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A phase I, pharmacokinetic, and pharmacodynamic evaluation of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine, administered with tetrahydrouridine

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Abstract

Purpose—Inhibitors of DNA (cytosine-5)-methyltransferases (DNMT) are active antineoplastic agents. We conducted the first-in-human phase I trial of 5-fluoro-2'-deoxycytidine (FdCyd), a DNMT inhibitor stable in aqueous solution, in patients with advanced solid tumors. Objectives were to establish the safety, maximum tolerated dose (MTD), pharmacokinetics, and pharmacodynamics of FdCyd + tetrahydrouridine (THU).

Methods—FdCyd + THU were administered by 3 h IV infusion on days 1–5 every 3 weeks, or days 1–5 and 8–12 every 4 weeks. FdCyd was administered IV with a fixed 350 mg/m²/day dose of THU to inhibit deamination of FdCyd. Pharmacokinetics of FdCyd, downstream metabolites and THU were assessed by LC–MS/MS. RBC γ -globin expression was evaluated as a pharmacodynamics biomarker.

Results—Patients were enrolled on the 3-week schedule at doses up to 80 mg/m²/day without dose-limiting toxicity (DLT) prior to transitioning to the 4-week schedule, which resulted in an MTD of 134 mg/m²/day; one of six patients had a first-cycle DLT (grade 3 colitis). FdCyd 40 mg/m²/day produced peak plasma concentrations >1 μ M. Although there was inter-patient variability, γ -globin mRNA increased during the first two treatment cycles. One refractory breast cancer patient experienced a partial response (PR) of >90 % decrease in tumor size, lasting over a year.

Conclusions—The MTD was established at 134 mg/m² FdCyd + 350 mg/m² THU days 1–5 and 8–12 every 4 weeks. Based on toxicities observed over multiple cycles, good plasma exposures, and the sustained PR observed at 67 mg/m²/day, the phase II dose for our ongoing multi-histology trial is 100 mg/m²/day FdCyd with 350 mg/m²/day THU.

Keywords

Fluorodeoxycytidine; DNA methylation; First-in-human; Phase I clinical trial; Pharmacokinetics; Pharmacodynamics

Introduction

A relationship between DNA methylation and neoplasia was recognized over 30 years ago [1]. The field of epigenetics (heritable changes not encoded by the primary DNA sequence) has been expanding ever since and exponentially so in the past decade [2]. Clusters of CpG's (CpG islands) are most often unmethylated in normal cells. The unmethylated status of the CpG's in 5'-regulatory regions is important for the expression of many housekeeping and tumor suppressor genes. Often, CpG islands that are unmethylated in normal tissues are methylated in both primary tumors and tumor cell lines. While the hypomethylation of specific CpG's in oncogenes may contribute to the neoplastic state and general hypomethylation may contribute to genome instability, the hypermethylation of CpG islands in the control regions of tumor suppressor genes may be of greater importance [3]. The intense interest in DNA methylation as a therapeutic target stems from the fact that unlike gene deletions or mutations, the aberrant hypermethylation is potentially reversible with small molecule therapeutic agents. 5-Azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine) are FDA-approved DNMT inhibitors [4–6]. Like decitabine and azacitidine, FdCyd inhibits DNMT by forming a covalent bond with the enzyme after incorporation into DNA [7]. It has been shown to inhibit DNA methylation and to induce differentiation in 10T½ cells [8]. Incorporation of FdCyd into DNA correlated with cytotoxicity in the cell line MCF-7, distinguishing the effects of FdCyd from the effects of fluorodeoxyuridine (FdUrd) and fluorouracil (FU) [9]. Nonetheless, as a fluoropyrimidine, FdCyd may differ from the azapyrimidines in its metabolic activation and mechanisms of action, e.g., through formation of the thymidylate synthase inhibitor fluorodeoxyuridylate (FdUMP). In addition, FdCyd may have cytotoxic effects due to steric influences of the DNMT protein covalently bound to DNA. Unlike the triazine compounds decitabine and azacitidine, FdCyd is chemically stable in aqueous environments. However, it is a known substrate for enzymatic deamination, both in vitro and in vivo, a process that can be inhibited with THU [10, 11]. Therefore, FdCyd was studied in combination with a fixed dose of THU previously shown to be safe in humans [12]. We report here the first-in-human clinical trial of FdCyd, administered together with the cytidine deaminase inhibitor THU to reduce enzymatic deamination, in patients with refractory solid tumors.

Patients and methods

Eligibility criteria

Patients (age ≥ 18 years) were eligible if they had an advanced, histologically confirmed solid tumor refractory to standard therapy or for which no standard therapy existed; a Karnofsky performance status ≥ 60 %; and adequate liver, kidney, and marrow function defined as bilirubin ≤ 1.5 mg/dl and aspartate aminotransferase and alanine aminotransferase ≤ 3 times the upper limits of normal (ULN), serum creatinine ≤ 2.0 mg/dl or creatinine clearance ≥ 50 ml/min, absolute neutrophil count ≥ 1,500/μL, and platelets ≥ 125,000/μL. The presence of measurable disease was not required.

Prior antineoplastic therapy had to have been completed at least four weeks before the patient's entry on this study, or patients had to have recovered from any expected side effects of the prior therapy. There was no limit on the number of cycles of prior

chemotherapy. Patients with any non-malignant intercurrent illness (e.g., cardiovascular, pulmonary, or central nervous system disease), which was either poorly controlled with currently available treatment, or which was of such severity that the investigators deemed it inappropriate to treat the patient on this protocol, were ineligible. Because FdCyd has been shown to be teratogenic in animals, pregnant patients were ineligible. All patients of childbearing potential, both male and female, were required to practice adequate contraception. A negative pregnancy test was required of premenopausal women prior to treatment.

This trial was conducted under a City of Hope-sponsored IND with institutional review board approval at each of the participating sites. The protocol design and conduct followed all applicable regulations, guidance, and local policies and was registered on

[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00378807), NCT00378807.

Investigational agents

FdCyd and THU were prepared according to the chemistry, manufacturing, and control data in IND #54,223, which were revised as the study progressed to higher doses. The final formulations were as follows. FdCyd Injection was supplied in sterile single-use vials, 100 mg in 5 ml water for injection (20 mg/ml), stored at controlled room temperature. The daily dose of FdCyd was diluted in 250 ml of D5W together with THU and infused over 3 h. THU Injection was supplied in sterile single-use vials containing 500 mg THU in 50 ml (THU—10 mg/ml, sodium phosphate dibasic, anhydrous, USP—1.5 mg/ml, and sodium phosphate monobasic, monohydrate, USP—0.4 mg/ml), stored at 2–8 °C. The total daily dose of THU was 350 mg/m². Each day, 20 % of the dose of THU was injected as an IV bolus (70 mg/m²). Within 10 min of the bolus, 80 % of the dose of THU (280 mg/m²) diluted in 250 ml of D5W along with the FdCyd was infused over 3 h. Using retention of 90 % of the initial potency as the criterion for stability, FdCyd and THU were stable when mixed together in D5W for at least 24 h, both at controlled room temperature and at 40 °C.

Trial design

This was a multicenter phase I study of FdCyd administered with a fixed dose of THU, using a standard 3 + 3 design. For the first six dose levels, FdCyd was infused over 3 h with THU daily for 5 days in a 3-week cycle. Interim pharmacokinetic analysis indicated that peak plasma concentrations were above the concentrations required in tissue culture for inhibition of DNA methylation [13]. Likewise, in vitro data suggested that longer exposures were more likely to be effective than were higher concentrations (Online Resource 1). For the remaining dose levels, FdCyd was co-infused over 3 h with a fixed dose of THU daily for 5 days in each of two consecutive weeks in a 4-week cycle. Thus, both schedules included a 2-week treatment-free period prior to the next cycle of chemotherapy. Adverse events were graded using NCI Common Toxicity Criteria 2.0; following standard practice at study initiation, this grading scale was used throughout the study. The dose of FdCyd was doubled in each cohort of patients until two moderate (grade 2 non-hematologic toxicity, grade 2 thrombocytopenia, or grade 3 neutropenia) or one serious (grade 3 non-hematologic toxicity, grade 3 thrombocytopenia, or grade 4 neutropenia) adverse event attributed to the investigational agent occurred. From that point on, dose escalation followed

a “modified Fibonacci scheme;” the planned dose increments were 1.67-, 1.5-, and 1.34-fold escalations, continuing at 1.34-fold escalations. All patients who did not experience a DLT were observed for a minimum of 2 weeks after the completion of the last day of treatment in the first cycle before the dose level was escalated. Intra-patient dose escalation was not allowed. Due to a limited drug supply during the initial period of the trial (through patient #25), patients with stable disease were removed from study after two cycles unless the treating physician, with the concurrence of the protocol chair, felt that the patient was deriving clinical benefit. This requirement limited duration of therapy and the potential for response in the early cohorts.

DLT was defined as a first-cycle adverse event possibly, probably or definitely attributed to the investigational agent and meeting one of the following criteria. Non-hematologic DLT was any toxicity grade 3 not reversible to grade 2 within 96 h or any grade 4 toxicity, except nausea and vomiting controllable by aggressive anti-emetic therapy or electrolyte disturbances corrected with supplementation. Hematologic DLT was grade 4 neutropenia associated with sepsis; or thrombocytopenia, neutropenia, or anemia, each of which must have been grade 4 and not reversible to grade 3 within 120 h. Leucopenia in the absence of neutropenia and lymphopenia were not DLT. All patients who received any amount of the investigational agents were followed for toxicity. To be considered in cohort dose-escalation decisions, a patient must have experienced a DLT or have received one complete cycle of therapy and followed to the end of the first cycle. The MTD was defined as the highest dose tested in which <33 % of patients experienced DLT, when at least six patients treated at that dose were evaluable for toxicity. Beginning with the dose level of 67 mg/m²/day on days 1–5 and 8–12, up to six evaluable patients could be accrued to obtain additional safety and correlative data at dose levels previously shown to be safe with fewer than six patients (i.e., no DLTs). However, cohort dose escalations were based on the first three or six patients treated at a dose level per standard 3 + 3 design. The phase II recommended dose was expanded further to 12 evaluable patients.

Safety and efficacy evaluations

History and physical examination, complete blood counts with differential, and serum chemistries were performed at baseline, weekly during the first cycle, and prior to each subsequent cycle. In patients with measurable disease, bidirectional tumor measurements were obtained every other cycle, and a patient had to have completed the first two cycles of treatment to be evaluable for response.

Pharmacokinetic evaluations

Samples for pharmacokinetics were obtained at the end of the infusion on days 1 and 5 of the first cycle for patients on the initial schedule. Having demonstrated that end-of-infusion levels were measurable in patients on the first schedule and that there were not significant difference between day 1 and 5 [13], a more extensive sample set was obtained on 1 day for patients on the revised schedule. For the first 35 patients, heparinized blood was obtained, placed on ice, and the plasma transferred into cryogenic vials containing THU (0.25 mg/ml final concentration) to prevent ex vivo deamination. Samples were stored at or below –20 °C until assayed by LC–MS/MS as previously described [13]. For the subsequent patients,

blood was transferred directly into heparinized vacutainer tubes containing zebularine (1 mg/ml final concentration) to prevent ex vivo deamination. Switching to zebularine as the additive to prevent ex vivo deamination made it possible to determine the pharmacokinetics of THU (to be reported elsewhere). Plasma was stored frozen until assayed by the same method. The maximum plasma concentration ($C_{\max}$) and the time to reach it ($T_{\max}$) were determined by visual inspection of the plasma concentration versus time data. Other pharmacokinetic parameters were calculated non-compartmentally using PK Solutions 2.0 (Summit Research Services, Montrose, CO; www.summitPK.com).

Pharmacodynamic evaluations

Beginning with patient 22 (and cycle 2 for patient 21), samples were obtained and processed for mRNA analysis prior to treatment, on the last day of treatment (day 12), and on a day in the first week following treatment in each cycle. Patients with sufficient samples (at least two of the three time points in a cycle) obtained in the first two cycles were included in the statistical analysis. Heparinized blood was separated over Ficoll-Hypaque; red blood cells were diluted 10fold in RNazol[®] (Molecular Research Center) or TRIzol[®] (Invitrogen) and frozen at -20°C until extracted. RNA was extracted according to the manufacturers' instructions, treated with DNase (Ambion), and re-extracted using TRIzol[®]. cDNA was prepared from total RNA using random primers and SuperScript[®] II or III reverse transcriptase (Invitrogen) and stored at -80°C until assayed. γ - and β -globin mRNA were quantified by RT-PCR on an Applied Biosystems 7900HT real-time PCR System against standard curves prepared from dilutions of cloned plasmids using primers and probes described by Smith, et al. [14]. The clones were a gift from Dr. Reginald D. Smith.

Statistical analysis

Overall, accrual and the numbers of patients evaluable for toxicity/dose escalation and response, as well as the numbers of patients experiencing grade 3 and 4 toxicities in the first cycle and in all subsequent cycles, are tabulated by dose level. Descriptive statistics are provided for patient characteristics.

Statistical analysis of the ratio of γ -globin to the sum of γ - and β -globin mRNA was performed using linear mixed effects models in the R package nlme [15, 16]. The initial model included the intercept, dose, cycle, linear days, quadratic days, and the associated two-way interactions, with days counted from the beginning of each cycle. The intercept and linear day effects were defined to be random and correlated both at the subject level and at the cycle within subject level. Nonsignificant fixed effects were removed based on p values from conditional *t* tests using restricted maximum likelihood estimates. For the purposes of illustration in figure, the ratios were normalized to the baseline value for each patient. However, the statistical analysis was performed using the raw ratio data.

Results

Demographics

Fifty-eight patients with advanced malignancies enrolled in the study (Table 1). Eight patients did not complete the first cycle for reasons other than DLT and were not evaluable

for the determination of cohort dose escalations and the MTD (Online Resource 2). Fifty-five of the 58 patients had been treated previously for their advanced disease. The median number of prior regimens was three.

Toxicity

Initial treatment schedule, days 1–5, every 3 weeks—There were no DLT and minimal grade 3 and 4 toxicities at any of the doses tested on this schedule (Table 2). The only grade 3 toxicities attributed to the study drugs were anemia and lymphopenia, which were not DLT as defined in the protocol.

Revised treatment schedule, days 1–5 and 8–12, every 4 weeks—Because the change in schedule doubled the number of days of treatment, the daily dose was reduced by half, and six patients were treated at the first dose level (40 mg/m²/day) before escalating to the next level. One patient at this dose experienced a first-cycle grade 3 hyponatremia, which resolved by 24 h with supplementation. Although not a DLT, this grade 3 toxicity triggered the planned change to the more conservative dose-escalation schedule. The predominant grade 3 non-DLT first-cycle toxicity at all dose levels on this schedule was lymphopenia. At the third dose level (100 mg/m²/day), one patient had grade 3 neutropenia and one patient had a grade 3 infection without neutropenia.

DLT, MTD, and recommended phase II dose—One of six patients at the dose of 134 mg/m²/day experienced a first-cycle DLT, grade 3 colitis. Two of two patients at the dose of 180 mg/m²/day experienced first-cycle DLT. In one patient, the DLT was grade 3 fatigue, accompanied by elevations in liver enzymes. In the other patient, the DLT was grade 4 neutropenia, accompanied by thrombocytopenia, leucopenia, and gastrointestinal toxicities. Therefore, the protocol-defined MTD is 134 mg/m²/day (one DLT in six evaluable patients). However, because of the nature of the toxicities observed at dose levels 134 and 180 mg/m²/day, and the clinical activity observed at dose levels 67 and 100 mg/m²/day (see below), the recommended phase II dose is 100 mg/m²/day of FdCyd in combination with 350 mg/m²/day of THU.

Toxicities in subsequent cycles—Because of the initial protocol requirement that patients have a response better than stable disease or subjective evidence of other clinical benefit to stay on treatment beyond two cycles, relatively few cycles beyond the first cycle were administered on the initial schedule and the first dose level of the revised schedule. More cycles were administered at the higher levels on the revised schedule. As was the case in the first cycle, the predominant grade 3 toxicity in subsequent cycles was lymphopenia. Anemia, thrombocytopenia, hyponatremia and elevations in liver enzymes were also observed (Table 2).

Efficacy

Forty patients were evaluable for response; 20 had a best response of stable disease ranging from 1.4 (censored with stable disease at last response evaluation) to 13.3 months (Table 3). One patient with metastatic breast cancer, who previously had failed multiple systemic therapies for metastatic disease, had a confirmed PR documented by CT scan (>90 %

reduction in the sum of the target lesions) for over a year. Although significant clinical improvement was noted after two cycles of treatment, the PR was obtained after four cycles, and continued shrinkage of the tumor was noted after six cycles. The patient maintained a PR for 15.2 months before progression. Figure 1 shows the pre- and post-treatment CT scans demonstrating the 90 % reduction in tumor size, as well as the observed improvement in skin involvement.

Pharmacokinetics

End-of-infusion plasma concentrations of FdCyd, FdUrd, and FU on the initial schedule have been reported previously and indicated that all patients treated with 40 mg/m²/day and above-obtained plasma concentrations of FdCyd 1 μ M (245 ng/mL) [13]. Data from patients on the revised schedule are reported here. Maximum plasma concentrations of FdCyd and its deaminated metabolites FdUrd and FU during the infusion are displayed in Fig. 2a. FdCyd concentrations increased linearly with the dose of FdCyd, at least through the 100 mg/m² dose. The concentrations of the deaminated metabolites FdUrd and FU also increased with dose. However, the concentrations of FdUrd were two orders of magnitude lower than the concentrations of FdCyd and the concentrations of FU were lower still (NOTE the different scales on the vertical axes.) No 5-fluorouridine and 5-fluorocytosine associated with IV administration of FdCyd + THU could be detected. The average FdCyd plasma concentration versus time curves is shown in Fig. 2b. The non-compartmental pharmacokinetic parameters are provided in Table 4.

γ -Globin mRNA expression

The γ -globin mRNA as a percentage of the sum of γ - and β -globin mRNA is presented in Fig. 3. Data were available for four dose levels [40 ($n = 5$), 67 ($n = 6$), 100 ($n = 12$), and 134 mg/m²/day ($n = 6$)]. (Graphs for the individual patients are included in Online Resource 3.) Because of the limited time on study for the two patients treated at 180 mg/m²/day, both of whom had DLT, samples were not obtained beyond baseline at that dose level. After implementing a random effects model that allowed three levels of variability (among patients, cycle within patient, and within cycle) for both the intercept and the slope, the final fixed effects model included the intercept, dose, cycle, and linear and quadratic day effects (Online Resource 3). Within the range of doses tested, there was no linear effect of dose on the percentage of γ -globin mRNA (coef = -0.0009 , SE = 0.0067 , p value = 0.9). The results were similar when the $C_{\max}$ or the AUC of FdCyd was substituted for dose in the model; neither had a significant linear effect. There is a significant increase in the percentage of γ -globin mRNA from cycle 1 to cycle 2 (coef = 0.82 , SE = 0.22 , p value = 0.0015). There was a significant quadratic day effect (coef = 0.016 , SE = 0.0031 , p value < 0.0001), so both the linear and quadratic day effects were kept in the model. The linear day coefficient (coef = -0.1 , SE = 0.049) represents the initial rate of change at day 0 for the percentage of γ -globin mRNA, and the quadratic day coefficient represents how the linear slope is changing over time. Thus, the negative linear coefficient indicates that at day 0, which included day 0 for cycle 2, the percentage of γ -globin mRNA was decreasing, whereas the positive quadratic coefficient indicates that between day 0 and day 14 the rate of change per day in the percentage of γ -globin mRNA increased. Not surprisingly, the variance among patients (intercept = 1.04 ; slope = 0.014 ; correlation = 0.63) is 3–4 times that of either the within-

patient variance (within patient between cycle intercept = 0.34; slope = 0.0034, correlation = -0.12) or the within-cycle variance (residual = 0.31).

Discussion

The intense interest in DNA methylation as a therapeutic target stems from the fact that tumor suppressor genes are silenced by DNA methylation in many tumors and that unlike gene deletions or mutations, the aberrant hypermethylation is potentially reversible with small molecule therapeutic agents. Two DNMT inhibitors, decitabine and azacitidine, are approved for the treatment of patients with myelodysplastic syndromes (MDS), but to date have not demonstrated similar activity in solid tumors. Based on preclinical data, the potential for differences from the azapyrimidines in additional sites of action, and favorable pharmaceutical properties of FdCyd, we initiated the first-in-human clinical trial of this agent.

The peak plasma concentrations exceeded 1 μM (245 ng/mL) in all patients treated with 40 mg/m²/day. The FdCyd volume of distribution of 30–40 L/m² suggests distribution of FdCyd in body water, consistent with its hydrophilic character. Although the range of doses was not sufficient to statistically test for nonlinearity, the observed FdCyd concentrations increased with dose. One μM FdCyd has been shown to inhibit in vitro DNA methylation in MCF-7 cells, both in a 5' CpG island (Online Resource 1) and overall [9], albeit after prolonged exposure in vitro. The plasma concentrations of the major metabolites FdUrd and FU were <10 % of the concentrations of FdCyd at all dose levels, suggesting that the activity was mostly due to FdCyd and not its circulating deaminated metabolites. However, it is possible that FdCyd is deaminated within the tumor tissue at either the nucleoside or the nucleotide level.

In clinical trials of new anticancer drugs, it is ideal to demonstrate a change indicative of the proposed mechanism of action in the tumor itself. The invasive procedures to obtain the specimens in solid tumors complicate this ideal approach, and the hypothesized biochemical effect of the agent may be measured in a greater number of patients using alternative specimens. We took advantage of the fact that the γ -globin gene is normally silenced by methylation in adult blood cells and can be reactivated by treatment with DNMT inhibitors. Several investigators have reported expression of fetal hemoglobin, normally present in low amounts in adults, after treatment with decitabine or azacitidine [17]. We found that FdCyd significantly increased γ -globin mRNA during each of the first two cycles of treatment. Although the levels dropped between the end of the first cycle and the beginning of the second, the levels in the second cycle were significantly higher than the levels in the first.

Several patients on the revised schedule had stable disease lasting beyond the first scheduled response evaluation (two cycles). One patient with breast cancer treated on the revised schedule with 67 mg/m²/day of FdCyd had a confirmed PR (>90 % reduction in indicator lesions) that lasted >1 year. It is noteworthy that this patient had received multiple chemotherapy and hormonal regimens prior to enrolling on this trial. In addition to primary treatment with tamoxifen, this patient had received eight regimens for advanced disease: high-dose chemotherapy (cyclophosphamide/paclitaxel/melphalan/etoposide) with

autologous hematopoietic stem cell rescue; capecitabine/docetaxel; paclitaxel/carboplatin/letrozole; radiation; and singleagent regimens with gemcitabine, irinotecan, vinorelbine, and liposomal doxorubicin. Anecdotally, the patient with the remarkable prolonged response had one of the highest post-treatment percentages of γ -globin (Online Resource 3, patient 67/27). This observation will prompt evaluation of this potential predictive biomarker in future clinical trials.

Although the MTD was established at 134 mg/m²/day of FdCyd on the revised schedule (treatment days 1–5 and 8–12, every 4 weeks), the nature of the toxicity observed in a patient at that dose (colitis) and the prolonged PR observed in a patient at a lower dose led us to recommend 100 mg/m²/day of FdCyd with 350 mg/m²/day of THU, days 1–5 and 8–12 every 4 weeks, for future studies. We are conducting a multi-histology phase II clinical trial with that regimen. Admittedly, daily infusions for five consecutive days in two consecutive weeks are not convenient. Other routes of administration should be explored, and we are currently conducting a phase I clinical trial of oral administration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A California Cancer Consortium Study. This manuscript is dedicated to the memory of Dr. Merrill J. Egorin, who would be a co-author if he were able to review the manuscript. Previous Presentation: Presented in part at the 38th Annual Meeting of the American Society of Clinical Oncology, May 18–May 21, 2002, Orlando, FL and the 102nd Annual Meeting of the American Association for Cancer Research, April 2–6, 2011 Orlando, FL. An interim pharmacokinetic analysis has been published [13]. Research Support: Supported in part by U01CA062505, UM1CA186717, P30CA033572, N01-CM-52202, U01CA099168, UM1CA186690, and P30CA047904.

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Fig. 1. Partial response in a heavily pre-treated patient pre- and post-treatment CT scans of a 61-year-old female with metastatic breast cancer who had received multiple chemotherapy and hormonal regimens including high-dose chemotherapy and autologous stem cell transplant prior to enrolling on the phase I trial of FdCyd + THU

Fig. 2.

Pharmacokinetics of FdCyd and its two major metabolites, FdUrd and FU **a** The maximum plasma concentrations of FdCyd, FdUrd, and FU are plotted as a function of the dose of FdCyd. Note the difference in scale between FdCyd (0–8000 ng/mL) and the metabolites (0–60 ng/mL). **b** Average FdCyd plasma concentration versus time profiles in patients after administration of 67 mg/m^2 FdCyd (*triangle* $N = 7$), 100 mg/m^2 FdCyd (*square* $N = 16$), and 133 mg/m^2 FdCyd (*circle* $N = 6$). Samples with concentrations below LLQ were given a value of 0 for the purpose of this graph

Fig. 3.

Relative fetal hemoglobin expression for the purpose of illustration, the data are normalized to each patient's cycle 1 pre-treatment sample and presented as the mean of the normalized data for each dose level

Table 1

Patient characteristics

Patients	58
Initial schedule	20
Revised schedule	38
Previously treated for advanced disease	55 (95 %)
Prior chemotherapy regimens, median (range)	3 (0–10)
Median age (range)	63 (30–85)
Median KPS (range)	80 (60–100)
Gender	
Male	32 (55 %)
Female	26 (45 %)
Race/ethnicity	
White non-hispanic	36 (62 %)
Asian	10 (17 %)
Hispanic	8 (14 %)
Black/African-American	4 (7 %)
Tumor site	
Colorectal	18 (31 %)
Lung	7 (12 %)
Breast	6 (10 %)
Stomach	4 (7 %)
Other (3 per site)	21 (36 %)
Unknown primary	2 (3 %)

Table 2

First-cycle grade 3/4 related toxicities^a

Days 1–5, every 3 weeks									
FdCyd dose level (mg/m ² /day)	2.5	5	10	20	40	80			
# Evaluable patients	3	3	3	3	3	3			
Anemia						1/0			
Lymphopenia	1/0								
Days 1–5 and 8–12, every 4 weeks									
FdCyd dose level (mg/m ² /day)	40	67	100	134	180				
# Evaluable patients	6	6 ^b	12 ^b	6	2				
Leucopenia					0/1				
Lymphopenia									
Neutropenia	2/0	2/0	1/0	1/0					
Thrombocytopenia					0/1 ^c				
Fatigue					1/0				
Colitis						1/0 ^c			
Diarrhea						1/0			
Mucositis–oral					0/1				
Vomiting					1/0				
Hemoptysis					1/0				
Henatochezia					1/0				
Infection w/o G3 ANC			1/0						
ALT					1/0				
AST					1/0				
Hyponatremia	1/0								
Grade 3/4 related toxicities in subsequent cycles ^{a,d}									
Days 1–5, every 3 weeks									
FdCyd dose level (mg/m ² /day)	2.5	5	10	20	40	80			
# Evaluable patients	3	3	3	2	1	3			
Median # cycles started after cycle 1	2	1	1	1	0	1			

Anemia	1/0								
Lymphopenia	1/0								
Days 1–5 and 8–12, every 4 weeks									
FdCyd dose level (mg/m ² /day)	40	67	100	134	180				
# Evaluable patients	6	6	12	5					
Median # cycles started after cycle 1	1	1	2	2					
Anemia	1/1								
Lymphopenia	1/0	1/0	1/0	1/0	1/0				
Thrombocytopenia	1/0								
ALT	1/0								
AST	1/0								
Hyponatremia	1/0								

^a Related: possibly, probably, or definitely related to FdCyd/THU treatment

^b Data from the first three evaluable patients used for the dose-escalation decision

^c Dose-limiting toxicity (DLT)

^d Grade is the maximum grade over all cycles in a given patient

Table 3

Patient accrual and response by dose level

Enrolled		Evaluable		Best response ^c		
		Toxicity ^a	Response ^b	PD	SD	PR
Days 1–5, every 3 weeks						
FdCyd dose level (mg/m ² /day)						
2.5	4	3	3	1	2	
5	3	3	3	2	1	
10	4	3	3	2	1	
20	3	3	2	2		
40	3	3	1		1	
80	3	3	3	2	1	
Days 1–5 and 8–12, every 4 weeks						
FdCyd dose level (mg/m ² /day)						
40	6	6	4	2	2	
67	7	6	5	3	1	1
100	17	12	11	3	8	
134	6	6	5	1	4	
180	2	2	0			
Time to progression		Best response ^c		Time to progression (months)		
Days 1–5, every 3 weeks						
FdCyd dose level (mg/m ² /day)						
2.5		SD	2.2, 3.3			
5		SD	8.4			
10		SD	1.5 + <i>d</i>			
20						
40		SD	1.7 + <i>d</i>			
80		SD	1.4 + <i>d</i>			
Days 1–5 and 8–12, every 4 weeks						
FdCyd dose level (mg/m ² /day)						

Time to progression	Best response ^c	Time to progression (months)
40	SD	1.9 + ^d 5.3 + ^d
67	PR	15.2
	SD	7.6
100	SD	1.6 + ^d 2.0 + ^d 2.7, 3.3, 3.5, 3.9, 4.1, 13.3
134	SD	2.1 + ^d 3.0, 3.5, 9.7

^a One complete cycle or DLT. At dose levels 67 and 100, only first three evaluable patients were considered in cohort dose escalations

^b Two complete cycles

^c PD progressive disease; SD stable disease; PR confirmed partial response

^d Off treatment for other than progression, censored at last disease evaluation

Non-compartmental pharmacokinetic parameters of FdCyd after a 3 h IV administration of FdCyd combined with 350 mg/m² THU

Table 4

FdCyd (mg/m ²)	C _{max} (ng/mL) (SD)	T _{max} (min) (SD)	AUC _{inf} (µg min/mL) (SD)	AUC _{0-last} (µg min/mL) (SD)	t _{1/2} (min) (SD)	V _{ss} (L/m ²) (SD)	Cl (mL/min/m ²) (SD)
67 (N = 7)	1672 (918)	150 (–)	284 (140)	271 (134)	42.5 (12.2)	47.7 (22.2)	291 (139)
100 (N = 16)	3302 (1312)	147 (29)	693 (312)	681 (312)	46.5 (16.0)	31.0 (14.2)	170 (71)
133 (N = 6)	3207 (696)	165 (23)	699 (155)	692 (152)	54.0 (26.4)	38.9 (11.2)	202 (55)