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Pharmacokinetics and ADME Characterization After Oral and Intravenous Administration of [^{14}C]-Ziftomenib in Healthy Male Participants

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ABSTRACT

Ziftomenib, a potent, selective inhibitor that binds menin at the lysine methyltransferase 2 interaction site, has demonstrated promising clinical activity with manageable toxicity in heavily pretreated patients with acute myeloid leukemia (AML) and *nucleophosmin 1* mutations. This phase 1, open-label study characterized the absorption, metabolism, excretion, and bioavailability of ziftomenib in healthy men and comprised two parts. In part A, a single oral dose of ziftomenib 400 mg (containing 250 μCi [^{14}C]-ziftomenib) was given to evaluate routes and rates of elimination, total radioactivity, and other pharmacokinetic parameters. In part B, a single oral dose of ziftomenib 400 mg followed by an intravenous dose of ziftomenib < 100 μg (containing 1 μCi [^{14}C]-ziftomenib) was administered to evaluate absolute bioavailability (both $n = 8$ patients). A median t_{max} of 3.5 h and an elimination $t_{1/2}$ of 61.5 h demonstrated rapid ziftomenib absorption and enabled once-daily dosing. Total radioactivity recovery was 89.7% in feces and 0.5% in urine over 480 h. Absolute bioavailability of 12.9% was observed. Ziftomenib was primarily metabolized by oxidation, *N*-demethylation, and *N*-dealkylation, with 19 metabolites recovered in plasma. All metabolites were considered minor (< 10% of total drug-related exposure). Ziftomenib was the most abundant plasma component (> 10% of total drug-related exposure). In conclusion, ziftomenib underwent limited metabolism following absorption and was primarily excreted as unchanged parent drug in feces. Ziftomenib was well tolerated with no new safety concerns in healthy men. Considering the pharmacokinetic profile and manageable safety outcomes, these findings support further clinical investigation of ziftomenib as treatment for AML.

1 | Introduction

Acute myeloid leukemia (AML) is the most common leukemia in adults and is characterized by the clonal expansion of myeloid precursors in the peripheral blood and bone marrow, which results in ineffective hematopoiesis and bone marrow failure [1].

Until recently, the management of AML has largely remained unchanged since the development of intensive induction chemotherapy and allogeneic stem cell transplantation therapies. However, advances in the molecular profiling of AML and identification of specific subpopulations by their mutational status have enabled the development of several targeted therapeutics against these identified driver mutations [2]. Approved targeted

Summary

- What is the current knowledge on the topic?
  - Ziftomenib is a selective inhibitor that binds menin at the lysine methyltransferase 2 interaction site with high potency and has demonstrated antileukemic effects in preclinical studies and promising clinical activity in patients with *nucleophosmin 1*-mutant acute myeloid leukemia. In vitro data demonstrated that ziftomenib was primarily metabolized by CYP3A4. An absorption, deposition, metabolism, and excretion (ADME) study of ziftomenib radiolabeled with [^{14}C] ([^{14}C]-ziftomenib) in mice demonstrated that ziftomenib was mildly metabolized and predominantly eliminated by fecal excretion, with minor urinary excretion. A human ADME study of [^{14}C]-ziftomenib was designed to characterize the pharmacokinetics and ADME profile of ziftomenib after oral and intravenous (IV) administration.
- What question did this study address?
  - Characterization of the pharmacokinetic and absorption, metabolism, and excretion (AME) profile of ziftomenib following a single oral and IV dose in healthy male participants.
- What does this study add to our knowledge?
  - Ziftomenib was characterized by rapid absorption (time to maximum concentration was 3.5 h) with an elimination half-life of 61.5 h. The absolute bioavailability of ziftomenib was 12.9%. Ziftomenib underwent minor metabolism and was predominantly eliminated as unchanged parent drug through fecal excretion. All metabolites of ziftomenib were minor (< 10% of total drug-related exposure) in circulation.
- How might this change clinical pharmacology or translational science?
  - This study demonstrates the utility of thorough characterization of the pharmacokinetic and ADME profile of drugs in development using oral and IV administration. In this case, these data conclusively demonstrated that ziftomenib undergoes minor metabolism, which might result in minimal drug–drug interactions such as with CYP modulators. Additionally, the characterized pharmacokinetic profile of ziftomenib (half-life and absolute bioavailability) provides valuable information for designing future clinical studies.

therapies include ivosidenib [3] and olutasidenib [4] for *isocitrate dehydrogenase 1* (*IDH1*) mutations, enasidenib for *IDH2* mutations [5], and gilteritinib for *FMS-like tyrosine kinase 3* (*FLT3*) mutations [6].

Among the most common mutations in AML, *nucleophosmin 1* (*NPM1*) gene mutations affect around one-third of AML patients [7] and *NPM1*-mutant (*NPM1*-m) AML is recognized as a distinct clinical entity in the World Health Organization classification of myeloid neoplasms [8]. AML with *lysine methyltransferase 2* rearrangement (*KMT2A*-r) is another distinct clinical entity that is characterized by chemotherapy resistance and high relapse rates [8, 9].

Menin is a nuclear protein that interacts with chromatin regulators and transcription factors to help regulate tissue-specific gene expression [10]. KMT2A is a transcriptional regulator that binds to menin and regulates *HOXA9* gene expression, a gene that is upregulated in *NPM1*-m AML [11]. Ziftomenib (KO-539) is a selective inhibitor that binds menin at the KMT2A interaction site with high potency and has demonstrated downregulation of *HOXA9* and *MEIS1* expression [12], myeloid terminal differentiation, antileukemic effects in preclinical studies (particularly within in vitro and in vivo models of *NPM1*-m and *KMT2A*-r AML) [12, 13], and promising clinical activity in patients with *NPM1*-m AML [14].

In this paper, the characterization of absorption, metabolism, and excretion (AME), mass balance, and absolute bioavailability of ziftomenib radiolabeled with [^{14}C] ([^{14}C]-ziftomenib) in healthy adult participants is presented. In addition, the quantitative metabolite profiles and the chemical structures of metabolites (where possible) in plasma, urine, and feces after administration of [^{14}C]-ziftomenib were determined. The safety and tolerability of a single oral dose of ziftomenib were also assessed.

2 | Materials and Methods

2.1 | Materials

Ziftomenib 400 mg capsules containing approximately 250 μCi of radiolabeled [^{14}C] were manufactured and labeled by licensed pharmacists at the study site using capsules containing ziftomenib 200 mg (supplied by Kura Oncology Inc., San Diego, California, USA) and the active pharmaceutical ingredient of radiolabeled powder (supplied by Quotient Sciences, Nottingham, UK). The intravenous solution of ziftomenib < 100 μg containing approximately 1 μCi of radiolabeled [^{14}C] was manufactured and labeled in the same way. Nonradiolabeled ziftomenib 200 mg capsules were supplied by the study sponsor (Kura Oncology Inc., San Diego, California, USA). Manufactured products were released by a good manufacturing practice quality auditor prior to participant administration.

2.2 | Study Design and Procedures

This was a phase 1, open-label, nonrandomized, single-center, two-part parallel group study evaluating ziftomenib in healthy men. As there were no data to suggest differences in ziftomenib pharmacokinetics and metabolism between sexes, female participants were excluded from the study to align with the US Food and Drug Administration (FDA)'s recommendation to keep radiation exposure in females of child-bearing potential as low as is reasonably achievable [15].

In part A of the two-part study, participants received a single oral dose of ziftomenib 400 mg containing approximately 250 μCi [^{14}C]-ziftomenib with 240 mL water on Day 1 (Figure 1a). In part B, participants received a single oral dose of ziftomenib 400 mg with 240 mL water on Day 1 and a single < 100 μg dose of approximately 1 μCi [^{14}C]-ziftomenib administered 3 h after the oral dose as an intravenous (IV) push over 2 min (Figure 1b).

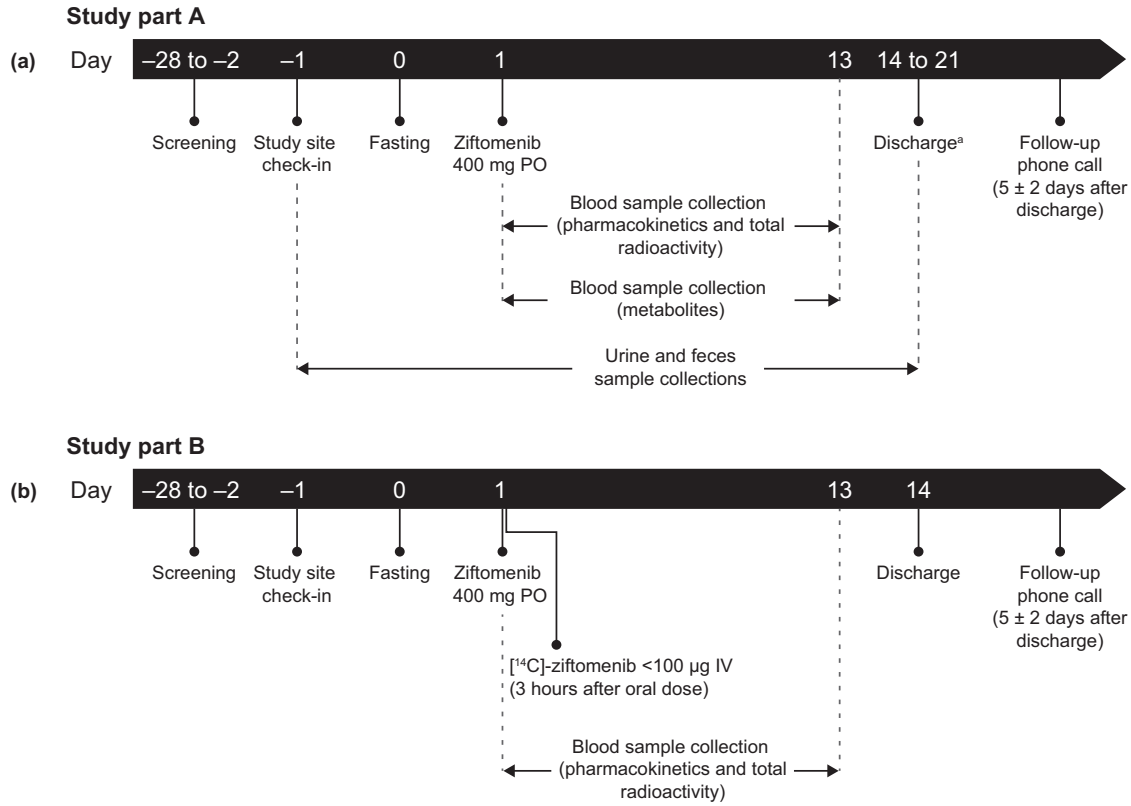

FIGURE 1 | Study designs of (a) part A and (b) part B. IV, intravenous; PO, oral. [a]Participants were discharged on day 14 if they met the discharge criteria. If participants did not meet the discharge criteria, they remained at the study site until they met all the discharge criteria up to day 21 to collect samples for total radioactivity.

The actual IV dose ranged from 84.67 µg to 88.30 µg (Table S1). In both study parts, drug administration was preceded by an overnight fast (≥10 h) from food. Participants also fasted from food for four or more hours after oral dose administration. Participants were not allowed water for 1 h prior and 2 h after oral dose administration in alignment with the ziftomenib fasting requirements.

The study was conducted in accordance with the Declaration of Helsinki, Council for International Organization of Medical Sciences International Ethical Guidelines, and International Council for Harmonisation E6 Good Clinical Practice guidelines. The study was conducted at Fortrea (formerly Labcorp) Clinical Research Unit (Madison, WI, USA) between January 17, 2022, and May 27, 2022, in accordance with local legal and regulatory requirements. The study protocol and informed consent form were reviewed and approved by an institutional review board (Salus IRB; Austin, TX, USA). All participants provided signed informed consent before starting any study-related procedures.

2.3 | Dosing Information

A pharmacokinetics study of ziftomenib in a mouse model using [^{14}C]-ziftomenib (data on file) was used to determine the dose for human administration. In part A, the planned radioactive dose of approximately 250 µCi of [^{14}C]-ziftomenib was expected to provide a sufficient radioactive signal to achieve the study objectives while minimizing radiation risk to participants. The whole-body radioactivity exposure was estimated to be 578 mrem, approximately 19% of the whole-body single study limit of 3000 mrem as per FDA guidance [15]. The estimated mrem exposures in active blood-forming organs, the lens of the eye, and gonads were estimated to be 65, 280, and 204 mrem, respectively. These estimates represent 2.2%, 9.3%, and 6.8%, respectively, of the single study limit of 3000 mrem. In part B, the radioactive dose of approximately 1 µCi of [^{14}C]-ziftomenib (representing 0.4% of the recommended whole-body dose limit of 3000 mrem for a single study) was expected to achieve the objectives and presented minimal radiation risk to healthy participants. The low levels of radioactivity planned in part B necessitated the use of accelerator mass spectrometry (AMS) as a highly sensitive analytical technique for quantifying the radioactivity in plasma samples.

An oral dose of ziftomenib 400 mg was selected based on Phase 1 dose escalation and expansion data and was considered sufficient to fully characterize the pharmacokinetics of a single oral dose of the parent compound. Furthermore, the safety profile of ziftomenib was acceptable in patients with relapsed or refractory AML when administered as multiple oral doses up to 800 mg once daily and was particularly well tolerated at a single oral dose of 400 mg in healthy volunteers [14]. Ziftomenib < 100 µg IV was chosen as a microdose to permit dosing without supporting IV toxicology data [16].

2.4 | Participants

Eligible participants were healthy men, aged 18–55 years, who weighed 50–100 kg and had a body mass index of 18.0–32.0 kg/m^2. Healthy status was determined by the absence of clinically significant findings from medical history and by physical examination, 12-lead electrocardiogram (EGC), vital signs, and clinical laboratory evaluations, as assessed by the investigator at screening and check-in. Important exclusion criteria included: history or presence of liver disease, pancreatitis, cholecystectomy, or respiratory or cardiovascular disease; abnormal liver function tests; estimated glomerular filtration rate < 90 mL/min/1.73 m^2; and any history of stomach or intestinal surgery/resection or use of medications/products that would affect drug absorption, metabolism, and/or excretion. A full list of inclusion and exclusion criteria is provided in Table S2.

2.5 | Outcome Measures

The primary objectives for part A were to determine the routes, rates of elimination, and mass balance of total radioactivity from [^{14}C]-ziftomenib, and to characterize the pharmacokinetics of ziftomenib and total radioactivity from [^{14}C]-ziftomenib. Secondary objectives were quantitative profiling and determination, where possible, of the chemical structure of metabolites in plasma, urine, and feces after [^{14}C]-ziftomenib administration. The primary objectives for part B were to determine the absolute bioavailability (F_{abs}) of oral ziftomenib relative to intravenous [^{14}C]-ziftomenib, and to characterize the pharmacokinetics of ziftomenib, [^{14}C]-ziftomenib, and total radioactivity following oral and intravenous administration.

The safety and tolerability of a single oral dose of ziftomenib were also assessed in both study parts as secondary objectives. Adverse events and the prior or concomitant use of medications were monitored throughout the study period. Laboratory abnormalities, 12-lead electrocardiography, vital sign measurements, and physical examination results were also assessed.

2.6 | Sampling

Up to eight participants were enrolled in each part (parts A and B) of the study to ensure that at least six participants completed each part of the study. All 16 participants were randomized, dosed, and completed the study. In part A, blood sampling for ziftomenib concentration and metabolites (in plasma), and for total radioactivity (in whole blood and plasma), was performed at predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h postdose, and every 24 h through day 13. Urine for ziftomenib concentration, total radioactivity, and metabolite profiling and identification was collected at predose (up to 12 h before the dose), and at 0–6, 6–12, 12–24, 24–48 h postdose, and every 24 h thereafter until the following discharge criteria were met: plasma radioactivity levels below the limit of quantitation for two consecutive collections, $\geq 90\%$ mass balance recovery, and $\leq 1\%$ of the total radioactive dose recovered in combined excreta (urine and feces) in two consecutive 24 h periods in which both samples were provided. Feces for total radioactivity and metabolite profiling and identification were collected at predose (between check-in and drug administration), and at 0–24 and 24–48 h postdose, and every 24 h thereafter until the discharge criteria were met. Blood sampling for metabolite profiling and identification (in plasma) was performed at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 216, 240, 264, 288, and 312 h postdose. Participants were discharged on day 14 if they met all discharge criteria.

In part B, blood sampling for ziftomenib concentration and metabolites (in plasma) was conducted as for part A relative to the oral dose, with the 3-h postdose measurement taking place just prior to the intravenous dose. Blood sampling for ziftomenib, [^{14}C]-ziftomenib, and total radioactivity (in plasma) was performed relative to the intravenous dose at predose (just prior to the intravenous dose); at 2 (end of intravenous infusion), 10, 20, 30, and 45 min; at 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120 h postdose; and every 24 h through Day 13. Blood samples were collected from the contralateral arm to IV administration. Urine for ziftomenib and metabolites was collected as per part A. Participants were discharged on Day 14.

2.7 | Sample Preparation and Analysis of Metabolites

Plasma samples were pooled to generate two 0–312-h samples. These pooled samples were combined with acetonitrile. The mixture was sonicated, vortexed, and centrifuged, and the supernatant was removed. Plasma extraction was repeated once with acetonitrile and once with methanol. All supernatants were combined, and the sample was evaporated to approximately 200 μL and was sonicated, vortexed, and centrifuged.

Urine samples were collected and pooled by participant to generate 0–48, 0–72, 0–96, 12–24, and 0–360-h pooled samples. Radioactivity was determined by liquid scintillation counting (LSC). Urine samples were processed using solid phase extraction (SPE) with a Strata C18-E 2 g SPE column (Phenomenex; Torrance, CA, USA). Columns were conditioned with methanol and reverse osmosis (RO) water. The sample was then applied to the column, with load recoveries determined by LSC and ranged between 0% and 4.82%. Columns were then washed with RO water and recovery ranged between 0% and 1.07%. Methanol elution was then performed, and recoveries ranged between 69.8% and 114%. An additional methanol elution for selected participants showed recoveries between 0.56% and 1.96%. The methanol fractions were combined and evaporated to dryness before reconstitution in 300 μL (RO water:methanol [4:1, volume/volume]). Reconstituted samples were sonicated, vortexed, and centrifuged, and recoveries ranged between 80.4% and 101%. The analysis of ziftomenib and its metabolites in plasma and urine samples was performed by Meriter Laboratories (Madison, WI, USA).

Feces samples were collected and pooled by participant to generate 24–72-, 24–96-, 48–72-, 0–96-, and 0–192-h pooled samples. Samples were dissolved in 1 N sodium hydroxide at 40°C

for radioactivity assessment by LSC. Pooled samples were combined with acetonitrile, and mixtures were sonicated, vortexed, and centrifuged. Supernatants were removed, and the extraction was repeated with the respective supernatants combined. Extraction recoveries determined by LSC ranged between 77.6% and 113%. Extraction was repeated with methanol, and recoveries ranged between 0.56% and 3.88%. The combined supernatants were evaporated to dryness before reconstitution in 300 μL methanol. Reconstituted samples were sonicated, vortexed, and centrifuged, and recoveries ranged between 91.5% and 111%.

The processed plasma, urine, and feces samples were analyzed by liquid chromatography-high-resolution mass spectrometry (LC-HRMS). Radioactivity was determined using MicroBeta2 analysis, and radiochromatogram profiles were generated based on radioactive counts. Quantitation of metabolites was based on the radiochromatograms. The limit of quantitation for radioactivity was set at 1% of the radiochromatographic analysis. The cutoff for identification of metabolites was 2% of the sample radioactivity for plasma, urine, and feces. Metabolite numbers (M1, M2, etc.) were assigned to all radiochromatogram peaks (except for the parent ziftomenib), regardless of their identity.

2.8 | Pharmacokinetic and Statistical Analyses

In part A, the area under the concentration–time curve (AUC) from time 0 to infinity ($AUC_{0-\infty}$), AUC from time 0 to last quantifiable concentration ($AUC_{0-tlast}$), maximum observed concentration (C_{max}), time of C_{max} (t_{max}), terminal elimination half-life ($t_{1/2}$), renal clearance (CL_R), and total clearance (CL/F) of ziftomenib in plasma and whole blood, and recovery of the administered dose over time (fe_{total}) of ziftomenib in urine were assessed. In part B, the F_{abs}, $AUC_{0-\infty}$, $AUC_{0-tlast}$, C_{max}, t_{max}, $t_{1/2}$, and CL_R for ziftomenib and [^{14}C]-ziftomenib in plasma and fe_{total} of ziftomenib in urine were assessed. Several other pharmacokinetic parameters, in addition to the ones listed above, were also assessed in both study parts.

Pharmacokinetic analyses were performed by Fortrea (Madison, WI, USA) using WinNonlin version 8.1.1.

The sample size chosen for this study is common in human radiolabeled studies and was considered sufficient to achieve the objectives of the study [17].

3 | Results

3.1 | Study Participants

Eight participants were evaluated in each part of the study, for a total of 16 participants. Their demographics are shown in Table S3. No statistical assessment of sample size was conducted. The mean (standard deviation [SD]) age of participants was 35.4 (7.4) years in part A and 33.6 (8.2) years in part B. In part A, six participants were White, one was Black or African American, and one was American Indian or Alaska Native. In part B, four participants were White, three were Black or African American, and one was Asian. Seven participants in each study part were non-Hispanic or non-Latino.

3.2 | Absorption

Following the administration of a single oral dose of ziftomenib 400 mg, a moderately rapid absorption phase was observed with a median t_{max} of 3.5 h (range 1.5–24 h) in plasma. The concentration–time profile of ziftomenib appeared to decline in a biphasic manner with a geometric mean $t_{1/2}$ of 61.5 h (range 48.5–107 h; Table 1 and Figure 2a). Ziftomenib pharmacokinetics were similar in parts A and B of the study; however, the minor differences between the two parts are likely due to interindividual differences in a parallel group study. The median t_{max} for total [^{14}C] radioactivity by AMS was 4.0 h (range 2.0–8.0 h) and the geometric mean $t_{1/2}$ was 212 h (range 101–366 h) in plasma (Table 2). Total radioactivity levels of [^{14}C] by AMS in plasma were quantifiable for all participants up to 312 h and for three participants up to 480 h.

3.3 | Bioavailability

The geometric mean F_{abs} of ziftomenib following a single oral dose of ziftomenib 400 mg was 12.9% (range 1.7%–32%) (Table 1 and Table S1). The individual oral $AUC_{0-\infty}$, IV $AUC_{0-\infty}$, and F_{abs} are shown in Table S1. Using $F = f_a \times f_G \times f_H$, where f_a is fraction absorbed, f_G is fraction escaping gut metabolism, and f_H is fraction escaping hepatic metabolism [18], f_a was estimated to be 30%–40%, depending on the clearance estimate. No gut metabolism was assumed in this calculation (i.e., $f_G \sim 100\%$), which was substantiated by negligible recovery of metabolites in excreta after oral [^{14}C]-ziftomenib administration. The absorption, deposition, metabolism, and excretion (ADME) pharmacokinetic parameters of ziftomenib following the oral administration of ziftomenib 400 mg were similar to findings in part A (Table 1).

Following the administration of a single oral dose of ziftomenib 400 mg and a single intravenous dose of [^{14}C]-ziftomenib < 100 μg, the median t_{max} of [^{14}C]-ziftomenib was observed at approximately 10 min (range 2–12 min) after the intravenous administration, and the $t_{1/2}$ of [^{14}C]-ziftomenib was 25.1 h (range 9.5–54.4 h; Table 1 and Figure 2b).

3.4 | Metabolic Profiling

Ziftomenib underwent minimal metabolism following the administration of a single oral dose of ziftomenib 400 mg. Of the 19 total metabolites observed in plasma, eight were identified and further characterized (KO-516, KO-739, M3, M4, M17, M23, M56, and M60; Table 3). Representative AMS radiochromatograms of pooled plasma, urine, and feces are shown in Figure 3. The remaining 11 metabolites represented < 2% of total radioactivity and were not characterized as these are not expected to have any meaningful impact on ziftomenib pharmacokinetics or ADME profile (Table S4). The proposed biotransformation pathways of ziftomenib, based on the eight identified metabolites, are presented in Figure 4.

TABLE 1 | Summary of ziftomenib pharmacokinetic parameters after oral and IV administration.

	Part A ($N=8$)	Part B ($N=8$)	
	Ziftomenib 400 mg PO[a]	Ziftomenib 400 mg PO + [^{14}C]-ziftomenib < 100 μg IV[b]	[^{14}C]-ziftomenib < 100 μg IV[b]
Plasma			
F_{abs}, %	N/A	12.9 (115.0) [8]	N/A
$AUC_{0-tlast}$, h•ng/mL	1170 (90.9) [8]	1890 (112.6) [8]	2.82 (28.7) [8]
$AUC_{0-tlast}$ plasma ziftomenib/plasma radioactivity by AMS ratio	0.154 (30.2) [8]	N/A	N/A
$AUC_{0-\infty}$, h•ng/mL	1200 (88.7) [8]	1950 (107.6) [8]	3.30 (31.5) [7]
$\%AUC_{extrap}$, %	2.07 (104.4) [8]	2.36 (83.7) [8]	13.3 (26.5) [8]
C_{max}, ng/mL	35.3 (185.0) [8]	61.2 (152.6) [8]	2.70 (56.4) [8]
t_{max}, h	3.50 (1.50–24.0) [8]	4.00 (2.00–12.0) [8]	0.167 (0.0333–0.200) [8]
t_{last}, h	312 (240–432) [8]	N/A	N/A
$t_{1/2}$, h	61.5 (26.3) [8]	53.5 (15.9) [8]	25.1 (53.0) [8]
CL_R, L/h	0.0857 (39.9) [8]	0.0867 (34.2) [8]	N/A
CL/F, L/h	339 (88.6) [8]	N/A	N/A
V_z/F, L	30,100 (98.4) [8]	N/A	N/A
Urine			
Ae_{total}, mg	0.278 (56.5) [3]	0.169 (92.3) [8]	N/A
fe_{total}, %	0.0684 (56.4) [3]	0.0423 (92.3) [8]	N/A

Note: Data are presented as geometric mean (CV) [*n*]; data for t_{max} and t_{last} are presented as median (range) [*n*].
Abbreviations: $\%AUC_{extrap}$, percentage of area under the concentration–time curve due to extrapolation from the last quantifiable concentration to infinity; Ae_{total}, amount of the ziftomenib dose administered recovered over the collection interval 0–480; AMS, accelerator mass spectrometer; $AUC_{0-\infty}$, area under the concentration–time curve from time 0 extrapolated to infinity; $AUC_{0-tlast}$, area under the concentration–time curve from time 0 to the time of the last quantifiable concentration; CL/F, apparent total clearance; CL_R, renal clearance; C_{max}, maximum observed concentration; CV, coefficient of variation; fe_{total}, percentage of the dose administered recovered over the collection interval 0 to 480 h; IV, intravenous; N/A, not assessed; PO, oral; $t_{1/2}$, terminal elimination half-life; t_{last}, time of the last quantifiable concentration; t_{max}, time to maximum observed concentration; V_z/F, apparent volume of distribution during the terminal phase.
[a]Contains approximately 250 μCi of [^{14}C]-ziftomenib.
[b]Contains approximately 1 μCi of [^{14}C]-ziftomenib.

Ziftomenib was the major and most abundant circulating plasma component and represented > 10% of total drug-related exposure (Table 3). All metabolites, including the two active metabolites, KO-739 and KO-516, were minor metabolites in plasma, as they represented < 10% of total drug-related exposure (Table S5) [19]. It is noted that there are some differences in the measured radioactivity and drug-related exposure between parts A and B. These differences could be due to interindividual differences in a parallel group study and bioanalytical variability due to differences in radioactive dose in part A (250 μCi) and part B (1 μCi). For KO-739 and KO-516, the t_{max} values were 2.5 and 3.0 h, respectively. KO-739 and KO-516 were recovered in urine and represented 0.02% and 0.01% of the administered dose, respectively. The metabolite-to-parent AUC_{0-inf} ratios for KO-739 and KO-516 were 0.21 and 0.10, respectively.

Ziftomenib was the major component (> 10% of dose) in feces and trace in urine (< 1% of dose) that accounted for 73.3% and 0.03% of the administered dose, respectively (Table 3). KO-516 was a minor component (< 10% of administered dose), and KO-739 was a trace component (< 1% of the administered dose) in feces. All other metabolites were trace components in feces. All metabolites in urine were trace components and cumulatively accounted for < 0.3% of the administered dose.

3.5 | Excretion

The cumulative amounts of total radioactivity recovered in feces and urine following a single oral dose of ziftomenib 400 mg were 365.0 mg equivalents (89.7%) and 2.1 mg equivalents (0.5%), respectively (Figure 2c), with most of the radioactivity (88.4%) recovered by 168 h postdose. The urine, feces, and cumulative recoveries for individual participants are shown in Table S6. At the time of study discharge, radioactivity levels were quantifiable in feces in all participants (ranging from 312 to 480 h postdose) and in the urine of two participants (456 and 480 h postdose). Unchanged ziftomenib in urine represented 0.07% of the administered dose (Table 1).

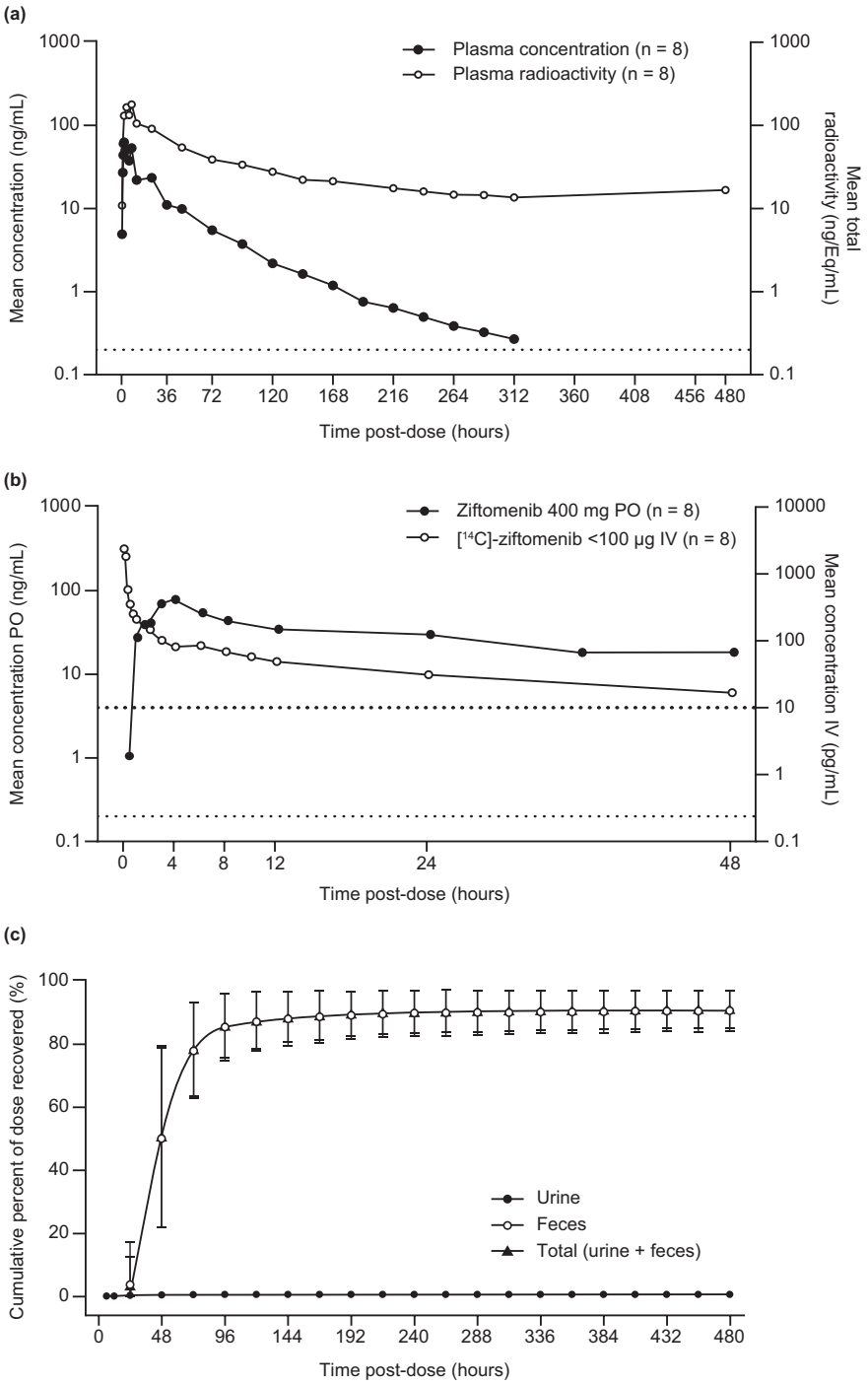

FIGURE 2 | Overlay of the arithmetic mean pharmacokinetic concentration–time profile of parent ziftomenib and total drug-related radioactivity after oral administration of ziftomenib 400 mg PO (containing 250 µCi [^{14}C]-ziftomenib) (a). Overlay of the arithmetic mean pharmacokinetic concentration–time profile after administration of oral ziftomenib 400 mg PO + [^{14}C]-ziftomenib < 100 µg IV (b). Arithmetic mean (± standard deviation) cumulative percentage of the radioactive dose recovered in urine and feces after oral administration of ziftomenib 400 mg PO (c). The dashed lines in (a) and (b) represent the lower limit of quantification of 0.2 ng/mL for ziftomenib. The upper dashed line in (b) represents the lower limit of quantification of 10 pg/mL for [^{14}C]-ziftomenib.

These findings resulted in a mean recovery of total radioactivity in urine and feces of 367 mg (90.3%; range 77.4%–94.8%) over the 480-h sampling period.

Compared with recovery data in feces and urine, total radioactivity was only quantifiable in blood in two participants and in plasma in four participants at 312 h postdose.

3.6 | Safety

Three participants reported a total of seven TEAEs; all TEAEs were mild in severity. These included diarrhea ($n = 2$), headache ($n = 2$), constipation, migraine, and dermal cyst ($n = 1$ each). Three TEAEs reported in two (25%) participants (headache, diarrhea, and migraine) were considered related to

ziftomenib by the investigator. No deaths or serious adverse events were reported. No TEAE led to study discontinuation. One participant in study part B reported myalgia of mild severity, which was not considered related to ziftomenib. No clinically significant findings were noted in clinical laboratory evaluations, vital signs, 12-lead ECGs, or physical examination data that would indicate any safety concerns with ziftomenib.

TABLE 2 | Summary of pharmacokinetic parameters for total [^{14}C] in plasma by accelerator mass spectrometry following a single oral administration of ziftomenib 400 mg.

	Ziftomenib 400 mg PO[a] (*N* = 8)
$AUC_{0-tlast}$, h•ngEq/mL	7610 (120.7) [8]
$AUC_{0-\infty}$, h•ngEq/mL	NC (NC) [2]
$\%AUC_{extrap}$, %	24.7 (44.0) [8]
C_{max}, ngEq/mL	100 (175.2) [8]
t_{max}, h	4.0 (2.0–8.0) [8]
t_{last}, h	312 (312–480) [8]
$t_{1/2}$, h	212 (51.9) [8]

Note: Data are presented as geometric mean (CV) [*n*]; data for t_{max} and t_{last} are presented as median (range) [*n*].
Abbreviations: $\%AUC_{extrap}$, percentage of area under the concentration–time curve due to extrapolation from the last quantifiable concentration to infinity; $AUC_{0-\infty}$, area under the concentration–time curve from time 0 extrapolated to infinity; $AUC_{0-tlast}$, area under the concentration–time curve from time 0 to the time of the last quantifiable concentration; C_{max}, maximum observed concentration; CV, coefficient of variation; NC, not calculated; PO, oral; $t_{1/2}$, terminal elimination half-life; t_{last}, time of the last quantifiable concentration; t_{max}, time to maximum observed concentration.
[a]Contains approximately 250 μCi of [^{14}C]-ziftomenib.

4 | Discussion

This ADME characterization of ziftomenib in humans was designed as a two-part study. In part A, the mass balance of [^{14}C]-ziftomenib after oral administration was evaluated, and in part B, the absolute bioavailability of ziftomenib after oral administration of nonradiolabeled ziftomenib and an intravenous microdose of [^{14}C]-ziftomenib was evaluated. Characterization of the pharmacokinetics and metabolism of [^{14}C]-ziftomenib after both oral and intravenous administration was important to quantify the absolute bioavailability and fraction absorbed of ziftomenib.

Following administration of a single dose of ziftomenib 400 mg, ziftomenib was characterized by a rapid absorption phase in plasma (median t_{max} of 3.5 h) and a long elimination half-life of 61.5 h, which would allow for once-daily dosing. The absolute bioavailability of ziftomenib following a single oral dose of 400 mg was 12.9%, with fraction absorbed (f_a) estimated to be 40%. Low f_a could be due to a combination of dissolution rate-limited absorption, and ziftomenib being a P-glycoprotein (P-gp) substrate, as determined in in vitro studies (PCT Patent Publ. No. WO2023/150635A1). However, the clinical relevance of any efflux due to P-gp on ziftomenib pharmacokinetics is currently unknown.

In this study, ziftomenib was determined to be the major component in plasma and feces and a trace component in urine. All circulating metabolites, including the active metabolites KO-739 and KO-516, were minor components (< 10% of the total drug-related exposure). Similarly, all metabolites were trace components in urine (cumulatively accounting for < 0.3% of the administered dose) and were minor components in feces (accounting for < 10%). Additionally, the metabolic profiles also demonstrated the absence of human-specific

TABLE 3 | Drug-related exposure of the total radioactivity of [^{14}C]-ziftomenib in the parent drug and eight identified metabolites.

			Drug-related exposure (%)			
Metabolite	**Retention time (min)[a]**	**Proposed identification**	**Plasma[b] (part A)**	**Plasma[b] (part B)**	**Urine[c]**	**Feces[c]**
Ziftomenib (parent)	38.0–40.7	Parent	15.1	13.4	0.0282	73.3
M1	36.3–37.0	KO-516	2.30	2.72	—	1.16
M9	35.8–36.0	KO-739	3.39	9.18	—	0.959
M23	34.5	Des(isopropyl-MSP)-oxy-ziftomenib	1.28	1.02	—	
M4	33.5–33.8	Desmethyl-oxy-ziftomenib	2.71	1.95	—	0.268
M60	32.7	Des(methyl-MSP)-oxy-ziftomenib	5.11	4.67	—	—
M3	31.2	Des(methyl-isopropyl-MSP)-ziftomenib	1.14	1.11	—	—
M56	24.5	Des(MSP)-ziftomenib	1.03	1.15	—	—
M17	23.7–23.8	Desethyl-oxy-ziftomenib	—	—	—	0.360

Note: The drug-related exposure of the total radioactivity of [^{14}C]-ziftomenib in the 11 unidentified metabolites is summarized in Table S4.
[a]Retention time ranges are from profiling analyses of plasma, urine, and feces. Part A = 6 participants (AUC_{0-312}). Part B = 2 participants (AUC_{0-312}).
[b]Based on the AUC pooled plasma concentration from time 0 to 312 h. Part A = 6 participants (AUC_{0-312}). Part B = 2 participants.
[c]Based on the percent of radioactive dose.

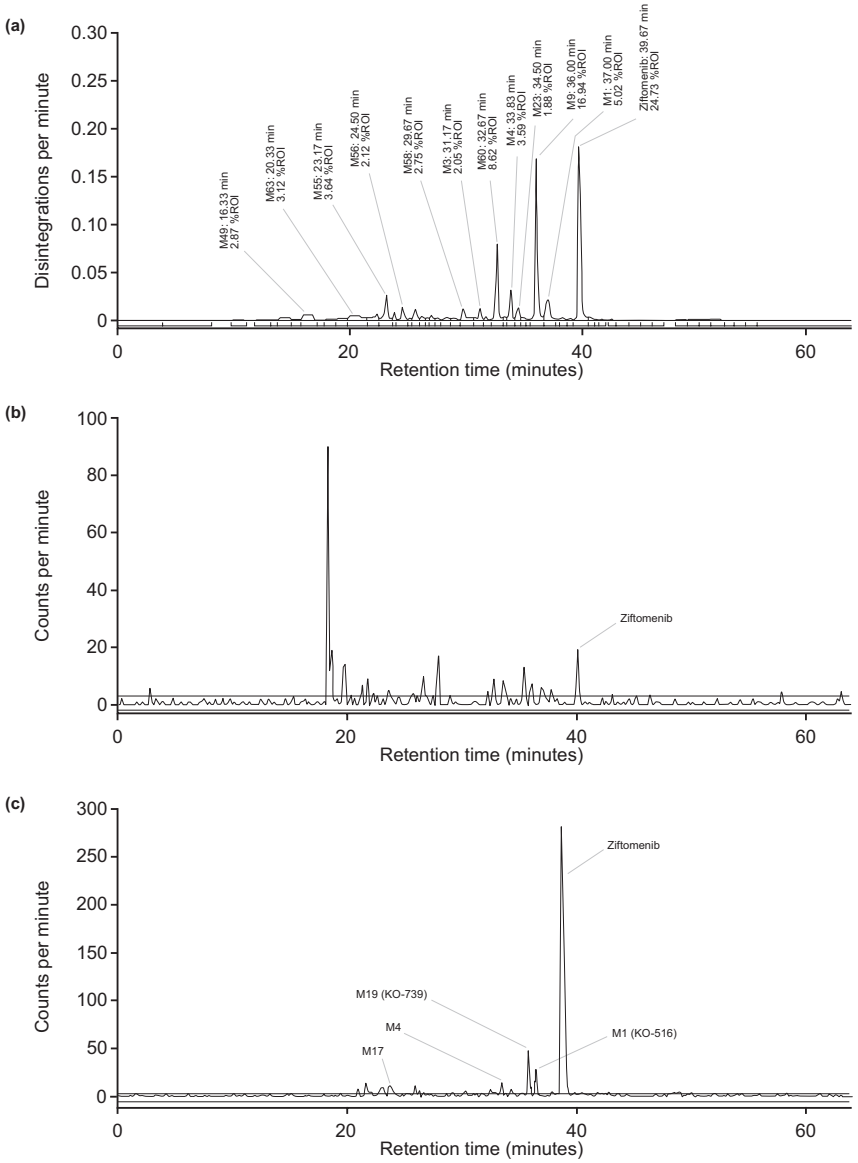

FIGURE 3 | Radiochromatogram profiles of pooled plasma (a), urine (b), and feces (c) samples by accelerator mass spectrometry following the administration of the ziftomenib 400 mg PO. %ROI, percentage of radioactivity for an individual region compared to the total radioactivity in the profile; CPM, counts per minute; DPM, disintegrations per minute.

metabolites. One goal of a radiolabeled human ADME study is the characterization of human in vivo metabolism to ensure that preclinical rodent and nonrodent toxicology species provide adequate coverage of the major human metabolites [19]. It is proposed that [^{14}C]-ziftomenib underwent metabolism primarily mediated by oxidation, N-demethylation, and N-dealkylation. Oxidation, N-dealkylation, and reduction are proposed secondary biotransformation pathways. No human-specific metabolites of ziftomenib were identified. Although ziftomenib is a CYP3A4 substrate in vitro, clinical data and physiologically based pharmacokinetic modeling demonstrated that inhibition of metabolic clearance by a coadministered strong or moderate CYP3A4 inhibitor is not expected to have a clinically meaningful impact on ziftomenib disposition or systemic pharmacokinetics [14].

Fecal excretion was the predominant route of elimination (total radioactivity recovery of ziftomenib was 89.7%). Metabolism was a minor elimination pathway for ziftomenib as all circulating metabolites were < 10% of total drug-related exposure. Ziftomenib was also a trace component in urine (0.5% of total radioactivity recovered), suggesting that renal elimination was minimal. As a result, ziftomenib pharmacokinetics are not expected to be impacted by renal impairment, and further clinical assessments in renal impairment are not scientifically justified. These data in healthy male participants are similar to data in a mouse absorption, deposition, metabolism, and excretion study of [^{14}C]-ziftomenib (Kura Oncology, data on file), which showed most radioactivity was recovered in feces (~86% of the total dose) and little in urine (~1.5% of the total dose).

The total radioactivity recovered in feces and urine (90.3%) in this study allowed for a reliable determination of the contribution of the elimination pathways of ziftomenib. This result aligns with US FDA and EMA guidance, which recommend that total radioactivity recovery in feces and urine should be

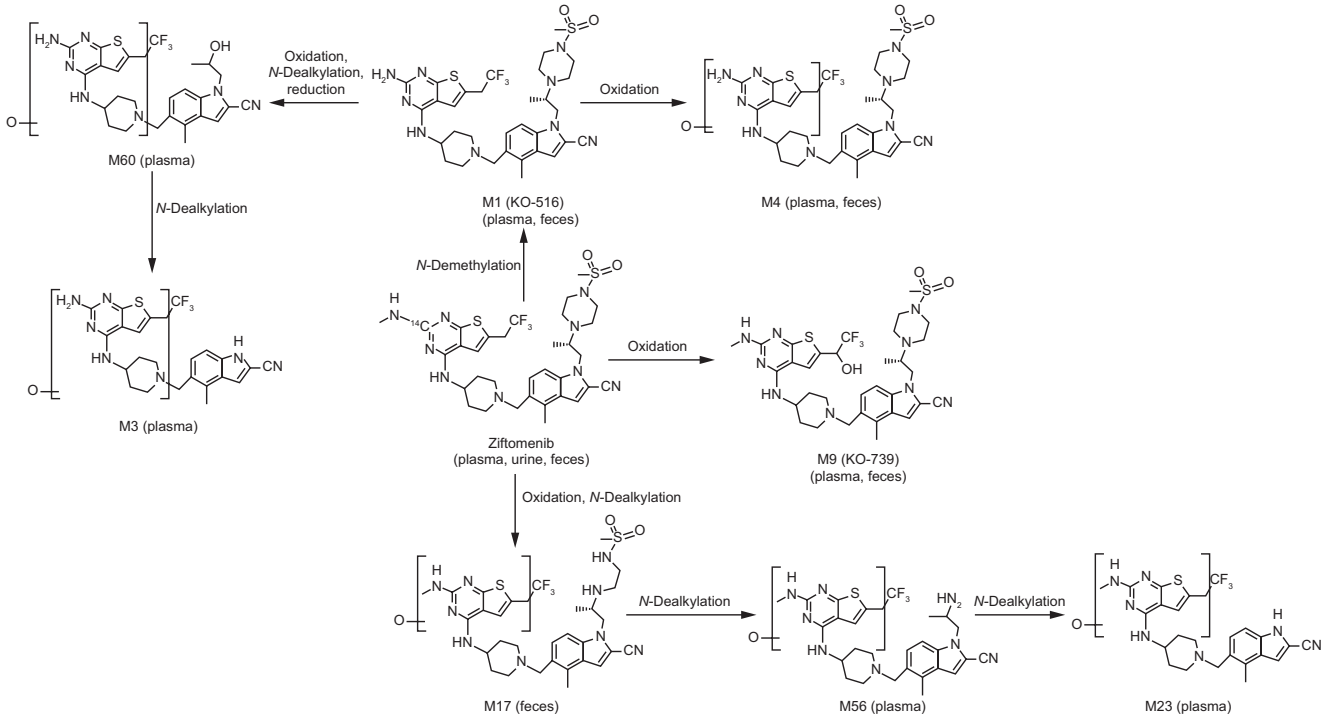

FIGURE 4 | The proposed biotransformation pathways of ziftomenib in humans. The location of C-14 in ziftomenib is shown.

> 90% [20, 21]. The high recovery indicates (1) appropriate selection of the C-14 position on ziftomenib, (2) adequate sample collection duration, especially given the long half-life of ziftomenib, and (3) appropriate use of sensitive bioanalytical methods.

Finally, a single dose of ziftomenib 400 mg in healthy male participants was tolerated with no new safety signals. Indeed, the TEAE incidence was low, and all treatment-related TEAEs were mild in severity.

In conclusion, this two-part ADME study of [^{14}C]-ziftomenib following oral and intravenous administration enabled the determination of the absolute bioavailability of ziftomenib and established that ziftomenib is amenable to once-daily dosing. Fecal excretion was the primary route of elimination, as unchanged parent drug, and ziftomenib underwent limited metabolism.

Author Contributions

Amitava Mitra, Julie Mackey Ahsan, Marilyn Tabachri, Taha El-Shahat, Mollie Leoni, and Stephen Dale wrote the manuscript. Amitava Mitra, Mollie Leoni, and Stephen Dale designed the research. Amitava Mitra, Julie Mackey Ahsan, Marilyn Tabachri, Taha El-Shahat, and Mollie Leoni performed the research. Amitava Mitra, Mollie Leoni, and Stephen Dale analyzed the data.

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Conflicts of Interest

Amitava Mitra employment with, owns stock and stock options with, and has patents and/or patent applications with Kura Oncology. Julie Mackey Ahsan employment with, owns stock and stock options with, and is reimbursed for conference fees, hotels, and travel expenses by Kura Oncology.

Marilyn Tabachri employment with, owns stock and stock options with, and reimbursed for conference fees, hotels, and travel expenses by Kura Oncology. Taha El-Shahat employment with Fortrea. Mollie Leoni employment with, owns stock or stock options with, has patents and/or patent applications with, and has other financial/nonfinancial interests in Kura Oncology. Stephen Dale employment with, owns stock or stock options, has patents and/or patent applications with, and has other financial or nonfinancial interests with Kura Oncology.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.