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PHASE I STUDIES

Two phase I, pharmacokinetic, and pharmacodynamic studies of DFP-10917, a novel nucleoside analog with 14-day and 7-day continuous infusion schedules

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Summary

Purpose DFP-10917 is a novel deoxycytidine analog with a unique mechanism of action. Brief exposure to high concentrations of DFP-10917 inhibits DNA polymerase resulting in S-phase arrest, while prolonged exposure to DFP-10917 at low concentration causes DNA fragmentation, G2/M-phase arrest, and apoptosis. DFP-10917 demonstrated activity in tumor xenografts resistant to other deoxycytidine analogs. *Experimental design* Two phase I studies assessed the safety, pharmacokinetic, pharmacodynamic and preliminary efficacy of DFP-10917. Patients with refractory solid tumors received DFP-10917 continuous infusion 14-day on/7-day off and 7-day on/7-day off. Enrollment required age > 18 years, ECOG Performance Status 0–2 and adequate organ function. *Results* 29 patients were dosed in both studies. In 14-day infusion, dose-limiting toxicities (DLT) consisting of febrile neutropenia and thrombocytopenia occurred at 4.0 mg/m^2/day. At 3.0 mg/m^2/day, 3 patients experienced neutropenia in cycle 2. The dose of 2.0 mg/m^2/day was well tolerated in 6 patients. In 7-day infusion, grade 4 neutropenia was DLT at 4.0 mg/m^2/day. The maximum tolerated dose was 3 mg/m^2/day. Other toxicities included nausea, vomiting, diarrhea, neutropenia, and alopecia. Eight patients had stable disease for >12 weeks. Paired comet assays performed for 7 patients showed an increase in DNA strand breaks at day 8. Pharmacokinetic data showed dose-proportionality for steady-state concentration and AUC of DFP-10917 and its primary metabolite. *Conclusion* Continuous infusion of DFP-10917 is feasible and well tolerated with myelosuppression as main DLT. The recommended doses are 2.0 mg/m^2/day and 3.0 mg/m^2/day on the 14-day and 7-day continuous infusion schedules, respectively. Preliminary activity was suggested. Pharmacodynamic data demonstrate biological activity at the tested doses.

Keywords DFP-10917 · Phase I · Solid tumor · Pharmacokinetic · Pharmacodynamic · Safety · Efficacy

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Translational relevance

DFP-10917 is a novel deoxycytidine analog with a unique DNA strand breaking mechanism. In vivo study, DFP-10917 was administered using various dosing schedules, and the results suggested that prolonged exposure of DFP-10917 by 14-day continuous infusion at a low concentration provides optimal anti-tumor activity. It takes a long period continuous infusion to produce the clinical effect expected from DNA strand breaks in vitro. On the basis of the preclinical data, the objectives of two phase I studies were to determine the maximum-tolerated dose, the dose recommended for phase II study and the dose-limiting toxicity of DFP-10917 given by 14-day continuous infusion or 7-day continuous infusion in patients with advanced solid tumors. These studies were to assess the safety pharmacokinetic and preliminary efficacy

of DFP-10917. In addition, the pharmacodynamic study utilized the Comet assay, allowing quantification of DNA strand breaks by using the blood sample.

Introduction

Many nucleoside analogs have been developed as anti-tumor agents that are effective through interruption with the synthesis of nucleic acids [1]. Among them, gemcitabine is a representative deoxycytidine analog used clinically as an anti-tumor agent in the treatment of various tumor types [2, 3]. Gemcitabine is activated through phosphorylation; the phosphorylated form inhibits the activity of DNA polymerase preventing DNA synthesis causing S-phase arrest [4, 5]. Since the great efficacy of gemcitabine in combination with radiotherapy or other antitumor agents for the treatment of solid tumors was shown, newer nucleoside antimetabolites have gained attention [6].

DFP-10917 [4-amino-1-(2-cyano-2-deoxy-β-D-arabinofuranosyl)-2(1H)-pyrimidinone or CNDAC] is a nucleoside analog similar to deoxycytidine [7, 8]. This compound formerly known as TAS-109 has a novel chemical property and was designed as a mechanism-based DNA self-strand-breaking nucleoside. DFP-10917 and its prodrug (Sapacitabine also known as CYC682) have been studied extensively *in vitro* tumor models and have a cytotoxic effect against a wide range of cancer cell lines. Similar to other deoxycytidine analogs (cytarabine and gemcitabine), this compound is activated by deoxycytidine kinase (DCK) and is inactivated by cytidine deaminase (CDA) [6]. Additionally, DFP-10917 induces DNA strand breaks by beta-elimination-mediated mechanism after incorporation into the DNA strand. CNddC [4-amino-1-(2-cyano-2,3-didehydro-2,3-dideoxy-ß-D-arabinofuranosyl)-2(1H)- pyrimidinone] was detected in the nucleosides extracted from cells treated with DFP-10917, where the presence of CNddC is indicative of DNA self-strand-breakage [9]. DFP-10917 is converted to DFP-10917-triphosphate, which is incorporated into DNA (without inhibiting DNA polymerase) causing the DNA strand breaks. As a result, DNA repair is then initiated by checkpoint regulators, which causes G2/M-phase arrest and ultimately results in cell apoptosis [10], unlike S phase arrest for similar deoxycytidine analogs. In this in vitro phenomenon, DFP-10917-triphosphate is incorporated into DNA strand as DFP-10917-monophosphate to a comparable level in a concentration- and time-dependent manner [11].

In vivo study using nude mice implanted with human colon adenocarcinoma was performed in order to determine the optimum dosing schedule. DFP-10917 was administered using various dosing schedules; anti-tumor activity was evaluated on each schedule and compared to that of gemcitabine administered as an intravenous bolus injection. The results suggested that prolonged exposure to DFP-10917 by 14-day continuous infusion at a low concentration provides an optimal anti-tumor activity that was superior to that of gemcitabine (Fig. 1, xenograft model). In addition, the least body weight loss was observed in mice on the 14-day continuous infusion schedule. Exposure to low dose DFP-10917 showed DNA strand break in comet assay (Fig. 1). Relative plasma concentrations of DFP-10917 were observed using the 14-day continuous infusion, while the myelosuppressive effects as seen in leukocyte and platelet count depressions were of lesser magnitude. The linear relationship between dose administered, and AUC and C_{max} was preserved across dose levels and infusion times. Thus, prolonged exposure of DFP-10917 at low concentrations was suggested to cause less myelosuppression while having greater tumor inhibitory efficacy than shorter high dose administration.

On the basis of these preclinical data, we planned phase I studies of DFP-10917 in patients with advanced solid tumors using a continuous infusion administration regimen at a low concentration. The objectives of two phase I studies were to determine the maximum-tolerated dose (MTD), the dose recommended for phase II study and the dose-limiting toxicity (DLT) of DFP-10917 given by 14-day continuous infusion or 7-day continuous infusion in patients with advanced solid tumors. In addition, these studies assessed the safety, pharmacokinetic, pharmacodynamic and preliminary efficacy of DFP-10917.

Patient and methods

Patient eligibility Patients had a pathologically-confirmed malignancy that was advanced or locally advanced solid tumor and was refractory to standard therapy or for which conventional therapy was not reliably effective or no effective therapy was available. They were required to be ≥18 years old, had an Eastern Cooperative Group performance status of 0–2. Patients with physiologically adequate organ function were eligible for this study. The following laboratory values, performed within 28 days prior to start of treatment must be satisfied [absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, plasma creatinine ≤1.5 × upper limit of normal (ULN) for the institution, bilirubin ≤1.5 × ULN, alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 × ULN (transaminase levels could be ≤5.0 × ULN if the patient had liver metastases)]. These patients had a measurable or non-measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) [12].

The study protocol, amendments, and informed consent form were reviewed and approved by the Institutional Review Board. The study was conducted in accordance with International Conference on Harmonization Good Clinical Practice, the protocol, all applicable regulatory requirements,

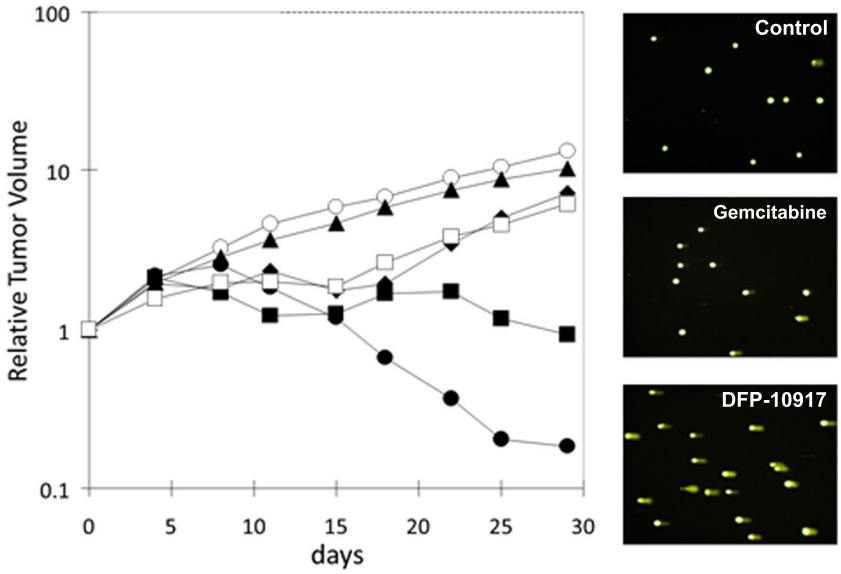

Fig. 1 Schedule-dependency of the antitumor activity of DFP-10917 against KM20C cell line in xenograft model (Dose: Gemcitabine iv; 300 mg/kg/day, DFP-10917 iv; 500 mg/kg/day, DFP-10917 1-day CI; 30 mg/kg/day, DFP-10917 3-day CI; 8 mg/kg/day, DFP-10917 14-day CI; 4.5 mg/kg/day) and DNA fragmentation of HeLa cells by treatment with DFP-10917 for 3 days in comet assay (Concentration: IC_{50} as a result of exposure for 10 days)

and the guiding principles of the Declaration of Helsinki. Written informed consent to participate in the study was obtained from each patient before study enrollment and the performance of any study-specific procedures. These studies received cooperation from Theradex (Princeton, USA).

Study drug DFP-10917 was supplied in vials as a lyophilized product and was stored between 2 and 8 °C. Patients received DFP-10917 via 14-day or 7-day continuous infusion using a central venous access device with an infusion pump. The appropriate dose was withdrawn from the vial and diluted with sodium chloride injection (United States Pharmacopeia) in a 250-mL container. The infusion solution was administered using an ambulatory pump at a temperature of not more than 25 °C. The solution was used immediately after reconstitution and the administration finished within 240 h. For single infusion bag administrations infused over a period of more than 4 days (96 h), an insulated pouch and a frozen 12-oz gel pack were used and replaced by the patient every 12 h. The dose was calculated based on the patient's actual weight at the beginning of each cycle.

Study design and treatment This was a phase I, multicenter, single arm, open-label study of DFP-10917 given by a continuous 14-day or 7-day central intravenous infusion to patients with advanced solid tumors. The first phase I study was to perform a treatment cycle for 3 weeks in duration, comprised of a 14-day continuous infusion followed by a 7-day rest period, and then a treatment cycle for 2 weeks in duration, comprised of a 7-day continuous infusion followed by a 7-day rest period (to assess for two cycles). The starting dose of 3.0 mg/m^2/day was based on the highest non-severely toxic dose observed in dogs (1.0 mg/kg/day) determined in a dosing study using a continuous infusion.

Patients were enrolled in ascending dose cohorts of 3 to 4 patients and monitored for evidence of DLT and plasma concentration. DLT was defined as any grade 3 or 4 non-hematologic toxicity; nausea/vomiting of grade 3 or greater despite maximal antiemetic therapy or grade 3 or greater diarrhea despite maximal antidiarrheal therapy; any grade 4 neutropenia of greater than 4 days duration or grade 3 associated with fever; grade 4 thrombocytopenia or any treatment delay of greater than 2 weeks due to drug-related side effects. The MTD was defined as one dose level below the dose at which DLT was observed in at least 2 patients. At least 6 patients were to be enrolled and treated at the MTD with no more than one of these patients experiencing DLT.

Dose modifications Dose escalation was guided by the observance of drug-related toxicities and dose-limiting toxicities (DLTs). Patients were treated in cohorts of at least three patients beginning at the starting dose level of 3.0 mg/m2/day. If no Cycle 1 DLT was observed in the first three patients, then the next cohort would be enrolled at an escalated dose level (dose doubling). If one patient in a cohort experienced a ≥ grade 2 drug-related toxicity (not dose-limiting), then dose escalation continued using 50% increments of the preceding dose. If more than one patient experienced a ≥ grade 2 drug-related toxicity or any non-related grade 3 or 4 toxicity (not dose-limiting), then dose escalation continued using 33% increments of the preceding dose. For both hematologic and non-hematologic clinically significant grade 3 or 4 toxicities, treatment ceased to allow for recovery. Toxicity had to recover to ≤ Grade 2 by study Day 21 for the patient treated with a subsequent cycle. If the patient recovered to ≤ Grade 2 by study Day 21, the next cycle began with the dose reduced by one dose level and the dose was not re-escalated for subsequent cycles for that patient. Any patient who had received a

dose ≤3.0 mg/m2/day and experienced an adverse event that required a dose modification for subsequent cycles would have his or her dose reduced by one dose level. In other words, those patients who experienced a Grade 3 or Grade 4 adverse event during the 14-day treatment cycle had to recover to Grade 2 by Day 21 (1 week after the cycle-recovery week) in order to be treated in the next cycle. If a patient did not recover to Grade 2 by Day 21, the patient could be allowed another week of recovery (up to Day 28). In this case, the patient had to recover to Grade 1 in order to be treated in the next cycle. In case of any Grade 3 or 4 toxicity after one dose reduction, patient was removed from the study. Also, if more than one dose reduction was required, the patient was removed from the study.

Patient evaluation All patients who received a dose of study medication are included in the safety analysis. Safety parameters included adverse events, vital signs, and clinical laboratory results. Adverse events were classified according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 [13].

Patients were evaluated every 2 cycles by 14-day infusion (or every 4 cycles by 7-day infusion) for tumor response and evidence of disease progression using the Response Evaluation Criteria in Solid Tumors (RECIST 1.0) methodology [12]. All patients who completed 2 cycles (or 4 cycles) of study therapy were considered evaluable for efficacy. For patients who received less than 2 cycles (or 4 cycles) of study therapy, clear evidence of clinical progression had to be present to be considered evaluable for efficacy. Patients with an initial positive response were to be confirmed at least 4 weeks later. Overall best response is defined as the highest-ranking response assessment (treating unconfirmed responses as SD). Valid response assessments are CR (complete response), PR (partial response), SD (stable disease) and PD (progressive disease).

Pharmacokinetics assessments Blood samples and urine specimens were collected for measurement of DFP-10917, the metabolite CNDAU, and the identification of unknown metabolites. Blood was collected at the following times: Day 1 at pre-dose, and 30 min, and 1, 2, 4, and 8 h post-infusion; on Day 8 (or Day 4 by 7-day infusion), before replacing the infusion bag; and on Day 15 (or Day 8 by 7-day infusion) immediately, and 5, 15, and 30 min, and 1 and 2 h post-infusion. Urine was collected during Cycle 1 on Day 1 for 0–24 h by 14-day infusion, and either on Day 4 or on Day 7 for 0–24 h by 7-day infusion. Pharmacokinetic parameters evaluated included: concentration at steady state (CSS), area under the concentration-time curve (AUC), drug half-life (T½), volume of distribution (Vd), and drug clearance (CL) for DFP-10917 and CSS for metabolite CNDAU at each dose level. Also evaluated were the urinary excretion rates of DFP-10917 and CNDAU at each dose level. Levels of DFP-109 and CNDAU in plasma and urine were quantitated in validated assays using a high-performance liquid chromatography coupled with a tandem mass spectrometer with electrospray ionization (LC/MS/MS). The pharmacokinetic samples were measured by Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The lower limit of quantification was 0.5 ng/mL for DFP-10917 and 2.5 ng/mL for CNDAU in plasma. PK parameters were calculated using WinNonlin (Version 4.1, Pharsight) software.

Pharmacodynamic assessments Optional blood samples were collected for the following exploratory pharmacogenomics studies: cytidine deaminase (CDA) levels and DNA genotyping [14], RNA gene expression analysis at pre-dose and at end of infusion. The CDA enzyme is involved in the metabolism of DFP-10917. In the DNA genotyping study, the presence of the CDA G208A allele was assayed by DNA sequencing. People with a novel polymorphism of CDA (G208A) may have a decreased ability to metabolize DFP-10917 possibly contributing to DFP-10917 sensitivity (toxicity). Briefly, a portion of the gene containing the polymorphism was amplified from blood samples using PCR and the amplified DNA was sequenced to determine the presence of the G208A polymorphism. The distribution of CDA polymorphisms among the tested patients was determined. In the RNA expression analysis of CDA, whole blood samples were assayed for the expression of CDA mRNA using RT-PCR. Relative levels of mRNA were analyzed using an Applied Biosystems (ABI) 7900 HT sequence detection system using TaqMan® chemistries. The cDNA samples were amplified and analyzed in triplicate. With the exception of one sample, triplicate data for all samples were within +/− 2 Cts. Assay limits of measurement were: all CDA data were analyzed at 35.0 Cts or less, and data for the reference gene ACTB were analyzed at 30.0 Cts or less. The pharmacodynamic samples were measured by Gentris Corporation (Morrisville, USA).

In this Proof of Concept study, whole blood sample specimens were collected for assessment of DNA strand breakage prior to and following DFP-10917 administration by 7-day infusion. The DNA strand breakage assessment (Comet Assay) was measured by BioReliance Corporation (Rockville, USA). Blood samples from the patients were collected at pre-dose (Day 1) and at the end of infusion (Day 8). After collecting each sample, they stored at 2–8 °C and transferred to the lab to be processed into slides and placed in lysis solution. The comet assay for the blood samples was performed under alkaline buffer condition (pH 13) with 20 min of unwinding time and 30 min of electrophoresis (0.7 V/cm) at room temperature. Two slides from each sample were used for scoring and fifty cells per slide for a total of 100 cells per sample were scored. DNA damage was measured using an automated scoring system (Comet Assay IV v 4.11). The

amount of DNA damage was represented as the length and number of fragmented DNA that migrated outside the cell nucleus (comet tail). Pair-wise comparison (t-test) was used to compare the % tail intensity from the group mean of the vehicle controls and % tail intensity from the group mean of the positive control to determine acceptance criteria of a valid test [15, 16].

Results

Patient characteristics A total of 29 patients were enrolled in the studies. The first patient was enrolled in the study August 2005, and the final patient was removed from the study in October 2007. The patient characteristics for each 14-day and 7-day infusion are summarized in Table 1. Fifteen patients were enrolled and treated in the 14-day infusion study. The median duration of disease was 25.6 months (range: 15–68 months). Fourteen patients were enrolled and treated in the 7-day infusion study. The median duration of disease was 20.2 months (range: 6–58 months).

Table 1 Patient baseline characteristics

Characteristics	14-day infusion	7-day infusion
No. of patients	15	14
Age (y)		
Median	61	60.5
Range	35–78	38–76
Body surface area (m^2)		
Median	1.92	1.90
Range	1.61–2.65	1.37–2.23
Sex		
Male	8	6
Female	7	8
ECOG performance status		
0	0	2
1	13	11
2	2	1
Primary site		
Colorectal	13	9
Pancreas	0	3
Lung	1	0
Bladder	1	0
Esophagus	0	1
Salivary gland	0	1
Prior therapy		
Chemotherapy	15	14
1 regimen	0	1
2 regimens	1	0
3 regimens	5	5
≥4 regimens	9	8
Surgery	15	14
Radiotherapy	5	5
Other	12	8

Toxicity All 29 patients were evaluated for safety, and 28 patients (93.3%) experienced at least one adverse event in these studies. A number of patients with DFP-10917 related adverse events for each 14-day and 7-day infusion are summarized in Table 2.

In 14-day infusion, cycle 1 dose-limiting toxicities (DLTs) were observed in all 3 patients treated at the 4.0 mg/m^2/day dose level and were primarily hematologic: grade 4 neutropenia (3 patients), grade 3 febrile neutropenia (2 patients), and grade 4 thrombocytopenia, grade 3 anemia, grade 3 leukopenia (1 patient each). grade 3 hyperbilirubinemia and grade 3 dyspnea were also reported for 1 patient. In addition to these DLTs, cycle 2 drug-related severe events were reported for 2 patients in the 3.0 mg/m^2/day cohort which included grade 3 or 4 neutropenia, grade 3 febrile neutropenia and grade 3 pneumonia. Serious adverse events were reported for 53.3% of patients; of these, 26.7% of patients had serious adverse events considered to be related to study therapy which included febrile neutropenia (20.0%), anemia (13.3%), and thrombocytopenia, pneumonia, or hyperbilirubinemia (6.7% each). One patient (6.7%) discontinued study therapy due to multiple study-related serious adverse events: neutropenia, thrombocytopenia, hyperbilirubinemia, anemia, and dyspnea. The only patient death reported was considered to be due to progressive disease and unrelated to study therapy. Regardless of relationship or severity the hematologic adverse events reported for ≥20% the patients were neutropenia (60.0% of patients), anemia (53.3%), febrile neutropenia (26.7%) and leukopenia (20.0%) and the non-hematologic adverse events were fatigue, nausea (66.7% each), alopecia, diarrhea (46.7% each), constipation (40.0%), vomiting (33.3%), pyrexia (26.7%), and back pain, dizziness, dyspnea, headache, hypokalemia, or stomatitis (20.0% each).

In 7-day infusion, during cycle 1, one patient treated at 4 mg/m^2/day DFP-10917 had a DLT of grade 3 febrile neutropenia lasting 5 days. During cycle 2, one patient treated at 4 mg/m^2/day DFP-10917 had a grade 4 neutropenia DLT lasting 8 days, and one patient treated at 3 mg/m^2/day DFP-10917 had a DLT of grade 3 neutropenic fever that lasted 10 days. Serious adverse events were reported for 64.3% of patients. 14.3% of patients had serious adverse events considered to be related to study therapy; those events were grade 3 febrile neutropenia reported by one patient, and grade 3 neutropenic fever and grade 3 anemia reported by the other patient. One patient had multiple acute brain infarcts that resulted in the patient's death; the event was considered unlikely to be related to study drug. Regardless of relationship or severity, the adverse events reported for ≥20% of the patients were anemia

Table 2 Number of patients with DFP-1097-related adverse events for >10% of patients overall

Adverse events	14-day infusion						7-day infusion				Total no. (%) $n = 29$
	2 mg/m^2/day		3 mg/m^2/day		4 mg/m^2/day		3 mg/m^2/day		4 mg/m^2/day		
	$n = 8$		$n = 4$		$n = 3$		$n = 10$		$n = 4$		
	All grades	Grades 3–4	All grades	Grades 3–4	All grades	Grades 3–4	All grades	Grades 3–4	All grades	Grades 3–4	
Hematological											
Neutropenia	3	3	2	2	3	3	4	4	3	3	15 (51.7)
Anemia	2	1			3	2	6	2	3	1	14 (48.3)
Leukopenia					2	2	3	2	1	1	6 (20.7)
Febrile neutropenia			1	1	3	3	1	1	1	1	6 (20.7)
Thrombocytopenia					2	2			1	1	3 (10.3)
Non-hematological											
Fatigue	4		2		2		8	3	1		17 (58.6)
Nausea	5		3				5	1	2	1	15 (51.7)
Vomiting	3		2				3		1	1	9 (31.0)
Diarrhea	2		2		2		3	1			9 (31.0)
Alopecia	2		2		2		3				9 (31.0)
Stomatitis	1				2						3 (10.3)
Neuropathy peripheral							3				3 (10.3)

(64.3% of patients), neutropenia and nausea (50.0% each), leukopenia and vomiting (28.6% each), and diarrhea, alopecia and neuropathy peripheral (21.4% each).

In the 7-day infusion cohort and out of 11 patients, 3 required dose reductions prior to cycle 3; 7 patients entered cycle 4 of which 1 had dose reduction; 3 patients entered cycle 5 and 1 required dose reduction. No further dose reduction was required. In the 14-day infusion cohort and out of 5 patients, 1 patient required dose reduction prior to cycle 3; All 5 patients entered cycle 4 without any dose reduction; 2 patients entered cycle 5 and no dose reduction was required; 1 patient entered cycle 7 and required dose reduction.

Maximum tolerated dose In a 14-day continuous central intravenous infusion followed by a 7-day rest, patients in the initial cohort received DFP-10917 administered at 3.0 mg/m^2/day. None of the patients in the cohort experienced cycle 1 DLTs, therefore the dose level was escalated and the next cohort sequentially enrolled at the 4.0 mg/m^2/day dose level. All 3 patients in the 4.0 mg/m^2/day cohort experienced cycle 1 hematologic DLTs which included grade 4 neutropenia (3 patients), grade 3 febrile neutropenia (2 patients), and grade 4 thrombocytopenia, grade 3 leukopenia, and grade 3 anemia (1 patient each). Cycle 1 non-hematologic DLTs of grade 3 hyperbilirubinemia and grade 3 dyspnea were observed in one patient. There were no DLTs in cycle 1 of the subsequent 3.0 mg/m^2/day cohort. However, based on the cycle 2 toxicities of grade 3 or 4 neutropenia (2 patients) and grade 3 febrile neutropenia (1 patient) seen in this cohort, the next patients were sequentially enrolled at the reduced dose level of 2.0 mg/m^2/day. None of the first 3 patients in the 2.0 mg/m^2/day cohort experienced cycle 1 DLTs, therefore additional patients were enrolled to confirm this dose level. None of the patients treated at the 2.0 mg/m^2/day dose level experienced cycle 1 DLTs and had only occasional severe related toxicities in subsequent cycles. Based on this experience, 2.0 mg/m^2/day was determined to be the MTD and recommended dose of DFP-10917 when administered as a continuous 14-day continuous central intravenous infusion in this population of patients.

In a 7-day continuous central intravenous infusion followed by a 7-day rest for two cycles, following treatment of the first 3 patients at a dose of 3.0 mg/m^2/day with no resulting DLT, the dose was increased to 4.0 mg/m^2/day for the next 4 patients. Following the development of DLTs in 2 patients, the dose was subsequently decreased to 3.0 mg/m^2/day for patients enrolled subsequently; at the 3.0 mg/m^2/day dose level, one patient experienced a DLT (grade 3 neutropenic fever). Therefore, based on this experience, 3.0 mg/m^2/day was determined to be the MTD and recommended phase II dose of DFP-10917 when administered as a continuous 7-day intravenous infusion in this population of patients.

Pharmacokinetics The pharmacokinetics parameters of DFP-10917 and urinary excretion rate for each 14-day and 7-day

infusion are summarized in Table 3. The CSS graphs for 7-day and 14-day infusions are shown in supplemental Fig. 1.

When DFP-10917 was intravenously infused continuously at doses of 2.0, 3.0 and 4.0 mg/m^2/day for 14 days, the mean areas under the concentration-time curve (AUC) were 720.8, 1140.4, and 1794.2 ng•hr./mL, respectively. Mean CSS at these doses were 2.06, 3.21, and 4.90 ng/mL, respectively, showing a dose-dependent increase (Fig. 2a). At each dose level, the half-life ($T_{1/2}$) was 0.8, 1.0 and 0.9 h, the volume of distribution (Vdss) 46.5, 46.8 and 40.3 L/m^2, and the clearance (CL) 38.9, 37.8 and 31.7 L/h/m^2. The mean urinary excretion rates of DFP-10917 until 24 h after starting infusion were 6.4, 8.4 and 6.5% at doses of 2.0, 3.0 and 4.0 mg/m^2/day. The mean rates of excretion of CNDAU (converted to the amount of DFP-10917) at the 3 dose levels were 33.8, 34.5 and 26.6%, and higher than the rates for DFP-10917.

In 7-day infusion, CSS of DFP-10917 were 3.26 ng/mL at the 3.0 mg/m^2/day dose and 5.54 ng/mL at the 4.0 mg/m^2/day dose; the AUC at each dose was 547.7 and 932.8 ng·hr./mL, respectively. Both parameters increased in a dose-dependent manner. The mean values of urinary excretion rates up to 24 h after the administration of DFP-10917 were 7.7% of dose at the dose of 3.0 mg/m^2/day, and 14.4% of dose at the dose of 4.0 mg/m^2/day which was somewhat higher probably due to the smaller number of patients evaluated at this dose. Furthermore, the mean values of the urinary excretion rate of CNDAU were 38.5% and 47.7% of the dose (converted to the amount of DFP-10917) at the doses of 3.0 and 4.0 mg/m^2/day, respectively, the results indicating no large difference between the doses.

Efficacy In 14-day infusion, the median number of DFP-10917 cycles administered was 2 (range: 1–8 cycles, Fig. 3) and the median duration of exposure was 36 days (range: 4–183 days). Of the 13 patients evaluable for response, none experienced a complete or partial response to therapy; 5 patients (38.5%) had SD as their best response (4 colorectal cancers and 1 non-small cell lung cancer). The median time to disease progression was 53 days.

In 7-day infusion, the median number of DFP-10917 cycles administered was 3 (range: 1–16 cycles, Fig. 3) and the median duration of exposure was 42 days (range: 8–237 days). Of the 9 patients evaluable for response, no objective tumor response was observed. Three patients (33.3%) had SD as their best response (2 colorectal cancers and 1 salivary gland cancer). Six patients (all treated at 3 mg/m^2/day) had progressive disease as their best response to treatment. The median time to disease progression was 62 days.

Pharmacodynamics In 14-day infusion, the genomic DNAs were purified from 15 whole blood samples and CDA genetic polymorphisms (G208A) were genotyped using DNA sequencing method. The genotypes of all samples were found to be GG (homozygous for the wild-type allele). Total RNAs were purified from 15 whole blood samples and the CDA gene expression levels measured using the RT-PCR method. CDA relative gene expression levels (CDA/ACTB) of all samples could be detected with the lowest value 0.0057 and the highest value 0.0167. Each CDA/ACTB of three patients with DLT was 0.0087, 0.0068 and 0.0102, or each CDA/ACTB of five patients with SD was 0.0072, 0.0095, 0.0080, 0.0066 and 0.0096.

In 7-day infusion, the genomic DNAs were purified from 11 whole blood samples and the genotypes of all samples were found to be GG (homozygous for the wild-type allele). Total

Table 3 Pharmacokinetic parameters of DFP-10917 and urinary excretion rate in the first treatment cycle

Duration	Dose levels (mg/m^2/day)	*n*	Pharmacokinetic parameters					Urinary excretion rate until 24 h (% of dose)		
			CSS (ng/mL)[a] mean ± SD	$T_{1/2}$ (h) mean ± SD	CL (L/h/m^2) mean ± SD	Vd (L/m^2) mean ± SD	AUC (ng·h/mL)[b] mean ± SD	DFP-10917 mean ± SD	CNDAU mean ± SD	Total mean ± SD
14-day infusion	2	4 or 5	2.06 ± 0.33	0.8 ± 0.3	38.9 ± 1.6	46.5 ± 18.9	720.8 ± 28.8	6.4 ± 2.1	33.8 ± 8.2	40.2 ± 10.0
	3	2	3.21	1.0	37.8	46.8	1140.4	8.4	34.5	42.9
	4	2	4.90	0.9	31.7	40.3	1794.2	6.5	26.6	33.1
7-day infusion	3	9 or 7	3.26 ± 1.04	0.67 ± 0.11	38.8 ± 9.3	35.7 ± 13.1	547.7 ± 176.2	7.7 ± 2.0	38.5 ± 6.6	46.2 ± 8.0
	4	3 or 2	5.54 ± 1.74	0.67	31.1 ± 9.7	25.7	932.8 ± 296.8	14.4 ± 6.8	47.7 ± 9.1	62.1 ± 2.5

[a] CSS: DFP-10917 concentration at steady-state from 8 to 336 h (14-day infusion) or from 8 to 168 h (7-day infusion)

[b] AUC: area under the time curve from time zero to infinity

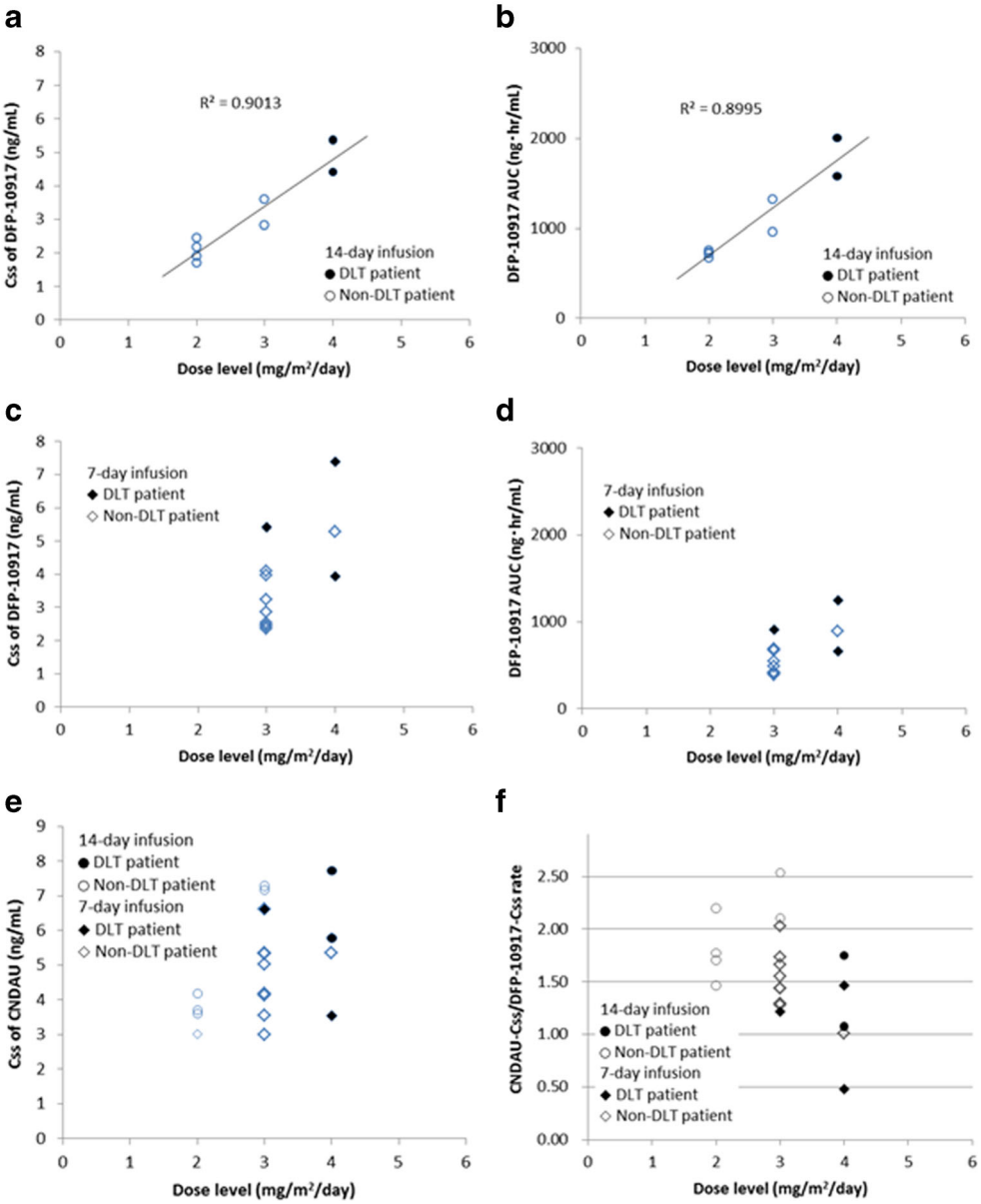

Fig. 2 Dose relationship for CSS and AUC of DFP-10917 or metabolite. **a** CSS of DFP-10917 with 14-day infusion ($n = 8$). **b** AUC of DFP-10917 with 14-day infusion ($n = 9$). **c** CSS of DFP-10917 with 7-day infusion ($n = 12$). **d** AUC of DFP-10917 with 7-day infusion ($n = 12$). **e** CSS of CNDAU as metabolite ($n = 20$). **f** CNDAU-CSS/ DFP-10917-CSS rate ($n = 20$).

RNAs were purified from 12 whole blood samples and the CDA relative gene expression levels (CDA/ACTB) of all samples could be detected with the lowest value 0.0079 and the highest value 0.0296. Each CDA/ACTB of three patients with DLT was 0.0296, 0.0105 and 0.0224, or each CDA/ACTB of three patients with SD was 0.0186, 0.0271 and 0.0296.

A total of 17 samples from 10 patients were assessed for DNA damage using the Comet Assay by 7-day infusion. Seven of ten patients had samples for Day 1 (time point 0) and Day 8 (the end of infusion). The percentage of tail intensity on Day 8 was 1.29 to 22.25 times greater than on Day 1. There were two patients for over the 40 of the % tail intensity at Day 8. The patient of value 47 observed DLT, and the patient of value 41 treated until 16 cycles (Fig. 3).

Discussion

DFP-10917 was generally well tolerated and was feasible administration despite 14-day or 7-day continuous infusion. The predominant drug-related and severe toxicities were hematologic; non-hematological events were infrequent in patients with solid tumors. DLTs and the most frequently observed adverse events were neutropenia, anemia, febrile neutropenia, leucopenia, and thrombocytopenia. Although, the most frequently observed non-hematologic drug-related adverse events were fatigue, nausea, vomiting, diarrhea, and alopecia. The adverse effects of DFP-10917 observed in these phase I studies were consistent with the finding of the preclinical toxicology studies. Based on the DLT profile seen at the DFP-10917 dose of 3.0 and 4.0 mg/m^2/day by continuous

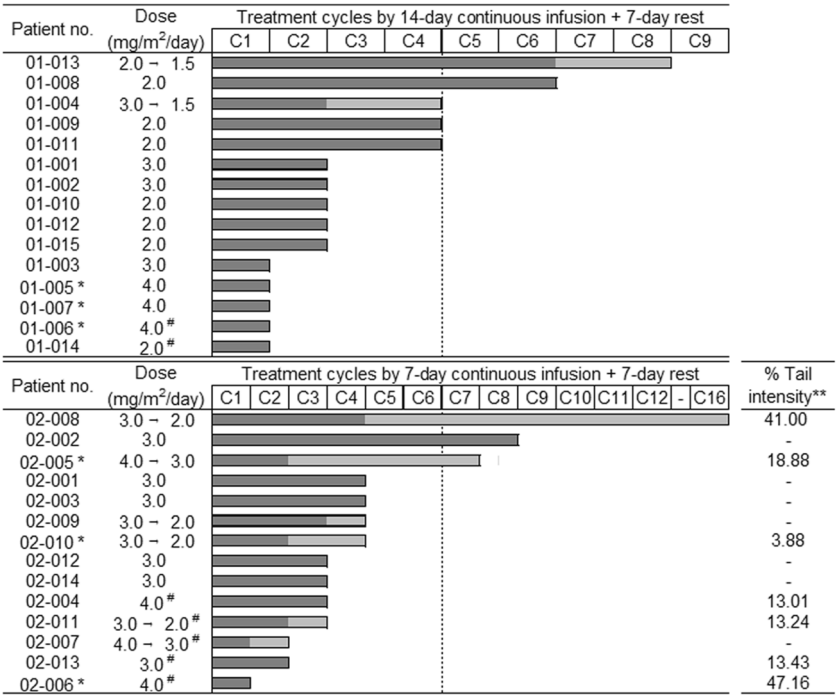

Fig. 3 Treatment cycles for each patient and DNA damage data in whole blood samples. * Patients with dose-limiting toxicities (DLT). # Not evaluable (NE) patient for tumor response. ** The measurement of Comet tail migration by Comet assay scoring at day 8 of the end of infusion

intravenous 14-day infusion, 2.0 mg/m^2/day was confirmed as the MTD. Meanwhile, in 7-day continuous infusion, 3.0 mg/m^2/day was selected as the MTD, however, a patient who was treated at 3.0 mg/m^2/day also had a DLT of grade 3 neutropenic fever. Moreover, in 14-day continuous infusion, no further DLTs were noted at 2.0 mg/m^2/day, and 5 of the 13 evaluable patients (38.5%) were experienced disease stabilization (Fig. 3). Therefore, the dose of 2.0 mg/m^2/day administered by a continuous 14-day central intravenous infusion was determined to be the recommended phase II dose/regimen in patients with solid tumors.

Correlation charts of DFP-10917 dose versus DFP-10917 concentration at steady-state (CSS) and versus the area under the concentration-time curve (AUC) for each continuous infusion are shown in Fig. 2. In a dose range of 2.0–4.0 mg/m^2/day with 14-day continuous infusion, the CSS concentration of DFP-10917 and the AUC appeared to increase in dose-dependent fashion and there was a good positive correlation. The differences in mean values among the 3 dose levels of other parameters ($T_{1/2}$, Vdss, and CL) by 14-day continuous infusion appeared not to be large. Pharmacokinetic parameters were calculated by 1-compartment model analysis, and the difference between the determined values of DFP-10917 concentration and values estimated after the model analysis was not large. These pharmacokinetic parameters were approximately similar by 7-day infusion and the results were likely to be consistent. Meanwhile, the CSS concentration of CNDAU for the DFP-10917 metabolite is provided insight into the inactivation of DFP-10917 and the influence of inactivating enzyme, CDA. The CSS concentration of CNDAU for a dose range of 2.0–4.0 mg/m^2/day had a tendency to increase in a dose-dependent fashion, and there was no connection with DLT patients (Fig. 2e). However, it was suggested that there was an association between the low rate of DFP-10917-CSS to CNDAU-CSS and the DLT patients (Fig. 2f). It was known that higher Cmax and AUC values of CNDAC (the same compound as DFP-10917) by the oral prodrug of CNDAC (Sapacitabine) were associated with greater percentage decrements in absolute neutrophil counts and the occurrence of the worst grade of neutropenia [17]. There was the same tendency for that association for this study, and it was estimated that the rate of DFP-10917-CSS to CNDAU-CSS was one of the monitoring markers for toxicity as low dose and long period continuous infusion of DFP-10917. Meanwhile, the mean urinary excretion rate of DFP-10917 until 24 h after starting infusion was lower than the rates for CNDAU of the DFP-10917 metabolite, and there was no significance ascribed to the difference of dose. These characters were also observed in Sapacitabine, the oral prodrug of DFP-10917 [18]. Therefore, these results indicate that after DFP-10917 administered in the continuous intravenous infusion was mainly metabolized in the liver, it was excreted in urine as the metabolite CNDAU or further metabolite, and there was no significant difference in the pharmacokinetic parameters or urinary excretion rates between 14-day and 7-day continuous intravenous infusion periods. (i.e., continuous intravenous infusion for).

The metabolic pathway of DFP-10917 is almost the same as cytarabine and gemcitabine. These deoxycytidine analogs are inactivated by the enzyme CDA. Homozygous G208A

alteration in CDA was reported to cause the severe drug toxicity by gemcitabine treatment in a Japanese cancer patient [19, 20], and the allelic frequency of the G208A polymorphism of the CDA gene in the Japanese population was 4.3% [14]. It was reported that the SNP G208A was not detected in Europeans, whereas the allelic frequency of 208A was 0.125 in Africans [21]. Frequencies of homozygous G208A individuals in the Japanese and African populations were estimated to be about 0.18% and 1.56%, respectively [19]. In these DFP-10917 studies, there were three African patients, and all patients were the wild-type allele. Therefore, the observed serious adverse events were not due to the G208A polymorphism of the CDA gene.

High activity of CDA, the degrading enzyme of deoxycytidine analogs, plays an important role in the development of resistance to these agents [22]. For example, CDA activity proved to be significantly higher in patients with cytarabine refractory AML than in untreated patients [23]. Furthermore, it was shown that most solid tumor cell lines had increased CDA activities, whereas 10 neuroblastoma lines showed extremely low activity, and low CDA activity may contribute high sensitivity to gemcitabine [24]. On the other hand, it was reported that there was no observation a correlation between CDA mRNA levels at a prognostic factor for survival either [25]. In small samples of these DFP-10917 studies, there was no detection a correlation with five times value range between CDA expression level and toxicity and efficacy. The correlation of CDA expression with sensitivity to DFP-10917 remains controversial and needs additional exploration.

The comet assay has become established as a highly sensitive technique for measuring DNA strand breaks [26, 27] and was set as a secondary pharmacodynamic endpoint in this trial. Several studies had examined blood of cancer patients who are undergoing chemotherapy compared to pre-treatment measurements, the tail moment for anti-neoplastic drugs were under 2 times or 3 times [28, 29]. Since all samples of the % tail intensity enhanced before and after DFP-10917 administration, 1.29 to 22.25 times, this result indicated that a DNA strand breakage may have occurred. However, the % tail intensity was not always observed at high levels in all patients. Based on historical controls, DNA damage data (% tail intensity) with saline, methyl methanesulfonate (10 μg/mL) and bleomycin (0.375 μg/mL) were 14, 89 and 56, respectively. Tail intensities greater than 40 were observed in two patients, one patient with prolonged SD and one patient with a DLT. This observation suggests that DNA double-strand break measurements by comet assay can potentially be used to evaluate response to DNA damage agents (such as DFP-10917). The comet assay can also possibly serve as a surrogate assay for efficacy and pharmacodynamics monitoring of such agents in future studies.

In conclusion, these studies investigated the differential toxicity and pharmacokinetics of 14-day and 7-day continuous infusions that were selected based upon preclinical studies. Continuous infusions of DFP-10917 are feasible and well tolerated with myelosuppression as main DLT. The recommended doses are 2.0 mg/m^2/day and 3.0 mg/m^2/day on the 14-day and 7-day continuous infusion schedules, respectively. Stable disease was the best antitumor response observed in this heavily pretreated population. Pharmacodynamic data demonstrate biological activity at the tested doses. Based on the results of the phase I studies, a phase II trial of DFP-10917 to evaluate the safety and efficacy of this agent in patients with advanced colorectal cancer refractory to cytotoxic chemotherapy was conducted (NCT00824161).

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Compliance with ethical standards

Conflict of interest All authors declare no potential conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent to participate in the study was obtained from each patient before study enrollment and the performance of any study-specific procedures.

References

1. Galmarini CM, Mackey JR, Dumontet C (2002) Nucleoside analogues and nucleobases in cancer treatment. Lancet Oncol 3:415–424
2. Burris HA 3rd, Moore MJ, Andersen J et al (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15:2403–2413
3. Sandler AB, Nemunaitis J, Denham C et al (2000) Phase III trial of gemcitabine plus cisplatin versus cisplatin alone in patients with locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 18:122–130
4. Hertel LW, Boder GB, Kroin JS et al (1990) Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). Cancer Res 50:4417–4422
5. Huang P, Chubb S, Hertel LW, Grindey GB, Plunkett W (1991) Action of 2′,2′-difluorodeoxycytidine on DNA synthesis. Clin Cancer Res 51:6110–6117
6. Matsuda A, Sasaki T (2004) Antitumor activity of sugar-modified cytosine nucleosides. Cancer Sci 95:105–111
7. Azuma A, Nakajima Y, Nishizono N et al (1993) Nucleosides and nucleotides. 122. 2′-C-cyano-2′-deoxy-1-beta-D-arabinofuranosylcytosine and its derivatives. A new class of nucleoside with a broad antitumor spectrum. J Med Chem 36:4183–4189

8. Azuma A, Hanaoka K, Kurihara A et al (1995) Nucleosides and nucleotides. 141. Chemical stability of a new antitumor nucleoside, 2′-C-cyano-2′-deoxy-1-beta-D-arabino- pentofuranosylcytosine in alkaline medium: formation of 2′-C-cyano-2′-deoxy-1-beta-D-ribo-pentofuranosylcytosine and its antitumor activity. J Med Chem 38: 3391–3397
9. Azuma A, Huang P, Matsuda A, Plunkett W (2001) 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine: a novel anticancer nucleoside analog that causes both DNA strand breaks and G(2) arrest. Mol Pharmacol 59:725–731
10. Matsuda A, Azuma A (1995) 2′-C-Cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC): A mechanism-based DNA-strand-breaking antitumor nucleoside. Nucleosides Nucleotides 14:461–471
11. Hanaoka K, Suzuki M, Kobayashi T et al (1999) Antitumor activity and novel DNA-self-strand-breaking mechanism of CNDAC (1-(2-C-cyano-2-deoxy-beta-D-arabino-pentofuranosyl) cytosine) and its N4-palmitoyl derivative (CS-682). Int J Cancer 82:226–236
12. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216
13. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 3.0, DCTD, NCI, NIH, DHHS. March 31, 2003 (http://ctep.cancer.gov), Publish Date: August 9, 2006
14. Yue L, Saikawa Y, Ota K et al (2003) A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 13(1):29–38
15. Hartmann A, Agurell E, Beevers C et al (2003) Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. Mutagenesis 18:45–51
16. Olive PL, Banáth JP, Durand RE (1990) Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. Radiat Res 122:86–94
17. Gilbert J, Carducci MA, Baker SD, Dees EC, Donehower R (2006) A Phase I study of the oral antimetabolite, CS-682, administered once daily 5 days per week in patients with refractory solid tumor. Investig New Drugs 24:499–508
18. Delaunoit T, Burch PA, Reid JM et al (2006) A phase I clinical and pharmacokinetic study of CS-682 administered orally in advanced malignant solid tumors. Investig New Drugs 24:327–333
19. Yonemori K, Ueno H, Okusaka T et al (2005) Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin Cancer Res 11:2620–2624
20. Sugiyama E, Kaniwa N, Kim SR et al (2007) Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. J Clin Oncol 25:32
21. Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL (2004) Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 4(5):307–314
22. Ge Y, Jensen TL, Stout ML et al (2004) The role of cytidine deaminase and GATA1 mutations in the increased cytosine arabinoside sensitivity of Down syndrome myeloblasts and leukemia cell lines. Cancer Res 64(2):728–735
23. Schröder JK, Kirch C, Seeber S, Schütte J (1998) Structural and functional analysis of the cytidine deaminase gene in patients with acute myeloid leukemia. Br J Haematol 103(4):1096–1103
24. Ogawa M, Hori H, Ohta T, Onozato K, Miyahara M, Komada Y (2005) Sensitivity to gemcitabine and its metabolizing enzymes in neuroblastoma. Clin Cancer Res 11(9):3485–3493
25. Galmarini CM, Thomas X, Calvo F et al (2002) Potential mechanisms of resistance to cytarabine in AML patients. Leuk Res 26(7): 621–629
26. Parker RJ, Gill I, Tarone R et al (1991) Platinum-DNA damage in leukocyte DNA of patients receiving carboplatin and cisplatin chemotherapy, measured by atomic absorption spectrometry. Carcinogenesis 12(7):1253–1258
27. Hartley JM, Spanswick VJ, Gander M et al (1999) Measurement of DNA cross-linking in patients on ifosfamide therapy using the single cell gel electrophoresis (comet) assay. Clin Cancer Res 5(3): 507–512
28. Kopjar N, Garaj-Vrhovac V, Milas I (2002) Assessment of chemotherapy-induced DNA damage in peripheral blood leukocytes of cancer patients using the alkaline comet assay. Teratog Carcinog Mutagen 22(1):13–30
29. Plummer ER, Middleton MR, Jones C et al (2005) Temozolomide pharmacodynamics in patients with metastatic melanoma: dna damage and activity of repair enzymes O6-alkylguanine alkyltransferase and poly(ADP-ribose) polymerase-1. Clin Cancer Res 11(9):3402–3409