Phase I Clinical Trials of Tezacitabine [(*E*)-2′-Deoxy-2′-(fluoromethylene)cytidine] in Patients with Refractory Solid Tumors[1]

Gladys I. Rodriguez,[2] Richard E. Jones, Elaine K. Orenberg, Maxine L. Stoltz,[3] and Donald J. Brooks,[4]

Cancer Therapy and Research Center, San Antonio, Texas 78207 [G. I. R.]; Matrix Pharmaceutical, Inc., Fremont, California 94555 [R. E. J., E. K. O.]; Hoechst Marion Roussel, Inc., Kansas City, Missouri 66160 [M. L. S.]; and Arizona Cancer Center, Tucson, Arizona 85750 [D. J. B.]

ABSTRACT

Purpose: **To evaluate safety, tolerability, and pharmacokinetics of a new nucleoside analogue, tezacitabine [(*E*)-2′-deoxy-2′-(fluoromethylene)cytidine (FMdC)] in patients with refractory solid tumors.**

Experimental Design: **Seventy patients were enrolled in four separate Phase I trials. Patients had metastatic or relapsed cancer of the colon, breast, pancreas, gastrointestinal tract, lung, and other sites. FMdC was administered by i.v. infusion over 30 min in one of four dose schedules—from once every 3 weeks to twice a week for 3 weeks, with dose escalation in each. Maximum doses ranged from 630 to 16 mg/m^2.**

Results: **Myelotoxicity, especially neutropenia, was the dominant toxicity and was generally dose-related. Grade 3 or 4 neutropenia occurred in 53% of patients but was of relatively short duration (1–8 days) in all of the patients. One patient experienced grade 3 thrombocytopenia and one patient grade 4 (duration 15 and 11 days, respectively). Transient febrile episodes were reported in 82% of patients with drug administration but were easily controlled. Drug-related gastrointestinal events were mild and appeared unrelated to dose. Pharmacokinetics were linear with dose, not appreciably affected by schedules, and not different after single or multiple doses. Terminal half-life was 3–4 h, and 23% of the administered drug was recovered in the urine as unchanged drug. The uridine analogue (FMdU), the deaminated metabolite of FMdC, was the primary metabolite. Objective antitumor activity was observed in eight patients: one exhibited a partial response and seven exhibited stable disease.**

Conclusions: **In general, FMdC was well tolerated. On the basis of the time to recovery from neutropenia, the recommended schedule for Phase II studies is one treatment every 2 weeks, at a minimum dose of 270 mg/m^2.**

INTRODUCTION

Tezacitabine (FMdC),[5] (Fig. 1) is a novel nucleoside with potent antiproliferative activity in a broad spectrum of preclinical tumor models. This nucleoside analogue is a potent irreversible inhibitor of RNR, an enzyme present at elevated levels in tumor tissue that catalyzes the biosynthesis of deoxyribonucleotides, key building blocks for DNA replication. Early members of this drug class such as ara-C are active in hematological malignancies but have been found ineffective against solid tumors (1). Unlike ara-C, gemcitabine (2′, 2′-difluorodeoxycytidine), a second-generation nucleoside analogue, is an RNR inhibitor and is used successfully to treat non-small cell lung cancer and pancreatic cancer, which suggests that a more potent RNR inhibitor might prove to be a more effective cancer treatment. FMdC was designed as an irreversible RNR inhibitor (2, 3), although subsequent studies demonstrated that it had other activities as well. In preclinical studies *in vitro*, it has potent cytotoxic activity against a broad spectrum of murine and human tumor cell lines; additionally, in animal studies it has activity against a wide variety of murine tumors and human xenografts representing all major types of cancer.

After uptake into cells, FMdC is phosphorylated by endogenous kinases to FMdC diphosphate and FMdC triphosphate. These two metabolites act in complementary fashion to inhibit DNA replication and, in turn, to induce apoptosis (4). The diphosphate irreversibly inhibits RNR activity (5), and the triphosphate acts as a substrate for DNA polymerase α, is incorporated into DNA as a fraudulent cytidine, and, thereupon, acts as a DNA chain terminator (6). FMdC also has antiangiogenic activity (7) and, unlike other

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[2] To whom requests for reprints should be addressed, at Cancer Therapy and Research Center, 315 North San Saba, Suite 1160, San Antonio, TX 78207. Phone: (210) 354-3838; Fax: (210) 354-3870; E-mail: grodrigu@saci.org.
[3] Present Address: Pharmaceutical Research Associates, Lenexa, KS 66160.
[4] Present Address: Hematology/Medical Oncology, Arizona Oncology Associates, Tucson, AZ 85750.

[5] The abbreviations used are: FMdC, (*E*)-2′-deoxy-2′-(fluoromethylene)cytidine; RNR, ribonucleotide reductase; ara-C, cytarabine; ULN, upper limit of normal; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; FMdU, deaminated metabolite of FMdC; HPLC-MS/MS, high-performance liquid chromatography/tandem mass spectroscopy; AUC, area under the plasma-concentration curve; NSCLC, non-small cell lung cancer.

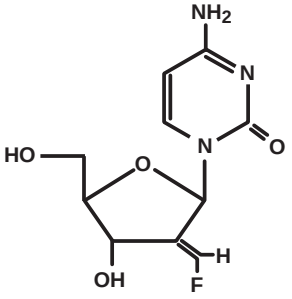

Fig. 1 Molecular structure of FMdC.

cytidine analogues, is relatively resistant to metabolic deactivation by cytidine deaminase (8).

In vitro studies demonstrated that FMdC is active against a wide variety of solid tumor and hematological murine and human tumor cell lines (2). These represent the major cancer sites, including lung, colon, breast, prostate, stomach, pancreas, ovary, brain, and hematopoietic tissue (2, 3, 9, 10). FMdC is also as active against multidrug-resistant murine cell lines (multidrug-resistant epidermoid carcinoma and multidrug-resistant leukemia) as it is against drug-sensitive parenchymal cell lines (3). IC_{50} values range from 10 to 90 nM (2). Finally, FMdC may be a radiosensitizer when used alone (11) or in combination with pentoxifylline in colon cancer cells *in vitro* (12).

In vivo studies demonstrated activity against murine tumors in normal mice and human tumor xenografts in nude mice and human breast, prostate, lung, and colon xenografts in nude mice (4, 5, 7, 9, 13, 14). Inhibition of intracerebral glioma xenografts in mice suggests that FMdC may penetrate the blood-brain barrier (10). Studies show *in vivo* synergistic activity in melanoma and lung cancer xenografts when FMdC is combined with ara-C or cisplatin (15, 16).

In toxicology studies conducted in mice, rats, and dogs, effects were dose and time dependent and were consistent with the expected cytotoxic effects of nucleoside analogues. Such expected effects included hematopoietic (leukopenia, anemia, and thrombocytopenia); reproductive (decreased testicular weights, testicular atrophy, or both); and gastrointestinal (diarrhea, intestinal crypt necrosis, and/or mild elevation of liver enzymes changes).[6] On the basis of these promising preclinical results, a series of four Phase I dose-escalation trials were conducted at three study sites to evaluate the safety, tolerability, and pharmacokinetics of FMdC in patients with refractory solid tumors.

PATIENTS AND METHODS

Patient Selection. Male and female patients were required to be at least 18 years old with histologically confirmed advanced solid malignancies and a life expectancy of at least 3 months. Their tumors had to be refractory to treatment or of a type for which no standard therapy existed. Patients' disease had to be measurable or evaluable; Southwest Oncology Group or Eastern Cooperative Oncology Group performance status had to be 0–2. Laboratory inclusion criteria required that patients have a WBC count of $\geq$3000 cells/μl, absolute neutrophil count of $\geq$2000 cells/μl, hemoglobin $\geq$9.0 g/dl, and platelets $\geq$100,000 cells/μl. Serum creatinine and bilirubin had to be within normal limits for the institution. Aspartate transaminase and alanine transaminase had to be $\leq 3 \times$ ULN or, if tumor-related, $\leq 5 \times$ ULN; if alkaline phosphatase was $>5 \times$ ULN, serum γ-glutamyl transferase had to be $\leq 1.5 \times$ ULN.

Patients were excluded if they were positive for HIV or hepatitis B; had chronic hepatitis; or were pregnant, lactating, or breast-feeding. Also excluded were patients with a history of major gastrointestinal illness, any other serious concomitant systemic disorder that would interfere with the conduct of the study, or bleeding diathesis. Patients who had used an investigational drug within 30 days before the screening visit, had a history of alcoholism or drug abuse within 60 days of the screening visit, or had a mental or psychiatric disorder that would interfere with consent or follow-up were not included in the study. Patients were also excluded if they had serious concomitant systemic conditions such as uncontrolled infection, a cardiac condition, or presence of central nervous system metastases not previously managed by surgery or radiotherapy and stable $<$4 weeks. Patients who had prior radiotherapy encompassing $\geq$20% of the bone marrow or prior pelvic radiotherapy were excluded. Studies II and IV (see below) excluded patients who required parenteral nutrition.

Study Design. Safety evaluation criteria included physical examination, vital signs, adverse medical experiences, and laboratory evaluations (hematology and blood chemistries). Drug-related toxicities were rated using the National Cancer Institute Common Toxicity Criteria (17). All of the patients signed an informed consent statement in accordance with the Institutional Review Board at each study center.

Dosing regimens for the four studies are summarized in Table 1. In Studies I (dosing every 3rd week) and III (dosing every week), doses were escalated based on a variation of the modified continual reassessment method (18, 19). This method is designed to identify the dose of a new drug that meets a prespecified, acceptable toxicity level. The identification is made through application of an algorithm: as each patient completes treatment, the presence or absence of toxicity at the assigned dose level is combined with toxicity information from previously treated patients to determine the dose level at which the next patient will be treated. The patient is assigned the dose closest to the updated MTD, subject to the following guidelines: the first three patients are all treated at the lowest dose level; a patient cannot be treated at a dose more than one level higher than the previous patient; if a patient suffers unacceptable toxicity, the next patient cannot be treated at a higher dose level; and if a patient suffers moderate toxicity, at least three patients must be treated at the dose level before treating subsequent patients at higher dose levels. The trial was to be terminated when the dose recommended for the next patient was a dose at which 10 patients had been treated, and this dose would be the suggested MTD for use in a Phase II trial. The starting dose in these studies was 16 mg/m^2, increasing in Study I by 33% at

[6] Data on file. Matrix Pharmaceutical, Inc., Fremont, CA 94555.

Table 1 FMdC dosing regimens

	Study I	Study II	Study III	Study IV
Schedule	Every 3 wk	Every 2 wk, days 1 and 15	Once a wk for 3 wk, 1 wk rest	Twice a wk for 3 wk, 1–2 wk rest
Dose level (mg/m^2, 30-min i.v. infusion)	16–630	32–270	16–112	16
Cycle duration	3 wk	4 wk	4 wk	4–5 wk
No. of patients	27	21	12	10

each dose level, and in Study III according to a modified Fibonacci series, *i.e.*, *n*, 2*n*, 3.3*n*, 5*n*, 7*n*, and so forth.

A different approach to dose escalation was used in Studies II and IV (with the same modified Fibonacci series). Cohorts of three patients were to be evaluated at each dose level. If there was no evidence of DLT after all three patients had completed their first cycles of treatment, then the next sequential dose level was initiated in a new cohort of patients. If DLT developed in one of three patients in a cohort, then an additional three patients were studied at that dose level before dose escalation resumed on a fresh cohort. If DLT occurred in two of six patients at any dose level, dose escalation was stopped. In all studies, the MTD was defined as the highest dose level at which fewer than two of six patients experienced a DLT.

DLT was defined as any grade 3 or 4 nonhematological toxicity (excluding nausea, vomiting, and nonneutropenic fever) during cycle 1, any grade 4 hematological toxicity that persisted for 5 days or more without resolving to grade 2 or less, the occurrence of neutropenic fever or thrombocytopenic bleeding at anytime during the study, or any toxicity (excluding alopecia) that delayed treatment for more than 2 weeks.

Among the criteria for removal of patients from the study were voluntary withdrawal, noncompliance, progressive disease, and unacceptable toxicity from treatment.

Drug Administration. Lyophilized FMdC was reconstituted and the appropriate dose diluted in 100 ml of 0.9% Sodium Chloride Injection, USP, and infused i.v. over a 30-min period. Doses were administered by hospital staff**,** and patients were monitored for 8–10 h after the first dose. Patients were seen at least once weekly throughout the study, including during rest periods.

Pharmacokinetic Analyses. For pharmacokinetic evaluation in Study I, serial blood samples were taken after the first dose; in Studies II–IV, serial blood and urine samples were obtained after the first and last doses in the first treatment cycle. Plasma and urine samples were analyzed for parent drug (FMdC) and for the deaminated metabolite (uridine analogue, FMdU).

For the majority of the plasma analyses, a HPLC-MS/MS method with ASTED dialysis sample cleanup was used to determine FMdC and FMdU concentrations. This method requires 200 μl of plasma and was validated over a range of 5–1000 ng/ml for FMdC and 20–4000 ng/ml for the metabolite. This method was also cross-validated against an earlier HPLC method with UV detection (plasma sample volume 1.0 ml; concentration range, 5–1000 ng/ml) used to determine FMdC levels in plasma samples from some early subjects.

For urine analyses, a similar method using HPLC-MS/MS was used to quantify FMdC and its metabolite in urine. This method requires 50 μl of urine and was validated over a range of 100–20,000 ng/ml.

Table 2 Patient characteristics

		n
Patients entered		70
Sex		
Male		39
Female		31
Median age, yr (range)	60.5 (24–83)	
Performance status		
0		15
1		52
2		3
Prior therapy		
Radiation		
Yes		31
No		39
Chemotherapy		
Yes		68
No		2

Stability studies (16) showed that the drug is stable in plasma and urine when stored frozen, without the need for adding a cytidine deaminase inhibitor.

Plasma concentration-time data were used to estimate FMdC and FMdU pharmacokinetic parameters using noncompartmental techniques. Maximum plasma concentrations (C_{max}) were observed from the raw data listings. Calculated pharmacokinetic parameters included terminal elimination rate constant and half-life; area-under-the-plasma-concentration-time curve (*AUC*) from time zero to the last quantifiable concentration [$AUC_{(0\text{-}z)}$]; *AUC* from time zero to infinity calculated as $AUC_{(0\text{-}z)}$ plus the plasma concentration at time *z* divided by the terminal elimination rate constant; systemic clearance; and steady-state volume of distribution. Urine concentrations and collection volumes were used to calculate the total mass of analyte excreted in urine over time and the renal clearance.

RESULTS

Patient Characteristics. A total of 70 patients were enrolled in the four studies. A subset of 10 patients from Study IV were described in an earlier report (20). These patients are included in this report to present the cumulative Phase I experience with the drug at various doses and schedules. Patient characteristics are listed in Table 2. Previous cancer therapies had failed in all of them, and all had a life expectancy of at least 3 months. Ninety-seven % (68 of 70) of the patients had had previous chemotherapy; 46% (32 of 70) had had three or more previous regimens. Southwest Oncology Group or Eastern Co-

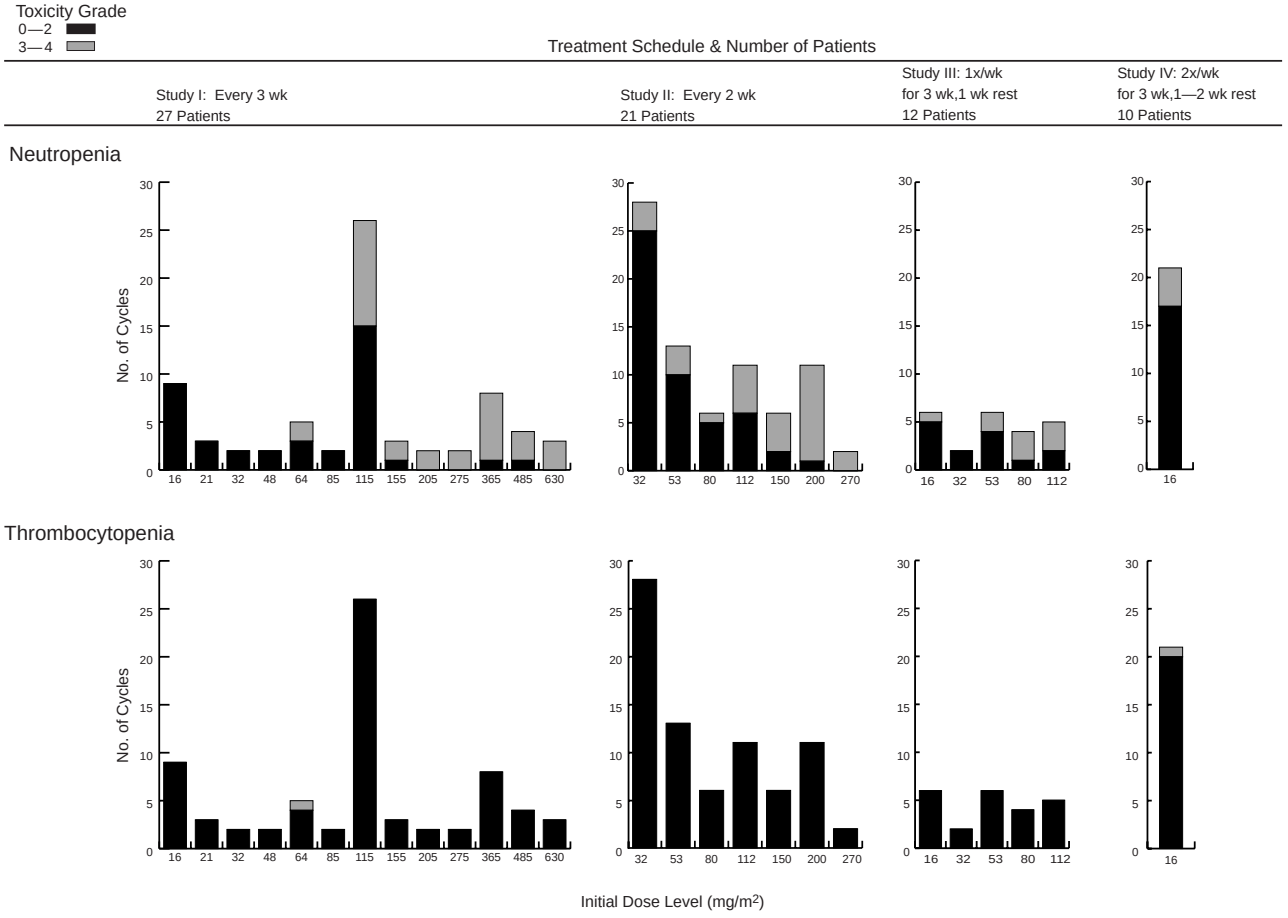

Fig. 2 Neutropenia and thrombocytopenia by Study and by toxicity grades in Phase I trials of FMdC.

operative Oncology Group performance status for all of the patients was ≤2. They had metastases or recurrences of the following cancers: colon (33 patients); breast (4 patients); pancreas (7 patients); gastric (3 patients); lung (non-small cell, 6 patients); and other (17 patients).

Toxicity. Myelotoxicity was the most notable toxicity and generally was more frequent with increased frequency of dosing and at higher doses (Fig. 2). Grade 3 or 4 neutropenia occurred in 37 (53%) of 70 patients. It was typically observed 5–7 days after dosing and resolved by 9–15 days after dosing. One patient experienced Grade 3 thrombocytopenia and one experienced Grade 4 thrombocytopenia with durations of 15 and 11 days, respectively. Both neutropenia and thrombocytopenia were rapidly reversible.

Nausea, vomiting, and diarrhea were mild, did not require dosage adjustments, and were not related to dose level or frequency of administration. Transient symptoms of fever (100.4° to 102.7°F) were reported in 82% (58 of 70) of patients within minutes to hours after the infusion of FMdC and was documented in 60% (42 of 70). Fever was easily controlled with acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), or both. Some patients received no antipyretic drug.

Other Grade 3 or 4 treatment-related events included transient urticaria (3 patients), anorexia (2), vomiting (2), and one patient each with asthenia, bilirubinemia, alopecia, stomatitis, malaise, hypertension, headache, visual field defect (peripheral vision loss), and hyperglycemia.

Serious Adverse Events. Fifteen patients died during the study, none of treatment-related causes. Death was attributed to progression of gastrointestinal cancer (6 patients), bladder cancer (1), or breast cancer (2), or of brain metastasis from an unknown primary cancer (1), leptomeningeal carcinomatosis (1), cerebral hemorrhage (1), myocardial infarction (1), pneumonia (1), or intestinal obstruction (1). Five patients experienced treatment-related adverse events (AEs) that required hospitalization, but none of these events were associated with serious sepsis or infection. Twenty-eight patients experienced 43 serious adverse events; in five of these patients, the events were treatment related. These events included one patient each with thrush (Study I at 115 mg/m^2), severe fever (Study II at 270 mg/m^2), mild febrile neutropenia (Study III at 112 mg/m^2), and anemia, unknown severity (Study IV at 16 mg/m^2). All four of these patients recovered. The fifth patient (Study IV at 16 mg/m^2) had severe asthenia, moderate anemia and fever, and a rash. This patient, who had metastatic colorectal cancer, was hospitalized and subsequently died of small bowel obstruction.

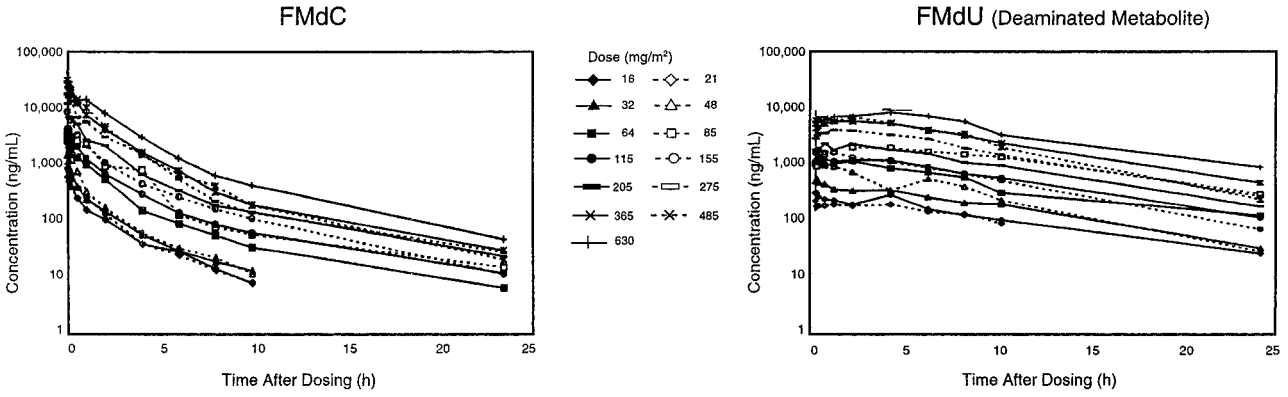

Fig. 3 Plasma concentration-time profiles after IV infusions of FMdC, Study I.

Table 3 Plasma pharmacokinetics of FMdC and its deaminated metabolite

Parameters	Study I	Study II	Study III	Study IV
No. of evaluable patients	27	19	9	7
Dose range (mg/m^2)	16–630	32–270	16–80	16
FMdC (mean ± SD)				
$t_{1/2}$ terminal (h)	4.4 ± 1.3	4.0 ± 0.8	3.0 ± 1.5	2.6 ± 0.8
Vdss[a] (liter)	80.2 ± 32.0	76.9 ± 19.6	74.8 ± 26.4	93.2 ± 24.8
CL (liter/h)	28.2 ± 8.8	29.6 ± 8.2	32.1 ± 7.7	42.5 ± 12.9
Metabolite (mean ± SD)				
$t_{1/2}$ (h)	5.6 ± 1.2	5.3 ± 1.1	5.9 ± 1.0	6.2 ± 2.7
CL (liter/h)	13.0 ± 4.2	14.3 ± 5.6	10.6 ± 3.8	18.4 ± 4.5

[a] *Vdss*, steady-state volume of distribution; *CL*, systemic clearance.

MTD. Neutropenia was the DLT and was both dose and schedule dependent. When the drug was administered twice a week (Study IV), the MTD was 16 mg/m^2. In contrast, in Study II, with drug administered once every 2 weeks, doses as high as 270 mg/m^2 were tolerated; patients who received the higher doses experienced Grade 4 neutropenia that resolved by 9–15 days after dosing. In Study I, with the drug administered once every 3 weeks, doses as high as 630 mg/m^2 were well tolerated, with the patients at the higher dose levels exhibiting readily reversible Grade 4 neutropenia.

Pharmacokinetics. Fig. 3 shows the plasma-concentration time profiles of FMdC and FMdU after a single FMdC dose in Study I. Table 3 compares the pharmacokinetic parameters derived from each of the four studies. FMdC declined biexponentially in plasma with mean terminal half-lives of about 3–4 h in all four of the studies. No differences were apparent in the pharmacokinetics after a single dose (Study I) as compared with multiple doses (Studies II–IV), although there was a slight trend toward shorter terminal half-life and higher clearance with more frequent dosing. For FMdU, peak concentrations in plasma occurred ~2–4 h after the end of infusion. The disappearance of FMdU from plasma was monoexponential with mean half-lives of 5–6 h across all studies. As shown in Fig. 4, the pharmacokinetics of both FMdC and FMdU were dose linear: *AUC* and C_{max} values increased proportionately with dose for both parent drug and metabolite.

Approximately 86% of the dose was recoverable in urine: 23% was eliminated as unchanged parent drug and 63% was recovered as FMdU. Mean renal clearance of FMdC was 113 ml/min.

Pharmacokinetic analyses showed no apparent drug or metabolite accumulation and no apparent enzyme induction in any of the study arms.

Response. Eight patients experienced either an objective improvement or long stabilization of disease. One patient in Study II with metastatic colon cancer who received doses of 200 mg/m^2 FMdC achieved a partial response after four treatment cycles (4 months) that was maintained for 5 additional months. This patient received a total of 10 treatment cycles (20 doses of FMdC). Seven patients had stable disease (increase in size of <25%); median duration was 6 months (range, 3–18 months). One patient in Study II with metastatic cholangiocarcinoma, who received 32 mg/m^2 FMdC twice a month, remained stable for 18 treatment cycles (18 months); this patient's disease progressed after 20 cycles.

DISCUSSION

In these Phase I trials that assessed the safety and pharmacokinetics of four different dose schedules, FMdC was found to be safe and tolerable. About one-half of the patients experienced myelosuppression, primarily Grade 3 or 4 neutropenia, which was observed 5–7 days after dosing and was resolved 9–15 days after dosing. Thrombocytopenia was infrequent. Both neutrope-

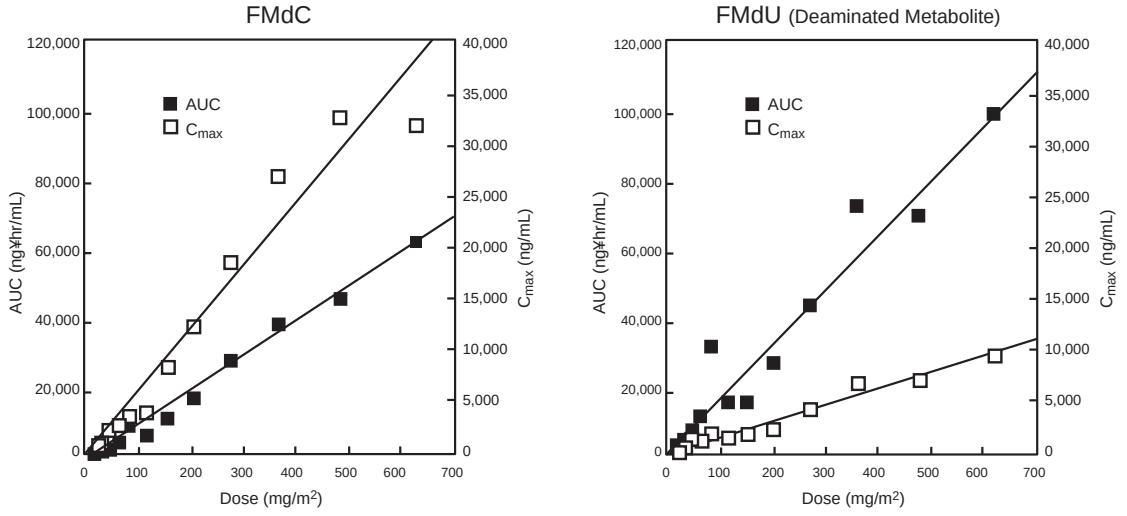

Fig. 4 Correlation of pharmacokinetic parameters with dose after a single IV infusion of FMdC, Study I.

nia and thrombocytopenia were reversible with cessation of dosing. Nonhematological events of Grade 3 or 4 were infrequent. Fever was usually low grade and controlled with acetaminophen, nonsteroidal anti-inflammatory drugs, or both. Gastrointestinal effects were mild, were not dose related, and did not require dosage adjustments. No drug-related deaths occurred.

Pharmacokinetic behavior was the same after single or multiple doses, with biexponential clearance of FMdC and monoexponential clearance of its metabolite, FMdU. The drug and its metabolite were not found to accumulate. Of the 86% of the administered drug that was recovered in the urine, 23% was recovered as the parent drug and 63% as the metabolite. This is consistent with the preclinical observations of the resistance of FMdC to cytidine deaminase and is in marked contrast to the metabolic behavior of gemcitabine, which is 95% or more metabolized to its uridine analogue (21). Because many tumors overexpress this enzyme (22), such resistance of FMdC to deamination could be therapeutically advantageous.

Although these were studies focused on tolerability and pharmacokinetics, evidence of drug efficacy was also observed. Eight patients showed evidence of drug activity: one partial response and seven stable disease. The partial response was maintained throughout the 4 months of treatment and for 5 additional months thereafter.

On the basis of the favorable safety profile of FMdC, promising evidence of activity in the Phase I studies (19), and the antitumor activity in preclinical studies, Phase II studies are now ongoing in patients with NSCLC and colorectal cancer. On the basis of the time course of observed neutropenia, the major DLT, a once-every-two-week dosage schedule has been chosen to allow optimum dose intensity without frequent dosing delays. Drug is being administered with this schedule at doses of 270 mg/m^2 and higher.

Preclinical studies indicate that FMdC is effective against multidrug-resistant cell lines (3, 13, 15) and in tumors grown in the brain (10). These findings point to opportunities for therapy in resistant cancers and brain cancers.

FMdC has a mechanism of action that suggests that it is noncompetitive with other cytotoxic agents. Moreover, preclinical studies in HeLa cells have indicated that FMdC has *in vitro* synergistic activity with ara-C, fluorouracil, and vinblastine, and *in vivo* synergism in B16 melanoma and Lewis lung carcinoma with ara-C (15). *In vivo* synergism was also found in A549 NSCLC and Calu-6 NSCLC xenografts in a sequence- and time-dependent manner, with combinations of FMdC and cisplatin (16). In addition, FMdC is a demonstrated radiopotentiator, showing *in vitro* synergism in HeLa cells (23) and *in vivo* synergism with xenografts of WiDr human colon carcinoma, C33-A human cervical cancer, and U-87 MG human glioblastoma (24, 25). The observed apparent synergy of FMdC with DNA-damaging agents such as cisplatin and radiation may be attributable to the recruitment of quiescent tumor cells (which typically make up a relatively large fraction of the total in a growing tumor) into active DNA synthesis, during which they become prone to the effects of FMdC.

On the basis of the novel mechanism of action, broad activity observed in preclinical trials, and the favorable safety profile in these Phase I studies on FMdC, additional studies are warranted to explore the activities of this novel nucleoside analogue in hematological and solid cancers, both as a single agent and in combination with other antineoplastic agents and treatment modalities.

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