The pharmacokinetics, metabolism, and excretion of dolutegravir, an unboosted, once-daily human immunodeficiency virus type 1 integrase inhibitor, were studied in healthy male subjects following single oral administration of [14C]dolutegravir at a dose of 20 mg (80 μCi). Dolutegravir was well tolerated, and absorption of dolutegravir from the suspension formulation was rapid (median time to peak concentration, 0.5 h), declining in a biphasic fashion. Dolutegravir and the radioactivity had similar terminal plasma half-lives (t1/2) (15.6 versus 15.7 h), indicating metabolism was formation rate limited with no long-lived metabolites. Only minimal association with blood cellular components was noted with systemic radioactivity. Recovery was essentially complete (mean, 95.6%), with 64.0% and 31.6% of the dose recovered in feces and urine, respectively. Unchanged dolutegravir was the predominant circulating radioactive component in plasma and was consistent with minimal presystemic clearance. Dolutegravir was extensively metabolized. An inactive ether glucuronide, formed primarily via UGT1A1, was the principal biotransformation product at 18.9% of the dose excreted in urine and the principal metabolite in plasma. Two minor biotransformation pathways were oxidation by CYP3A4 (7.9% of the dose) and an oxidative defluorination and glutathione substitution transformation product at 18.9% of the dose excreted in urine and the principal metabolite in plasma. Two minor biotransformation pathways were oxidation by CYP3A4 (7.9% of the dose) and an oxidative defluorination and glutathione substitution (1.8% of the dose). No disproportionate human metabolites were observed.

Since zidovudine was first approved in the United States in 1987 for the treatment of human immunodeficiency virus type 1 (HIV-1) infection, the incorporation of multiple agents into combination antiretroviral therapy (ART) that targets the different phases of the HIV replication cycle has enhanced the management of HIV infection. Inhibitors of HIV-1 integrase have been the last of the three virus-encoded enzymes (reverse transcriptase, integrase, and protease) for which therapeutic agents have been developed, with raltegravir in 2007 being the first licensed HIV-1 integrase inhibitor. The HIV integrase enzyme coordinates the insertion of viral DNA into the host chromosome (1). The 2-metal binding class of integrase inhibitors targets the binding of the divalent metal ions Mg$^{2+}$ and Mn$^{2+}$ (primarily Mg$^{2+}$ under physiological conditions) to prevent the strand transfer step in the integration process, thereby inhibiting viral replication (2-4).

Dolutegravir (S/GSK1349572) (DTG) is a potent tricyclic carbamoyl pyridine integrase inhibitor (5) possessing subnanomolar antiviral 50% effective concentrations (EC$_{50}$) in vitro and a distinct resistance profile designed to retain activity against raltegravir- and elvitegravir-resistant strains (A. Sato, M. Kobayashi, T. Seki, C. W. Morimoto, T. Yoshinaga, T. Fujiwara, B. A. Johns, and M. Underwood, presented at the 8th European HIV Drug Resistance Workshop, Sorrento, Italy, 2010). Furthermore, there is a potential for a higher barrier to resistance based on serial-passage experiments, do lutegravir activity against HIV-1 with single and multiple integrase mutation combinations (6), and prolonged binding to mutant integrase proteins in integrase-Mg$^{2+}$-DNA complexes (7). Dolutegravir has been generally well tolerated, with in vivo efficacy demonstrated in a phase Iia 10-day mono- therapy study (8), at 48 weeks of a phase Ib antiretroviral-naïve-adult study (9), and at 24 weeks of a phase Ib treatment-experienced-subject pilot study (10). Dolutegravir exhibits rapid oral absorption and dose-proportional kinetics (2 to 100 mg) from a suspension formulation, and its low apparent clearance and oral terminal half-life (t1/2) of approximately 15 h supports once-daily dosing without the need for a boosting agent (11). Dolutegravir has demonstrated low to moderate pharmacokinetic variability, with a predictable exposure-response relationship (8). In vitro, dolutegravir is primarily metabolized by UDP-glucuronosyltransferase (UGT) 1A1 and cytochrome P450 (CYP) 3A4 and at clinically relevant concentrations is not an inhibitor of CYP or UGT enzymes (12). DTG has demonstrated a limited number of clinically significant drug-drug interactions without dose adjustment for most ART and other commonly coadministered drugs in integrase-naïve subjects (I. Song, J. Borland, S. Chen, A. Peppercorn, P. Savina, T. Wajima, S. Min, G. Nichols, and S. Piscitelli, presented at the 13th International Workshop on Clinical Pharmacology of HIV Therapy, Barcelona, Spain, 2012).

This mass balance study identifies the major metabolic profile in humans for the evaluation of cross-species comparisons to assess the systemic exposure to dolutegravir and its metabolites. This comparison provides support for the selection of animal species used in the nonclinical safety assessment. Data from human mass balance studies also provide information on excretory routes that can be used to guide studies in special patient populations, such as individuals with hepatic or renal impairment. In addition, the assessment of exposure to total drug-related material can aid in the design of pharmacodynamic studies, such as a cardiac repolarization study, by helping to identify time points for collecting...
selecting the radioactive dose were based on balancing the analytical requirements for meeting the study objectives, minimizing the radioactivity exposure of the volunteers, and a radioactivity exposure not to exceed approximately one-third of the annual average background effective dose to the whole body (including radon) of ~3 mSv. The effective radioactive dose of 0.96 mSv was selected following review of the effective dose and effective-dose equivalent calculations performed independently of GSK using the Medical Internal Radiation Dose system (15) and the MIRDOS 3.1 software package obtained from the Radiation Internal Dose Information Center at Oak Ridge, TN. The 20-mg dose was selected to be within the linear range established from a previous study (11) to provide sufficient mass and a characteristic ratio of labeled to unlabeled material to aid in metabolite identification efforts. Except for an hour before and an hour immediately after dosing, subjects were allowed water ad libitum and were provided breakfast following the 4-hour-postdose sample collection.

As part of a separate drug interaction study with efavirenz, informed consent was received from a group of 12 nonsmoking fasted adult male subjects who received 50 mg of unlabeled dolutegravir alone as two 25-mg tablets once daily for 5 days. After 5 days of dosing, residual urine collected over 24 h was used to isolate the target metabolite, M4, for further characterization.

Sample collection. Following single-dose 13C-dolutegravir administration, blood and plasma samples were collected (EDTA anticoagulant) predose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 h postdose, with sample collection continuing until radioactivity in 2 successive samples was less than or equal to twice the background radiation. Blood was stored at 2 to 8°C before analysis, and plasma was stored at −20°C or colder.

Urine was collected predose and at 0 to 6, 6 to 12, and 12 to 24 h and at 24-hour intervals until discharge criteria were met. Urine was stored at 2 to 8°C during collection and pooled over the collection interval, and then portions were subsequently frozen at −20°C or colder. Feces were collected predose and pooled at 24-hour intervals until the subject was discharged. Fecal samples were homogenized with 20% ethanol in deionized water (approximately 3 times the sample weight) by using a probe-type homogenizer, and then portions of the homogenate were stored frozen at −20°C or colder. Scintillation-counting data (counts per minute) were automatically corrected for counting efficiency using the external-standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards.

Measurement of radioactivity. Plasma (in duplicate) and urine (in triplicate) sample radioactivity was determined by liquid scintillation counting (LSC). Portions of each sample were mixed with Ultima Gold XR scintillation cocktail and analyzed for radioactivity using a Model 2900TR liquid scintillation counter (Packard Instrument Co., Meriden, CT) for at least 5 min or 100,000 counts.

Whole-blood (in duplicate) and fecal-homogenate (in triplicate) samples were combusted in a Model 307 Sample Oxidizer (Packard Instrument Co.), and the resulting 14CO2 was trapped in a mixture of Perma Fluor and Carbosorb and then analyzed by LSC. Oxidation efficiency was evaluated on each day of sample combustion by analyzing a commercial radiolabeled standard both directly in scintillation cocktail and by oxidation. Acceptance criteria included combustion recoveries of between 95% and 105%.

Quantification of dolutegravir in plasma. Plasma samples were analyzed for dolutegravir using a validated liquid chromatography and tandem mass spectrometry (LC–MS–MS) method. Plasma samples were extracted by protein precipitation with acetonitrile containing [13C6]dolutegravir as the internal standard. The extract was injected onto an Acquity HPLC system (Waters Associates, Milford, MA), and a mobile phase of 39% acetonitrile in aqueous 0.1% formic acid was used to elute components from a 2.1- by 50-mm 3.5-µm XBridge C18 column (Waters Associates). The eluate was detected by using a Sciex API-4000 (AB Sciex, Framingham, MA) equipped with a TurboSpray ionization source using positive ion mode and multiple reaction monitoring (dolutegravir, ion transition m/z 420 to 277; internal standard, ion transition...
Data acquisition and processing were performed with Analyst 1.4.1 software (AB Sciex). The calibration range for dolutegravir was 5 to 5,000 ng/ml. Quality control samples, prepared at three different analyte concentrations and stored with study samples, were analyzed with each batch of samples against separately prepared calibration standards. For the analysis to be acceptable, no more than one-third of the total quality control results and no more than one-half of the results from each concentration level were allowed to deviate from the nominal concentration by more than 15%. The applicable analytical runs met all predefined run acceptance criteria.

Pharmacokinetic analysis of dolutegravir. All pharmacokinetic parameters were calculated by using standard noncompartmental analysis and WinNonlin Pro 5.2 software (Pharsight Corp., Mountain View, CA) based on actual sampling times. After single-dose administration, the following pharmacokinetic parameters were calculated for total radioactivity in whole blood and plasma and for dolutegravir in plasma: area under the concentration-time curve from time zero to the last quantifiable time point (AUC0–\( \infty \)), area under the concentration-time curve from time zero to infinity (AUC0–\( \infty \)), maximum observed concentration (\( C_{\text{max}} \)), time to \( C_{\text{max}} \), absorption lag time, and terminal-phase \( t_{1/2} \). The additional parameters apparent oral clearance and apparent volume of distribution were calculated for plasma dolutegravir. Total-radioactivity/dolutegravir ratios were calculated for the terminal-phase \( t_{1/2} \) values and for plasma AUC0–\( \infty \) values. The blood-to-plasma ratio of total radioactivity (\( C_{\text{p}}/C_{\text{p}} \)) was calculated at each time point, and the percent association with blood cellular components (%Assoc) was calculated as follows: %Assoc = 100 - \([C_{\text{p}}/C_{\text{p}} - (1 - \text{Hct})]/C_{\text{p}} \times 100\), where Hct is the hematocrit expressed as a fraction, \( C_{\text{p}} \) is the concentration of radioactivity in blood, and \( C_{\text{p}} \) is the concentration of radioactivity in plasma. Plasma concentration-time profiles for dolutegravir were compared with those for total radioactivity to estimate how much of the total measured radioactivity was due to metabolites. Descriptive statistics were provided for pharmacokinetic parameters.

Sample preparation for radiochemical profiling. (i) Sample pooling. Three individual pools of plasma were created from the 6th, 24th, and 48-hour post-dose samples by combining equal volumes of plasma from each subject at each time point. Representative samples of urine and fecal homogenates from each subject were pooled proportionally by weight to obtain a pool containing 85% or more of the radioactivity excreted via that route.

(ii) Sample pretreatment. To enhance extraction of radioactivity, each plasma and fecal-sample portion was mixed with a half portion of EDTA disodium salt solution (10 mg/ml in water) before initial extraction.

(iii) Plasma and fecal-homogenate extraction. A portion of each pooled plasma or fecal-homogenate sample was extracted by adding 1 sample volume of methanol and vortex mixing, followed by the addition of 3 sample volumes of acetonitrile. The extract was centrifuged, and the supernatant was transferred into a fresh tube. The extraction procedure was repeated twice on the residual pellet, and the resulting supernatants were combined with the supernatant from the first extraction. The fecal pellet was further extracted twice with acetonitrile-water-formic acid (50:50:0.1 by volume), and the resulting supernatants were combined with the previous extractions. The total weight of each combined extract was determined, and weighed portions were removed for LSC to determine the efficiency of extraction of radioactivity. The combined supernatants were dried under a stream of nitrogen and reconstituted by adding methanol (200 \( \mu \)l) and water containing EDTA (2 mg/ml; 1,000 \( \mu \)l). The sample extract was centrifuged, and then a portion of each supernatant was removed to determine the recovery of radioactivity upon reconstitution. A portion was analyzed for the metabolic profile by using HPLC with radiochemical detection.

(iv) Urine extraction. A pooled urine sample from each subject was centrifuged, and a portion was analyzed by using HPLC with radiochemical detection.

Conditions for HPLC radiochemical profiling. Radiochemical profiles of plasma, urine, and feces were generated by using an Agilent (Palo Alto, CA) 1200 HPLC system. Radiochromatographic peaks were separated following injection onto a Symmetry C18 column (100 by 4.6 mm; 3.5 \( \mu \)m; Waters Associates) at 40°C and eluted under gradient conditions with a mobile phase consisting of 2 solvents: solvent A, 0.1% ammonium acetate in water; and solvent B, 0.1% ammonium acetate in acetonitrile. The gradient conditions at a flow rate of 1.5 ml/min were as follows. Solvent B started at 5% and increased linearly to 25% from 3.3 to 40 min and then to 35% from 40 to 43.3 min and to 95% from 43.3 to 46.7 min. The gradient was held at 95% for 1.3 min and returned to the initial conditions. The eluate was analyzed with a model 625TR series radiochemical flow detector (PerkinElmer, Waltham, MA) and Ultima Flo M scintillation fluid (3 ml/min). For samples requiring greater sensitivity, radiochemical profiles were generated offline by collecting column effluent fractions into Deepwell Lumaplate 96-well plates (PerkinElmer), drying under a stream of nitrogen, and then analyzing with a TopCount NXT microplate scintillation counter (PerkinElmer). The limit of detection and quantification for offline analysis was set to 2 times background.

Metabolic isolation and identification. The HPLC method described above for radiochemical profiling was also applied to metabolic analyses. An Agilent 1100 HPLC System was interfaced with an LQT-Orbitrap hybrid mass spectrometer (ThermoFisher Scientific, San Jose, CA), and LC–MS–MS spectra were acquired by electrospray ionization in the positive ion mode using data-dependent scanning from a mass list, which consisted of all known and likely metabolites. During the LC separation, a postcolumn split was used to direct 20% of the sample to the mass spectrometer. A full-scan mass spectrum (resolution, 30,000) was collected, and the data were interrogated in real time to identify mass peaks (\( \pm 5 \) ppm). If present, the metabolite mass peaks were selected as target peaks for subsequent MS–MS scans using either high- or low-resolution settings.

The remaining 80% of LC effluent from the postcolumn split was fractionated by a Collect Pal fraction collector (LEAP Technologies, Carrboro, NC) at 20 s per well. A portion (25 \( \mu \)l) from each fractionated well was transferred into a deep-well Lumaplate-96 solid-scintillant microplate (PerkinElmer) and dried under a stream of nitrogen. The radioactivity in each well was measured by using a Topcount NXT microplate scintillation counter (PerkinElmer). The data from the Topcount NXT were transferred to Excel (Microsoft, Redmond, WA) and used to generate reconstructed radiochemical profiles. The reconstructed radiochemical profiles were compared with the LC–MS–MS data and the definitive radiochemical profiles to ensure proper peak assignment.

Urine from the repeat dose administration was used to isolate adequate amounts of M4 for nuclear magnetic resonance (NMR) characterization. Urine was pooled by volume across all subjects to create a pool of 2.3 liters and then was filtered using a 0.45-\( \mu \)m nylon filter(s) before being freeze-dried in an Advantage Lyophilizer (Virtis, Gardiner, NY). The dried urine sample was reconstituted in 0.1% ammonium acetate in water-0.1% ammonium acetate in acetonitrile, (90:10 [vol/vol]; 230 ml), and portions were centrifuged at ambient temperature before metabolite isolation. To ensure adequate purity for NMR analysis, the isolation of M4 was performed using three separate LC methods. During each LC separation, a postcolumn split was used to direct 10% of the sample to an LTQ mass spectrometer (ThermoFisher Scientific). Data-dependent scanning with a mass list was used to initiate time-based fraction collection for the remaining 90% of the LC effluent. Fractions were combined and reduced to dryness using a rotary concentrator (Genevac Inc., Gardiner, NY). Before each cleanup, LC–MS was performed to provide mass confirmation of M4. Liquid chromatography method 1 employed an Agilent 1100 HPLC System with a Symmetry Prep C18 column (7.8 by 150 mm; 7 \( \mu \)m; Waters Associates) at 40°C and eluted under gradient conditions with a mobile phase consisting of two solvents: solvent A, 0.1% ammonium acetate in water, and solvent B, 0.1% ammonium acetate in acetonitrile. The gradient conditions at a flow rate of 5.0 ml/min were as follows. Solvent B started at 10% and increased linearly to 22% from 0 to 15 min and then to 95% from 15 to 15.1 min. The gradient was held at 95% for 3 min and finally at 10% for 5 min. The remaining 90% of the LC effluent was transferred into a deep-well Lumaplate-96 solid-scintillant microplate (PerkinElmer) and dried under a stream of nitrogen. The radioactivity in each well was measured by using a Topcount NXT microplate scintillation counter (PerkinElmer). The data from the Topcount NXT were transferred to Excel and used to generate reconstructed radiochemical profiles. The reconstructed radiochemical profiles were compared with the LC–MS–MS data and the definitive radiochemical profiles to ensure proper peak assignment.
TABLE 1 Summary of percent radioactivity recovered in urine and feces from healthy male human subjects after a single oral 20-mg (80-μCi) suspension dose of [14C]dolutegravir

<table>
<thead>
<tr>
<th>Subject</th>
<th>% of administered dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces</td>
</tr>
<tr>
<td>531001</td>
<td>72.0</td>
</tr>
<tr>
<td>531002</td>
<td>65.2</td>
</tr>
<tr>
<td>531004</td>
<td>61.0</td>
</tr>
<tr>
<td>531005</td>
<td>59.4</td>
</tr>
<tr>
<td>531006</td>
<td>65.7</td>
</tr>
<tr>
<td>Mean</td>
<td>64.0</td>
</tr>
<tr>
<td>SD</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*Subject 531006 withdrew from the study after 144 h postdose. The subject was excluded from descriptive statistics after this time point.*

RESULTS

Subjects. Six healthy, nonsmoking white males aged between 32 and 46 years (mean, 37.5 years) with body mass indices between 23.7 and 28.4 (mean, 26.6) participated in the study.

Safety and tolerability. All subjects completed the study portion as planned and received the correct treatment in the fasting state. The treatments were well tolerated, and no deaths, nonfatal serious adverse events, pregnancies in female partners of male subjects, or withdrawals due to AEs were reported. Five out of six subjects (83%) reported at least one AE during the study, with diarrhea (2 subjects; 33%) being the most commonly reported drug-related AE. All AEs were mild (grade 1) to moderate (1 subject with grade 2 vomiting) in intensity. No grade 4 or drug-related grade 3 laboratory abnormalities were reported. No clinically significant trends in postdose clinical laboratory values, vital signs, or electrocardiograms were observed.

Excretion and recovery of radioactivity. After oral administration of [14C]dolutegravir, the recovered radioactivity, which was principally detected in feces, accounted for a mean of 64.0% of the administered dose (range, 59.4% to 72.0%) (Table 1). Urinary excretion accounted for a mean of 31.6% of the administered dose (range, 24.0% to 36.7%). The mean total recovery of radioactivity (Fig. 2) was 95.6% of the dose (range, 93.2% to 97.6%) by 216 h postdose. Most of the dose (94.5%) was recovered in feces and urine by 144 h postdose. Variability in recovery of radioactivity was very low, with coefficient of variation values of 1.5% (total recovery), 7% (feces), and 15% (urine). The radioactivity recovery measurements were based on the actual radioactive dose administered to each volunteer.

Pharmacokinetics of radioactivity and dolutegravir. The recovery of radioactive material from the 6-, 24-, and 48-hour pooled plasma samples following solvent extraction ranged from 99.8% to 104%, indicating no noticeable irreversible binding to or sequestration by plasma components.

The mean concentration-time profiles of total radioactivity in blood and plasma of and dolutegravir in plasma are presented in Fig. 3. A summary of select pharmacokinetic parameters is presented in Table 2. Absorption of radioactivity and dolutegravir from the suspension formulation was rapid, with a median time to peak concentration ranging from 0.5 h (plasma dolutegravir and radioactivity) to 1.25 h (blood radioactivity). Plasma concentrations of radioactivity and dolutegravir declined biphasically, falling below the limit of quantification by 168 h postdose. Dolutegravir represented the predominant drug-related component in the plasma, essentially accounting for all the plasma radioactivity through 4 h postdose, with the plasma dolutegravir AUC∞–t∞ accounting for greater than 97% of the total plasma radioactivity AUC∞–t∞. The mean blood/plasma radioactivity concentration ratios through 72 h postdose ranged from 0.441 to 0.535, and the association with blood cellular components (hematocrit range, 43.1 to 46.6) was less than 5% at all time points in all subjects, indicating minimal association of dolutegravir or metabolites with blood cells. The estimated terminal t1/2 values were similar.
among plasma dolutegravir (15.6 h), plasma radioactivity (15.7 h), and blood radioactivity (14.6 h). The geometric mean $t_{1/2}$ ratios of dolutegravir to blood radioactivity and of dolutegravir to plasma radioactivity were 1.07 and 0.99, respectively.

**Metabolite profiling.** Representative radiochromatograms from pooled 24-hour plasma samples, the 0- to 72-hour urine collection pool, and the 0- to 96-hour fecal homogenate pool are presented in Fig. 4. A summary of the mean relative abundances of the principal quantifiable radiochromatographic peaks is presented in Table 3. Recovery of radioactivity from each excreta sample following the extraction procedures ranged from 95% to 101%.

(i) **Plasma.** Dolutegravir accounted for 95.2%, 96.8%, and 99.8% of the radioactivity in the 6-, 24-, and 48-hour pooled plasma radiochromatograms, respectively. The glucuronide, M2, was a minor component, corresponding to 2.4% of the 6-hour and 1.5% of the 24-hour plasma pool radiochromatograms, and was not quantifiable in the 48-hour plasma pool radiochromatogram. Combined, dolutegravir and M2 accounted for approximately 98.6% of the total radioactivity in each of the three plasma pools.

(ii) **Urine.** The principal quantifiable radiochromatographic peaks identified (Table 3) were unchanged dolutegravir, its glucuronide conjugate (M2), and a product of oxidation (M3) with its hydrolysis product (M1). The predominant component in urine was M2, which represented a mean of 62.5% of the radioactivity (18.9% of the dose). The renal excretion of unchanged dolutegravir was low (0.7% of the dose). Small radiochromatographic peaks present, which collectively accounted for <5% of the administered dose, were not fully characterized.

![FIG 3 Mean concentration-time profiles of plasma dolutegravir and total radioactivity in blood and plasma after a single 20-mg (80-μCi) oral dose of [14C]dolutegravir to six healthy male subjects. (Inset) Data using log scale on the y axis.](image)

![FIG 4 Representative radiochromatograms of plasma, urine, and feces after a single oral 20-mg (80-μCi) dose of [14C]dolutegravir to an individual healthy male subject. (A) Plasma at 24 h. (B) Urine at 0 to 72 h. (C) Feces at 0 to 96 h.](image)

**TABLE 2** Summary of selected pharmacokinetic parameters for plasma dolutegravir, plasma radioactivity, and blood radioactivity in healthy male human subjects after a single oral 20-mg (80-μCi) dose of [14C]dolutegravir as a suspension

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC_{0–t} (μg · h/ml)</th>
<th>AUC_{0–∞} (μg · h/ml)</th>
<th>CL/F (liter/h)</th>
<th>Vz/F (liter)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma dolutegravir</td>
<td>6</td>
<td>2.57 (24)</td>
<td>0.50 (0.50–2.00)</td>
<td>35.7 (12)</td>
<td>35.9 (12)</td>
<td>0.56 (12)</td>
<td>12.5 (9)</td>
<td>15.6 (16)</td>
</tr>
<tr>
<td>Plasma radioactivity</td>
<td>6</td>
<td>2.46 (24)</td>
<td>0.50 (0.50–1.50)</td>
<td>35.9 (11)</td>
<td>36.1 (11)</td>
<td>NR</td>
<td>NR</td>
<td>15.7 (14)</td>
</tr>
<tr>
<td>Blood radioactivity</td>
<td>6</td>
<td>1.13 (25)</td>
<td>1.25 (0.50–2.00)</td>
<td>17.7 (15)</td>
<td>18.4 (13)</td>
<td>NR</td>
<td>NR</td>
<td>14.6 (12)</td>
</tr>
</tbody>
</table>

*Values are geometric mean (percent coefficient of variation) unless otherwise stated; NR, not reported. CL/F, apparent oral clearance; $T_{\text{max}}$, time to $C_{\text{max}}$; Vz/F, apparent volume of distribution.

*Median (range).*
The pharmacokinetics, metabolism, and excretion of dolutegravir after a single oral-suspension dose (20 mg or 80 μCi) of [14C]dolutegravir were investigated in this study. Dolutegravir was well tolerated in these healthy male subjects, with a safety profile consistent with other phase I dolutegravir studies and which have been previously reported in patients who have received a dose of...
<table>
<thead>
<tr>
<th>ID</th>
<th>RT (min)</th>
<th>Proposed structure</th>
<th>Parent ion [M+H]$^+$ (error) (ppm)</th>
<th>Fragment ion(s)</th>
<th>$^1$H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTG</td>
<td>44</td>
<td><img src="image1" alt="DTG Structure" /></td>
<td>420.1377 (2.7) 295, 277</td>
<td></td>
<td>$^1$H NMR (600 MHz, DMSO-d$_6$) δ ppm 1.33 (d, $J = 6.88$ Hz, 3H) 1.54 (d, $J = 13.75$ Hz, 1H) 1.97–2.04 (m, 1H) 3.16 (d, $J = 5.23$ Hz, 1H) 3.89 (dd, $J = 11.69$, 2.89 Hz, 1H) 4.35 (dd, $J = 13.76$, 5.78 Hz, 1H) 4.54 (d, $J = 6.05$ Hz, 2H) 5.45 (t, $J = 6.60$ Hz, 1H) 7.06 (dd, $J = 8.53$, 2.48 Hz, 1H) 7.25 (d, $J = 2.48$ Hz, 1H) 7.38 (d, $J = 8.80$ Hz, 1H) 8.50 (s, 1H) F29 = −116.4 ppm, F30 = −113.4 ppm</td>
</tr>
<tr>
<td>M1</td>
<td>14</td>
<td><img src="image2" alt="M1 Structure" /></td>
<td>294.1087 (0.8) 277</td>
<td></td>
<td>$^1$H NMR (600 MHz, DMSO-d$_6$) δ ppm 1.26 (d, $J = 6.59$ Hz, 3H) 1.49 (d, $J = 13.17$ Hz, 1H) 1.93 (m, 1H) 3.79–3.88 (m, 1H) 3.90–3.95 (m, 2H) 4.23 (dd, $J = 14$, 4 Hz, 1H) 4.41 (dd, $J = 14$, 2 Hz, 1H) 4.74 (m, 1H) 5.34 (br m, 1H) 8.29 (s, 1H)</td>
</tr>
<tr>
<td>M2</td>
<td>26.5</td>
<td><img src="image3" alt="M2 Structure" /></td>
<td>596.1674 (−2.2) 420</td>
<td></td>
<td>$^1$H NMR (600 MHz, DMSO-d$_6$) δ ppm 1.16 (d, $J = 6.96$ Hz, 3H) 1.48 (d, $J = 13.18$ Hz, 1H) 1.92 (td, $J = 12.45$, 6.22 Hz, 1H) 3.10–3.19 (m, 1H) 3.21–3.28 (m, 1H) 3.29–3.35 (m, 1H) 3.40 (d, $J = 7.69$ Hz, 1H) 4.51 (d, $J = 3.30$ Hz, 1H) 4.61–4.70 (m, 1H) 5.14 (d, $J = 7.69$ Hz, 1H) 5.27 (dd, $J = 7.32$, 3.66 Hz, 1H) 7.00 (d, $J = 8.42$, 2.20 Hz, 1H) 7.15 (td, $J = 9.79$, 2.38 Hz, 1H) 7.35 (dd, $J = 15.38$, 8.79 Hz, 1H) 8.46 (s, 1H) 10.22 (t, $J = 5.86$ Hz, 1H)</td>
</tr>
<tr>
<td>M3</td>
<td>39 and 42</td>
<td><img src="image4" alt="M3 Structure" /></td>
<td>436.1304 (−2.4) 418, 294</td>
<td></td>
<td>$^1$H NMR (600 MHz, DMSO-d$_6$) δ ppm 1.27 (d, $J = 7.03$ Hz, 1H) 1.46–1.54 (m, 1H) 1.95 (none, 1H) 3.80–3.88 (m, 1H) 3.91–4.02 (m, 1H) 4.23–4.34 (m, 1H) 4.47 (td, $J = 13.40$, 3.95 Hz, 1H) 4.74 (dd, $J = 12.30$, 6.15 Hz, 1H) 5.37 (q, $J = 4.83$ Hz, 1H) 6.54 (d, $J = 8.35$ Hz, 1H) 7.07 (t, $J = 8.35$ Hz, 1H) 7.15 (t, $J = 9.45$ Hz, 1H) 7.56 (dd, $J = 15.38$, 8.35 Hz, 1H) 8.38 (s, 1H) 10.67 (d, $J = 8.35$ Hz, 1H)</td>
</tr>
<tr>
<td>M4$^b$</td>
<td>26</td>
<td><img src="image5" alt="M4 Structure" /></td>
<td>537.1470 (3.8) 294, 277, 244</td>
<td></td>
<td>$^1$H NMR (600 MHz, methanol-d$_4$) δ ppm 1.40 (d, $J = 7.00$ Hz, 3H) 4.29 (m, 1H) 4.51 (m, 1H) 4.49 (m, 1H) 6.65 (s, 1H) 7.54 (s, 1H) 8.38 (s, 1H) H5-H6, cysteine resonances not assigned. $^{13}$C (HSQC) δ ppm C2-75.8, C7-51.6, C9-139.9, C15-13.4, C22-35.7, C25-102.9, C28-136.8 F29 = −117.1 ppm</td>
</tr>
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$^a$RT, retention time.  
$b$A putative structural isomer is shown.
50 mg that resulted in a significant drop in plasma HIV-1 RNA (8–10). Mass balance was achieved, with most of the radioactivity recovered in urine and feces within 6 days after oral administration, consistent with the observed oral $t_{1/2}$ and indicative of the expected metabolic stability of the radiolabel. By its mode of action, dolutegravir binds multivalent cations, and the mass balance that was attained, in addition to the complete extraction from sample matrices, indicates no notable sequestration or covalent binding of dolutegravir or metabolites. During an initial rising-dose pharmacokinetic study, the AUC of dolutegravir increased proportionally with single oral-suspension doses of 2 to 100 mg and showed time-independent pharmacokinetics during administration of repeat oral-suspension doses of 10 to 50 mg (11). The results from this initial pharmacokinetic study, combined with the pharmacokinetics from our current study, indicate that a 20-mg dose can be extrapolated directly to the anticipated clinical dose of 50 mg.

Recovery from excreta and radiochromatograms showed low intersubject variability, with only small quantitative differences across each of the six subjects complementing the low pharmacokinetic variability of dolutegravir. Fecal excretion was the primary route of elimination and reflects both elimination of unabsorbed material and biliary secretion of dolutegravir or its metabolic products.

Absorption of dolutegravir from the suspension formulation was rapid, consistent with earlier observations (11). Based on the fractions of the dose renally eliminated (31.6%) and excreted in the feces as oxidative metabolic products (3.1%), at least 34% of the administered dose was absorbed. The expected biliary secretion of dolutegravir glucuronide, the principal metabolite, suggests that the absorption of dolutegravir is higher. Although bile was not collected as part of this study, biliary secretion of dolutegravir and dolutegravir conjugates (i.e., M2) in these subjects is inferred from the analysis of bile samples collected from rats and monkeys (unpublished data) and from molecular weight hypotheses of biliary secretion (16). The mean whole-gut (colonic) transit time (the time from ingestion to passage) in humans has been reported to be approximately 30 to 40 h, with an upper limit of normal of 70 h and a lower value of 14 h (17). Therefore, if the mean percentages of the dose at 48 and 72 h postdose recovered in the feces of 16% and 31%, respectively, represent unabsorbed material, then the 33% to 48% remaining to be excreted after this time would be indicative of dolutegravir conjugates that undergo biliary secretion and are excreted as parent dolutegravir. Thus, absorption of dolutegravir is expected to be higher than that reflected by fecal elimination, and the total systemic burden of absorbed material would be approximately 70% to 80%. Food was noted to modestly increase systemic exposure to dolutegravir, but the effect is not clinically significant, and dolutegravir can be taken without regard to meals (11, 18). Therefore, the results from this study are applicable to the nonfasted or fasted state, as food is not expected to alter the metabolic profile of dolutegravir.

The similarity of the dolutegravir concentration and the radioactivity concentration plasma profiles indicates that unchanged dolutegravir was the predominant drug-related component in the plasma, accounting for more than 97% of the total plasma radio-carbon AUC$_{0\rightarrow\infty}$. This was confirmed by LC–MS–MS analysis, where unchanged dolutegravir represented the entire radioactivity content at $C_{\text{max}}$. Metabolic products appeared at later time points, consistent with low to negligible presystemic clearance. The parallel terminal-phase $t_{1/2}$ values of radioactivity and of dolutegravir indicated that the products that were present in the systemic circulation were formation rate limited and did not persist. This observation supported the electrocardiographic sampling scheme for a cardiac repolarization study, which primarily focused on the anticipated maximum concentration and duration in plasma of the parent compound (14).

The pharmacokinetic parameters from the suspension dose indicated low variability and were consistent with the parameter estimates previously reported for doses of 2 to 100 mg when administered as a suspension (11). The mean concentration, 0.502 µg/ml, of dolutegravir at 24 h after a single 20-mg dose was 8-fold higher than the protein-adjusted 90% inhibitory concentration (0.064 µg/ml) and, together with the observed oral dolutegravir $t_{1/2}$ (15.6 h), further supports a once-daily regimen. The low exposure to total circulating metabolites representing less than 5% of the dolutegravir-related components would be expected not to contribute significantly to either the pharmacological activity of or intolerance for dolutegravir.

Dolutegravir was extensively metabolized with observed biotransformations following three primary pathways: glucuronidation of dolutegravir principally by UGT1A1, carbon oxidation via CYP3A4 (12), and what appears to be a sequential oxidative de-fluorination and glutathione conjugation (Fig. 6). The ether glucuronide metabolite, M2, was the only metabolite characterized in plasma and represented the predominant metabolism pathway based on the mean percentage of the dose recovered (18.9% in urine). The formation of the ether glucuronide disrupts the ability of the molecule to bind to metal ions, and thus, the metabolite is inactive.

The oxidation of the benzyl methylene generated two diastereomeric hemiaminal intermediates (M3) detected in urine. The reduced basicity of the amide nitrogen prevents complete chemical decomposition to the corresponding amide, M1, and the corresponding difluorobenzaldehyde. Consequently, the significance of this biotransformation is best reflected in the sum of the M3 diastereomers and M1 as a mean percentage of the dose (6.6%). It was noted during the course of sample manipulation and repeated chromatographic runs that the radio peak areas corresponding to

![FIG 5 Nuclear magnetic resonance characterization of M4 regioisomers.](attachment:image.png)
the two diastereomers changed, suggesting that some decompo-
sition to M1 could occur during sample processing.

Refinement beyond isomer I or III to a definitive structure
could not be achieved for M4 due to the limited amount of sample
and ambiguous $^{19}$F NMR data. From a mechanism perspective,
isomer I can be rationalized as the result of an initial enzyme-
mediated addition of an oxygen atom to generate either a fluoro-
hydroxyl cyclohexyl diene or the corresponding epoxide interme-
diate, followed by glutathione addition and HF elimination
(Fig. 7). In contrast, formation of isomer III would require two

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FIG 6 Metabolic scheme for dolutegravir after a single oral 20-mg (80-$\mu$Ci) dose of [14C]dolutegravir to six healthy male subjects.

FIG 7 Proposed mechanism for the formation of M4 isomer I.
separate biotransformations: oxidative defluorination to generate a stable 2-hydroxy-4-fluorobenzyl group and glutathione opening of an epoxide intermediate where water rather than HF is eliminated. Both biotransformations in the formation of isomer III appear to involve epoxide intermediates. The mechanism leading to isomer III is more difficult to rationalize based on the biotransformation literature (19–21). Thus, although there are not enough spectroscopic data from this study to definitively characterize M4 as isomer I, mechanistic considerations favor this structure. Although the proposed mechanism of formation for M4 proceeds through an arene oxide, the low fractional clearance through this pathway, lack of evidence for covalent binding, and the low to moderate dose administered suggest the risk for drug-induced toxicities via this potential bioactive species is low. This analysis indicating the low body burden of systemic metabolites is consistent with the strategic analysis recently reviewed (22).

In conclusion, following oral administration, the recovery of dolutegravir and dolutegravir-related components from urine and feces was nearly complete, with feces being the principal route of excretion (Fig. 8). Dolutegravir was the predominant circulating compound in plasma and was consistent with minimal presystemic clearance. Radioactivity was minimally associated with the blood cellular components. At least 34% of the dose was absorbed, with an additional 33% to 48% fraction undergoing enterohepatic recirculation. Dolutegravir was extensively metabolized, and the disposition kinetics of the metabolites was formation rate limited, indicating no long-lived metabolites. An inactive ether glucuronide, formed primarily via UGT1A1, was the principal biotransformation pathway (at least 18.9% of the dose, with an additional amount secreted in bile and deconjugated), and minor biotransformation pathways included oxidation by CYP3A4 (7.9% of the dose) and a correlated oxidative defluorination and glutathione substitution (1.8% of the dose). These biotransformations were also observed in nonclinical studies, thereby supporting selection of the nonclinical species for safety coverage in humans. Thus, this study provides an understanding of the clearance pathways via the routes of metabolism and excretion of dolutegravir, underpinned by the nonclinical safety assessment and the low risk of idiosyncratic reactions by bioactive metabolites. The metabolic profile and fractional clearances obtained from this study, in conjunction with the in vitro investigations (14), provide the mechanistic basis for understanding potential effects when dolutegravir is coadministered with other therapeutic products.

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FIG 8 Summary of the mean excretion and mass balance after a single oral administration of [14C]dolutegravir to six healthy male subjects at a target dose of 20 mg (80 µCi).
REFERENCES