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A Single-Center Retrospective Study of Acute Kidney Injury Incidence in Patients With Advanced Malignancies Treated With Antimitochondrial Targeted Drug

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Introduction: Mitochondrial dysfunction plays an important role in the pathophysiology of kidney disease. Inhibitors of mitochondrial metabolism are being developed for the treatment of solid organ and hematologic malignancies. We describe the incidence and clinical features of acute kidney injury (AKI) in patients treated with the antimitochondrial drug CPI-613.

Methods: We identified 33 patients with relapsed or refractory malignancy, previously enrolled in 3 open-label phase II studies, who received single-agent CPI-613 chemotherapy. AKI was defined by the Kidney Disease Improving Global Outcomes serum creatinine criteria. Participants were followed for a median (25th–75th percentile) of 120.0 (74.0–301.0) days. Risk factors for AKI were assessed by proportional hazards regression using univariate and multivariate analyses.

Results: Participants had baseline mean (SD) age of 63.8 (11.6) years and serum creatinine 0.9 (0.3) mg/dl. AKI developed in 9 (27%) patients; chart review failed to identify a potential cause of AKI other than CPI-613 administration in 5 (15%) patients, of whom 1 had AKI stage 1, 1 had AKI stage 2, and 3 experienced AKI stage 3. Time from initiation of CPI-613 treatment to AKI was 51.0 (16.0–58.0) days. Age, per 5-year increase, was associated with higher risk of AKI (adjusted hazard ratio 2.01, 95% confidence interval 1.06–3.79, $P = 0.03$). Follow-up serum creatinine was available in 4 participants 174.8 (139.6) days after the episode of AKI; 3 patients had complete recovery in kidney function and 1 had partial recovery.

Conclusion: AKI is a possible complication during treatment with mitochondria-targeted chemotherapy.

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Cells with a high proliferative index, such as neoplastic cells, can undergo adaptive metabolic changes whereby alterations in mitochondrial metabolism occur to uphold cellular proliferation. Utilization of glutamine to replenish tricarboxylic acid intermediates is an example of a metabolic adaptation frequently present in neoplastic cells.[1] As a result, enzymes with key roles in mitochondrial metabolism, such as the pyruvate dehydrogenase complex, which is regulated by pyruvate dehydrogenase regulatory kinases, and α-ketoglutarate dehydrogenase complex (KGDH), are upregulated in cancer cells, particularly in the context of hypoxia.[2] These metabolic transformations render the neoplastic cells particularly vulnerable to inhibition of mitochondrial metabolism and have presented therapeutic opportunities.

CPI-613 is the first agent from a novel class of lipoate analogs developed as potential antineoplastic drugs. Lipoic acid is a cofactor necessary for the activity of pyruvate dehydrogenase and KGDH. *In vitro* and *in vivo* studies show that in tumor cells, CPI-613 inhibits KGDH function and activates lipoate-sensitive pyruvate dehydrogenase regulatory kinases, which in

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turn phosphorylate and inhibit pyruvate dehydrogenase. The net effect is a disruption in the tumor's energy supply and biosynthetic intermediates with resultant cell death.[3,4] To date, several clinical trials have evaluated the safety, tolerability, efficacy, and pharmacokinetics of CPI-613 in patients with malignancy refractory to standard chemotherapeutic regimens. CPI-613 is well tolerated, with relatively few adverse events reported, but a targeted analysis of renal outcomes has not been undertaken.[5,6]

Renal tubular epithelial cells represent one of the most metabolically active epithelia in the human body. Research over past years revealed striking pathological changes in the mitochondria (i.e., mitochondrial fragmentation) of the tubular epithelium in experimental models of AKI. Mitochondrial distress accompanying AKI (mitochondrial fragmentation, disruption of the mitochondrial cristae, decreased expression and activity of electron-transport chain enzymes) is not just a morphological change but also contributes to the generation of reactive oxygen species and cell death.[7–9] Therefore, it is important to evaluate the effects of pharmacological agents that target mitochondrial metabolism on kidney function and electrolyte homeostasis. This retrospective study of data collected from 3 previous phase II open-label studies analyzed the incidence and severity of AKI following administration of CPI-613.

MATERIALS AND METHODS

Study Population

Patients enrolled in this study participated in 1 of the 3 phase II open-label oncology trials involving CPI-613 conducted at the Wake Forest Baptist Comprehensive Cancer Center between August 2013 and December 2016. Enrollment criteria included inoperable, locally advanced, or metastatic bile cancer (NCT01766219); pancreatic adenocarcinoma (NCT01839981); and myelodysplastic syndrome for patients who failed previous therapy (NCT01902381). As part of the inclusion criteria, patients were required to have an expected survival of more than 2 or 3 months, Eastern Cooperative Oncology Group performance status of 0 to 2, and serum creatinine $\leq$1.5 or 2.0 mg/dl within the 2 weeks preceding the study. Across all 3 studies, exclusion criteria included uncontrolled bleeding or bleeding diathesis, HIV infection, active heart disease, recent myocardial infarction or congestive heart failure, and receipt of cancer immunotherapy within 4 weeks or chemotherapy with stem cell support within 6 months before enrollment. To limit confounding by administration of other potential nephrotoxic agents, the study included only patients who did not receive any additional chemotherapy medication at the time of CPI-613 administration. The studies were all approved by the Wake Forest Institutional Review Board.

Treatment With CPI-613

For all 3 phase II oncology protocols, CPI-613 was administered through a central line over 120 minutes. In protocols NCT01766219 and NCT01839981, CPI-613 was administered on days 1 to 5 as an initial "precycle" followed by a 1-week break. For all remaining cycles, CPI-613 was given on days 1 and 4 of weeks 1 to 3 in a 4-week cycle. These trials consisted of several dosing cohorts. Cohort 1: No "precycle" and the dose of CPI-613 was 2300 mg/m^2. Cohort 2: No "precycle" and the dose of CPI-613 was 3000 mg/m^2. Cohort 3: The dose of CPI-613 for the precycle 1 week was 600 mg/m^2 per day with same dose as cohort 2 for all other cycles. Protocol NCT01902381 for patients with myelodysplastic syndromes having failed previous therapies did not have "precycle" drug administration, and the dose of CPI-613 was 2940 mg/m^2. Following the ninth patient participant, the protocol was amended due to the incidence in AKI, and CPI-613 was given at a dose of 2500 mg/m^2 on days 1 to 5 every 28 days. In all 3 phase II oncology protocols, toxicity was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0, which classifies AKI as grade 1 if elevation in serum creatinine $>$1.0 to 1.5 times the upper limit of normal; grade 2 if elevation in serum creatinine $>$1.5 to 3.0 times upper limit of normal; grade 3 if elevation in serum creatinine $>$3.0 to 6.0 times upper limit of normal and hospitalization indicated; grade 4 for elevation in serum creatinine $>$6.0 times upper limit of normal and life-threatening consequences or dialysis indicated; and grade 5 AKI in the event of death related to kidney injury.[10] All 3 phase II trials followed a protocol of CPI-613 dose adjustment for events of AKI suspected to be related to CPI-613: for AKI grade 1, CPI-613 dose was reduced by 15%; for AKI grade 2, dose was reduced by 25%; and for AKI grade 3 or 4, dose was decreased by 50% or treatment with CPI-613 was discontinued.[5,6]

Data Collection

Demographic (age, sex, race) and medical data were obtained through review of inpatient and outpatient medical records. Comorbidities collected included diabetes mellitus, hypertension, and history of coronary artery disease (history of myocardial infarction or coronary revascularization). Laboratory data included serum creatinine, blood urea nitrogen (BUN), serum potassium, serum bicarbonate, serum magnesium, serum phosphorus, serum lactate dehydrogenase,

serum uric acid, hemoglobin, and platelet count. When available, albuminuria (urine albumin by urinalysis dipstick, mg/dl), spot urine protein-to-creatinine ratio (mg/g), and hematuria (urine red blood cells per high power field) were recorded. Each laboratory variable was recorded at baseline (collected at initiation of therapy), during treatment, end of treatment, and at study completion. The dose of CPI-613 (in mg, mg/kg, and mg/m^2) across the first 5 doses was documented; afterward, the dose was collated at intervals of 0 to 4 weeks, 5 to 15 weeks, 16 to 24 weeks, 25 to 36 weeks, 37 to 48 weeks, 49 to 72 weeks, and 73 to 96 weeks following initiation of therapy. For those who developed AKI, the CPI-613 dose accumulated up to the date of peak serum creatinine was logged. For those who did not have AKI, the CPI-613 dose accumulated by the observed median days to kidney injury was logged. Peak values for serum chemistries and nadir values for blood cell counts (hemoglobin and platelets) per each time interval were logged for each patient. In patients who developed AKI, the peak eosinophil blood count noted in the 14 days before or after the date of peak serum creatinine was logged. In patients who did not develop AKI, the peak eosinophil blood count noted in the 14 days before or after the observed median time to kidney injury relative to the first dose of CPI-613 was logged. When available, urine studies were recorded. We reported hematuria as mild (presence of 4 to 10 red blood cells per high power field), moderate (10–30 red blood cells per high power field), and severe (>30 or too numerous to count red blood cells per high power field); and albuminuria as mild (trace protein on urine dipstick), moderate (30 mg/dl protein on urine dipstick), and severe (≥100 mg/dl protein on urine dipstick). Blood pressure values (systolic blood pressure, diastolic blood pressure, mean arterial pressure [MAP]) were recorded at baseline (obtained the day of treatment before initiation of CPI-613 infusion) and at each treatment visit. We defined hypotension using an absolute MAP threshold (i.e., MAP less than 65 mm Hg) and a relative MAP threshold (i.e., greater than 20% decrease in MAP from baseline).[11] Medical charts were reviewed for occurrence of intercurrent illness, hospitalization, and administration of i.v. iodinated contrast; these events were recorded and evaluated when they occurred up to 14 days before an AKI event. To assess whether the kidney function was declining before CPI-613 treatment initiation, pretreatment serum creatinine levels, 7 to 14 days before first dose of CPI-613, were logged and compared with baseline serum creatinine. Data were collected to the last laboratory measurement available at the time of the study or death. Data were anonymized at the time of collection and before analysis.

Outcomes

All AKI events that occurred during treatment with CPI-613 were recorded. We defined AKI based on fold serum creatinine elevation from baseline using the Kidney Disease Improving Global Outcomes Clinical Practice Guideline, according to the highest serum creatinine level documented during treatment with CPI-613.[12] AKI severity was categorized as stage 1 (rise in serum creatinine 1.5–1.9 times baseline or ≥0.3 mg/dl increase); stage 2 (rise in serum creatinine 2.0–2.9 times baseline); or stage 3 (rise in serum creatinine ≥3.0 times baseline, or serum creatinine ≥4.0 mg/dl, or initiation of renal replacement therapy). To calculate the time to AKI event, dates of first CPI-613 administration and peak serum creatinine during treatment with CPI-613 were recorded. To evaluate the recovery of kidney function (complete, partial, or lack of recovery), we analyzed data in patients who developed AKI during treatment with CPI-613, had CPI-613 treatment discontinued, and had serum creatinine measured at least 7 days after the AKI event. Complete recovery of kidney function following AKI was defined as a return of serum creatinine to <0.5 mg/dl above the baseline value, partial recovery as a return of serum creatinine to ≥0.5 mg/dl but less than twice the baseline value, and lack of kidney function recovery as last serum creatinine twice or more above the baseline value. Events of AKI for which chart review failed to identify a potential cause of AKI other than CPI-613 administration are referred to as unexplained AKI.

Statistical Analysis

Normally distributed continuous variables were expressed as the mean (SD), and groups were compared using an independent *t* test. Non-normally distributed continuous variables were presented as the median (25th–75th percentile), and groups were compared using the Wilcoxon rank-sum test. Categorical variables were expressed as counts (percentages) and analyzed using the Fisher exact test. Cox proportional hazard regression models were used to identify associations between patient characteristics and CPI-613 therapy with AKI occurrence. Hazard ratios are reported with their 95% confidence intervals. Multivariate Cox proportional hazard regression models were used to identify variables with a *P* value of less than 0.2 in descriptive analysis; these variables were further examined in multivariate analysis to identify independent risk factors for AKI. The covariates included in multivariate regression analysis of AKI risk included age (risk expressed as change per 5 years), diabetes mellitus, serum lactate dehydrogenase (risk expressed as change per 10 units), and CPI-613 dose (initial dose and cumulative dose, expressed as mg, mg/kg, mg/m^2,

and mg/body mass index) and infusion rates. A P value of less than 0.05 was deemed significant.

RESULTS

Patients

Between August 2013 and December 2016, 33 patients with a diagnosis of malignancy were eligible to receive the investigational chemotherapy drug CPI-613 at our institution. Baseline characteristics of the study participants are summarized in Table 1. Participants had mean (SD) age at initiation of treatment with CPI-613 of 63.8 (11.6) years, 39% (13/33) were women, 85% (28/33) were white, 36% (12/33) had diabetes, and 30% (10/33) had history of hypertension. Baseline renal function parameters, available in all patients immediately before initiation of CPI-613, were serum creatinine 0.9 (0.3) mg/dl and BUN 15.9 (5.4) mg/dl. Urine studies before CPI-613 treatment were available in 13 of the 33 study patients; of these, none had hematuria and 2 had mild albuminuria.

Table 1. Baseline demographic and clinical characteristics of study participants

Parameter	Mean or *n*	SD or %
Age, yr (at malignancy diagnosis)	63.8	11.6
Age, yr (at first CPI-613 administration)	65.4	11.2
Female	13	39.4
Black	4	12.1
Hispanic	1	3.0
White	28	84.8
Body mass index, kg/m^2	28.1	6.5
Diabetes mellitus (yes)	12	36.4
Hypertension (yes)	10	30.3
Coronary artery disease (yes)	1	3.0
Systolic blood pressure (mm Hg)	130.0	16.8
Diastolic blood pressure (mm Hg)	70.9	10.7
Mean arterial pressure (mm Hg)	90.6	10.2
Serum creatinine (mg/dl)	0.9	0.3
BUN (mg/dl)	15.9	5.4
Hemoglobin (g/dl)	11.5	4.5
Platelets ($\times$ 10^3/μl)	193.4	137.3
Potassium, serum (mEq/l)	4.2	0.4
Bicarbonate, serum (mEq/l)	26.6	2.2
Magnesium, serum (mg/dl)	1.9	0.2
Phosphorus, serum (mg/dl)	3.6	0.6
lactate dehydrogenase, serum (IU/l)	181.2	53.6
Uric acid, serum (mg/dl)	5.1	1.3
Malignancy diagnosis		
Myelodysplastic syndrome	10	30.3
Cholangiocarcinoma	10	30.3
Pancreatic cancer	10	30.3
Gallbladder adenocarcinoma	1	3.0
Bile duct neuroendocrine carcinoma	1	3.0
Moderately differentiated adenocarcinoma	1	3.0

Data are presented as *n* (%) for categorical variables and mean (SD) for continuous variables. Data are compiled on all 33 participants, with the exception of serum lactate dehydrogenase level available in 26 participants, and serum uric acid level available in 7 participants.
n, number of patients.

CPI-613 Therapy

Patients were followed for a median of 120.0 (74.0–301.0) days. By the end of this study, 23 (69.7%) participants died, with time to death of 115.0 (58.0–136.5) days. Per patient, the mean (range) number of CPI-613 administrations was 21.5 (1.0–132.0) and total dose was 100,840.1 (2400.0–666,900.0) mg (54,493.2 [1200–392,294.1] mg/m^2); of these, 1 patient received only 1 dose and 2 patients received 2 doses of CPI-613 therapy. Four patients were still receiving single-agent CPI-613 chemotherapy by the end of this study and had median treatment duration of 113.0 (102.5–135.8) days; in the remaining patients, the median duration of treatment with single-agent CPI-613 was 45.0 (15.5–93.5) days. The average dose of CPI-613 at first administration was 3975.0 (1842.2) mg (2071.1 [880.9] mg/m^2), and each treatment dose was infused over 120 minutes. The per-patient dose and infusion rate of CPI-613 at various treatment intervals are displayed in Table 2.

Incidence of AKI

Mean (SD) and median values for peak serum creatinine, BUN at the time of peak serum creatinine, nadir complete blood cell counts, and peak electrolyte levels per each time interval are summarized in Table 3. Acute kidney injury developed in 9 (27.3%) participants; of these, 1 (11.1%) patient had AKI stage 1, 4 (44.4%) patients had AKI stage 2, and 4 (44.4%) patients experienced AKI stage 3 (Table 4). Ten episodes of intercurrent illness and hospitalization were noted during the study period; of these, 4 episodes occurred in patients who developed AKI and for which the etiology of kidney injury could arguably be attributed to the acute illness (bacteremia, sepsis, acute respiratory failure, and arrhythmia). The remaining 6 episodes of acute illness (caused by bacterial infection, fungal infection, anasarca, or respiratory failure) were not accompanied by AKI.

Five of the 9 patients who developed AKI had no apparent renal insult identified based on medical chart review; among these, 1 patient had AKI stage 1, 1 patient had AKI stage 2, and 3 patients experienced AKI stage 3. At peak serum creatinine, mean (range) BUN:serum creatinine ratio in the selected 5 cases of AKI was 13.0 (7.0–18.6) mg/dl. Median time to AKI (time from first administration of CPI-613 to peak serum creatinine) was 51.0 (16.0–58.0) days.

Urine studies were available in 3 of the 5 patients who developed unexplained AKI, 2 of whom had AKI stage 3 and 1 had AKI stage 2. Of these, 1 patient developed 1046 mg/g proteinuria and mild hematuria, 1 patient had 300 mg/dl albuminuria and no hematuria, and 1 patient did not have hematuria or albuminuria

Table 2. CPI-613 dose and infusion rate

CPI-613 dose per time interval	*n*	mg	m/kg	mg/m^2
First dose	33	3975.0 (1842.2), 4300.0	50.6 (21.5), 58.4	2071.1 (880.9), 2311.6
First 5 doses	27	19,013.0 (9622.8), 21,250.0	240.2 (109.1), 291.1	9811.6 (4549.2), 11,494.3
First 4 weeks	26	26,107.6 (12,887.4), 27400.0	326.6 (147.8), 364.6	13,370.8 (6107.7), 14,845.4
Weeks 5 through 15	21	49,550.0 (28,354.9), 49550.0	538.5 (306.6), 537.7	21,770.0 (11,888.9), 22,988.5
Weeks 16 through 24	9	44,988.9 (30,160.3), 29000.0	573.9 (456.1), 385.7	23,427.8 (17,804.5), 15,025.9
Weeks 25 through 36	2	101,250.0 (8273.2), 101250.0	1442.9 (230.8), 1442.9	57,307.3 (6506.6), 57,307.3
Weeks 37 through 48	2	83,000.0 (12,445.1), 83000.0	1186.0 (269.6), 1186.0	47,029.0 (8534.1), 47,029.0
Weeks 49 through 72	1	202,500.0	3036.8	117,052.0
Weeks 73 through 96	1	76,800.0	1151.7	44,393.1
CPI-613 infusion rate	*n*	mg × min^{-1}	m/kg × min^{-1}	mg/m^2 × min^{-1}
First dose	33	33.1 (15.4), 35.8	0.4 (0.2), 0.5	17.2 (8.0), 19.2
First 5 doses	27	158.4 (80.2), 177.1	2.0 (0.9), 2.4	81.8 (37.9), 95.8

Data presented as mean (SD), median. *n*, number of patients.

(Figure 1). Two patients who did not develop AKI (as defined by changes in serum creatinine) had urine studies obtained during treatment with CPI-613 and experienced new-onset moderate albuminuria. Of these 2 patients, 1 had mid hematuria and 1 had severe hematuria.

Factors Associated With AKI

Cox proportional hazards regression models were used to test for association between baseline demographics, comorbidities, laboratory data, and CPI-613 initial and cumulative dose and infusion rate with AKI risk (Table 5). Patients who developed AKI thought to be attributable to other causes ($n = 4$) were excluded. The mean (SD) CPI-613 dose accumulated to the date of peak serum creatinine in patients who developed unexplained AKI ($n = 5$) was 24,949.2 (17,462.6) mg/m^2; for those who did not develop AKI ($n = 24$) the mean CPI-613 dose administered to day 58.0 of treatment was 24,845.4 (18,420.1) mg/m^2 ($P = 0.9$). In multivariate analysis, age was associated with AKI development (hazard ratio 2.01 for a 5-year increase in age, 95% confidence interval 1.06–3.79, $P = 0.03$). No associations were detected between sex, race, diabetes, baseline laboratory data (serum creatinine, BUN, hemoglobin, serum lactate dehydrogenase), and initial CPI-613 dose and infusion rate in this cohort.

A total of 727 vital signs were recorded during CPI-613 treatment. Hypotension defined as a MAP decrease by >20% from baseline occurred in 3 patients who did not develop AKI and 3 of the 5 patients who developed unexplained AKI. Among each of these patients, the proportion of events of MAP drops by >20% (out of total vitals recorded per patient) was 41.4%, 16.3%, and 78.3% in those who did not develop AKI, and 11.1%, 6.0%, and 3.7% in those who developed AKI. Hypotension defined as a MAP <65 mm Hg occurred in 1 patient (1 event) who did not develop AKI and 2 patients (3 events) who developed unexplained AKI. Neither form of hypotension (a MAP decrease by >20% from baseline or a MAP <65 mm Hg) occurred within 7 days before the incipient rise in serum creatinine in those who developed AKI. I.v. iodinated contrast was administered to 1 of the 4 patients who developed AKI during an acute illness, 9 days before achieving peak serum creatinine; none of the 5 patients who developed unexplained AKI received i.v. iodinated contrast.

To evaluate whether patients who developed AKI related to CPI-613 therapy had preexisting occult kidney injury, serum creatinine levels obtained 12.1 (4.9) days before initiation of therapy were compared with baseline serum creatinine recorded at the start of treatment. The mean (range) pretreatment serum creatinine levels were 0.9 (0.8–1.0) mg/dl, and the ratio between pretreatment and baseline serum creatinine was 1.2 (1.1–1.2). The absolute eosinophil blood count was compared between those with and without AKI. The mean (SD) absolute eosinophil blood count was 260.0/ml (194.9) in the 5 patients who developed unexplained AKI and 158.9/ml (135.5) in the patients who did not develop AKI ($P = 0.16$).

Renal Outcomes

Follow-up laboratory data were available in 4 of the 5 patients with unexplained AKI. Analyses of kidney function recovery were performed based on the last available serum creatinine measurements (Figure 1). In this subset, mean (range) age at initiation of CPI-613 therapy was 77 (67–85) years, baseline serum creatinine was 0.8 (0.7–0.8) mg/dl, peak serum creatinine was 3.5 (1.2–5.3) mg/dl, and serum creatinine at last check was 1.0 (0.8–1.6) mg/dl obtained 174.8 (25.0–337.0) days after peak serum creatinine. Of these patients, 3 experienced complete recovery of kidney function; whereas 1 patient had partial recovery at the last serum creatinine measurement available 337 days after AKI (Figure 1).

Table 3. Laboratory parameters by time interval following initiation of CPI-613 chemotherapy

Parameter	Weeks 1–4		Weeks 5–12		Weeks 13–24		Weeks 25–36		Weeks 37–48		Weeks 49–72		Weeks 73–96	
	n	Mean (SD), median	*n*	Mean (SD), median	*n*	Mean (SD), median	*n*	Mean (SD), median	*n*	Mean (SD), median	*n*	Mean (SD), median	n	Mean (SD), median
Creatinine, serum (mg/dl)[a]	33	1.3 (1.2), 0.9	27	1.2 (0.8), 1.0	17	1.3 (1.0), 1.0	7	0.9 (0.0), 0.4	5	0.9 (0.1), 1.0	5	0.9 (0.2), 0.9	4	0.8 (0.1), 0.8
BUN (mg/dl)[b]	33	20.8 (13.0), 16.0	27	24.5 (18.2), 22.0	17	21.1 (18.7), 18.0	7	18.0 (4.6), 17.0	5	18.0 (3.2), 20.0	5	15.8 (4.8), 17.0	4	16.5 (3.0), 17.0
Hemoglobin (g/dl)[c]	33	9.7 (2.0), 10.1	27	9.2 (2.1), 9.3	17	9.6 (2.3), 8.1	7	8.4 (2.2), 7.6	5	8.2 (2.1), 7.9	5	7.6 (1.6), 7.2	4	6.7 (0.8), 6.8
Platelets ($\times 10^3$/μl)[c]	33	151.6 (112.3), 142.0	27	133.1 (111.3), 124.0	17	119.2 (85.2), 117.0	7	80.2 (93.4), 50.0	5	49.0 (56.9), 15.0	5	50.6 (50.3), 28.0	4	28.0 (42.8), 9.0
Potassium, serum (mEq/l)[a]	33	4.3 (0.6), 4.3	27	4.5 (0.5), 4.4	17	4.5 (0.4), 4.5	7	4.3 (0.3), 4.3	5	4.2 (0.1), 4.3	5	4.5 (0.1), 4.6	4	4.2 (0.2), 4.3
Bicarbonate, serum (mEq/l)[a]	33	28.2 (2.3), 28.0	27	28.6 (2.1), 28.0	17	27.8 (3.6), 28.0	7	29.0 (3.5), 29.5	5	29.0 (2.8), 28.0	5	28.6 (2.8), 28.0	4	28.0 (2.4), 27.5
Magnesium, serum (mg/dl)[a]	31	2.0 (0.2), 2.0	24	2.0 (0.1), 2.0	12	2.0 (0.2), 2.0	6	2.1 (0.2), 2.1	4	1.9 (0.2), 1.9	4	2.1 (0.05), 2.1	3	2.0 (0.1), 2.1
Phosphorus, serum (mg/dl)[a]	30	3.7 (0.8), 3.6	23	3.9 (0.7), 3.9	11	4.1 (1.2), 3.6	3	3.9 (0.6), 3.7	3	4.0 (0.2), 4.0	2	4.4 (0.2), 4.4	1	4.6 (0.1), 4.6
Lactate dehydrogenase, serum (IU/l)[a]	21	498.9 (1267.2), 201.5	14	222.0 (109.2), 188.5	8	273.1 (149.1), 235.5	5	344.8. (289.6), 222.0	4	207.5 (77.1), 188.5	2	192.0 (14.1), 192.0	4	286.7 (210.5), 213.0
Uric acid, serum (mg/dl)[a]	3	8.0 (3.4), 6.9	4	5.1 (0.5), 5.2	1	3.9 (1.0), 4.5	3	4.6 (0.4), 4.4	2	4.2 (0.7), 4.3	1	4.9	3	5.2 (0.2), 5.1

BUN, blood urea nitrogen; *n*, number of patients.
Data presented as mean (SD), median. Values listed based on [a]peak measurements, [b]concomitant level at peak serum creatinine, and [c]nadir measurements.

DISCUSSION

In this study, 27.3% (9 of 33) of the patients receiving treatment with tumor-targeted antimitochondrial agent CPI-613 developed AKI, of whom 5 patients had CPI-613 agent as the possible cause of AKI, with the larger proportion (4 of 5) experiencing moderate or severe AKI (AKI stage 2 or 3). To date, this is the only study to analyze the incidence of AKI in patients who received CPI-613, an antineoplastic agent with antimitochondrial properties.

Mitochondrial dysfunction is recognized as an important cause of, or contributor to, kidney disease.[13] Primary mitochondrial cytopathies caused by mutations in mitochondrial DNA can cause tubular dysfunctions (e.g., Fanconi syndrome, renal tubular acidosis, aminoaciduria, glycosuria, hypermagnesuria), tubulointerstitial fibrosis and chronic kidney disease (CKD) with low-grade proteinuria; and glomerular disorder with focal segmental glomerulosclerosis and high-grade proteinuria.[14,15] Variants in the promoter region of mitochondrial DNA or in transfer RNA itself inhibit cellular respiration and manifest with chronic tubulointerstitial kidney disease while having normal function in other organs.[16,17] Disease-causing mutations in nuclear DNA can also generate mitochondrial dysfunction with consequential kidney disease. In experimental models of apolipoprotein L1–associated kidney disease, *APOL1* G1 and G2 renal-risk variants induced marked downregulation of enzymes in mitochondrial complexes I to V and suppressed mitochondrial respiration in the tubular epithelium.[18] Coenzyme Q10 is an essential component of the mitochondrial electron-transport chain; its synthesis involves at least 10 different enzymes termed coenzyme Q1 through coenzyme Q10.[19] To date, nuclear DNA mutations in 8 genes (*PDSS1, PDSS2, COQ2, COQ4, COQ6, ADCK3, ADCK4,* and *COQ9*) have been associated with coenzyme Q10 deficiency, which can manifest with encephalomyopathy, ataxia, lactic acidosis, deafness, retinitis pigmentosa, hypertrophic cardiomyopathy, and steroid-resistant nephrotic syndrome.[20]

Besides the pathophysiologic role played by primary mitochondrial dysfunction in CKD, secondary (or acquired) mitochondrial dysfunction also plays a critical role in the pathophysiology of AKI.[7,9] Depletion of adenosine triphosphate in renal tubular epithelial cells as a result of mitochondrial dysfunction was shown to play a critical role in the development of AKI.[21] Experimental therapies that used mitochondria-targeted antioxidants,[22,23] inhibition of mitochondrial fragmentation,[24] and regeneration of mitochondrial mass following AKI[25] point toward a potential future mitochondria-rescue therapy as a therapeutic approach in AKI.

Table 4. AKI incidence in patients with advanced malignancy treated with CPI-613

	AKI, all cases	AKI, probable etiology CPI-613
Outcome, *n* (%)		
Any AKI	9/33 (27)	5/33 (15)
AKI stage 1	1/33 (3) or 1/9 (11)	1/33 (3) or 1/5 (20)
AKI stage 2	4/33 (12) or 4/9 (44)	2/33 (6) or 2/5 (40)
AKI stage 3	4/33 (12) or 4/9 (44)	2/33 (6) or 2/5 (40)
Peak serum creatinine, mg/dl		
Any AKI	2.4 (1.7–5.1)	2.4 (1.8–5.2)
AKI stage 1	1.2	1.2
AKI stage 2	1.9 (1.7–2.1)	1.8 and 2.4
AKI stage 3	5.2 (5.0–5.4)	6.0 and 5.2
Time to AKI, d[a]		
Time to first rise in serum creatinine by >0.3 mg/dl	66.0 (4.0–116.0)	3.0 (1.0–4.0)
Time to peak serum creatinine	74.0 (51.0–118.0)	51.0 (16.0–58.0)
Incidence rate (number of cases per patient-days)		
Any AKI	9/2669 or 0.34/100	5/2340 or 0.21/100
AKI stage 1	1/2117 or 0.05/100	1/2117 or 0.05/100
AKI stage 2	4/2338 or 0.17/100	2/2146 or 0.09/100
AKI stage 3	4/2082 or 0.19/100	2/1945 or 0.10/100

AKI, acute kidney injury; *n*, number of patients.
AKI stage defined using Kidney Disease Improving Global Outcomes criteria. Data presented as n (%) for categorical variables; and median (25th–75th percentile) or raw values for continuous variables.
[a]Time calculated from first dose of CPI-613.

CPI-613 is a novel antineoplastic agent that targets mitochondrial energy metabolism in tumor cells causing apoptosis, necrosis, and autophagy of tumor cells.[3] Side effects previously reported in patients treated with CPI-613 included dysgeusia, hyponatremia, hypocalcemia, lymphopenia, nausea, and vomiting.[5,6] This study specifically analyzed the events of AKI following CPI-613 administration by using the Kidney Disease Improving Global Outcomes guidelines to identify and classify the AKI events, which differ from those based on National Cancer Institute Common Terminology Criteria for Adverse Events criteria.[10,12] After excluding 4 AKI cases in which a potential etiology other than chemotherapy was identified, 5 cases of AKI remained unexplained. If these AKI events were secondary to CPI-613 therapy, it translates into a potential incidence rate of AKI secondary to CPI-613 antimitochondrial therapy of 0.21 per 100 patient-days. Importantly, patients with history of CKD or pretreatment serum creatinine ≥1.5 mg/dl were excluded from enrollment in CPI-613 oncology trials, and none of the 5 patients who developed AKI

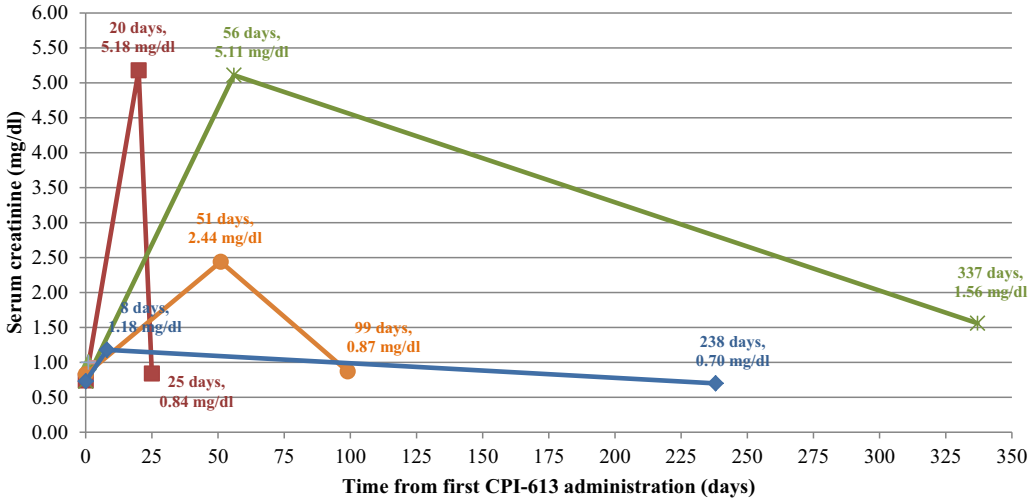

Figure 1. Serum creatinine trend over time in 4 patients who developed unexplained acute kidney injury (AKI). Urine studies obtained at the time of AKI were available in 3 patients. Urine microscopy red blood cell count per high power field (RBC/hpf), white blood cell count per high power field (WBC/hpf), and dipstick urine albumin (albumin mg/dl) or spot urine protein-to-creatinine ratio (UPCR mg/g) were as follows: 4 to 8 RBC/hpf, 0 to 5 WBC/hpf, UPCR 1046 mg/g (red line); 0 to 3 RBC/hpf, 0 to 5 WBC/hpf, albumin 300 mg/dl with specific gravity 1024 (green line); and 0 to 3 RBC/hpf, 0 to 5 WBC/hpf, albumin negative (orange line).

Table 5. Association among baseline demographics, laboratory parameters, CPI-613 dose, and infusion rate with AKI

Parameter	Hazard ratio	95% confidence interval	*P*
Age at first CPI infusion (per 5 years)	2.01	1.06–3.79	0.03
Female	2.42	0.40–14.5	0.33
Black	2.06	0.23–18.6	0.52
Diabetes mellitus	1.03	0.17–6.22	0.97
Serum creatinine (per 0.1 unit)	0.83	0.54–1.28	0.41
Blood urea nitrogen (per 1 unit)	0.97	0.81–1.16	0.74
Hemoglobin (per 1 unit)	0.93	0.71–1.23	0.62
Lactate dehydrogenase (per 10 units)	0.95	0.75–1.20	0.64
First dose CPI-613, mg (per 1000 mg)	0.81	0.51–1.28	0.37
First dose CPI-613, mg/kg (per 10 units)	0.91	0.62–1.33	0.62
First dose CPI-613, mg/m^2 (per 100 units)	0.97	0.88–1.06	0.49
First dose CPI-613, mg/body mass index (per 10 units)	0.96	0.84–1.09	0.50
Infusion rate first CPI-613 dose, mg/kg $\times$ min^{-1} (per 0.1 units)	1.10	0.80–1.51	0.56
Infusion rate first CPI-613 dose, mg/m^2 $\times$ min^{-1} (per 5 units)	1.27	0.64–2.51	0.49
Cumulative CPI-613 dose, mg (per 1000 mg)	0.99	0.97–1.01	0.41
Cumulative CPI-613 dose, mg/kg (per 10 units)	0.99	0.98–1.01	0.47
Cumulative CPI-613 dose, mg/m^2 (per 100 units)	0.998	0.994–1.003	0.43
Cumulative CPI-613 dose, mg/body mass index (per 10 units)	0.998	0.991–1.004	0.46

presumed secondary to CPI-613 therapy had a reported intercurrent illness, hospitalization, or received i.v. iodinated contrast before AKI development. When events of hemodynamic instability were compared, no difference was seen between the AKI and non-AKI groups, suggesting that AKI was not of ischemic etiology. In addition, BUN:serum creatinine ratio calculated for each patient at the time of peak serum creatinine was not elevated, suggesting that the selected AKI events seen during treatment with CPI-613 were not of prerenal etiology.

Although this study does not establish a causal link between CPI-613 administration and AKI, other potential nephrotoxic factors were not identified (e.g., i.v. iodinated contrast, sepsis, hemodynamic compromise, hospitalization, or volume depletion) in 5 of the AKI cases. Two mechanisms through which this drug can lead to kidney injury can be postulated (Figure 2). First, inhibition of KGDH by CPI-613 leads to accumulation of excess reactive oxygen species. In high levels, mitochondrial reactive oxygen species have been implicated in the pathogenesis of toxic, ischemic, and immunologically mediated kidney injury.[26] Second, tricarboxylic acid cycle inhibition by CPI-613 may increase glutamine flux within mitochondria, followed by glutamine deamination and increased ammoniagenesis.[27] Inhibition of KGDH by CPI-613 could lead to excess mitochondrial levels of α-ketoglutarate, which could directly exert cellular toxic effects via apoptosis or indirectly via modification in glutamine and ammonia synthesis in a feedback loop. Increased ammonia generation by nephrons can lead to activation of the alternative complement pathway and increased endothelin-1 and aldosterone levels in the kidney, which may cause renal tubule-interstitial injury.[28] Of note, the activity of KGDH is pH-regulated, being promoted on environment acidification.[29] One study showed that the concentration of α-ketoglutarate in rat kidneys significantly decreased in an acidic milieu, owing to a pH-induced increase of KGDH activity.[30] In addition, there is variation along the nephron in energy generation, with tubular epithelial cells generating adenosine triphosphate mainly via oxidative phosphorylation, whereas podocytes and endothelial cells have more flexibility in their glycolytic capacity to generate energy.[31,32] Therefore, renal tubular epithelial cells might be more susceptible to imbalanced levels of α-ketoglutarate, glutamine, and adenosine triphosphate synthesis than other tissues due to innate metabolic and acidic conditions present in the renal tissue. The heterogeneity in generating adenosine triphosphate and the reliance on glutamine influx and oxidative phosphorylation to meet varying metabolic activities in different segments of the kidney might play an important role in the development of CPI-613–mediated mitochondrial dysfunction in the kidneys.

Our study had limited ability to describe the trajectory of kidney function following AKI events during treatment with CPI-613. Four (of the 5) selected cases of AKI had complete or partial recovery of kidney function following withdrawal of CPI-613 treatment. The seemingly good recovery rate of kidney function is encouraging. Nevertheless, AKI, and particularly moderate or severe AKI, poses a risk of future CKD development.[33,34] This consideration would be of relevance should antimitochondrial agents be used more often in patients with less advanced malignancies and longer survival, potentially introducing the risk of

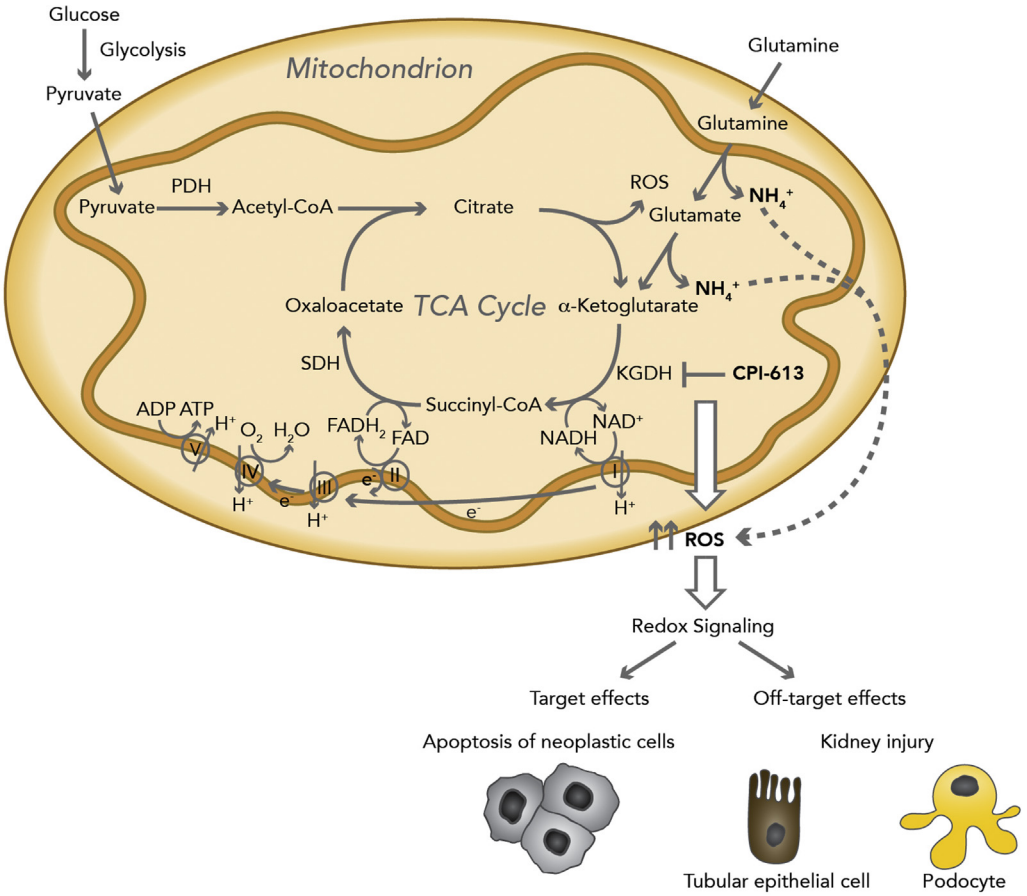

Figure 2. Schematic of hypothetical mechanism for CPI-613–induced kidney injury. Under physiologic conditions, the tricarboxylic acid (TCA) cycle is coupled with oxidative phosphorylation in the mitochondria to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. Most fuel molecules enter the TCA cycle as acetyl coenzyme A (Acetyl-CoA); glutamine may also enter the TCA cycle by converting into α-ketoglutarate. Reactive oxygen species (ROS) form as a byproduct of mitochondrial cellular respiration. At low levels, mitochondrial ROS have physiological functions by regulating cellular proliferation and cellular survival in response to stress conditions. We hypothesize 2 synergistic mechanisms through which CPI-613 can lead to kidney injury: increased ROS and ammonia (NH_4^+) production. Inhibition of α-ketoglutarate dehydrogenase (KGDH) by CPI-613 leads to excess ROS production, which in turn causes cellular damage by inducing nuclear and mitochondrial DNA damage and promoting cell apoptosis. These effects are desirable within neoplastic cells, but are deleterious for the surviving nephrons. Furthermore, TCA cycle inhibition with CPI-613 may increase glutamine flux through the mitochondrial pathway and promotes ammoniagenesis. The process of ammoniagenesis occurs throughout most tubular epithelial cells from glutamine metabolism, which produces 2 NH_4^+ for each glutamine metabolized. Excess NH_4^+ production can lead to tubulointerstitial injury via alternative complement pathway activation and increased endothelin-1 levels in the kidneys. Increased NH_4^+ levels within renal tubular epithelial cells may increase the susceptibility to ROS-mediated cellular damage, creating an amplification loop of cellular toxicity. FAD, flavin adenine dinucleotide; $FADH_2$, flavin adenine dinucleotide high-energy electron carrier; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide high-energy electron carrier; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase.

CKD development. In this study, age was the only factor associated with unexplained AKI during treatment with CPI-613. Our study lacked power to detect other potential risk factors due to the small cohort size. Moreover, the oncology protocols had amended the dosing scheme for CPI-613 in reaction to AKI events, which further limited our ability to detect an association between the CPI-613 dose and development of unexplained AKI. Importantly, all patients treated with CPI-613 had advanced malignancy that was refractory to previously administered standard chemotherapy agents. Although not evaluated in this study, a 2-hit mechanism for the development of AKI can be speculated, with the first hit corresponding to (subclinical) kidney insult during previous chemotherapy regimens and/or acute maladies, which primed the renal cellular metabolism to the development of clinical AKI at a second-hit exposure during treatment with mitochondria-targeted agents. In addition, urine studies were available in very few patients and lacked studies of interest (e.g., urine eosinophils), which limited the ability to comprehensively characterize the risk of AKI during treatment with CPI-613. In particular, information on *de novo* proteinuria would have been of interest, because many of the renal phenotypes associated with primary mitochondrial cytopathies manifest with focal

segmental glomerulosclerosis and proteinuria. We note that the peak absolute eosinophil blood count was not significantly different between those with and without AKI, but the interpretation of eosinophil count in this study is impeded by concomitant leukopenia, which was present in most patients. Finally, none of these patients underwent kidney biopsy, likely due to the advanced nature of underlying malignancy.

In conclusion, we discovered incident AKI in 9 patients treated with the chemotherapy drug CPI-613 during 3 open-label phase II trials at our medical center. Of these, 5 cases lacked an evident cause of AKI based on chart review. CPI-613 is an antimitochondrial agent with potential adverse effects on kidney function and could not be ruled out as the cause of AKI in these 5 patients. Future studies with larger cohorts, better biochemical phenotyping, kidney biopsy, and longer follow-up are warranted to further analyze the association between CPI-613 and incident AKI.

DISCLOSURE

All the authors declared no competing interests.

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