mg/m^2 cohorts.

Establishing the MTD as 75 mg/m^2 did not follow the procedure defined in the protocol. The DMC became concerned with the pattern of serious AEs reported for patients treated in the 105 mg/m^2 dose level. Although these AEs could not be ascribed to the drug with certainty, the DMC ruled that the dose should not be escalated further and recommended a dose of either 75 or 90 mg/m^2 for continued

Table 2 Most common (≥10 %) all grade adverse events and drug-related adverse events

Adverse event	Drug-related AEs		All AEs								
			Dose level							Total ($n = 38$)	
	# of pts	%	30 mg/m^2 ($n = 6$)	45 mg/m^2 ($n = 6$)	60 mg/m^2 ($n = 4$)	75 mg/m^2 ($n = 10$)	90 mg/m^2 ($n = 6$)	105 mg/m^2 ($n = 4$)	120 mg/m^2 ($n = 2$)	# of pts	%
Any AEs	31	82	6	6	4	10	6	4	2	38	100
Hematologic											
Anemia	5	13	1	2	0	3	4	1	0	11	29
Leukopenia	5	13	1	0	0	1	3	0	0	5	13
Non-hematologic											
Nausea	10	26	3	2	1	3	1	2	1	13	34
Vomiting	9	24	3	3	1	2	0	2	1	12	32
Fatigue	9	24	4	3	2	7	2	1	1	20	53
Diarrhea	5	13	3	2	2	1	2	1	1	12	32
Asthenia	4	11	1	1	0	1	1	1	0	5	13
Peripheral edema	3	8	2	1	2	1	2	1	0	9	24
Abdominal pain	2	5	3	2	1	1	0	1	0	8	21
Constipation	2	5	0	1	0	3	2	0	1	7	18
Pyrexia	2	5	2	0	1	2	1	2	0	8	21
Dehydration	2	5	1	0	0	2	0	2	0	5	13
Dyspnea	2	5	0	2	1	2	0	1	0	6	16
Laboratory abnormalities											
AST-SGOT	6	16	1	1	1	2	3	1	2	11	29
ALT-SGPT	4	11	0	0	0	0	3	1	1	5	13
Hyperbilirubinemia	4	11	1	0	1	3	1	1	1	8	21
Alkaline phosphatase	4	11	0	1	1	2	2	1	2	9	24
Serum creatinine increased	4	11	0	1	0	0	2	2	0	5	13
Hyperglycemia	4	11	0	0	0	2	2	1	1	6	16
Hypokalemia	4	11	2	0	1	0	2	1	0	6	16
Hyponatremia	2	5	0	0	1	0	2	2	0	5	13

AEs adverse events, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

treatment. Subsequently, after joint discussion between the sponsor and investigators, it was decided that 75 mg/m^2 would be declared the MTD and additional patients were enrolled with a goal to evaluate a total of 15 patients at this dose. Ultimately, only 10 patients were enrolled because 9 of these 10 patients developed clinical or radiological progression. Five (50 %) of the 10 patients treated with 75 mg/m^2 experienced a serious AE, but none of these events was considered to be drug related. The 75 mg/m^2 dose was considered to be relatively well tolerated and not directly associated with any major treatment-related safety concerns.

Antitumor activity

All 38 treated patients were evaluable for efficacy assessments as the intent to treat population. None of the 38 patients achieved a complete or partial response. Seven (18 %) of 38 patients achieved stable disease for ≥16 weeks as their best response: 2 patients each at the 30 and 45 mg/m^2 dose levels and 1 patient each at the 60, 75, and 90 mg/m^2 dose levels. The other patients had either progressive disease (20, 53 %), removal from study due to AE (10, 26 %), or withdrawal from study (1, 3 %).

Pharmacokinetics

Plasma concentration–time profiles for DENSPM that were amenable to pharmacokinetic analysis were obtained from 16 patients for the day 1 infusion and from 15 of these same patients for the day 12 infusion during cycle 1. Mean values of the pharmacokinetic variables for

Table 3 Most common (≥ 5%) grade 3 and 4 adverse events

Adverse event	Dose level							Total ($n = 38$)	
	30 mg/m^2 ($n = 6$)	45 mg/m^2 ($n = 6$)	60 mg/m^2 ($n = 4$)	75 mg/m^2 ($n = 10$)	90 mg/m^2 ($n = 6$)	105 mg/m^2 ($n = 4$)	120 mg/m^2 ($n = 2$)	# of pts	%
Patients with at least one ≥grade 3 AE	4	3	2	5	5	4	1	24	63
Hematologic	2	0	0	1	3	0	0	6	15
Anemia	0	0	0	1	1	0	0	2	5
Leukopenia	1	0	0	0	1	0	0	2	5
Neutropenia	1	0	0	0	1	0	0	2	5
Non-hematologic toxicities	3	1	1	4	1	2	0	12	32
Fatigue and asthenia	1	0	0	2	1	1	0	5	13
Renal failure	0	1	0	1	0	1	0	3	8
Abdominal pain	1	0	1	0	0	0	0	2	5
Vomiting	1	0	0	1	0	0	0	2	5
Dyspnea	0	0	1	0	0	1	0	2	5
Laboratory Abnormalities	2	2	3	2	2	3	4	18	47
AST increased	1	1	1	0	0	1	1	5	13
Hyperbilirubinemia	1	0	1	1	0	0	1	4	11
Hyperglycemia	0	0	0	1	1	0	1	3	8
Serum alkaline phosphatase increased	0	1	0	0	1	0	0	2	5
Hyperkalemia	0	0	0	0	0	1	1	2	5

AE adverse event, *AST* aspartate aminotransferase

Table 4 Mean pharmacokinetic variables for DENSPM

Dose (mg/m^2)	Day	No. of patients	C_0 (nM)	C_{max} (nM)	AUC_2 (nM·h)	$t_{1/2,z}$ (h)	CL (L/h/m^2)	V_{ss} (L/m^2)
30	1	2	<LOQ	9,662 ± 9,142	2,740 ± 1,843	0.26 ± 0.06	27.9 ± 18.7	9.3 ± 5.9
	12	2	6.4 ± 9.1	4,117 ± 701	757 ± 442	2.29 ± 0.81	93.7 ± 51.5	66.5 ± 28.3
45	1	1	<LOQ	3,488	1,009	0.20	113.9	17.4
	12	1	<LOQ	4,953	815	2.23	133.1	70.4
60	1	2	<LOQ	14,693 ± 7,239	3,105 ± 664	0.36 ± 0.04	49.4 ± 10.5	4.8 ± 1.2
	12	2	13.6 ± 6.7	8,284 ± 2,427	1,779 ± 98	0.49 ± 0.69	86 ± 4.4	19.6 ± 2.6
75	1	6	<LOQ	7,571 ± 3,075	2,129 ± 693	0.57 ± 0.29	89.6 ± 29.2	15.1 ± 8.3
	12	5	11.0 ± 9.4	6,848 ± 2,295	1,896 ± 271	3.17 ± 0.65*	101.7 ± 16.6	36.3 ± 10.5*
90	1	1	<LOQ	19,111	5,469	0.71	41.9	7.2
	12	1	<LOQ	8,415	2,369	1.90	95.8	21.1
105	1	3	<LOQ	11,088 ± 1,529	4,286 ± 1,237	0.88 ± 0.64	61.8 ± 16.3	23.7 ± 2.3
	12	3	8.0 ± 8.3	13,853 ± 5,832	3,795 ± 757	0.94 ± 0.63	68.6 ± 14.5	20.8 ± 8.9
120	1	1	<LOQ	11,362	2,370	1.71	126.1	38.5
	12	1	<LOQ	4,254	1,679	1.36	175.7	83.5

C_0 concentration in plasma prior to dosing, C_{max} maximum concentration in plasma, AUC_2, area under the plasma concentration time profile from time zero to 2 h, $t_{1/2,z}$, apparent terminal phase half-life, *CL* total body clearance, V_{ss} apparent volume of distribution at steady-state, *<LOQ* less than the lower limit of quantitation (6.4 nM)

* Statistically significant difference between mean values of the parameter on days 1 and 12 ($P < 0.05$)

DENSPM at each dose level are presented in Table 4. Pharmacokinetic data were obtained from at least one patient at each dose level and from a group of six patients at the 75 mg/m^2 MTD. The mean plasma concentration–time profiles for DENSPM in the patients treated with the MTD are shown in Fig. 1.

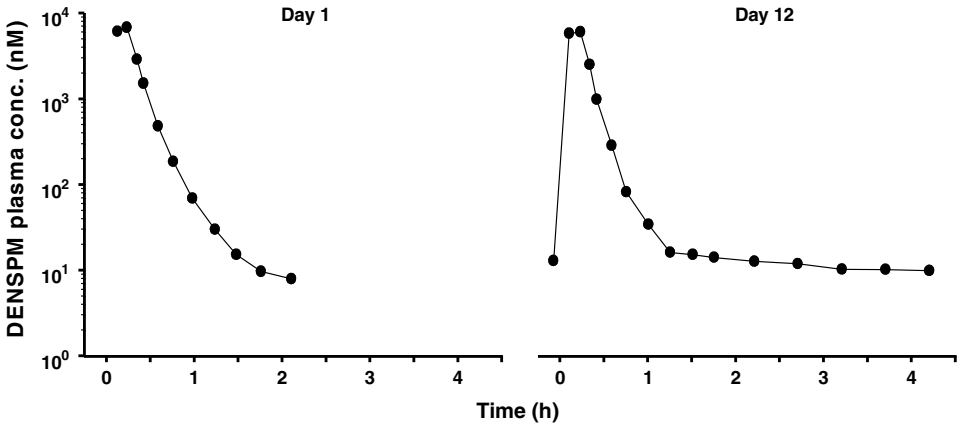

Fig. 1 DENSPM plasma concentration. Mean plasma profiles for DENSPM on days 1 and 12 in the group of patients treated with the 75 mg/m^2 MTD

The plasma pharmacokinetics of DENSPM was highly variable between patients. Neither the maximum concentration of drug in plasma ($r = 0.21$) nor the area under the plasma concentration–time curve ($r = 0.25$) was significantly correlated with the dose. The C_{max} for the day 1 infusion in the group of patients treated at the MTD ranged from 4.7 to 12.3 μM. DENSPM plasma levels decayed very rapidly after ending the infusion, decreasing nearly 1,000-fold on average from the C_{max} within 2 h and remained measurable (i.e., ≥6.4 nM) in most patients for only 2 h after the end of the infusion at doses ≤90 mg/m^2. With few exceptions, the concentration of DENSPM became unmeasurable before the true terminal disposition phase was achieved after the day 1 infusion, precluding accurate estimation of apparent biological half-life ($t_{1,2/z}$).

Evidence of prolonged persistence of the drug in plasma at concentrations exceeding 10 nM was evident in the plasma profiles for the day 12 infusion. DENSPM was present at measurable concentrations in the preinfusion samples obtained on day 12 from 11/15 patients. The mean $t_{1,2/z}$ estimated from the terminal log-linear region of the plasma profiles for the day 12 infusion in a group of five patients treated at the MTD was 3.2 ± 0.7 h. For the patients evaluated at the MTD, the mean CL of DENSPM determined for the day 1 (89.6 ± 29.2 L/h/m^2) and day 12 (101.7 ± 16.6 L/h/m^2) infusions was similar ($P = 0.81$).

Discussion

The polyamine pathway has been implicated as a potential novel target for antineoplastic therapy. Historically, prior efforts to treat cancer by inhibiting this pathway have focused on enzymatic targets involved in polyamine synthesis. As with polyamine analogs, the goal has been to deplete intracellular polyamine pools. Compounds that have reached clinical trials include difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC), and methylglyoxal-bis (guanylhydrazone) (MGBG), an inhibitor of *S*-adenosylmethionine decarboxylase (SAMDC). Although these and other polyamine synthesis inhibitors have demonstrated antiproliferative effects in vitro, their clinical application as anticancer agents has been largely unsuccessful [30, 31]. The reason for this is most likely due to the short half-lives and rapid re-synthesis of these enzymes and the existence of homeostatic mechanisms that serve to restore intracellular polyamine pools, thereby maintaining functionality. A further reason is that rapid drug clearance may lead to insufficient concentrations of drug being delivered to the tumor tissue and lack of sustained inhibition of tumor growth.

In this phase I study of patients with advanced HCC, the polyamine pathway was targeted with DENSPM, a dysfunctional polyamine analog. The novel administration schedule used in this trial was derived from the experience of previous phase I trials of the drug. Dosing regimens using multiple daily divided doses in patients with refractory solid tumors demonstrated significant dose-limiting central nervous system (CNS) toxicity [21]. An initial phase I study, which evaluated a daily 5× dosing schedule repeated every 28 days, also resulted in dose-limiting CNS toxicity [22]. Improved tolerability was demonstrated for the daily 5× regimen on a 21-day cycle upon excluding patients with an abnormal baseline brain MRI, although efficacy was limited [19]. Preclinical studies demonstrate that >96 h of exposure to DENSPM at tissue levels of at least 500 nmol/g [17] was necessary for significant antitumor activity for inducing cell death [32]. It was speculated that the limited antitumor activity observed in previous clinical trials may have been due to the inability to achieve adequate intratumoral levels of the drug for a sufficient duration of time. In an effort to enhance exposure to potentially effective drug levels, a phase I trial was performed to evaluate the administration of DENSPM as a 5-day daily intravenous loading dose followed by thrice-weekly maintenance dosing in patients with advanced solid tumors.

However, intensification of the maintenance dose led to dose-limiting nephrotoxicity [17]. Therefore, in the current trial, the loading dose was eliminated, and DENSPM was administered thrice weekly for the first 2 weeks of each 28-day cycle to sustain exposure of the tumor to drug and minimize toxicity.

The novel administration schedule of DENSPM evaluated in this phase I trial led to reasonable tolerability of the regimen. On this schedule, only 11 doses of DENSPM were missed and 3 dose reductions occurred across all patients at all dose levels. The CNS toxicity seen in multiple phase I studies with other administration schedules was not seen on this schedule. The nephrotoxicity seen with the 5-day daily loading dose followed by the thrice-weekly dosing was seen in a limited number of patients on this schedule (11 % all grades, 8 % grades 3 and 4); however, one patient did have a drug-related fatal adverse event due to acute renal failure at a dose of 105 mg/m2. DENSPM otherwise had a similar toxicity profile on this schedule as it did on phase I trials where DENSPM was given daily for 5 days of each 21-day cycle. The most frequently reported drug-related AEs across all cohorts were nausea (26 %), vomiting (24 %), and fatigue (24 %). The most common grade 3 and 4 adverse events were fatigue/asthenia (13 %), hyperbilirubinemia (13 %), and increased AST (11 %).

The plasma pharmacokinetics of DENSPM has not been studied in patients with HCC or liver dysfunction. The pharmacokinetics of DENSPM in HCC patients exhibited a high degree of variability, both between different patients and within the same patient. Peak plasma concentrations of the drug provided by the short intravenous infusion were relatively high, ranging from 1 to 20 μM. Plasma concentrations decreased very rapidly after the end of the infusion, and the overall mean (±SD) CL for the initial dose was 66.3 ± 35.9 L/h/m^2 (n = 16). These findings are in good agreement with previous reports of DENSPM pharmacokinetics in cancer patients with normal to near normal hepatic function. In particular, values of the mean CL of DENSPM calculated from data provided in the two most recently reported studies were 72.0 ± 19.3 and 68.6 ± 15.9 L/h/m^2 [10, 19]. Pharmacokinetic data have only been reported for the initial dose of DENSPM in these prior clinical investigations. A unique finding of our study was that DENSPM persisted in plasma at concentrations exceeding 10 nM for several days after dosing, remaining measurable in samples obtained before dosing on day 12 in 11/16 patients. In addition, for the majority of patients, the day 12 plasma profiles showed evidence of rebound peaks that are characteristic of enterohepatic cycling.

Despite the lack of tumor response, we did observe stable disease in 7 of the 38 evaluable patients (18 %). The contributing factors that led to the observed modest antitumor activity of DENSPM in HCC remain unknown. First, despite the rationale of testing DENSPM in HCC outlined above, there were no robust studies assessing the optimal dosing schedule, antitumor activity, and mechanism of action in preclinical HCC models. Second, the MTD may have been subtherapeutic. It is well known that underlying liver dysfunction in HCC patients can limit dose escalation due to safety and toxicity concerns. Despite the stringent criteria for liver function used in our study in which 84 % of patients enrolled had Child-Pugh A cirrhosis, the MTD in our study was 75 mg/m^2, which is less than the phase II dose of DENSPM tested in other advanced solid tumors such as breast carcinoma, where 100 mg/m^2 [20] has been tested, and non-small-cell lung cancer, where 185 mg/m^2 has been tested [19]. Our study reinforces the importance of testing novel agents in phase I studies in an HCC-specific population given the presence of confounding underlying hepatic dysfunction [33].

In conclusion, this phase I study demonstrated that DENSPM can be safely administered intravenously at the MTD of 75 mg/m^2 on a novel schedule of thrice-weekly dosing during the first 14 days of each 28-day cycle in patients with advanced HCC. Treatment with DENSPM at the MTD for this schedule provides prolonged systemic drug exposure at concentrations above 10 nM. Although no objective responses were seen, 18 % of patients achieved stable disease. Our study demonstrated that the toxicity profile of DENSPM in patients with mild-to-moderate liver dysfunction was acceptable and that the plasma pharmacokinetic parameters were found to be similar to those in patients with normal hepatic function as reported previously.

Conflict of interest None.

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