

Phase I Study of ABT-751 in Combination With CAPIRI (Capecitabine and Irinotecan) and Bevacizumab in Patients With Advanced Colorectal Cancer

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Abstract

ABT-751 is an orally bioavailable sulfonamide with antimetabolic properties. A nonrandomized phase I dose-escalation study of ABT-751 in combination with CAPIRI (capecitabine and irinotecan) and bevacizumab was conducted to define the maximum tolerated dose, dose-limiting toxicity (DLT), and pharmacokinetics in patients with advanced colorectal cancer. Patients were treated with ABT-751 daily for 7 days (alone) and then began 21-day cycles of treatment with ABT-751 daily and capecitabine twice daily for 14 days plus irinotecan on day 1 intravenously. Bevacizumab was added as standard of care at 7.5 mg/kg on day 1 after the first 2 dose levels. Because of intolerance to the regimen, a reduced dose of ABT-751 was also explored with reduced-dose and full-dose CAPIRI with bevacizumab. ABT-751 and irinotecan pharmacokinetics, ABT-751 glucuronidation, and protein binding were explored. Twenty-four patients were treated over 5 dose levels. The maximum tolerated dose was ABT-751 125 mg combined with full-dose CAPIRI and bevacizumab 7.5 mg/kg on day 1. DLTs were hypokalemia, elevated liver tests, and febrile neutropenia. ABT-751 is metabolized by UGT1A8 and to a lesser extent UGT1A4 and UGT1A1. Irinotecan and APC exposure were increased, SN-38 exposure was similar, and SN-38 glucuronide exposure was decreased. Clinically relevant alterations in ABT-751 and irinotecan pharmacokinetics were not observed. Despite modest efficacy, the combination of ABT-751, CAPIRI, and bevacizumab will not be studied further in colorectal cancer.

Keywords

colorectal cancer, tubulin inhibitor, toxicity, pharmacokinetics

Colorectal cancer is a leading cause of cancer-related deaths with an estimated incidence of over 140,000 cases and caused an estimated 51,000 deaths in 2010. In spite of extensive research patients with advanced colorectal cancer continue to have disappointingly poor outcomes with 5-year survival rates of 5% to 6%.¹

Microtubules play a vital role in many cell functions including cell movement, intracellular transport, and cell division. Disruption of microtubules arrests the cell cycle at the G₂M checkpoint, leading to apoptosis.² ABT-751 (N-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide) (Abbott Laboratories, Abbott Park, Illinois) is an orally bioavailable sulfonamide molecule that binds to the colchicine binding site on β -tubulin and inhibits microtubule polymerization and functioning.³ In addition, ABT-751 appears to be a vascular disrupting agent, reducing blood flow to tumors and thereby enhancing cytotoxic effects with little effect on normal vasculature, suggesting possible synergistic activity with vascular endothelial growth factor (VEGF)-directed therapies such as bevacizumab.⁴ Preclinical

evidence suggests significant activity across tumor types including colon cancer both in vitro in cell lines⁵ and in vivo in xenografts and syngeneic models.^{6–9} Furthermore, other mouse xenograft experiments also suggest that ABT-751 has additive effects with 5-FU in colon cancer.¹⁰ Early phase I clinical trials suggest that ABT-751 has a favorable pharmacokinetic profile with

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rapid absorption, the maximum concentration (C_{max}) being reached within 3 hours of administration, a half-life ranging from 4.4 to 16.6 hours, and concentrations associated with preclinical efficacy.¹¹ With prolonged administration schedules (ie, 7- and 21-day schedules with once- or twice-daily dosing) the most common toxicities were peripheral neuropathy, ileus, and fatigue. Notably, no significant myelosuppression was observed, favoring a combination with other more myelosuppressive regimens.^{9,12-14}

Irinotecan in combination with infusional 5-FU (FOLFIRI) with or without bevacizumab, a monoclonal antibody against human VEGF, is a standard regimen for metastatic colon cancer. At the time of initiation of this trial, capecitabine was being considered as a possible replacement for infusional 5-FU in FOLFIRI (ie, CAPIRI) based on several phase 1/2 trials, and the role of bevacizumab in combination drug regimens using capecitabine was still being explored. Finally, the role of continuing bevacizumab in patients who had progressed on first-line therapy was still unclear. Given the promising preclinical data, nonoverlapping toxicities, and possible synergistic activity in the current clinical trial, we treated metastatic colorectal cancer patients with the combination of CAPIRI, bevacizumab, and ABT-751.

Patients and Methods

Approval by the institution's institutional review board was obtained, and written informed consent was mandatory prior to enrollment.

Patient Eligibility

Patients with advanced, unresectable, histologically confirmed metastatic or locally advanced colorectal adenocarcinoma were eligible if they were untreated or had been treated with 1 prior non-irinotecan-containing regimen for advanced disease, were ≥ 18 years of age and had measurable disease, a Karnofsky performance score of ≥ 60 , a life expectancy of ≥ 12 weeks, an absolute granulocyte count of more than $1500/\mu\text{L}$, a platelet count of more than $100,000/\mu\text{L}$, a serum bilirubin level of ≤ 2 mg/dL, normal renal function, transaminases less than $2.5 \times$ the upper limit of normal (ULN), and $ULN < 5 \times$ the ULN (in case of liver metastases). Patients should not have had chemotherapy and/or radiotherapy in the 3 weeks prior to enrollment. Patients were excluded if they had brain metastases, peripheral neuropathy $>$ grade 1, severe comorbid health conditions, or lacked the ability to comply.

Drug Dosage and Administration

ABT-751 was administered orally once daily as monotherapy from day -14 to day -8 during the lead-

Table 1. Patient Characteristics (n = 24)

Characteristic	n	%
Gender		
Male	17	71
Female	7	29
Age, years		
Median	57.5	
Range	34-82	
ECOG performance status		
0	15	63
1	9	37
Cancer diagnosis		
Colon	15	63
Rectal	3	12
Colorectal	6	25
Prior treatment ^a		
None	2	4
1 Chemotherapy regimen	13	54
2 Chemotherapy regimens	5	21
Radiotherapy	5	21
Hormonal therapy	1	4

ECOG, Eastern Cooperative Oncology Group.

^aIncluding adjuvant therapy.

in period and in combination with capecitabine and irinotecan (CAPIRI) with or without bevacizumab from days 1 to 14 of each 21-day cycle, along with standard antiemetics (5-HT₃ antagonist and dexamethasone). CAPIRI consists of capecitabine administered orally in 2 divided doses on days 1 to 14 and irinotecan administered intravenously over 2 hours on day 1. The dose levels for ABT-751 and CAPIRI are presented in Table 1. During the course of the study, bevacizumab became the standard of care and was incorporated into the regimen.^{15,16} Bevacizumab 7.5 mg/kg administered intravenously over 90 minutes on day 1 was added to the regimen on day 1 from dose level 2b. In the absence of treatment delays as a result of adverse events, treatment continued until disease progression, intercurrent illness, unacceptable adverse events, or withdrawal of consent.

Assessments, Follow-Up and Monitoring

Before study entry, patients had a clinical history and physical examination, performance status and toxicity assessment, CBC, chemistries, PT/aPTT, urinalysis, pregnancy test (if applicable), chest x-ray, ECG, and disease assessment by CEA, computed tomography (CT), and/or magnetic resonance imaging (MRI). A history and physical examination, performance status and toxicity assessments, CBC, and chemistry panel were performed on day -14 and day 1 of each cycle. CT scans of disease sites were performed at baseline within 2 to 3 weeks of study entry and every 2 cycles thereafter or sooner if clinically indicated. Additional

assessments for adverse effects were done around day -8, weekly during cycle 1, and on day 15 of subsequent cycles. Adverse events were classified and graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3). Response was evaluated by Response Evaluation Criteria in Solid Tumors 1.0.¹⁷

Definition of Dose-Limiting Toxicity, MTD, and Dose-Escalation Plan

Dose-limiting toxicity (DLT) was determined by toxicities seen prior to the end of the first cycle only and defined as any drug-related grade 4 neutropenia ≥ 5 days; grade 3 to 4 neutropenia with fever; grade 4 thrombocytopenia; any other grade 3 or 4 nonhematological toxicity excluding nausea/vomiting, diarrhea, and constipation unless an appropriate preventive regimen was already in use; grade 2 symptomatic toxicity persisting for longer than 7 days despite appropriate supportive treatment or delay of treatment ≥ 3 weeks due to toxicity. Each dose level cohort consisted of 3 patients, and nonevaluable subjects who received $< 85\%$ of cycle 1 dosing were replaced. If $< 33\%$ of patients developed a DLT, the dose was escalated to the next level. The maximum tolerated dose (MTD) was defined as the dose level below which $\geq 33\%$ of patients develop the same or similarly grouped DLT. A total of 6 to 12 patients were to be treated at the MTD for better toxicity and pharmacology characterization.

Pharmacokinetic Sampling and Analytic Assay for ABT-751

Pharmacokinetic studies were performed during the lead-in phase and during cycle 1 for ABT-751. Serial sampling of venous blood was performed on day -14 prior to treatment, at steady state during the lead-in phase (day -9), and in combination with CAPIRI (day 8) prior to treatment and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 24 hours posttreatment. Blood samples were collected in tubes containing EDTA and were processed by centrifugation for 10 minutes at 1000g at 4°C. Plasma was frozen at -70°C until analysis. Total plasma concentrations of ABT-751 and the sulfate and glucuronide metabolites were determined using a high-performance liquid chromatography with mass spectrometric detection (LC/MS/MS).¹⁸ Calibration curves were generated over the range of 20 to 5000 ng/mL for ABT-751 and metabolites, with a 1:10 dilution allowing for quantitation up to 50,000 ng/mL. Quality assurance samples were assayed with each analytic run and were within 15% of the nominal concentration. Unbound plasma concentrations of ABT-751 were determined using an equilibrium dialysis method using 96-well microdialysis plates that was optimized and validated for determining the fraction unbound (f_u)

in human plasma.¹⁹ The degree of ABT-751 protein binding was assessed in isolated protein solutions containing either human serum albumin (HSA) or human α_1 -acid glycoprotein (AAG) as described above.

Pharmacokinetic Sampling and Analytic Assay for Irinotecan

Pharmacokinetic studies were performed on cycle 1 day 1 for irinotecan. Blood samples were collected at the following times: prior to treatment; at 0.5 hour during the irinotecan infusion; 5 minutes before the end of infusion; and postinfusion at 0.17, 0.5, 1, 2, 4, 7.5, 24, and 48 hours after the end of the infusion. Blood samples were collected in heparinized tubes and were processed by centrifugation for 10 minutes at 1000g at 4°C. Plasma was frozen at -70°C until the time of analysis. Total plasma concentrations (ie, the total of lactone and carboxylate) of irinotecan and its metabolites SN-38, SN-38 glucuronide (SN-38G), and APC were determined by reverse-phase high-performance liquid chromatography with fluorescence detection using a modification of a procedure described previously.^{20,21} Irinotecan, SN-38, SN-38G, and APC were quantitated over the range of 10 to 3000, 2 to 600, 100 to 600, and 2 to 600 ng/mL, respectively. SN-38G was measured indirectly by quantitation of the peak area at the retention time of SN-38G using the SN-38 calibration curve as previously described.^{20,21} A limited amount of SN-38G was available to confirm the retention time of this metabolite (Aventis Pharma, Vitro-sur-Seine Cedex, France). Quality assurance samples were assayed with each analytic run and were within 15% of the nominal concentration.

Pharmacokinetic Data Analysis

Individual plasma concentrations of total and unbound ABT-751, ABT-751 sulfate, ABT-751 glucuronide, irinotecan, SN-38, SN-38G, and APC were analyzed using noncompartmental methods as implemented in the computer software program WinNonlin version 5.3 (Pharsight, Inc, Mountain View, California). Reported pharmacokinetic parameters included the maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration-time curve (AUC) value during the dosing interval at steady-state (AUC_{τ}) for ABT-751 or extrapolated to infinity (AUC_{∞}) for irinotecan, and AUC ratios demonstrate relative extent of metabolism of ABT-751 and irinotecan. If the correlation coefficient (r) for λ_z was less than 0.9, the AUC could not be extrapolated to infinity (AUC_{∞}) by using the equation, $AUC_{\infty} = AUC_{last} + C_{last} / \lambda_z$, where C_{last} was the final quantifiable concentration. Additionally, if the percentage extrapolated was greater than 20%, the AUC_{∞} was not

reported. The observed exposure parameters (ie, C_{max} and AUC_{∞}) for irinotecan and metabolites was dose normalized to 250 mg/m² without applying further correction (normalized parameter = observed parameter \times [250/actual dose]).²²

Drug Metabolism

In vitro metabolism studies were conducted using recombinant UDP glucuronosyltransferase (UGT) isozymes using standard techniques.²³ The initial screen for activity was performed using a 0.5-mL reaction incubated at 37°C for 60 minutes containing 1 mg/mL protein, 2 mM uridine diphosphoglucuronic acid (UDPGA), 8 mM magnesium chloride, 25 μ g/mL alamethicin (0.25% methanol), 9 μ M ABT-751 in 50 mM Tris (pH 7.5). All reactions to determine K_m and V_{max} for UGT1A1, UGT1A4, and UGT1A8 were conducted at 37°C under conditions that produced glucuronidation products that were linear with respect to time and protein concentration. ABT-751 and ABT-751 glucuronide were quantitated in extracts from in vitro drug metabolism studies using LC/MS/MS.¹⁸ Microsomal kinetic data were fit to the Michaelis-Menten model. Apparent K_m , V_{max} , and intrinsic clearance (V_{max}/K_m) were calculated accordingly.

Statistical Analysis

Descriptive statistics were used to summarize patient characteristics, efficacy, and safety data. Pharmacokinetic parameters were summarized by descriptive statistics using dose-normalized parameters for dose-dependent parameters and actual values for dose-independent parameters. A Kruskal-Wallis analysis of variance by ranks was used to compare ABT-751 pharmacokinetic parameters over dose levels. A Wilcoxon matched-pairs signed-rank test was used to compare ABT-751 pharmacokinetic parameters when administered alone or in combination with CAPIRI. In addition, for unbound ABT-751 pharmacokinetic parameters, a Wilcoxon matched-pairs signed-rank test was used to compare parameters when administered with or without bevacizumab. The a priori level of significance was set at $P < .05$. Statistical analysis was performed using JMPTM statistical discovery software (SAS Institute, Cary, North Carolina).

Results

Patient Characteristics

Thirty patients with advanced colorectal cancer were consented, and 24 patients started treatment in this study conducted at 2 US centers. Four patients were only treated during the lead-in phase for reasons including toxicity, poor compliance, discovery of brain metastases, and needing surgery for a noncancer diag-

nosis. There were 17 males and 7 females with a median age 57.5 (range 34-82). Most patients had colon cancer as their primary site (15 colon/3 rectal/6 colorectal) and had been treated with at least 1 chemotherapy regimen previously (n = 10) and some had received at least 2 regimens (n = 5).

Safety and Tolerability

A total of 106 treatment cycles were administered to 20 patients through 5 dose levels. The median number of treatment cycles per patient was 5.5 (range: <1-25). Only 21 patients were evaluated for toxicity because 4 patients came off study prior to initiation of combination therapy. Treatment-related toxicities per dose level observed in at least 10% of patients are summarized in Table 2.

The most common toxicities were mainly gastrointestinal including nausea and/or vomiting in 91% (grade 3/4 in 5%), diarrhea in 72% (5%), abdominal pain in 48% (10%), and elevated liver function tests in 39%. Constitutional symptoms such as fatigue (52%), anorexia (19%), and neutropenia (59%) were also noticed commonly. The most common grade ≥ 3 toxicities included neutropenia in 29%, abdominal pain in 10%, and bowel obstruction in 10%. Of the patients developing neutropenia, 2 had febrile neutropenia.

Dose escalation is summarized in Table 1. One patient at dose level 2 developed several grade 2 gastrointestinal toxicities including nausea, vomiting, and diarrhea 3 days after her first irinotecan infusion and was noted to have grade 3 hypokalemia on day 8, requiring intravenous replacement. On day 15 of cycle 1, she was also noted to have grade 3 elevation in ALT, which resolved to grade 1 by day 20. She was subsequently dose reduced to dose level 1 on which she was treated for an additional 4 cycles. Another patient at dose level 2b developed grade 4 febrile neutropenia without a clear infectious source 1 week after initiating CAPIRI that responded to intravenous antibiotics. This patient subsequently was taken off the study per his request. Given the DLTs noted on dose levels 2 and 2b (3 DLTs in 6 patients), we decided against enrolling 3 additional patients on dose level 2b due to safety concerns. Therefore, dose level 1b with reduced-dose CAPIRI, ABT-751 150 mg orally once daily days 1 to 14 in combination with bevacizumab was tested, and no further DLTs were observed. However, multiple, intolerable grade ≥ 3 toxicities were noted that were not DLT because they occurred after cycle 1. They were mostly related to the peripheral neuropathic effects of ABT-751 including bowel obstruction (1), constipation (1), abdominal pain (2), and grade 1 to 2 neuropathy (2). Therefore, the protocol was amended to test full-dose CAPIRI and bevacizumab with a reduced dose

Table 2. Dose Levels Explored

Dose Level	Number of Patients (lead-in/combination phase)	ABT-751 ^a	Irinotecan ^b	Capecitabine ^c	Bevacizumab ^b
1	3/3	150 mg	200 mg/m ²	1600 mg/m ²	–
2	4/3	150 mg	250 mg/m ²	2000 mg/m ²	–
2b	4/3	150 mg	250 mg/m ²	2000 mg/m ²	7.5 mg/kg
1b	5/4	150 mg	200 mg/m ²	1600 mg/m ²	7.5 mg/kg
3	8/8	125 mg	250 mg/m ²	2000 mg/m ²	7.5 mg/kg

^aOral once daily days 1–14.^bIntravenous day 1.^cOral divided into twice-daily dosing days 1–14.**Table 3.** Treatment-Related Side Effects During Treatment Phase per Dose Level in at Least 10% of Total Patients

Toxicity (by G)	DL 1 (n = 3)		DL2 (n = 3)		DL2b (n = 3)		DL 1b (n = 4)		DL3 (n = 8)		Total (%), n = 21	
	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4
Neutropenia				1		3	2	1	4	1	8 (38)	6 (29)
Fever									1		1 (5)	
Abdominal pain	1		1		1			2	5		8 (38)	2 (10)
Nausea/vomiting	1		1		3		6		7	1	18 (86)	1 (5)
Diarrhea	2		1		2		3	1	6		14 (67)	1 (5)
Bowel obstruction							1	1		1	1 (10)	2 (10)
Constipation			1					1	1		2 (10)	1 (5)
Anorexia/Wt loss			1		1		1		1		4 (19)	
Elevated ALT/AST				1	1				1		2 (10)	1 (5)
Elevated Alk Phos									3		3 (14)	
Hyperbilirubinemia									2		2 (10)	
Hypokalemia								1	1		1 (5)	1 (5)
Hypophosphatemia								1	1		1 (5)	1 (5)
Hyperglycemia							3		2	1	5 (24)	1 (5)
Proteinuria									2		2 (10)	
Mucositis					1				1		2 (10)	
Fatigue	1		2		2		2		4		11 (52)	
Neuropathy	1		1		1		2			1	5 (24)	1 (5)
Rash	1							1			1 (5)	1 (5)
Headache							2				2 (10)	
Pain							2		1		3 (14)	
Alopecia									4		4 (19)	
Anemia								1	2		3 (14)	
Hand-foot syndrome	2				2		1		2		7 (33)	

DL, dose level; G, toxicity grade per the NCI CTC version 3 criteria. Toxicities are worst-grade, treatment-related, and occurring during any cycle.

of ABT-751 125 mg orally once daily, days 1 to 14 in cohort 3. No DLTs were noted in the first 3 patients plus 5 additional subjects treated at this dose level and deemed MTD.

Response Evaluation

Of the 30 patients who consented for the trial, 24 were eligible and started treatment and were included in an intent-to-treat efficacy evaluation. One previously untreated patient with an isolated liver lesion had a complete radiographic response (CR) after 5 cycles and underwent surgical resection. Two patients (8%) had partial responses (PR, 1 confirmed, 1 unconfirmed). In addition, 10 patients (41%) had stable disease with a duration of 2 to 25 cycles.

Pharmacokinetics

ABT-751 pharmacokinetic sampling was evaluable in 20 patients when ABT-751 was administered alone and 15 patients when ABT-751 was administered in combination with CAPIRI (Table 3). There appeared to be a decrease in the extent (AUC_{τ}) and a slight increase in the rate (C_{max}) of formation of ABT-751 glucuronide when ABT-751 was administered with CAPIRI. The altered glucuronidation of ABT-751 did not affect exposure to active parent compound or ABT-751 sulfate. Because there were alterations in ABT-751 glucuronide pharmacokinetics, we determined that ABT-751 undergoes glucuronidation by UGT1A8 and 1A4 and to a lesser extent by UGT1A1, UGT1A7, and UGT2B7 (Table 4, Table 5).

Table 4. ABT-751 and Irinotecan Pharmacokinetic Parameters

	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	AUC ^a ($\mu\text{g} \cdot \text{h/mL}$)	AUC ratio (%) ^b
ABT-751 Pharmacokinetics ^c				
Total ABT-751				
Alone	8.6 ± 3.1 (20)	1.7 (0.5-5.0, 20)	37.4 ± 5.7 (15)	NA
Combination	8.5 ± 2.4 (15)	2.0 (0.1-4.1, 15)	41.5 ± 17.2 (14)	NA
Unbound ABT-751				
Alone	0.8 ± 0.4 (20)	1.7 (0.5-6.0, 20)	3.2 ± 0.9 (15)	8.3 ± 1.4% (15)
Combination	0.7 ± 0.2 (15)	2.0 (0.1-4.1, 15)	3.4 ± 1.7 (14)	8.0 ± 1.0% (14)
ABT-751 Sulfate				
Alone	6.4 ± 3.0 (20)	3.4 (2.0-6.4, 20)	74.8 ± 37.9 (15)	163.7 ± 69.7% (15)
Combination	7.7 ± 3.3 (15)	3.1 (0.5-5.5, 15)	77.8 ± 36.4 (14)	168.2 ± 91.2% (14)
ABT-751 Glucuronide				
Alone	5.2 ± 1.9 [†] (20)	4.0 (2.0-6.4, 20)	63.9 ± 23.8 [†] (15)	116.1 ± 36.6% [†] (15)
Combination	5.4 ± 2.4 [†] (15)	4.3 (1.0-8.5, 15)	54.1 ± 18.5 [†] (14)	96.4 ± 37.9% [†] (14)
Irinotecan Pharmacokinetics ^d				
Irinotecan	3.1 ± 0.7 (17)	1.9 (1.4-3.8, 17)	29.5 ± 14.4 (14)	NA
SN-38	0.1 ± 0.0 (17)	2.9 (0.5-26.0, 17)	0.8 ± 0.33 (8)	2.6 ± 2.3% (8)
SN-38 glucuronide ^e	0.3 ± 0.2 (10)	3.7 (1.9-5.9, 10)	NC	NC
APC	0.6 ± 0.3 (17)	4.2 (1.9-7.0, 17)	7.9 ± 5.1 (16)	26.8 ± 35.4% (16)

Data are presented as the mean ± SD (n) or median (range, n) for T_{max} . AUC, area under the concentration-time curve; C_{max} , maximum plasma concentration; NA, not applicable; NC, not calculated; T_{max} , time of C_{max} ; SD, standard deviation.

^aFor ABT-751, AUC_∞ is reported. For irinotecan, AUC_∞ is reported.

^bRatio of unbound ABT-751, ABT-751 sulfate, or ABT-751 glucuronide to ABT-751 and SN-38, APC, or SN-38 glucuronide to irinotecan expressed as a percentage.

^c C_{max} and AUC_∞ were dose normalized to 150 mg.

^d C_{max} and AUC_∞ were dose normalized to 250 mg/m².²¹

^eSeven patients had undetectable SN-38 glucuronide concentrations. Of the 10 with detectable levels, either the half-life or percentage extrapolated for AUC was poor; thus, AUC_∞ was not calculated.

[†] $P < .05$ for matched pair comparison when administered alone or in combination with CAPIRI.

Table 5. Kinetic Parameters of ABT-751 Glucuronide Formation by UGT1A4 and UGT1A8^a

	Glucuronidation		
	K_m (μM)	V_{max} (pmol/[min · mg P450])	V_{max}/K_m (mL/[min · mg])
UGT1A4	83.7 ± 30.9	59.9 ± 20.2	0.7
UGT1A8	27.0 ± 7.3	197.0 ± 23.8	7.3

Results are expressed as the mean ± SD (n = 3 experiments; n = 3 replicates per concentration).

^aDue to analytical assay limits, accurate kinetic parameters of ABT-751 glucuronide formation were unable to be calculated for UGT1A1, UGT1A7, and UGT2B7.

ABT-751 was extensively bound in human plasma obtained from healthy volunteers with a mean unbound fraction value of $8.2 \pm 0.6\%$. The unbound fraction was determined to be $8.4 \pm 1.4\%$ in patients on this study and was not altered over the concentration-time profile or when administered alone or in combination. ABT-751 bound almost exclusively to albumin (unbound fraction of $5.6 \pm 0.1\%$) rather than AAG ($84.4 \pm 1.1\%$). Seventeen patients had irinotecan pharmacokinetic sampling (Table 3). Irinotecan and APC exposure were increased, SN-38 exposure was similar, and SN-38 glucuronide exposure was decreased compared to historical controls.²⁴

Discussion

ABT-751 is an orally available sulfonamide that causes a G₂M cell cycle arrest and subsequent apopto-

sis through microtubule inhibition by acting at the colchicine-binding site on β -tubulin. ABT-751 is not a substrate for the multiple drug resistance (MDR) transporter.^{2,3,8,12} Subsequent research has also suggested that ABT-751 also acts as a vascular disrupting agent through disruption of the tubulin cytoskeleton of endothelial cells, ultimately leading to decreased blood flow and tumor necrosis.²⁵ The primary objectives of this trial were to determine the MTD and toxicity profile of ABT-751 in combination with bevacizumab and CAPIRI in advanced colorectal cancer.

Several DLTs observed during the original escalation were deemed related to CAPIRI; thus, the next cohort explored reduced-dose CAPIRI in combination with ABT-751 150 mg and added bevacizumab. Non-DLT intolerable toxicities occurred related to the peripheral neuropathic effects of ABT-751, and a still lower dose level of ABT-751 was explored and ultimately deemed

the MTD. However, other combination studies evaluating a similar dosing schedule of ABT-751 of days 1 to 14 of a 21-day cycle have suggested higher MTDs of 200 mg in combination with pemetrexed in lung cancer²⁶ and with docetaxel in prostate cancer.¹⁴ The reason for reduced tolerability to ABT-751 in the current trial is unclear.

In order to assess the interaction, we looked not only at unbound ABT-751 but also at how the drug was glucuronidated, which was unknown at the time the clinical trial was conducted. Our *in vitro* studies have been confirmed by Innocenti and colleagues, who also demonstrated the highest affinity for UGT1A8 then UGT1A4 followed by minor clearance by UGT1A1 and UGT2B7.³¹ More importantly, functional polymorphisms in UGT1A8 and UGT1A4 and SULT1A1 copy number can alter the pharmacokinetics of ABT-751. Unfortunately, we did not collect pharmacogenomic samples to explore the variability in our patient population. The active metabolite of irinotecan is SN-38 which is primarily eliminated via glucuronidation by UGT1A1, UGT1A7, and UGT1A9.³² Because the UGT isozymes responsible for metabolism of irinotecan and ABT-751 are different, the alteration is unlikely to be competitive due to the isozymes but likely due to substrate depletion.²⁹ A limitation of the current study is that we did rely on historical control comparisons for irinotecan pharmacokinetics. During the initial development of CAPIRI, it was noted that capecitabine decreased the rate of formation of SN-38 and SN-38 glucuronide during a transient period at the beginning of coadministration.³⁰ It therefore cannot be ruled out that some of the alterations observed with ABT-751 glucuronide may indeed be due to capecitabine. However, the alteration of ABT-751 pharmacokinetics by CAPIRI is not significant and does not explain the increased toxicity.

At the time of initiation of this trial, capecitabine was being evaluated as a replacement for 5-FU in the FOLFIRI regimen. However, several large trials since then have convincingly shown increased toxicity with this combination due to overlapping toxicity profiles of capecitabine and irinotecan.^{31,32} The randomized BICC-C trial compared FOLFIRI, modified IFL with irinotecan plus bolus FU/LV, and CAPIRI with a later amendment adding bevacizumab to all arms. In this trial the CAPIRI arm was associated with significantly higher rates of severe vomiting, diarrhea, and dehydration and was discontinued.³³ Since the results of these trials were published, CAPIRI has fallen out of favor, with 5-FU being the fluoropyrimidine of choice in combination therapies with irinotecan.

Given that only 2 patients had ≥ 2 prior chemotherapy regimens and the rest were either chemotherapy naive or had ≤ 1 prior systemic chemotherapy

regimen, the activity of this regimen is modest (1 CR, 1 confirmed PR). The BICC-C trial convincingly showed inferior outcomes with CAPIRI as compared to FOLFIRI.³³ Also, the early clinical evidence for the antitumor activity of ABT-751 has not been encouraging.

In conclusion, the MTD of the combination of ABT-751, CAPIRI, and bevacizumab in advanced colorectal cancer is ABT-751 125 mg orally days 1 through 14, irinotecan 250 mg/m² intravenous day 1, capecitabine 2000 mg/m² orally daily in 2 divided doses, and bevacizumab 7.5 mg/kg IV day 1 and every 3 weeks. Although this combination does have some activity in colorectal cancer, previous trials showed decreased efficacy of CAPIRI compared to FOLFIRI, and increased toxicity of ABT-751 was noted in this trial for unclear reasons. Therefore, this combination will not be evaluated further in colorectal cancer.

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