Original Article

A Phase 1, Open-Label, Dose Escalation Study of Intravenous Paricalcitol in Combination With Gemcitabine in Patients With Advanced Malignancies

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BACKGROUND: Calcitriol, the active analogue of vitamin D, is antiproliferative and enhances the cytotoxicity of several anticancer agents, including gemcitabine. The vitamin D receptor (VDR) is expressed in the tumor stroma and treatment with VDR ligands results in stromal remodeling and increased intratumoral gemcitabine delivery. Furthermore, calcitriol can decrease the activity of the gemcitabine deactivating enzyme cytidine deaminase (CDD). Because hypercalcemia has been the most worrisome calcitriol-related adverse event, the less hypercalcemic agent paricalcitol may be preferred for further investigation. **METHODS:** The authors undertook a phase 1 study of gemcitabine in combination with escalating doses of paricalcitol administered weekly intravenously in patients with advanced cancers. A standard 3+3 dose escalation schema was used. Pharmacokinetic assessment of gemcitabine and its metabolite 2',2'-difluorodeoxyuridine (dFdU) was performed. Pharmacodynamic assessment of paricalcitol was performed by measurement of CDD activity in peripheral blood mononuclear cells. **RESULTS:** A total of 44 patients were enrolled. Somnolence was the main dose-limiting toxicity. The highest dose of paricalcitol administered was 10.5 μg/kg. Hypercalcemia was infrequent and mild in severity. Paricalcitol did not appear to affect the pharmacokinetics of gemcitabine and dFdU. Evaluation of CDD activity was available for 9 patients; no clear trend for CDD activity after treatment with paricalcitol was established. The overall response rate was 4%; the rate of disease control was 67% in patients who were pretreated with gemcitabine. Progression-free and overall survival were 3.4 months and 6.5 months, respectively. **CONCLUSIONS:** Paricalcitol can be administered safely in doses up to 7 μg/kg weekly with fixed dose rate gemcitabine without dose-limiting hypercalcemia. To the best of the authors' knowledge, the maximum tolerated dose has not been formally established to date. Preliminary clinical activity deserves further exploration. ***Cancer* 2018;124:3890-3899**.

KEYWORDS: cytidine deaminase, 2',2'-difluorodeoxyuridine (dFdU), gemcitabine, paricalcitol, phase 1.

INTRODUCTION

Calcitriol (1,25 OH-cholecalciferol) is the biologically active form of vitamin D. Its main role is maintenance of calcium homeostasis, mediated mainly through binding the nuclear vitamin D receptor (VDR).[1] The presence of VDRs has been described in many benign and malignant tissues, and there is evidence for the role of calcitriol as an inhibitor of cellular proliferation and differentiation, as well as a mediator of immunoregulation.[2,3] The mechanism of calcitriol activity includes decreased expression of epidermal growth factor receptor, dephosphorylation of the retinoblastoma protein, and induction of p21/p27–mediated G_1 cell cycle arrest.[4–9] Calcitriol has been tested as a single agent in several phase 1 studies using oral or parenteral formulations.[10–12] Hypercalcemia has been the most common adverse event (AE), and is potentially dose-limiting. 19-nor-1,25-OH-vitamin D2 (paricalcitol) is a synthetic vitamin D analog of calcitriol with less hypercalcemic potential and thus is preferred to calcitriol for development in cancer therapeutics.[13,14]

Synergy between VDR ligands and several cytotoxic agents has been documented in preclinical models[15,16]; inhibition of cell cycle checkpoint function, promotion of apoptosis, and inhibition of angiogenesis are some of the proposed mechanisms.[17–21] Gemcitabine is an antimetabolite with a broad spectrum of action against both solid and hematologic tumors. Synergistic apoptotic activity was noted in preclinical pancreatic cancer models when calcitriol was administered sequentially, 24 hours prior to gemcitabine.[22] Furthermore, calcitriol may also modify gemcitabine metabolism. Cytidine deaminase (CDD) is one of the key enzymes for degradation and subsequent inactivation of gemcitabine to 2',2'-difluorodeoxyuridine (dFdU).[23] Subcutaneous calcitriol in doses up to 10 μg for 3 consecutive days decreased

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

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peripheral blood mononuclear cell (PBMC) CDD activity when combined with carboplatin in a phase I study.[24]

Therefore, we conducted a phase 1 study of gemcitabine in combination with escalating doses of paricalcitol in patients with advanced cancer. In this paper, we report the final results of this phase 1 trial.

MATERIALS AND METHODS

Study Design

The current study was a phase 1, open label, dose escalation study of intravenous (iv) paricalcitol in combination with gemcitabine in patients with advanced, refractory malignancies.

We followed a 3+3 phase 1 trial design. In this trial design, the maximum tolerated dose (MTD) is the dose level at which 33% of the patients experience a dose-limiting toxicity (DLT). The DLT was defined as any hematologic toxicity of grade 3 lasting >7 days, any hematologic toxicity of grade 4, any episode of febrile neutropenia, persistent (duration of > 8 weeks) grade 2 neuropathy/neurotoxicity, and other nonhematologic toxicity of grade 3. Hypercalcemia and its complications (kidney stones, pancreatitis, renal failure, etc) were paricalcitol-specific DLTs of interest. Dose-limiting hypercalcemia was defined as symptoms of hypercalcemia at any serum calcium level >11.5 mg/dL on 2 consecutive calcium levels at least 1 week apart, corrected calcium ≥12.5 mg/dL even if the patient was asymptomatic confirmed on 2 consecutive calcium levels at least 1 week apart, and any calcium level ≥14 mg/dL confirmed immediately even if the patient was asymptomatic. A recurrent decrease in creatinine clearance to <50% of baseline or an increase in serum creatinine to > 2 times from baseline also was defined as a DLT. The duration of DLT monitoring was 4 weeks. Patients were monitored for hypercalcemia-related complications throughout the duration of the study (treatment and follow-up).

Patients were enrolled in escalating paricalcitol dose cohorts of 3 patients each. Dose escalation in the initial 5 dose levels followed a modified Fibonacci sequence with subsequent dose escalations at 25% increments above the preceding dose. If no patient experienced a DLT, patients were enrolled in the next dose cohort. If one patient experienced a DLT, 3 additional patients were enrolled in the same dose cohort. If no more DLTs were observed, 3 more patients could be enrolled in the next dose cohort. If at least 2 of 6 patients experienced a DLT, dose escalation stopped and the MTD was the dose at the previously tested lower dose level.

Patients were enrolled in a single center (Roswell Park Comprehensive Cancer Center [RPCI]). The current study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. The RPCI institutional review board approved the study protocol. Written informed consent was obtained from all patients.

Eligibility

Patients aged >18 years were considered eligible for participation if they had a confirmed histologically or cytologically advanced malignancy; adequate bone marrow, kidney, and liver function (total leukocytes ≥3000/μL, absolute neutrophil count ≥1500/μL, platelet count ≥100,000/μL, bilirubin<5 mg/dL, transaminase levels ≤2.5 times the institutional upper limit of normal, and creatinine ≤2.0 mg/dL); and corrected serum calcium ≤10.5 mg/dL. An estimated life expectancy of >3 months and good performance status (≤2 in the Eastern Cooperative Oncology Group scale or ≥60% on the Karnofsky Performance Score) were additional inclusion criteria. Patients previously exposed to gemcitabine were eligible for participation.

Key exclusion criteria were the presence of renal, ureteral, or urinary bladder stones on imaging before study entry or curative therapy for a condition associated with risk of renal stones and no stone formulation for ≤5 years prior to study entry or a single episode of renal lithiasis <5 years prior to study entry.

Objectives and Endpoints

The primary objective of the current study was to determine the MTD of the combination of gemcitabine and paricalcitol in patients with advanced malignancies. Secondary objectives were to evaluate toxicity, the effect of paricalcitol on gemcitabine pharmacokinetics (PK) and pharmacodynamics (PD), the overall response rate (ORR), progression-free survival (PFS), and overall survival (OS).

Toxicity evaluation was performed using the Common Terminology Criteria for Adverse Events (version 3.0). Response evaluation was performed with the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.0). Patients were evaluable for response if they had completed the first cycle of combination therapy. The ORR was defined as the rates of complete and partial response (PR) in all evaluable patients. PFS was defined as the time from study treatment initiation to disease progression or treatment termination for any cause. OS

was defined as the time from study treatment initiation to death from any cause.

Study Treatment and Procedures

Gemcitabine was administered weekly on a 3-week-on/1-week-off schedule at a dose of 1000 mg/m^2 as a fixed dose rate infusion at a rate of 10 mg/m^2/minute. Paricalcitol was administered as a rapid infusion over 15 minutes weekly 24 hours before gemcitabine, starting on week 2 (day 7). The starting paricalcitol dose was 0.24 μg/kg. This dose is one-third of the highest dose that has been reported to have been administered to patients with end-stage renal disease and caused no toxicity (dose of 0.24 μg/kg administered 3 times per week). The duration of 1 cycle was 4 weeks. Treatment was continued until disease progression, DLT, or death.

Treatment was withheld for AEs of ≥grade 2. The dose of gemcitabine was modified or suspended for toxicity (see Supporting Table 1). In general, after the first occurrence of any grade 3 toxicity, calcitriol and gemcitabine were withheld until resolution to a grade ≤1 or baseline. Patients who experienced any persistent or recurrent toxicity of ≥grade 3 were taken off the study permanently and considered to have experienced a DLT. Treatment was withheld when serum creatinine increased 2-fold from baseline; reinitiation was allowed at the time of recovery of the creatinine to <1.5 times from baseline. Paricalcitol was withheld if gemcitabine was withheld for any reason.

Assessments

Patients were evaluated for toxicity at every clinic visit. Computed axial tomography or magnetic resonance imaging was performed at baseline and every 8 weeks thereafter. The same type of imaging was used consistently for each patient. Peripheral blood for PK/PD studies was drawn on day 1 (after gemcitabine therapy), day 7 (after paricalcitol therapy), and day 8 (after gemcitabine therapy) on cycle 1.

PK and PD Studies

Paricalcitol PK

The plasma concentrations of paricalcitol were determined at Abbott Laboratories (Lake Bluff, Illinois) using a specific high-performance liquid chromatography/radioreceptor assay with a lower limit of quantification of 40 pg/mL. Blood samples (5 mL each) were collected in heparinized tubes at baseline and at the end of the 15-minute infusion, and then 0.5 hours, 1 hour, 2 hours, 4 hours, and 6 hours after infusion on day 7.

TABLE 1. Baseline Characteristics (N = 44)

Characteristic	No. (%)
Median age (range), y	62 (26-80)
Sex	
Male	27 (61)
Female	17 (39)
Race/ethnicity	
White	38 (86)
African American	5 (12)
American Indian/Alaskan Native	1 (2)
ECOG performance status	
0	26 (59)
1	17 (39)
2	1 (2)
Prior gemcitabine therapy	
No	34 (77)
Yes	10 (23)
Tumor type	
Lung	9 (20)
Pancreas	8 (18)
Esophageal/gastric	8 (18)
Hepatocellular	4 (9)
Colorectal	3 (7)
Prostate	2 (5)
Gallbladder	1 (2)
Bladder	1 (2)
Ampulla of Vater	1 (2)
Breast	1 (2)
Larynx	1 (2)
Thymus	1 (2)
Pleura (mesothelioma)	1 (2)
Mediastinum (germ cell)	1 (2)
Endocrine	1 (2)
Unknown primary	1 (2)

Abbreviations: ECOG, Eastern Cooperative Oncology Group.

Gemcitabine PK

The plasma concentrations of gemcitabine were determined at RPCI. Blood samples (5 mL each) were collected in heparinized tubes containing 5 μmole of the CDD inhibitor tetrahydrouridine. The drug and its deaminated metabolite dFdU were extracted from plasma with acetonitrile. Measurement of gemcitabine and dFdU in plasma was performed using a validated reverse-phase high-performance liquid chromatography procedure using Econosphere C8 column and a gradient of 0.5 M of ammonium acetate (pH 5.9) to methanol in 20 minutes at a flow rate of 1.5 mL/minute. Detection was made by ultraviolet at 272 nanometers. 2'-deoxycytidine was used as the internal standard. Samples were obtained at baseline; 75 minutes into the infusion; at the end of the infusion; and 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, and 8 hours after injection on days 1 and 8.

CDD activity

A CDD activity assay was performed at RPCI. Serum samples (14 mL in a special CPT tube on ice) were drawn on days 1 and 8 prior to administration of gemcitabine. PBMC lysates were prepared by thawing and

centrifugation at 14,000 × g for 10 minutes at 4°C, and the supernatant fluid subsequently was assayed for protein content and CDD activity. Protein concentrations were determined by the Bio-Rad protein binding assay (Bio-Rad Laboratories, Hercules, California) using bovine serum albumin as the standard.[25] CDD activity was assayed in 10 μg to 25 μg of protein using 100 μM of cytidine as a substrate. Determination of cytidine to uridine was monitored spectrophotometrically by change in absorbance at 286 nanometers at room temperature as previously described.[26] The initial rate of absorbance without the substrate was subtracted from that of the assay with cytidine, and CDD was expressed as nmol of uridine formed per minute/mg of protein.

Statistical Analysis

The current study was a phase 1, open-label, dose escalation study following a classic 3+3 dose escalation schema. The paricalcitol MTD was defined as the paricalcitol dose at which greater than one-third of the patients experienced a DLT. Noncompartmental analysis was conducted to determine PK parameters for paricalcitol, gemcitabine, and dFdU. Preparicalcitol and post-paricalcitol gemcitabine and dFdU PK were compared. Summary statistics of the PK parameters are presented. The estimated PFS and OS distributions were obtained using the Kaplan-Meier method. Using this distributional estimate, summary descriptive statistics such as the median survival and a 95% confidence interval (95% CI) of the median survival were obtained. SAS statistical software (version 9.4; SAS Institute Inc, Cary, North Carolina) was used for all statistical analyses.

RESULTS

Patients

A total of 44 patients with advanced, refractory malignancies were enrolled in the current trial. Baseline patient characteristics are presented in Table 1. The median patient age was 62 years (range, 26-80 years), and the majority of patients were white. Ten patients (23%) had received prior therapy with gemcitabine. The most common primary disease type was lung (20%), pancreatic (18%), and gastroesophageal (18%) cancers. Patients had received a median of 7 prior systemic therapies (range, 0-21 prior systemic therapies).

Treatment

Patients were enrolled in 10 paricalcitol dose cohorts (Table 2); the highest paricalcitol dose tested was 10.5 μg/kg. The median number of cycles delivered was 2 (range, 1-21 cycles) (see Supporting Table 2). Three patients were treated at a gemcitabine dose of 1000 mg/m^2. Because at this dose thrombocytopenia was frequent, all subsequent patients received gemcitabine at a dose of 800 mg/m^2. Greater than 80% of the patients received up to 4 treatment cycles. The MTD of paricalcitol was not formally established (see below).

Safety

Three DLT events were observed; a grade 2 thrombocytopenia in the cohort receiving 3 μg/kg and 2 episodes of grade 3 somnolence, one in the cohort receiving 7 μg/kg and one in the cohort receiving 10.5 μg/kg. In both cases, this event was self-limited and fully resolved within 48 hours. The episode of thrombocytopenia was related to gemcitabine use, and both somnolence episodes were related to the paricalcitol solvent propylene glycol. The MTD was not formally assessed because only 2 patients were treated at the dose of 10.5 μg/kg. The window of safety for propylene glycol at this dose of paricalcitol was not deemed to be safe by the local institutional review board and data safety and monitoring board and therefore further dose escalation was stopped. The highest dose of paricalcitol that was determined to be safe was 7 μg/kg. There were 2 deaths on study, both secondary to disease progression. Detailed reports of AEs by system and severity are presented in Table 3 and Supporting Table 3. The most common AEs were fatigue, decreased appetite, diarrhea, nausea/vomiting, constipation, peripheral edema, neutropenia/leukopenia, thrombocytopenia, anemia, hepatic function enzyme and albumin abnormalities, hyperglycemia, hypercalcemia, and hyponatremia. The majority of AEs (82%) were grade 1/2 in severity. There were 12 cases of hypercalcemia (7 of grade 1 and 5 of grade 2) and 7 episodes of hypocalcemia were recorded (5 of grade 1 and 2 of grade 2). No cases of symptomatic hypercalcemia or complications of hypercalcemia such as nephrolithiasis were reported to occur during study treatment and follow-up (median, 6 months).

Pharmacokinetics/Pharmacodynamics

The half-life and systemic clearance of paricalcitol were similar to those observed in healthy subjects. The maximum concentration (C_{max}) and area under the curve (AUC) increased linearly with the dose (see Supporting Table 4) (see Supporting Fig. 1). The mean C_{max} for gemcitabine was 18 μg/mL prior to paricalcitol and 16 μg/mL after paricalcitol. The mean $AUC_{0-\infty}$ was similar prior to and after administration of paricalcitol (17.5 hour*μg/mL vs 17.8 hour*μg/mL) (Table 4) (see Supporting Table 5).

TABLE 2. Patient Distribution per Dose Cohort

Cohort Dose, μg/kg	No. of Patients	Tumor Type (No.)
0.24	3	Lung (1) Pancreas (1) Colorectal (1)
0.72	3	Lung (1) Pancreas (1) Colorectal (1)
1.20	4	Pancreas (2) Esophageal/gastric (1) Unknown primary (1)
1.80	3	Lung (2) Bladder (1)
2.40	4	Esophageal/gastric (2) Colorectal (1) Prostate (1)
3.00	8	Lung (3) Esophageal/gastric (2) Prostate (1) Pleura (1) Hepatocellular (1)
3.75	4	Pancreas (1) Esophageal/gastric (1) Ampulla of Vater (1) Mediastinum (1)
4.69	6	Pancreas (2) Esophageal/gastric (1) Gallbladder (1) Lung (1) Endocrine (1)
7.00	7	Hepatocellular (3) Esophageal/gastric (1) Larynx (1) Thymus (1) Breast (1)
10.50	2	Pancreas (1) Lung (1)

Neither gemcitabine nor dFdU systemic exposure were altered by the coadministration of paricalcitol at the dose levels tested (Fig. 1 Top and Bottom, respectively).

Estimation of CDD was feasible in only 9 patients due to a lack of adequate PBMCs in treated patients. Six patients had samples for both days 1 and 8. CDD activity is presented in Supporting Figure 2 and Supporting Table 6.

Efficacy

A total of 27 patients were evaluable for response. One PR (4%) was noted (ORR, 4%). This patient had non–small cell lung cancer with neuroendocrine features and had received prior treatment for his disease but was gemcitabine-naive. The duration of response for this patient was 48.2 months. Eleven patients had stable disease and 15 patients had disease progression. The median duration of disease stability for the patients with stable disease as their best response (defined as the time from study treatment initiation to date of disease progression or death) was 8.4 months (range, 3.3-91.5 months). Of the 10 patients previously treated with gemcitabine, 6 were evaluable for response assessment. The best response was stable disease in 4 patients, for a disease control rate of 67% in the population pretreated with gemcitabine. The median duration of stable disease for these patients was 10.1 months (range, 4.1-12.8 months). In general, disease control was noted across different dose cohorts and different disease types (see Supporting Table 7). The median PFS was 3.4 months (95% CI, 2-5.4 months) and the median OS was 6.5 months (95% CI, 3.9-8.9 months) (Fig. 2 Top and Bottom).

TABLE 3. Most Common Adverse Events by Grade[a].

	Grade					
Adverse Event	1	2	3	4	5	Total
Leukopenia	1	14	19	1	0	35
Neutropenia	3	10	11	6	0	30
Anemia	5	14	8	2	0	29
Thrombocytopenia	11	8	10	0	0	29
Fatigue	5	23	0	0	0	28
Hyperglycemia	7	7	6	0	0	20
Hypoalbuminemia	7	11	0	0	0	18
Nausea	9	9	0	0	0	18
Increased AST	13	2	2	0	0	17
Hyperkalemia	12	3	0	0	0	15
Lymphopenia	0	10	4	1	0	15
Increased ALP	6	6	1	0	0	13
Increased ALT	9	3	0	0	0	12
Decreased appetite	2	10	0	0	0	12
Hypercalcemia	7	5	0	0	0	12
Vomiting	7	4	1	0	0	12
Constipation	6	5	0	0	0	11
Diarrhea	8	3	0	0	0	11
Peripheral edema	4	6	1	0	0	11
Hyponatremia	9	0	2	0	0	11

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a]Toxicity evaluation was performed using the Common Terminology Criteria for Adverse Events (version 3.0).

DISCUSSION

This paper presents the final results of a phase 1 study of gemcitabine in combination with paricalcitol in patients with advanced, refractory malignancies. The MTD for paricalcitol could not be formally assessed because dose escalation was terminated before enrollment of the third patient in the cohort receiving 10.5 μg/kg based on the recommendations of the institutional review board and data safety monitoring board. The highest dose that was administered safely was 7 μg/kg iv weekly. There were 12 episodes of hypercalcemia. All episodes were mild in severity and improved with supportive care alone, with no need for withholding or dose reduction of paricalcitol. Furthermore, no episodes of symptomatic hypercalcemia were observed. The presence of bone metastasis did not appear to increase the risk of hypercalcemia

TABLE 4. Gemcitabine Pharmacokinetics Before and After Paricalcitol.

Gemcitabine Dose, mg/m^2		$T_{1/2}$, Hours	T_{max}, Hours	C_{max}, ug/mL	$AUC_{0\text{-}2.17}$, Hour*ug/mL	AUC_{∞}, Hour*ug/mL	AUC%, Extrap	V_Z, l/m$_2$)	Cl, l/hour/m$_2$)
Before paricalcitol									
800	No.	30	39	39	38	30	30	30	30
	Mean	3.726	1.093	17.725	16.577	17.507	0.350	333.823	59.689
	SD	2.017	0.304	14.590	8.651	8.610	0.318	291.461	35.416
	Minimum	1.44	0.33	3.47	4.21	4.34	0.03	77.81	19.85
	Median	2.85	1.00	15.00	15.79	16.28	0.21	231.28	49.14
	Maximum	8.24	1.67	70.61	40.05	40.31	1.07	1206.04	184.52
	CV%	54.1	27.8	82.3	52.2	49.2	90.8	87.3	59.3
1000	No.	0	3	3	3	0	0	0	0
	Mean		1.390	7.253	7.865				
	SD		0.242	2.026	0.532				
	Minimum		1.25	5.44	7.27				
	Median		1.25	6.88	8.02				
	Maximum		1.67	9.44	8.30				
	CV%		17.4	27.9	6.8				
After paricalcitol									
800	No.	27	33	33	33	27	27	27	27
	Mean	4.550	1.062	16.118	15.541	17.752	0.513	396.981	59.422
	SD	3.682	0.325	14.605	9.486	9.548	0.687	430.472	32.176
	Minimum	1.10	0.33	2.81	3.63	6.63	0.03	88.12	17.57
	Median	3.71	1.00	11.64	13.66	16.64	0.26	251.38	48.08
	Maximum	17.99	1.83	67.13	44.63	45.53	2.79	2230.45	120.66
	CV%	80.9	30.6	90.6	61.0	53.8	133.9	108.4	54.1
1000	No.	0	3	3	3	0	0	0	0
	Mean		1.197	8.103	9.795				
	SD		0.502	1.701	2.671				
	Minimum		0.67	6.23	6.77				
	Median		1.25	8.53	10.80				
	Maximum		1.67	9.55	11.82				

Abbreviations: AUC, area under the curve; Cl, clearance; C_{max}, maximum concentration; CV, coefficient of variation; Extrap, extrapolated; SD, standard deviation; $T_{1/2}$, half-life, T_{max}, time to C_{max}, V_Z, volume of distribution.

because there were no episodes noted in patients with documented disease in the bones. The main DLT related to paricalcitol treatment was somnolence secondary to propylene glycol, the paricalcitol solvent. Propylene glycol is metabolized by alcohol (ADH) and aldehyde (ALDH) dehydrogenases; it is likely that propylene glycol toxicity in the current study may have been affected by the individual genetically determined alcohol tolerance based on polymorphisms of *ADH* and *ALDH*[27] as well as differences in baseline liver function between patients. In addition, patients with metastatic disease to the liver and who were exposed to multiple prior cytotoxic therapy regimens may have a lower threshold for toxicities secondary to propylene glycol. The inclusion of treatment-naive patients with nonmetastatic disease potentially can allow for the delivery of higher doses of paricalcitol in future clinical trials.

In general, the combination was found to be safe and the majority of AEs were related to gemcitabine use. Paricalcitol did not appear to exacerbate gemcitabine-related AEs. In terms of antitumor efficacy, the combination resulted in response or disease stabilization in approximately 44% of the evaluable patients. There was only one confirmed response (PR) in this heavily pretreated population. It is interesting to note that approximately two-thirds of evaluable patients who were pretreated with gemcitabine achieved disease stabilization with the combination therapy. Approximately 38% of the evaluable gemcitabine-naive patients achieved disease control. Disease control was noted across different dose cohorts and disease types. Disease stabilization appeared to be relatively durable, with the median duration of stable disease being 8.4 months (10.1 months for patients pretreated with gemcitabine). Of the 22 patients with a disease type known to be responsive to gemcitabine (ie, cancers of the lung or pancreas), 9 achieved disease control, including the 1 patient with a PR. Four of these patients had received treatment with gemcitabine in the past.

It is possible that paricalcitol can restore tumor sensitivity to gemcitabine. Calcitriol can augment gemcitabine-induced apoptosis through enhanced caspase-8, caspase-6, caspase-9, and caspase-3 activation and significantly decreased Akt phosphorylation.[22] The fixed

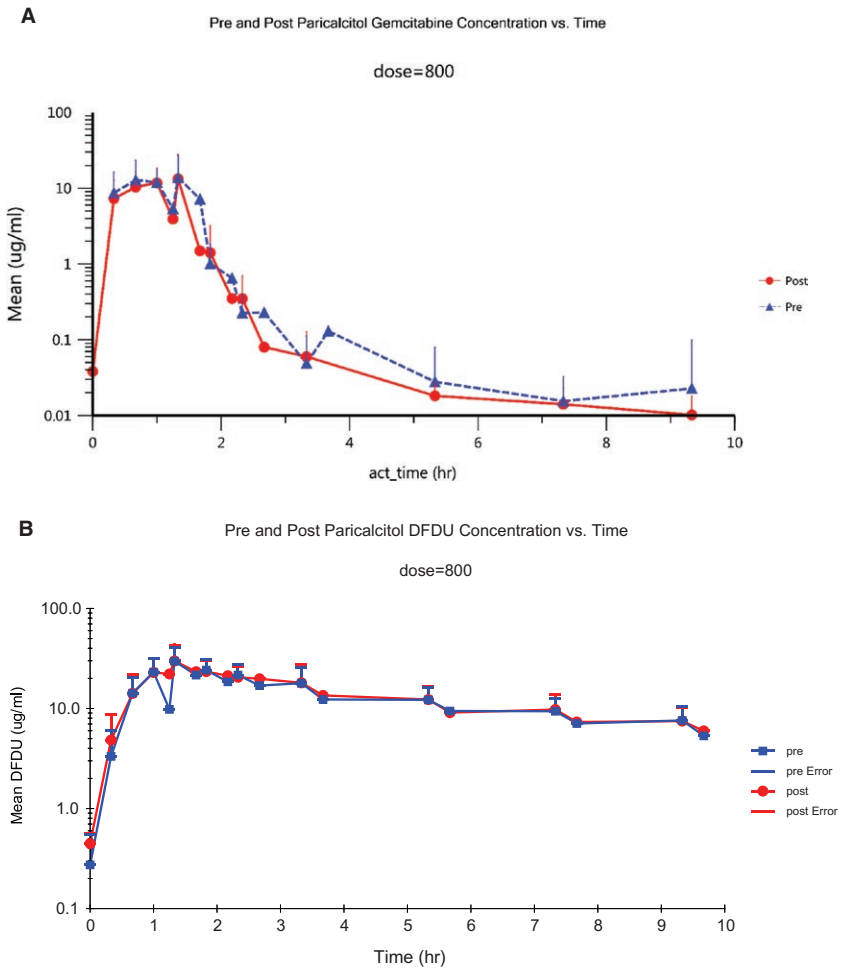

Figure 1. (*Top*) Gemcitabine and (*Bottom*) 2',2'-difluorodeoxyuridine (dFdU) concentrations before (pre) and after (post) administration of paricalcitol. act_time indicates time.

dose rate of gemcitabine administration also may have contributed to disease stabilization in patients who were pretreated with gemcitabine through improved intracellular accumulation of gemcitabine monophosphate[28,29]; the optimal gemcitabine concentration for the optimization of active metabolite formation has been established at 15 to 20 μmol/L (3.5-4.7 μg/mL). The gemcitabine C_{max} achieved in the current study exceeded this level, and as such it is unlikely the method of gemcitabine administration was by itself responsible for the observed efficacy in pretreated subjects. Gemcitabine triphosphate levels in PBMCs were not measured in the current study.

We also tested the hypothesis that CDD activity is suppressed in PBMCs from treated patients, resulting in higher gemcitabine concentrations. We previously have shown that CDD activity is suppressed 48 hours after subcutaneous administration of calcitriol.[24] Unfortunately, PBMC isolation was not feasible for the majority of the patients treated in the current study. Of the 6 patients with PBMC samples for both days 1 and 8, CDD activity was decreased in 3 patients on day 8, increased in 2 patients, and stable in 1 patient. The sample size was too limited for statistical comparison. Gemcitabine and dFdU PK before and after treatment with paricalcitol essentially were unchanged, making CDD activity modulation by paricalcitol unlikely in the doses tested in the current trial. Furthermore, to the best of our knowledge, it is unknown whether paricalcitol also is a potent inhibitor of CDD similar to calcitriol or whether there is intertumor variability.

In the current study, single-nucleotide polymorphism (SNP) studies were not performed to explore whether other patient-related and tumor-related factors

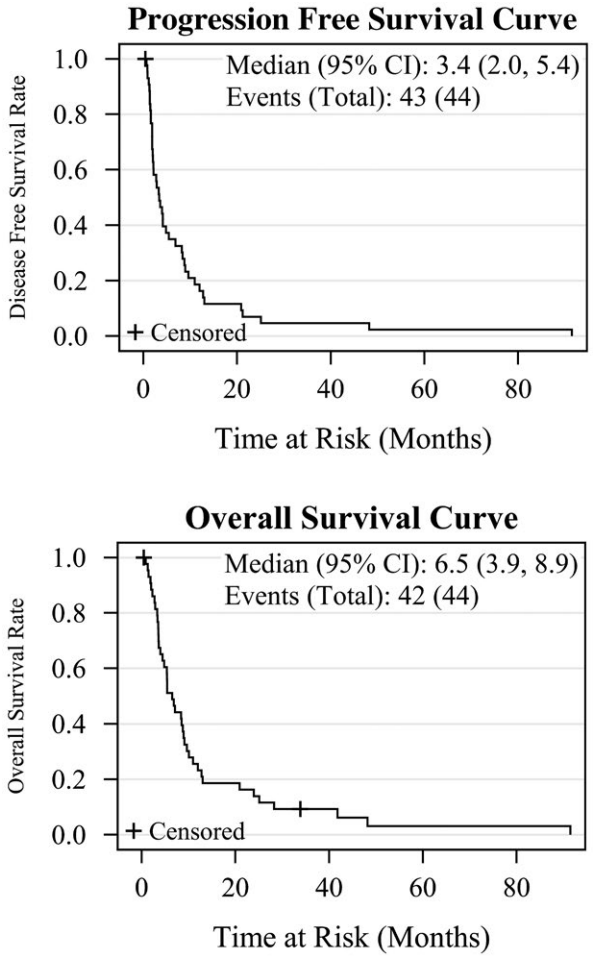

Figure 2. (*Top*) Progression-free survival and (*Bottom*) overall survival estimates for the entire study population. 95% CI indicates 95% confidence interval.

also may play a role in paricalcitol/calcitriol metabolism and hence affect its antitumor activity. The cytochrome P450 family 24 subfamily A member 1 (*CYP24A1*) gene encodes 24-hydroxylase, an enzyme responsible for 1,25 OH-D3 degradation.[30] It can be overexpressed in malignant cells, leading to escape from vitamin D–induced growth control.[31,32] In a phase 1/2 study of iv calcitriol and the combination of cisplatin and docetaxel in patients with treatment-naive non–small cell lung cancer, the addition of calcitriol at a dose of 60 μg/m^2 (the recommended phase 2 dose) did not improve the response rate compared with historical controls.[33] Germline *CYP24A1* gene polymorphism analysis in the current study revealed that SNP rs3787554 (C>T) correlated with disease progression. There was a trend toward an association between SNP rs2762939 (C>G) and disease control. No association between *CYP24A1* SNPs and calcitriol PK was noted. Genomic studies in ongoing trials of paricalcitol in patients with pancreatic cancer may indicate whether this adds significant predictive information.

Modulation of supportive cells and stroma within the tumor microenvironment can be potentially responsible for the antitumor activity of VDR agonists. Vitamin D appears to be a master regulator of the transcriptional activity of pancreatic stellate cells in pancreatic ductal adenocarcinoma, inducing a quiescent state.[34] In preclinical models, treatment with a VDR agonist and gemcitabine resulted in a 500% increase in the intratumoral gemcitabine triphosphate (dFdCTP; the main active metabolite of gemcitabine) concentration, and significantly decreased tumor growth and fibrosis.[34] In the current study, 4 of the 8 patients with pancreatic cancer had received prior treatment with gemcitabine. Stable disease was noted in 2 patients, 1 of whom had received prior treatment with gemcitabine. Three patients had progressive disease as their best response and 3 were not evaluable for response. There are ongoing studies evaluating paricalcitol in patients with pancreatic cancer. A preoperative trial of paricalcitol (50 μg iv weekly in arm A and 12 μg orally daily in arm B) in patients with resectable pancreatic cancer with toxicity as the primary endpoint is currently enrolling patients at the University of Pennsylvania (ClinicalTrials.gov identifier NCT03300921). The same center also is planning a randomized pilot PD genomic study of neoadjuvant paricalcitol (fixed dose of 25 μg administered on day 1 of each cycle) with standard gemcitabine and nanoparticle albumin–bound paclitaxel to target the pancreatic cancer microenvironment (ClinicalTrials.gov identifier NCT02030860). A second neoadjuvant pilot study will assess 2 cycles of concurrent pembrolizumab at a dose of 200 mg iv on day 1 and paricalcitol at a dose of 7 μg/kg on days 1, 8, and 15 of each 3-week cycle followed by surgery after the last dose of paricalcitol (ClinicalTrials.gov identifier NCT02930902). More potent VDR agonists with less hypercalcemic potential currently are in development for patients with prostate cancer.[35]

CONCLUSIONS

The results of the current study demonstrate that paricalcitol can be combined safely with gemcitabine in patients with advanced cancer in doses up to 7 μg/kg iv weekly. Hypercalcemia was not found to be a DLT in the current study for the doses tested. Somnolence related to the

propylene glycol solvent was the main DLT. Gemcitabine PK were unaffected by paricalcitol for the doses tested. Future clinical trials including paricalcitol in combination with gemcitabine alone or gemcitabine and nab-paclitaxel warrant further investigation.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Christos Fountzilas: Data curation, formal analysis, writing–original draft, and writing–review and editing. **Milind Javle**: Conceptualization, data curation, formal analysis, methodology, investigation, and writing–review and editing. **Wei Tan**: Data curation, formal analysis, methodology, software, and writing–review and editing. **Yingyu Ma**: Conceptualization, data curation, formal analysis, investigation, methodology, and writing–review and editing. **Gerald Fetterly**: Data curation, formal analysis, investigation, methodology, and writing–review and editing. **Renuka Iyer**: Conceptualization, data curation, formal analysis, investigation, methodology, and writing–review and editing. **Candace Johnson**: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, and writing–review and editing.

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