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Brincidofovir Is Not a Substrate for the Human Organic Anion Transporter 1: A Mechanistic Explanation for the Lack of Nephrotoxicity Observed in Clinical Studies

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Background: Brincidofovir (BCV) is an orally bioavailable lipid conjugate of cidofovir (CDV) with increased in vitro potency relative to CDV against all 5 families of double-stranded DNA viruses that cause human disease. After intravenous (IV) administration of CDV, the organic anion transporter 1 (OAT1) transports CDV from the blood into the renal proximal tubule epithelial cells with resulting dose-limiting nephrotoxicity.

Objective: To study whether OAT1 transports BCV and to evaluate the pharmacokinetic and renal safety profile of oral BCV compared with IV CDV.

Methods: The cellular uptake of BCV and its major metabolites was assessed in vitro. Renal function at baseline and during and after treatment in subjects in BCV clinical studies was examined.

Results: In OAT1-expressing cells, uptake of BCV and its 2 major metabolites (CMX103 and CMX064) was the same as in mock-transfected control cells and was not inhibited by the OAT inhibitor probenecid. In human pharmacokinetic studies, BCV administration at therapeutic doses resulted in detection of CDV as a circulating metabolite; peak CDV plasma concentrations after oral BCV administration in humans were <1% of those observed after IV CDV administration at therapeutic doses. Analysis of renal function and adverse events from 3 BCV clinical studies in immunocompromised adult and pediatric subjects indicated little to no evidence of associated nephrotoxicity. Over 80% of subjects who switched from CDV or foscarnet to BCV experienced an improvement in renal function as measured by maximum on-treatment estimated glomerular filtration rate.

Conclusions: The lack of BCV uptake through OAT1, together with lower CDV concentrations after oral BCV compared with IV CDV administration, likely explains the superior renal safety profile observed in immunocompromised subjects receiving BCV compared with CDV.

Key Words: brincidofovir, cidofovir, OAT1, renal function, dsDNA virus

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INTRODUCTION

Brincidofovir (BCV) is an orally bioavailable lipid conjugate of cidofovir (CDV) (Fig. 1) that preferentially delivers the antiviral moiety CDV diphosphate to the intracellular space, avoiding the significant nephrotoxicity that limits the effectiveness of CDV. CDV is administered by intravenous (IV) infusion and provides high concentrations of circulating drug in the plasma. CDV is then actively transported into renal proximal tubule epithelial cells by the human organic anion transporter 1 (OAT1).[1–3] Dose-limiting nephrotoxicity after IV administration of CDV has been demonstrated in both animals and humans.[1–3] Severe nephrotoxicity resulting in renal failure and death has occurred after a single IV administration of CDV.[4] Recommendations to ameliorate CDV-associated nephrotoxicity include prehydration with normal saline and coadministration of probenecid, an inhibitor of OATs.[4–8] Despite these protective measures, nephrotoxicity remains a significant risk and limits the clinical utility of CDV.

The proprietary BCV molecule, with its lipid moiety, is designed to mimic the natural lipid lysophosphatidylcholine and to use endogenous lipid uptake pathways to achieve intracellular concentrations of BCV that provide antiviral activity against a broad range of double-stranded DNA (dsDNA) viruses. In cell-based assays, BCV demonstrated activity against all 5 families of dsDNA viruses that cause human disease, including orthopoxviruses, polyomaviruses, human herpesviruses, human papillomaviruses, and adenoviruses (AdVs).[9] BCV has also demonstrated antiviral activity in animal models of dsDNA virus infection, including

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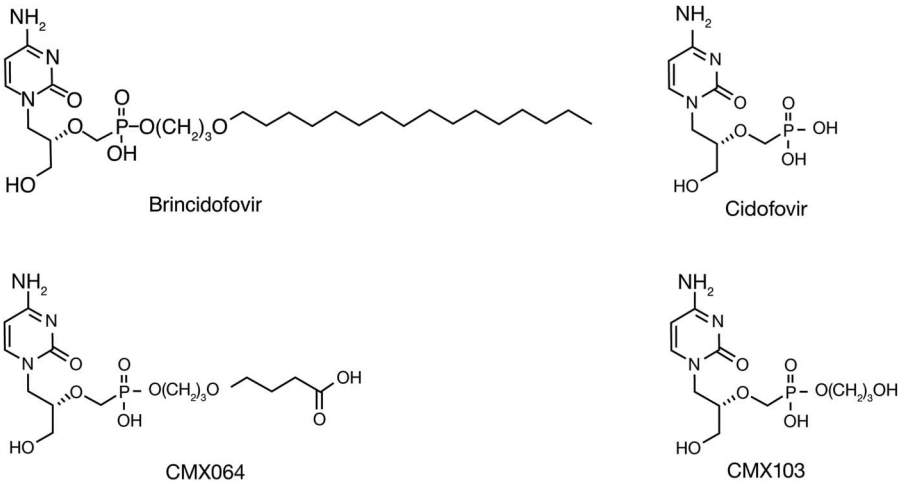

FIGURE 1. Chemical structures of BCV and metabolites.

herpesviruses,[10,11] AdVs,[12] and poxviruses.[13–16] BCV (Chimerix, Durham, NC) is in late-stage clinical development for the treatment of serious AdV infection [the AdVise study, CMX001-304 (NCT02087306)] and for the prevention of cytomegalovirus (CMV) infection in hematopoietic cell transplant (HCT) recipients [the recently completed SUPPRESS study CMX001-301 (NCT01769170)], and is in pivotal efficacy studies for the treatment of smallpox in well-described animal models under the Food and Drug Administration's Animal Rule.

Herein, we present data confirming the lack of in vitro uptake of BCV and its major metabolites by OAT1 and summarize the pharmacokinetic (PK) and renal safety profiles observed after BCV administration in clinical studies.

MATERIALS AND METHODS

Human OAT-Mediated Cellular Uptake of BCV and Metabolites

Epithelial Madin–Darby canine kidney type 2 (MDCK-II) cells were grown on semipermeable filters and transiently transfected with OAT1, OAT3, or vector only (Optivia Biotechnology, Menlo Park, CA). BCV and its major metabolites CMX103 (3-hydroxypropyl ester of CDV) and CMX064 [4-(3-propoxy) butanoic acid ester of CDV][17] or CDV (Gilead Sciences, Foster City, CA) were added to the basolateral side of the cell monolayer ($n = 3$–4 replicates/condition), with or without probenecid (100 μM). A higher incubation concentration of each of the metabolites (25 versus 5 μM) was used to obtain detectable concentrations of these more polar compounds in mock-transfected cells. After a 5-minute incubation, drugs were removed from cells, and the cells were rinsed, extracted, and analyzed using high-performance liquid chromatography–tandem mass spectrometry.

Net OAT-mediated uptake was determined from total uptake in OAT-expressing cells minus uptake in vector-treated control cells. The minimum established acceptance criteria for OAT1 activity of >0.71 pmol·min^{-1}·cm^{-2} for *p*-aminohippurate, with >70% inhibition in the presence of probenecid, were met in these studies. The minimum established acceptance criteria for OAT3 activity of >0.62 pmol·min^{-1}·cm^{-2} for estrone-3-sulfate, with >76% inhibition in the presence of probenecid, were also satisfied. Statistical significance ($P < 0.05$) of the cellular uptake of compounds in transfected versus control cells, or ± probenecid, was assessed using an unpaired *t* test. Statistical analysis of multiple parameters was performed using analysis of variance (see **additional assay details**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A167).

Clinical Studies

All studies were approved by an institutional review board, and informed consent was obtained before performing any study procedures.

Study CMX001-201 (NCT00942305; phase 2 CMV Prevention Study)[18] enrolled high-risk adult allogeneic HCT recipients who had antibody evidence of previous CMV infection. In a double-blind fashion, dosing in 5 sequential BCV dose cohorts [40, 100, or 200 mg once weekly (QW), or 100 or 200 mg twice weekly (BIW)] with embedded placebo was initiated after confirmed engraftment within 30 days of HCT, and dosing was continued for up to 11 weeks. One hundred seventy-one subjects received BCV and 59 received placebo.

Study CMX001-202 (NCT01241344; phase 2 AdV Preemptive Treatment Study)[19] enrolled adult (18–70 years old) and pediatric (7–17 years old) allogeneic HCT recipients who had asymptomatic AdV viremia. Thirty subjects were randomized to receive one of 2 BCV dosing regimens (4 mg/kg QW or 2 mg/kg BIW for pediatric subjects, and 200 mg QW or 100 mg BIW for adult subjects), and 18 subjects were randomized to receive placebo. Randomization to study treatment (BCV versus placebo) was blinded; randomization to dosing frequency was unblinded. Subjects received between 6 and 12 weeks of blinded study treatment, followed by 4 weeks of post-treatment follow-up. Subjects who experienced an increase in AdV viral load or who developed probable or definitive AdV disease were offered up to 12 weeks of open-label BCV BIW. Subjects originally assigned to placebo but who switched to open-label BCV therapy were included in the BCV analysis set.

Study CMX001-350 (NCT01143181; Expanded Access Study) was a multicenter, open-label, expanded access study of the safety and antiviral activity of BCV. The study enrolled 210 subjects who had serious or immediately life-threatening diseases or conditions caused by dsDNA viruses for which there was no alternate therapy; most subjects were treated for CMV or AdV infection. Sixty-eight pediatric subjects (3 months–12 years old) received BCV 4 mg/kg QW or 2 mg/kg BIW (not to exceed 200 mg/wk) as a solution or tablet. A total of 142 adolescent and adult subjects (13–78 years old) received BCV. Doses were adjusted during the study from 150 to 300 mg BIW in the original protocol, to 200 or 300 mg BIW in amendment 1, and subsequently to 200 mg/wk (either 200 mg QW or 100 mg BIW; not to exceed 4 $mg \cdot kg^{-1} \cdot wk^{-1}$) in amendment 2. Subjects were treated for up to 6 months. Most subjects were HCT (72%) or solid organ transplant (16%) recipients, and 25% were enrolled on the basis of nephrotoxicity attributed to their previous antiviral medication (CDV or foscarnet).

Pharmacokinetics

The absolute bioavailability of BCV is not currently known but is estimated to be approximately 25% (allometric scaling; data on file). The in vitro plasma protein binding was >99%, with no observed change in plasma binding in subjects with hepatic impairment (data on file). Metabolism is the primary route of elimination for BCV, with metabolites approximately equally excreted in urine and feces within 3 days after oral administration.[17] The major metabolites in excreta included the oxidative metabolites CMX103 and CMX064 and the hydrolysis metabolite CDV. The major circulating metabolites [>10% of total drug-related area under the curve (AUC)] included CMX103 and CMX064.[17]

BCV and CDV plasma concentrations were measured using a validated high-performance liquid chromatography–tandem mass spectrometry method.[20] Calibration standard concentrations for BCV ranged from 5 ng/mL to 1500 ng/mL. The interassay accuracy of the analytical quality controls (% bias) was −0.8%–9.0% with an interassay precision (% coefficient of variation) of 3.7%–25.9%. Calibration standard concentrations for CDV ranged from 2.5 to 750 ng/mL. The interassay accuracy of the analytical quality controls (% bias) was −2.7% to 6.7% with an interassay precision (% coefficient of variation) of 3.7%–30.4%. Incurred sample reanalysis was performed on approximately 10% of the total number of samples analyzed. The incurred sample reanalysis was acceptable, with >98% of the BCV and CDV concentrations meeting the acceptance criterion (see **additional assay details**, **Supplemental Digital Content 2**, http://links.lww.com/TDM/A168).

Serial PK blood samples (predose, and 1, 4, 8, 12, 24, 48, and 72 hours postdose) were drawn on day 1 week 1 (AdV Preemptive Treatment and Expanded Access studies) and at week 6 (AdV Preemptive Treatment Study) during randomized treatment or open-label therapy. Noncompartmental plasma PK parameters were calculated using Phoenix WinNonlin, version 6.2 or 6.3 (Certara, St. Louis, MO). For purposes of BCV and CDV exposure comparisons, the following PK parameters were used: for BCV, the partial AUC that captured most of the detectable plasma concentrations from time 0–24 hours (AUC_{0-24}), which captured an average of 87% of AUC from time 0 extrapolated to infinity (AUC_{inf}) in subjects for whom both AUCs were available; and for CDV, the partial AUC from time 0–72 hours (AUC_{0-72}), which captured an average of 79% of AUC_{inf} in subjects for whom both AUCs were available. Molar concentration comparisons were converted using a molecular weight of 561 and 279 daltons (Da) for BCV and CDV, respectively.

Assessment of Renal Function

Parameters of renal function [ie, serum creatinine and estimated glomerular filtration rate (eGFR)] and adverse events (AEs) relating to renal toxicity were compiled. Changes from baseline (BL) for serum creatinine and eGFR were used to describe renal function. eGFR was determined based on age [<18 years old, Schwartz formula[21]; and ≥18 years old, Modification of Diet in Renal Disease equation (4 inputs; MDRD4[22])]. Renal function was assessed at BL, during the treatment phase (frequency every 2 weeks), and during the posttreatment follow-up period.

For the phase 2 CMV Prevention Study, renal function was assessed at BL, during the treatment phase, and 1 week posttreatment. A comparison was then performed between treatments (BCV and placebo). Subjects administered BCV 200 mg BIW had a higher frequency of gastrointestinal AEs, primarily diarrhea, leading to early treatment discontinuation.[18] Enrollment in this cohort was discontinued; thus, subjects from this cohort were not included in this analysis.

For the phase 2 AdV Preemptive Treatment Study, the minimum on-treatment eGFR and last on-treatment eGFR were compared with BL values in 26 pediatric subjects. eGFR was calculated for each subject and assigned to one of the 5 stages of chronic kidney disease (CKD) per National Kidney Foundation guidelines.[23] Stages 1 and 2 were combined, as were stages 4 and 5, resulting in 3 eGFR categories for analysis: ≥60 (stages 1 and 2), 30–59 (stage 3), and ≤29 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (stages 4 and 5). This analysis was not performed in adult subjects because of the small number of subjects with available eGFR data ($n \leq 5$) in this study.

In subsets of pediatric and adolescent (<18 years old; $n = 52$) and adult ($n = 120$) subjects from the Expanded Access Study, changes in renal function were assessed based on changes in CKD stage as described for the AdV Preventive Treatment Study. In a subset of subjects who had received CDV or foscarnet before enrollment, improvement in renal function was assessed by comparing maximum on-treatment eGFR to BL eGFR. Renal AEs consistent with possible nephrotoxicity (ie, renal impairment or failure, and azotemia) were also examined.

All AEs were graded according to the National Institutes of Health/National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.02. An AE was considered serious (SAE) if it resulted in death, was life threatening, required or prolonged in-patient hospitalization, resulted in a congenital anomaly or birth defect, or led to persistent or significant disability.

RESULTS

Human OAT-Mediated Cellular Uptake of BCV and Metabolites

Incubation of BCV with cultured OAT1-expressing MDCK-II cells resulted in a rate of uptake similar to that observed in incubation with mock-transfected (control) cells (mean ± SD: 15.3 ± 5.4 versus 15.6 ± 4.3 pmol·min^{-1}·cm^{-2}). Accordingly, the net BCV uptake due to OAT1 was approximately 0 (Fig. 2). Uptake of BCV by OAT1-expressing cells was not affected by coincubation with probenecid (Fig. 2). In contrast, significantly greater uptake of CDV, approximately 8-fold, was observed in OAT1-expressing cells compared with uptake in control cells (10.7 ± 1.56 versus 1.93 pmol·min^{-1}·cm^{-2}; $n = 2$; $P < 0.05$), resulting in a net CDV uptake due to OAT1 of approximately 9 pmol·min^{-1}·cm^{-2} (Fig. 2). In the presence of probenecid, uptake of CDV was significantly reduced by approximately 75% (Fig. 2) compared with uptake in OAT1-expressing cells without probenecid ($P < 0.05$), confirming that CDV uptake was mediated by OAT1.

No significant difference in uptake was observed in OAT1-expressing cells for the major BCV metabolites CMX103 and CMX064 relative to control cells (CMX103: 3.03 ± 1.18 versus 2.18 ± 1.41 pmol·min^{-1}·cm^{-2}; CMX064: 2.01 ± 1.18 versus 1.91 ± 0.907 pmol·min^{-1}·cm^{-2}; Fig. 2). Uptake rates of CMX103 and CMX064 by OAT1-expressing cells were not significantly affected by coincubation with probenecid (Fig. 2).

There was no difference in BCV uptake between cultured OAT3-expressing MDCK-II cells and mock-transfected control cells (12.8 ± 2.4 versus 12.1 ± 2.9 pmol·min^{-1}·cm^{-2}). Similar results were found for the BCV metabolites CMX103 and CMX064. CDV uptake was also not significantly enhanced in OAT3-expressing MDCK cell monolayers relative to mock-transfected cells (14.8 ± 2.9 versus 11.8 ± 4.8 pmol·min^{-1}·cm^{-2}), nor was uptake in OAT3-expressing cells reduced by probenecid.

Clinical Studies

Pharmacokinetics

In the phase 2 AdV Preemptive Treatment Study, plasma concentrations of BCV and CDV were assessed after administration of a single oral dose of BCV in pediatric subjects (Table 1). Peak BCV concentrations were approximately 20-fold higher (10-fold higher on a molar basis) than mean CDV maximum concentration (C_{max}), although differences in partial AUCs were not as substantial (~30%–70% lower CDV $AUC_{0–72}$ on a molar basis compared with BCV $AUC_{0–24}$). In general, BCV PK parameters after multiple administrations to pediatric subjects determined at week 6 were similar to those obtained at week 1 (data not shown). For CDV, a slight trend toward increasing C_{max} and $AUC_{0–72}$ was observed at week 6 relative to day 1. The week 6 to day 1 ratios of C_{max} or AUC values were <2.

For the Expanded Access Study, BCV and CDV PK parameters were assessed after a single administration of BCV to pediatric and adult subjects (Table 1). Mean BCV C_{max} and AUC increased in proportion to dose over the range of BCV doses administered to pediatric subjects (2–4 mg/kg: 1.8- to 2.4-fold increase) and adult subjects (100–200 mg: 1.7- to 1.8-fold increase). Median BCV time-to-maximum concentration (T_{max}) was 4 hours and plasma half-life ($t_{½}$) was 8–10 hours in both pediatric and adult subjects. In general, BCV PK parameters determined in pediatric subjects after administration of 2 and 4 mg/kg BCV doses were similar to those determined in adult subjects after administration of 100 and 200 mg BCV, respectively.

As observed in the phase 2 AdV Preemptive Treatment Study, the mean BCV C_{max} was approximately 10- to 20-fold higher than CDV C_{max} (5- to 10-fold higher on a molar basis), although little to no difference in partial AUC was observed (on a molar basis). Median T_{max} for CDV was 12–24 hours in both dose groups in pediatric and adult subjects. CDV $t_{½}$ was estimated in a small subset of pediatric subjects and ranged

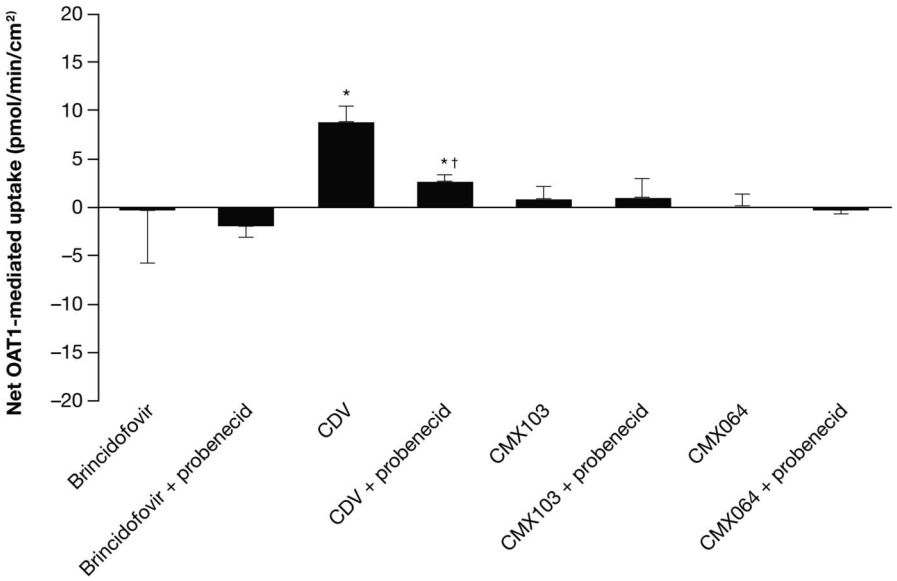

FIGURE 2. Net OAT1-mediated uptake of BCV, CDV, CMX103, and CMX064 in the absence or presence of probenecid. Net OAT1-mediated uptake was determined from total uptake in OAT1-expressing cells minus uptake in mock-transfected control cells after incubation with 5 μM BCV, 25 μM CDV, 25 μM CMX103, or 25 μM CMX064 for 5 minutes in the presence or absence of 100 μM probenecid. Data are mean and SD of triplicate or quadruplicate samples from one experiment. *$P < 0.05$ versus mock-transfected cells; †Significantly lower uptake in the presence of probenecid, a nonspecific inhibitor of OAT1.

TABLE 1. Summary of PK Parameters for BCV and CDV After a Single Administration of BCV to Pediatric and Adult Subjects

			BCV					
Study	**Age Group**	**BCV Dose**	***n***	**C_{max}, ng/mL**	**T_{max},* h**	**$t_{1/2}$, h**	**AUC_{0-24}, ng·h·mL^{-1}**	**AUC_{inf}, ng·h·mL^{-1}**
AdV preemptive treatment	Ped.	2 mg/kg	17	318 (296)	4.0 (1.0–12)	8.9† (4.2)	2808 (2100)	4273† (3010)
		4 mg/kg	9	542 (451)	4.0 (1.0–8.0)	8.3‡ (3.6)	4130 (3380)	5771‡ (5140)
Expanded access	Ped.	2 mg/kg	29	259 (312)	4.0 (1.0–13.5)	7.8 (4.7)	2593 (2150)	3311 (3450)
		4 mg/kg	25	621 (551)	4.0 (1.0–14)	7.8 (5.3)	4836 (3643)	5735 (4277)
	Adult	100 mg	65	290 (275)	4.1 (1.2–25)	8.0 (5.2)	2802 (2545)	2962 (3129)
		200 mg	26	531 (351)	4.1 (3.9–49)	9.5 (4.9)	4768 (3327)	5496 (4387)
			CDV					
Study	**Age Group**	**BCV Dose**	***n***	**C_{max}, ng/mL**	**T_{max},* h**	**$t_{1/2}$, h**	**AUC_{0-72}, ng·h·mL^{-1}**	**AUC_{inf}, ng·h·mL^{-1}**
AdV preemptive treatment	Ped.	2 mg/kg	13	18.1 (12.0)	12 (8.0–72)	NC	1093 (805)	NC
		4 mg/kg	7	25.2 (9.98)	12 (8.0–48)	NC	1190 (594)	NC
Expanded access	Ped.	2 mg/kg	19	21.8 (13.6)	24 (7.9–72)	34.9§ (11.4)	1228 (807)	NC
		4 mg/kg	22	25.7 (10.4)	12 (4.0–48)	44.3¶ (18.4)	1125 (393)	NC
	Adult	100 mg	60	30.4 (25.3)	24 (7.9–72)	NC	1668‖ (1522)	NC
		200 mg	22	43.4 (25.8)	23 (6.8–96)	NC	2155 (1308)	NC

Values are mean (SD) except where noted otherwise. For purposes of PK comparisons, pediatric subjects were defined as <18 years old. Adult subjects were defined as ≥18 years old. Also, *n* indicates the number of subjects in the PK analysis set for each dose group; *n* for individual parameters within each group may have been lower.

*Values are median (range).

†Value obtained from 10 subjects.

‡Value obtained from 5 subjects.

§Value obtained from 4 subjects.

¶Value obtained from 12 subjects.

‖Value obtained from 34 subjects.

AUC_{0-24}, AUC from time 0–24 hours; AUC_{0-72}, AUC from time 0–72 hours; AUC_{inf}, AUC from time 0 extrapolated to infinity; C_{max}, maximum concentration; NC, not calculated due to insufficient data points to characterize the terminal elimination phase; Ped., pediatric; $t_{½}$, half-life; T_{max}, time-to-maximum concentration.

from 35–44 hours, but CDV $t_{½}$ was not calculable in most subjects because of insufficient duration of sampling.

Assessment of Renal Function

Parameters of AEs related to renal function were reviewed from the 3 completed clinical studies with BCV discussed above. Seventy kidney-related AEs (renal events) were reported in 62 of 420 (14.8%) subjects who received BCV. Most renal events (48, 69%) were not reported as SAEs, and 32 (46%) were mild or moderate in severity (CTCAE grade 1 or 2). Five (7%) renal events in 5 (8%) subjects resulted in early discontinuation of BCV. Four (6%) renal failure events were considered possibly related to BCV by the reporting investigator. The Data Safety Monitoring Boards reviewed all cases of renal events reported and did not recommend changes to study conduct.

In the phase 2 CMV Prevention Study, adult recipients of an allogeneic HCT who were at high risk of CMV reactivation received BCV or placebo. BCV-treated subjects experienced a dose-related improvement in renal function parameters (serum creatinine and eGFR) during study drug administration as compared with placebo recipients. Compared with BL, serum creatinine levels decreased in subjects receiving BCV 100 mg BIW and increased in placebo recipients at treatment week 10 (−5.6 versus 14.0 μM; P = 0.03, Satterthwaite t test); this improvement continued through posttreatment week 1 (−4.7 versus 10.8 μM; P = 0.03). Mean change from BL in eGFR showed statistically significant improvement for subjects receiving BCV 100 mg BIW compared with pooled placebo recipients at treatment weeks 8 and 10, and at 1 week posttreatment (Fig. 3). There were 17 renal events reported in 16 subjects receiving BCV, and 6 renal events reported in 6 subjects receiving placebo. None of the renal events were considered related to study treatment by the reporting investigator, and most events (12 of 17) in subjects receiving BCV were mild to moderate in severity (grade 1 or 2). Four (24%) of the 17 renal events reported for subjects receiving BCV were severe (grade 3), and 1 (6%) severe event (acute renal failure, grade 3) resulted in early discontinuation of BCV 40 mg QW. Two severe events resolved with continued BCV dosing of 200 mg QW and 100 mg BIW. The remaining severe event occurred approximately 4 weeks after the last dose of BCV 40 mg QW. There was a serious, life-threatening (grade 4) case of renal failure reported 2 weeks after the last dose of BCV in a subject receiving 100 mg BIW.

For the phase 2 AdV Preemptive Treatment Study, laboratory values and changes from BL for renal parameters including blood urea nitrogen, serum creatinine, eGFR, and urinalysis demonstrated no clear patterns within or between treatment groups (intention-to-treat population, n = 48). There were 12 renal events reported in 10 subjects receiving BCV, and 1 renal event reported in the placebo group. None of the events were considered possibly related to study treatment by the reporting investigator, and there were no early discontinuations for subjects receiving BCV. Six (50%) of 12 renal

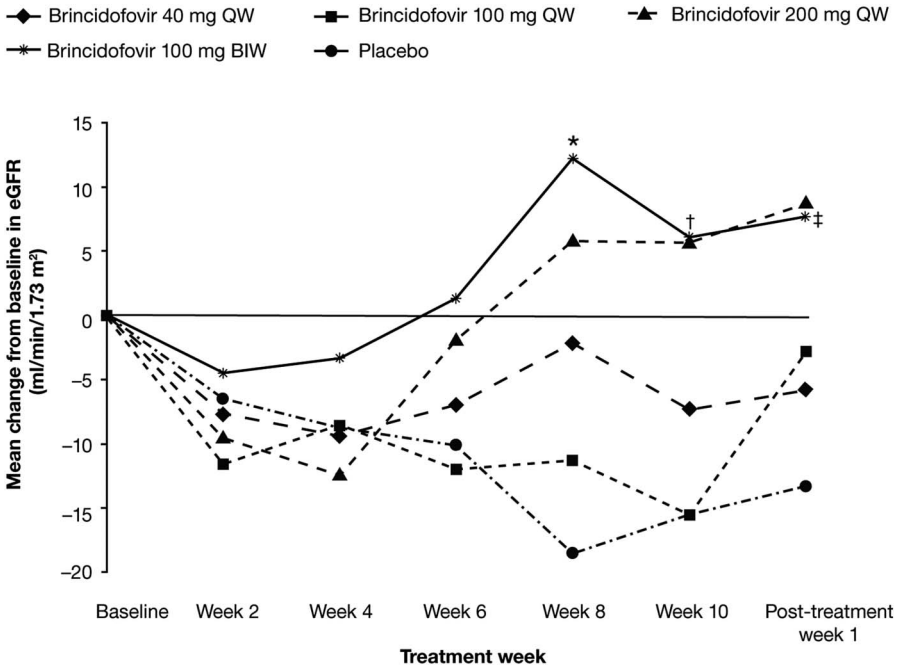

FIGURE 3. Mean change from BL in eGFR in virally infected subjects administered BCV (CMX001) or placebo (CMV Prevention Study). Mean change from BL in eGFR as calculated through the Modification of Diet in Renal Disease equation (4 inputs) is presented by visit and dose. *P*-values (Satterthwaite *t* test) for CMX001 100 mg BIW relative to pooled placebo: *Week 8, $P = 0.0013$ (CMX001 $n = 31$, placebo $n = 36$); †week 10, $P = 0.0103$ (CMX001 $n = 21$, placebo $n = 21$); ‡posttreatment week 1, $P = 0.0025$ (CMX001 $n = 49$, placebo $n = 57$).

events in subjects receiving BCV were mild to moderate in severity (grade 1 or 2). Four renal failure events in subjects receiving BCV 200 mg QW or 100 mg BIW were severe (grade 3), but none were reported as SAEs. Additionally, 2 serious, life-threatening (grade 4) acute renal failure events were reported in subjects receiving BCV 200 mg QW or 100 mg BIW. The subject receiving BCV 100 mg BIW had the serious grade 4 renal event approximately 4 weeks after the last dose.

To further examine the effects of BCV on renal function in pediatric subjects, an analysis of eGFR from 26 pediatric subjects enrolled in the AdV Preemptive Treatment Study was conducted; all enrolled subjects had evidence of AdV replication in the blood. A decrease of at least one CKD stage was used as a surrogate for improved renal function.[23] As shown in Figure 4, >80% of the pediatric subjects experienced stable or improved renal function as demonstrated by an on-treatment CKD stage that remained the same or improved (ie, lower than BL) during the study or at the last on-treatment assessment.

In the Expanded Access Study, a large percentage of pediatric and adult subjects experienced stable or improved renal function (ie, same or lower CKD stage). Of the 52 pediatric subjects with on-treatment eGFR measurements, >80% demonstrated stable or improved renal function with an on-treatment CKD stage that remained the same as or

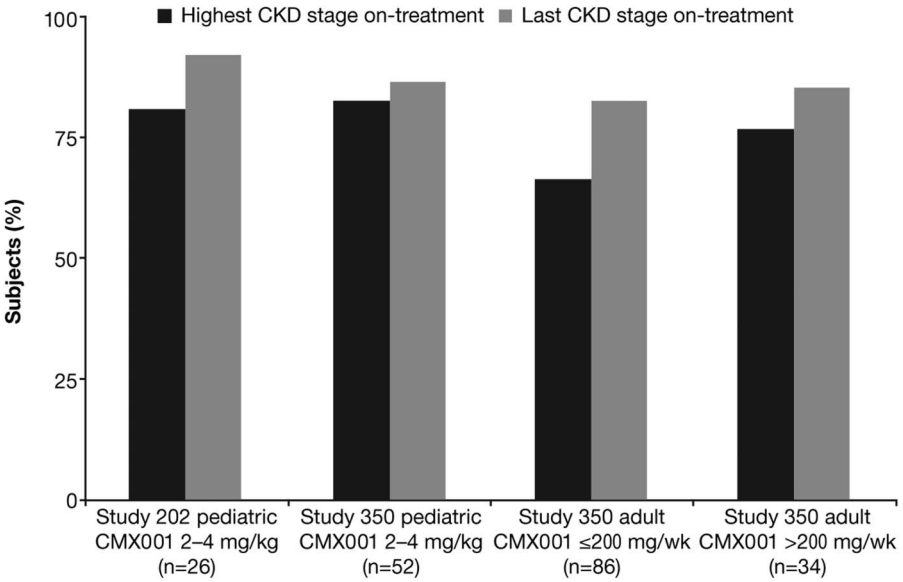

FIGURE 4. Percentages of subjects with stable or improved renal function based on CKD stage after multiple administrations of BCV (CMX001). eGFR was calculated for each subject and assigned to one of the 5 stages of CKD: stages 1 and 2, eGFR ≥60 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$; stage 3, 30–59 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$; and stages 4 and 5, ≤29 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$. Subjects were judged to have stable or improved renal function if the highest on-treatment CKD stage or last on-treatment CKD stage was the same as or lower than BL. Data are from the AdV Preemptive Treatment Study (Study 202 Pediatric) and the Expanded Access Study (Study 350 Pediatric, Study 350 Adult). Data from the Expanded Access Study are separated by dose for the adult subjects. wk, week.

lower than BL (Fig. 4). In the 34 adult subjects who received BCV >200 mg/wk, >75% of subjects demonstrated stable or improved renal function and approximately two-thirds of the 86 subjects who received BCV dose ≤200 mg/wk demonstrated stable or improved renal function (Fig. 4).

In the Expanded Access Study, 41 renal events were reported in 36 (17%) of 210 subjects enrolled. Subjects were required to have a life-threatening viral infection with no alternative therapy, and thus had often been treated with antivirals with known nephrotoxicity, such as CDV and foscarnet, before initiating treatment with BCV. Enrolled subjects also frequently received multiple nephrotoxic medications, including ganciclovir, foscarnet, and tacrolimus, during the BCV dosing period. Four (10%) renal failure events resulted in early discontinuation of BCV: 2 were serious renal failure events and were considered possibly related to BCV by the reporting investigator. There were 2 additional renal events that did not result in discontinuation but were considered possibly related to BCV by the reporting investigator. There was 1 serious renal event that was fatal and was considered not related to BCV treatment by the investigator.

Renal safety parameters in subjects who had received CDV or foscarnet treatment before BCV initiation in the Expanded Access Study were compared with renal parameters in subjects who had not reported CDV or foscarnet use. For the 37 subjects switching from CDV to BCV, the median BL eGFR was 87 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$. Approximately 85% had a maximum on-treatment eGFR greater than BL after switching to BCV, with more than 50% of subjects showing a clinically significant improvement of $>20\ mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (Fig. 5). When this subject group was subdivided into subjects who had renal impairment at BL (eGFR <60 or eGFR $<30\ mL \cdot min^{-1} \cdot 1.73\ m^{-2}$), similar trends in the percentage of subjects exhibiting improved eGFR were observed (Fig. 5). Of the 37 subjects switching from CDV to BCV, 9 (24%) reported events possibly associated with kidney dysfunction, with 2 events (acute renal failure and renal failure) in 2 (5%) subjects considered by the investigator to be related to use of BCV.

Improvements in eGFR relative to BL were observed for 66 subjects switching from CDV or foscarnet to BCV. Fifty-five (83%) of the 66 subjects had a maximum on-treatment eGFR greater than BL. Of these subjects, clinically significant increases from BL of $>20\ mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ were observed in 13 (45%) of 29 subjects with BL eGFR $<60\ mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (CKD stages 3–5) and 5 (39%) of 13 with BL eGFR $<30\ mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (CKD stages 4 and 5). Of the 66 subjects switching from CDV or foscarnet use, 13 (20%) reported events possibly associated with renal dysfunction, with events in 4 (6%) subjects considered by the investigator to be related to use of BCV.

DISCUSSION

Despite the potential for antiviral activity against multiple DNA viruses, the utility of CDV has been limited by toxicity, including renal failure and death after a single IV dose.[4] Mechanistically, renal injury associated with CDV use is due to uptake of CDV by the transporter OAT1.[1,3] In our study, no detectable uptake of BCV was observed in OAT1-expressing cells, indicating that BCV is not transported into the proximal tubule through this pathway. This finding is consistent with the general structure–activity relationships of known substrates of this transporter. Substrates of OAT1 and its related family member OAT3 are type I anions: relatively low-molecular weight (<400 Da) polar compounds.[24] The higher molecular weight of BCV of >500 Da and the increase in lipophilicity compared with CDV resulting from the conjugation of the hexadecyloxypropyl moiety (Fig. 1) likely lead to reduced uptake of BCV by OAT1. Of note, the major circulating human metabolites of BCV—CMX064 and CMX103 (Fig. 1)—are more polar and have a lower molecular weight (423 Da and 337 Da, respectively) as compared with BCV, and yet were also not transported by OAT1.

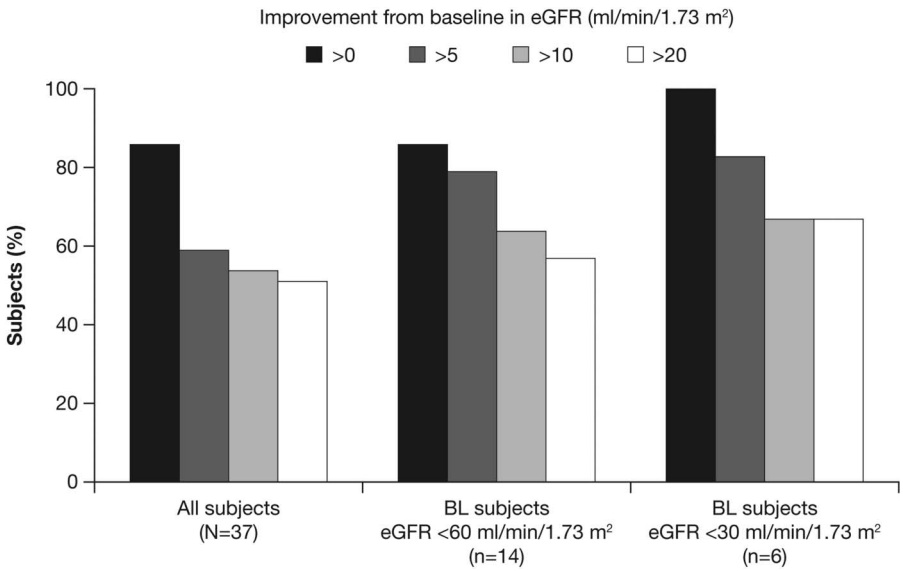

FIGURE 5. Percentages of subjects with improved eGFR from BL after multiple administrations of BCV in virally infected subjects with previous CDV use (Expanded Access Study). BL and on-treatment eGFR were estimated using the Modification of Diet in Renal Disease equation (4 inputs) formula in adult subjects and the Schwartz formula in pediatric subjects.

OAT3 has been shown to have overlapping substrate specificity with OAT1, contributing to accumulation of some toxic organic anions in the kidney.[6,25] In our study, neither BCV uptake nor its metabolites, including CDV, were found to be enhanced in OAT3-expressing cells. Our results are consistent with reports from other investigators who also did not observe OAT3-mediated uptake of CDV under similar conditions.[26] Although conflicting results for OAT3 have been reported,[27] our results and consistent results reported in the literature[26] suggest that OAT1 is the OAT transporter that most efficiently transports CDV from blood into proximal kidney tubule cells, leading to high kidney concentrations of CDV and the resulting significant nephrotoxicity.

Although little is currently known about the specific efflux transporters responsible for transport of CDV out of the kidney into urine, it is postulated that the initial rapid OAT1-mediated uptake of CDV into kidney cells exceeds its efflux into urine and results in accumulation of CDV and subsequent nephrotoxicity.[5,28] The reported K_m value of OAT1 for CDV (46 μM)[1] indicates a low likelihood of functional saturation of this transporter, even with very high plasma concentrations of CDV observed after IV administration. The capacity of OAT1 to mediate uptake of CDV into the kidney is supported by the reported 30-fold higher CDV concentrations in the kidney relative to plasma after IV administration in monkeys.[29] The inability of BCV and 2 of its metabolites (CMX064 and CMX103, together comprising approximately 75% of the circulating drug-related material in the 0- to 24-hour postdose period) to be taken up by OAT1 provides a mechanistic explanation for the lack of CDV-like nephrotoxicity after BCV administration.

CDV is a BCV-derived metabolite. A single oral dose of BCV 200 mg results in a CDV C_{max} as high as 43 ± 26 ng/mL, and BIW administration may result in a slight increase (1.5-fold higher) at steady state. In comparison, the peak CDV plasma concentration post-IV CDV (5 mg/kg, without probenecid) is approximately 11,500 ng/mL (41 μM).[7] Therefore, peak concentrations of CDV after oral administration of BCV in humans are approximately <1% of peak concentrations observed after IV CDV administration. In addition, after BCV administration, CDV C_{max} is reached slowly, with mean T_{max} values ranging from 12 to 24 hours. The PK profile of the metabolite CDV after BCV administration as compared with the CDV PK profile observed after IV CDV administration may contribute to the lack of nephrotoxicity observed with BCV in multiple controlled clinical studies and the >400 patients who have received BCV in the Expanded Access Study and through Emergency Investigative New Drug requests. We were unable to compare the relative risk of nephrotoxicity based on AUC values resulting from IV CDV administration and after oral administration of BCV due to large differences in the bioanalytical assay sensitivities (~100-fold better sensitivity in the BCV studies). Although AUC cannot be entirely ruled out as a PK parameter that relates to kidney concentrations of CDV, it is likely that C_{max} drives the high kidney concentrations after IV CDV administration because C_{max} remains below the reported K_m for OAT1.[1] Therefore, CDV C_{max} remains the optimal plasma PK parameter on which to base comparisons of the relative risk of kidney accumulation of CDV and associated nephrotoxicity.

Coadministration of probenecid has been shown to partially ameliorate the nephrotoxicity of CDV in animals and humans. In animals, the reduction in CDV kidney concentrations has been shown to correspond to a reduction in nephrotoxicity, although human studies have not been conducted.[7] Although not designed to predict the impact of probenecid on CDV accumulation, the cell uptake experiments presented herein demonstrate that CDV accumulation was reduced by 75% in the presence of probenecid. From a physiologically based PK model, it is estimated that CDV kidney concentrations are reduced by 40%–95% in the presence of probenecid.[30] Based on the OAT1 uptake experiments presented and observed peak CDV plasma concentrations after oral BCV administration, kidney CDV concentrations are estimated to be much lower than kidney CDV concentrations after IV CDV administration, even with concomitant probenecid. Further work will be required to establish the relationship between peak plasma and kidney CDV concentrations. The altered PK profile for CDV observed after BCV administration adds to the mechanistic hypothesis for the improved renal safety observed clinically with BCV, with over 1000 subjects exposed in the BCV development program to date.

Nephrotoxicity is a common dose-limiting toxicity for the antivirals foscarnet and CDV.[18] In contrast, there was little to no evidence of CDV-like renal toxicity in BCV-exposed subjects who had participated in the 3 clinical studies summarized in this article. The majority of renal AEs observed were considered by the reporting investigator to be unrelated to treatment with BCV, and alternative etiologies were identified.

Limitations of our analyses include post hoc analyses of categorical shifts in CKD stage and inconsistent eGFR data for all subjects. In addition, the contribution of other nephrotoxic medications was not evaluated, nor was the impact of specific infections treated (some of which have renal involvement and can therefore themselves lead to kidney dysfunction); therefore, drug treatment of the infection may have contributed to improvement in renal function. Moreover, the design of the Expanded Access Study was single arm and open-label, although the comparison of renal function after switching from foscarnet or IV CDV to BCV did allow within-subject comparisons.

Nevertheless, most subjects in the AdV Preemptive Treatment and the Expanded Access studies experienced stable or improved renal function parameters after administration of BCV. The higher weekly BCV dose in the Expanded Access Study resulted in a higher number of subjects with stable or improved renal function, further supporting a lack of nephrotoxicity in doses of BCV higher than those currently being explored in the phase 3 SUPPRESS trial of BCV for the prevention of clinically significant CMV infection in HCT recipients. Subjects receiving BCV in the phase 2 CMV Prevention Study had a statistically significant dose-related improvement in eGFR compared with the recipients receiving placebo, suggesting that BCV at minimum does not cause renal injury. The impact

of BCV on observed improvements in eGFR in BCV-treated subjects may be related to the prevention of CMV reactivation or prevention of other viral infections and is being explored in larger controlled clinical trials.

The analysis of renal function in subjects participating in the Expanded Access Study suggests that BCV allows for recovery of renal function after discontinuation of known nephrotoxic antivirals. eGFR remained stable or improved in more than two-third of subjects across the treatment groups during BCV treatment.

The effects of BCV on renal function in the AdV Preemptive Treatment and Expanded Access studies were similar in pediatric and adult subjects. Lack of BCV-related nephrotoxic effects was not study specific; the percentage of pediatric subjects with stable or improved renal function was similarly high in both studies.

CONCLUSIONS

Unlike CDV, BCV and its major metabolites, CMX103 and CMX064, were not found to be substrates for OAT1, the primary transporter responsible for accumulation of CDV in the kidney. PK analysis indicated that, after BCV administration, peak plasma concentrations of the metabolite CDV are $<1\%$ of peak concentrations observed after IV CDV administration. After oral administration of BCV in virally infected subjects, there was no evidence of drug-associated nephrotoxicity. Furthermore, 80% of subjects who were previously administered CDV or foscarnet experienced improvement in renal function parameters during treatment with BCV. The potential for BCV to have a positive impact on renal function will be further evaluated in future studies of BCV.

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