Bioequivalence of the Once-Daily Single-Tablet Regimen of Darunavir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide Compared to Combined Intake of the Separate Agents and the Effect of Food on Bioavailability

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Abstract
The effect of food on the bioavailability of the components of the once-daily, single-tablet human immunodeficiency virus (HIV) type 1 regimen containing darunavir (DRV 800 mg), cobicistat (COBI 150 mg), emtricitabine (FTC 200 mg), and tenofovir alafenamide (TAF 10 mg) (D/C/F/TAF) (NCT02475135) and the bioequivalence of D/C/F/TAF versus combined intake of the separate agents (NCT02578550) were evaluated. These were 2 phase 1, open-label, randomized, 2-period crossover studies (7-day washout between treatments) in HIV-negative healthy volunteers. Twenty-four participants each received a single dose of D/C/F/TAF in fasted conditions (test) or after a standardized high-fat breakfast (reference). Ninety-six participants each received a single dose of D/C/F/TAF (test) or combined intake of a single DRV 800-mg tablet, a COBI 150-mg tablet, and an FTC/TAF 200/10-mg tablet (reference), both after a standardized regular-calorie, regular-fat breakfast. Pharmacokinetic profiles for all D/C/F/TAF components, safety, and tolerability were assessed. Following D/C/F/TAF in fasted conditions, DRV peak concentration, area under the concentration-time curve from time of administration until the last time point with a measurable concentration (AUC)last, and extrapolated to infinity (AUCinf) were lower by 45%, 34%, and 30%, respectively, compared with fed conditions, with no clinically relevant differences in COBI, FTC, or TAF exposures between fed and fasted conditions. In the bioequivalence study 90% confidence intervals of the geometric mean ratios of all main pharmacokinetic parameters were within the 80.00% to 125.00% bioequivalence limits for DRV, COBI, FTC, and TAF. No grade 3/4 adverse events (AEs), serious AEs, deaths, or discontinuations due to AEs occurred. D/C/F/TAF is bioequivalent to combined administration of the separate agents. Consistent with other (co)formulations of DRV, DRV exposure was lower in fasted than in fed conditions as evaluated when taken with food, so D/C/F/TAF should be taken with food.

Keywords
bioavailability, bioequivalence, cobicistat, darunavir, emtricitabine, tenofovir alafenamide

Previous studies have shown that once-daily single-tablet regimens for the treatment of human immunodeficiency virus type 1 (HIV-1) improve treatment adherence, patient satisfaction, and virologic outcomes compared with multitablet regimens.¹⁻⁴ A once-daily, single-tablet antiretroviral regimen, D/C/F/TAF, combines the protease inhibitor (PI) darunavir (DRV, D, 800 mg) with cobicistat (COBI, C, 150 mg), and the nucleoside reverse transcriptase inhibitors emtricitabine (FTC, F, 200 mg), and the tenofovir prodrug, tenofovir alafenamide (TAF, 10 mg).³ DRV is extensively metabolized by cytochrome P450 (CYP), mainly CYP3A.³ COBI is a potent mechanism-based inhibitor of CYP3A and thus a pharmacoenhancer of DRV.¹⁻⁹
Once-daily boosted DRV 800 mg has demonstrated a high and durable virologic response in a range of patient populations and a high genetic barrier to the development of resistance. Current treatment guidelines include DRV/COBI combined with 2 nucleoside reverse transcriptase inhibitors, or administered as the D/C/F/TAF single-tablet regimen, as a recommended treatment option for HIV-1 treatment-naive patients or recommended either when integrase inhibitors are not an option or in certain clinical situations.

TAF is a prodrug predominantly hydrolyzed to tenofovir intracellularly by cathepsin A5, resulting in higher intracellular levels of the active moiety tenofovir diphosphate and 90% lower plasma tenofovir concentrations relative to tenofovir disoproxil fumarate (TDF). Therefore, TAF provides comparable efficacy to TDF at one-tenth of the dose, with improved renal and bone safety. TAF does not undergo CYP-mediated interactions, but it is a substrate of P-glycoprotein. Coadministration of COBI or PI-based regimens with TAF resulted in a range of 6% to 183% increases in TAF and 105% to 316% increases in tenofovir exposure. However, in an exploratory phase 2 trial in 153 treatment-naive adults, intracellular tenofovir diphosphate levels in patients receiving D/C/F/TAF were 6.5-fold higher than in those receiving DRV plus COBI and F/TDF. FTC is also not an inhibitor of human CYP450 and is mainly eliminated via the kidney (86%), with 13% recovered as metabolites.

The efficacy and safety of D/C/F/TAF in HIV-1–infected patients is under investigation in 2 large, international, randomized phase 3 studies: AMBER in treatment-naive, HIV-1–infected adults (NCT02431247) and EMERALD in adults with virologically suppressed HIV-1 (NCT02269917). D/C/F/TAF showed noninferior virologic efficacy compared to DRV/COBI plus F/TDF in AMBER or switching from a boosted PI plus FTC/TDF in EMERALD through week 48, with significantly improved bone and renal biomarker safety and no development of DRV, primary PI or TDF/TAF resistance-associated mutations.

Two phase 1 studies evaluated the relative oral bioavailability and bioequivalence of the D/C/F/TAF single-tablet regimen versus combined intake of the separate agents in healthy volunteers. Given the impact of food on the systemic exposure of DRV the effect of food on the single-dose pharmacokinetics of the D/C/F/TAF components was also evaluated in the relative bioavailability study. The food-effect data are reported herein, along with the results of the bioequivalence study.

Methods

Study Locations and Ethics

Both studies were conducted according to the Declaration of Helsinki, Good Clinical Practice principles and applicable regulatory requirements. The study protocols and amendments were reviewed and approved by an independent ethics committee. The relative oral bioavailability study (TMC114FD2HTX1002; ClinicalTrials.gov: NCT02475135; EudraCT No. 2015-001213-27) was conducted at SGS Life Science Services Clinical Pharmacology Unit (Antwerp, Belgium), and the Institutional Review Board was the ZNA Medical Ethics Committee in Antwerp, Belgium. The bioequivalence study (TMC114FD2HTX1001; ClinicalTrials.gov: NCT02578550; EudraCT No. 2015-001264-18) was conducted at QPS Netherlands BV (Groningen, The Netherlands), and the Institutional Review Board was the Foundation for the Assessment of Ethics for Biomedical Research in Assen, The Netherlands. All volunteers provided written informed consent before starting the studies.

Study Populations

Both studies had similar inclusion and exclusion criteria and included male or female subjects (of non–child-bearing potential or who agreed to use a highly effective contraceptive method), aged ≥18 to 55 years, with a body mass index of 18.5–30.0 kg/m², who were nonsmokers for ≥3 months before selection, and healthy based on physical examination, medical history, vital signs, 12-lead electrocardiograms, and clinical laboratory tests. Key exclusion criteria for both studies included a positive screening test for HIV-1, HIV-2, hepatitis A, B, and/or C; history or evidence of drug and/or alcohol abuse; significant and active diarrhea, nausea, or constipation, which would affect drug absorption/bioavailability; a clinically significant disease (eg, gastrointestinal, cardiovascular, neurologic disease) or history of renal insufficiency.

Volunteers were not allowed to take any medication ≥14 days before first study drug administration except for paracetamol (no more than 3 × 500 mg/d or 3 g/wk) or ibuprofen (no more than 1 × 400 mg/d), hormone replacement therapy in postmenopausal women, and hormone-based contraceptives.

October 23–26, 2016 (abstract and poster P310). Bioequivalence results were presented at the 9th IAS Conference on HIV Science, Paris, France, July 23–26, 2017 (abstract and poster MOPEB0335).
**Study Designs and Treatments**

These were 2 phase 1, single-center, open-label, randomized, 2-sequence, 2-period crossover studies.

The relative bioavailability study comprised 3 separate panels; however, only the panel evaluating the impact of food on the single-dose pharmacokinetics of the D/C/F/TAF components is presented.

In both studies, volunteers were screened within 21 days before first administration of study drugs, admitted to the study site in the morning of day −1, and fasted overnight for ≥10 hours (water was permitted until 2 hours before dosing). Volunteers were randomized (with a computer-generated randomization schedule using permuted blocks) on day 1 of the first treatment session, before intake of a single dose of study drug. All study drug intakes were with 240 mL of noncarbonated water and were observed within the units. The washout period between the 2 treatment sessions was ≥7 days.

To evaluate the impact of food on the bioavailability of the components of D/C/F/TAF, 24 volunteers were randomized to receive a single oral dose of D/C/F/TAF under either fasted (test) or fed (within 30 minutes after the start of a standardized high-fat breakfast) (reference) conditions. The standardized high-fat breakfast (928 kCal; 56 g fat) consisted of 2 eggs fried in butter, 2 strips of bacon, 2 slices of white bread with butter, 1 croissant with 1 slice of cheese, and 240 mL (8 oz) of whole milk (or its equivalent).

In the bioequivalence study 96 volunteers were randomized to receive a single oral dose of the D/C/F/TAF tablet (test) or a single oral dose of DRV as 1 × 800-mg tablet, FTC/TAF as 1 × 200/10-mg fixed-dose combination (FDC) tablet, and COBI as 1 × 150-mg tablet (as combined intake, reference), under fed conditions (within 5 minutes after completing a standardized regular breakfast). TAF was approved at a dose of 10 mg in Europe in the presence of a booster and 25 mg in the absence of a booster, so a 10-mg dose in combination with FTC 200 mg as a single-tablet regimen was available when the trial was conducted. However, in the United States, only the single-tablet regimen containing 200 mg FTC and 25 mg TAF was approved when the trial was conducted. D/C/F/TAF 800/150/200/10 mg is now approved in Europe and in the United States. The standardized regular breakfast (533 kCal; 21 g fat) consisted of 4 slices of bread, 2 slices of ham and/or cheese, butter, fruit preserve, and 2 cups (up to 480 mL) of decaffeinated coffee or tea with milk and/or sugar (or its equivalent).

Water and food were permitted from 2 and 4 hours, respectively, after taking the study drugs.

**Sample Sizes**

**Impact of Food on the Bioavailability of the Components of D/C/F/TAF.** From previous studies, intrasubject variability (log-transformed) was estimated to be a maximum of 0.36, which was then the highest observed variability across exposures and maximum plasma concentrations (Cmax) for the different components of D/C/F/TAF. Based on this intrasubject variability of 0.36, and a target sample size of 22 volunteers completing the food-effect study, the precision range, expressed as the percentage of the true value, for the geometric mean ratio (GMR) (test over reference) of the primary pharmacokinetic parameters was expected to be within the 83% and 120% bounds with 90% confidence. An additional 2 volunteers were recruited to account for premature discontinuations, making the total sample size 24 participants.

**Bioequivalence Study.** The Cmax of TAF was found to be the most variable pharmacokinetic parameter among those for the components of D/C/F/TAF. As such, a within-subject log standard deviation of 0.456, corresponding to a coefficient of variation of 48%, was used for the sample size calculation. Based on a within-subject log standard deviation of 0.456 and using a “two-one-sided t-tests” (TOST) procedure at a significance level of 5% each, the total number of volunteers needed to demonstrate bioequivalence between the D/C/F/TAF single-tablet regimen and combined administration of DRV, an FTC/TAF FDC, and COBI separate agents, with an overall 80% power when the true difference between treatments is 5%, was 91 participants. Recruitment of 96 volunteers was planned to account for premature discontinuations.

Given the uncertainty around the variability for the TAF Cmax, a blinded (for treatment) sample size reestimation was performed by an independent party after all volunteers had been dosed, to reevaluate the sample size and statistical power based on the actual observed variability of TAF Cmax; however, no additional volunteers were required to maintain sufficient statistical power.

**Pharmacokinetic Assessments**

In the food-effect relative bioavailability study, full pharmacokinetic profiles for DRV, COBI, FTC, and TAF were determined over 72, 72, 48, and 12 hours, respectively, after administration in each treatment session. In the bioequivalence study, full pharmacokinetic profiles for these drugs were determined over 72, 72, 72, and 8 hours, respectively.

Validated, specific, and sensitive high-performance liquid chromatography-tandem mass spectrometry assays were used to determine plasma concentrations of DRV, COBI, FTC, and TAF with lower limits of quantification of 5.0 ng/mL for FTC, DRV, and COBI, and 1.0 ng/mL for TAF. DRV and COBI were measured with a combined method in which protein precipitation with acetonitrile was done on 50 μL of
plasma, with separation on a Waters XBridge C18 column (4.6 × 30 mm, 3.5 μm particles; Waters, Milford, Massachusetts) at 30°C. Isocratic elution was with a mobile phase consisting of 40% acetonitrile in 0.1% formic acid, followed by a step gradient at 95% acetonitrile to clean the column. The flow rate was 1.5 mL/min. A triple quadrupole mass spectrometer (ABSciex Triple Quad 5500) equipped with a turbo ion spray source was used for detection in positive-ion mode. Transitions monitored were 548.4 → 392.2 and 552.3 → 396.3 for DRV and internal standard DRV-d4; 776.4 → 562.3 and 784.5 → 570.4 for COBI and internal standard GS-427990. A linear regression with a 1/2 weighting factor was used for COBI, and log/log regression with no weighting was used for DRV. For DRV, the percentage coefficient of variation ranged from 2.5% to 6.0%, and the overall bias ranged from −1.6% to 1.8%. For COBI, the percentage coefficient of variation ranged from 2.4% to 3.5%, and the overall bias ranged from −5.9% to 3.9%.

Safety and Tolerability Assessments
Safety and tolerability assessments included evaluation of adverse events (AEs), clinical laboratory parameters (serum chemistry, hematology, urinalysis), vital signs, electrocardiograms, physical examination, and follow-up on specific toxicities including rash, acute systemic allergic reactions, aspartate aminotransferase/alanine aminotransferase elevations, clinical hepatitis, renal complications, nausea, and diarrhea. AEs and concomitant medications were monitored throughout the studies. In the food-effect bioavailability study, electrocardiograms, vital signs, serum chemistry and hematology (10 hours after fasting), and urinalysis were assessed at screening, on day 1 (within 2 hours before intake of study drug), and on day 4. In the bioequivalence study, the electrocardiogram was measured only at screening, and vital signs were measured at screening, on day 1 (within 2 hours before intake of study drug), and on day 4. Clinical laboratory assessments were made at screening and on day −1 or 1 and on days 2 and 4. The follow-up period was 7 to 10 days after the last intake of study medication or after study discontinuation. The severity of AEs and laboratory abnormalities were evaluated using the Division of AIDS grading table. All AEs were coded according to the Medical Dictionary for Regulatory Activities Version 18.0 (food-effect bioavailability study) or Version 18.1 (bioequivalence study).

Data Analyses
For both studies the following key pharmacokinetic parameters for each of the component drugs were determined using noncompartmental analysis (WinNonlin version 6.2.1, Pharsight Corporation, Mountain View, California): \( C_{\text{max}} \), time to \( C_{\text{max}} \), area under the plasma concentration-time curve (AUC, calculated from linear-linear trapezoidal summation) from time of administration up to the last time point with a measurable concentration post-dose (AUC\(_{\text{last}}\)) and extrapolated to infinity (AUC\(_{\text{inf}}\) calculated as AUC\(_{\text{last}}\) plus \( C_{\text{last}}/\lambda Z \)), where \( C_{\text{last}} \) is the last time postdose with a quantifiable concentration and \( \lambda Z \) is the apparent terminal elimination rate constant), and the terminal half-life (calculated as 0.693/\( \lambda Z \)). All observations for test and reference, paired and unpaired, were included in the statistical analyses.

The least squares (LS) means of natural logarithm–transformed \( C_{\text{max}} \), AUC\(_{\text{last}}\), and AUC\(_{\text{inf}}\) for each component of D/C/F/TAF were estimated using a linear mixed-effects model, controlling for treatment, sequence, and period as fixed effects, and subject as a random effect (SAS version 9.3, SAS Institute, Cary, North Carolina).

In the food-effect relative bioavailability study, a 90% CI was constructed around the difference between the LS means of test and reference, back-transformed using the exponential function, and compared with the 80% to 125% boundaries of no effect. In the bioequivalence study an adjusted CI of 90.14% (as opposed to a 90.00% CI) was constructed around the difference between the LS means of test and reference and retransformed to the original scale. The CI was adjusted to control the nominal type I error rate as a result of a blinded (for treatment) sample size reestimation (given the uncertainty of the TAF \( C_{\text{max}} \) variability). Bioequivalence of the D/C/F/TAF tablet compared to combined intake of the separate agents was met if the 90.14% CIs for \( C_{\text{max}} \), AUC\(_{\text{last}}\), and AUC\(_{\text{inf}}\) for DRV, FTC, and TAF were within the predefined limits of 80% to 125%.

Results

Participant Disposition and Baseline Characteristics
In the food-effect relative bioavailability study, 24 participants were randomized, of whom all completed the study and were included in the pharmacokinetic and safety analyses. For the bioequivalence study, 96 volunteers were randomized. Two volunteers withdrew consent but completed the safety follow-up visit and were considered as having completed the study. All volunteers were included in the pharmacokinetic and safety analyses except for 1 volunteer, who vomited on day 1 of both treatment sessions (within 2 times the median \( t_{\text{max}} \) for DRV, COBI, and FTC in 1 or both treatments), and for whom pharmacokinetic data for DRV, COBI, and FTC (test treatment only)
were therefore excluded from the pharmacokinetic analysis.

In both studies, there was an even distribution of women and men; the majority of volunteers were white and not Hispanic or Latino (Table 1). Demographic data and baseline characteristics were comparable across treatment sequences (data not shown).

**Effect of Food on the Bioavailability of the Components of D/C/F/TAF**

Results of the food-effect relative bioavailability study showed that when D/C/F/TAF was administered under fasted conditions (test), DRV $C_{\text{max}}$, $AUC_{\text{last}}$, and $AUC_{\text{inf}}$ were 45%, 34%, and 30% lower, respectively, compared with administration in fed conditions (standardized high-fat breakfast; reference) (Table 2). Also, mean maximum DRV concentrations were reached earlier in fasted than in fed conditions (Figure 1A).

The COBI $C_{\text{max}}$, $AUC_{\text{last}}$, and $AUC_{\text{inf}}$ were, respectively, 23%, 29%, and 16% lower in fasted compared with fed conditions (Table 2). Also, mean maximum COBI concentrations were reached earlier in fasted than in fed conditions (Figure 1B).

For FTC, $C_{\text{max}}$ was 26% higher in fasted compared with fed conditions, whereas $AUC_{\text{last}}$ was comparable under both conditions (90% CI of the GMR within the 80% to 125% interval) (Table 2). Also, the mean maximum FTC concentrations (Figure 1C) were reached earlier in fasted compared with fed conditions.

For TAF, the $C_{\text{max}}$ was 82% higher, and $AUC_{\text{inf}}$ was 20% lower, in fasted than in fed conditions. The TAF $AUC_{\text{last}}$ was comparable under both conditions (90% CI of the GMR within the 80% to 125% boundaries of no effect) (Table 2). Also, the mean maximum TAF concentrations (Figure 1D) were reached earlier in fasted compared with fed conditions.

**Bioequivalence Study**

DRV, COBI, FTC, and TAF plasma concentration-time profiles (Figures 2A to 2D) were similar for D/C/F/TAF (test) and combined administration of the separate agents (reference), under fed conditions (standardized regular breakfast). The 90.14% CIs of the GMRs of all main pharmacokinetic parameters were within the bioequivalence range of 80.00% to 125.00% for all 4 components (DRV, COBI, FTC, and TAF) (Table 3).

**Safety and Tolerability**

For both studies, administration of D/C/F/TAF was generally well tolerated. The most frequently reported AEs were headache and nausea (Table 4). The incidence of these AEs was generally comparable in each treatment group for each study (Table 4). No grade 3 or 4 or serious AEs or deaths occurred. There were no discontinuations due to AEs. There were no clinically relevant or consistent changes in laboratory parameters (clinical chemistry, hematology, or urinalysis). Most treatment-emergent laboratory abnormalities were grade 1 in severity and not reported as AEs. There were no relevant or clinically significant changes in electrocardiogram parameters, vital signs, or physical examination. The most frequently observed vital signs abnormality was a low supine pulse.

**Food-Effect Relative Bioavailability Study.** Grade 2 AEs were reported by 3 volunteers. One case of grade 2 irritable bowel syndrome occurred following D/C/F/TAF under fasted conditions and was considered not related to the study medication by the investigator. Grade 2 nausea and headache reported following D/C/F/TAF with a standardized high-fat breakfast were considered possibly and doubtfully related, respectively, to the study medication. A grade 3 treatment-emergent increase in low-density lipoprotein cholesterol occurred in 1 volunteer, which was transient and was not reported as an AE.

Overall, 6 volunteers (25%) in fasted conditions and 5 volunteers (21%) in fed conditions experienced at least 1 AE that was considered by the investigator to be at least possibly related to D/C/F/TAF, including nausea, erythema, vomiting, diarrhea, and pruritus.

**Bioequivalence Study.** All reported AEs were grade 1 in severity. One grade 3, isolated, transient, treatment-emergent increase in lipase (195 U/L) was observed on day 4 following treatment with the separate agents (DRV plus COBI plus FTC/TAF), but this was not considered clinically significant by the investigator. Lipase levels for this participant were within normal ranges...
Table 2. Food-Effect Relative Bioavailability Study: Pharmacokinetic Parameters and Statistical Analysis of DRV, COBI, FTC, and TAF Following Administration of a Single Oral Dose of D/C/F/TAF 800/150/200/10 mg Under Fed (Standardized High-fat Breakfast) and Fasted Conditions

| Parameter, Mean (SD) | DRV (Test) | DRV (Reference) | COBI (Test) | COBI (Reference) | FTC (Test) | FTC (Reference) | TAF (Test) | TAF (Reference) |
|----------------------|------------|-----------------|------------|-----------------|------------|----------------|------------|----------------|
|                      | N = 23b    | N = 24b         | N = 23c    | N = 24          | N = 24d    | N = 24         | N = 24    | N = 24         |
| C<sub>max</sub>, ng/mL | 4089 (1846)| 6629 (1543)     | 704 (368)  | 711 (164)       | 2247 (573) | 1785 (486)     | 180 (90.6)| 107 (65.2)     |
| t<sub>max</sub>, h   | 3.00       | 5.00            | 3.00       | 5.00            | 1.00       | 2.00           | 0.50     | 0.88           |
| AUC<sub>last</sub>, ng·h/mL | 67,504 (35,642) | 93,541 (39,730) | 5771 (3206) | 6168 (2260) | 11,593 (2573) | 11,499 (2055) | 106 (44.7) | 117 (51.5) |
| AUC<sub>inf</sub>, ng·h/mL | 72,147 (36,009) | 94,686 (40,882) | 6136 (3064) | 6258 (2268) | 12,286 (2729) | 10,029 (1079) | 109 (47.7) | 125 (57.3) |
| t<sub>1/2</sub>, h   | 7.0 (2.3)  | 7.8 (3.5)       | 4.1 (0.9)  | 3.9 (0.6)       | 10.8 (1.2) | 10.7 (1.2)     | 0.3 (0.2) | 0.5 (0.1)      |

Geometric mean ratio, % (90% CI)

|                | DRV 23 vs 24 | COBI 23 vs 24 | FTC 24 vs 24 | TAF 24 vs 24 |
|----------------|--------------|--------------|-------------|-------------|
| C<sub>max</sub> | 54.99 (46.73–64.71) | 76.96 (55.70–106.33) | 125.99 (112.85–140.65) | 182.29 (140.50–236.50) |
| AUC<sub>last</sub> | 65.65 (56.76–75.92) | 70.90 (51.13–98.30) | 100.12 (96.29–104.10) | 89.54 (81.20–98.72) |
| AUC<sub>inf</sub> | 70.25 (59.49–82.95) | 84.39 (68.52–103.95) | ... | 80.38 (73.04–88.45) |

AUC<sub>inf</sub> indicates area under the plasma concentration-time curve from time of administration to infinity; AUC<sub>last</sub>, AUC from time of administration up to the last time point with a measurable concentration post-dose; CI, confidence interval; C<sub>max</sub>, maximum plasma concentration; COBI, cobicistat; DRV, darunavir; FTC, emtricitabine; TAF, tenofovir alafenamide; t<sub>1/2</sub>, terminal half-life; t<sub>max</sub>, time to C<sub>max</sub>.

<sup>a</sup>Except t<sub>max</sub> = median (range).
<sup>b</sup>n = 20.
<sup>c</sup>n = 22.
<sup>d</sup>n = 16.
<sup>e</sup>n = 7.
<sup>f</sup>n = 21 for AUC<sub>inf</sub> and t<sub>1/2</sub>.
<sup>g</sup>Accurate determination not possible for more than 50% of participants; interpret with caution.
<sup>h</sup>Test vs reference.
<sup>i</sup>n = 20 for test and reference.
<sup>ii</sup>n = 22 for test.
<sup>iii</sup>n = 21 for test and n = 16 for reference.
at baseline (29 U/L) and at all assessments before day 4 of treatment with the separate agents (range: 27–41 U/L) as well as at follow-up after 7–10 days (31 U/L). Concurrent transient grade 2 increases in total amylase (156 U/L) and pancreatic amylase (113 U/L) also occurred on day 4 following treatment with the separate agents but were within normal ranges at all other time points during the study. No AEs related to this laboratory abnormality were reported for this volunteer. The levels of ALT and AST were within normal ranges at all time points during the study.

Overall, 28 volunteers (30%) experienced at least 1 AE that was considered to be possibly related to D/C/F/TAF by the investigator, most frequently nausea, headache, vomiting, abdominal pain, dizziness, somnolence, and diarrhea.

Discussion

The data demonstrated bioequivalence of the D/C/F/TAF tablet to combined administration of the separate agents DRV, the FTC/TAF FDC, and COBI. The relative bioavailability study demonstrated a food effect for DRV absorption following administration of the D/C/F/TAF 800/150/200/10-mg single-tablet HIV-1 regimen, similar to other DRV-containing regimens.

Results from the food-effect relative bioavailability study showed that the $C_{\text{max}}$, $AUC_{\text{last}}$, and $AUC_{\text{inf}}$ for DRV decreased under fasted conditions compared with fed conditions. This observation is consistent with the findings of previous food-effect studies for DRV. In a study of DRV coadministered with ritonavir, a 32% decrease in DRV $AUC_{\text{last}}$ was seen in fasted versus fed conditions.31 In another study, for DRV boosted with COBI, a 39% to 56% decrease in DRV pharmacokinetic parameters was seen in fasted versus fed conditions.30 The food effect for DRV has been previously shown to be similar for different types of food.30,31 Consistent with other DRV formulations,30,31 it is recommended that D/C/F/TAF be taken with food. Also in the ongoing phase 3 studies, the recommended intake for the D/C/F/TAF single-tablet regimen and other DRV-containing regimens is with food.28,29
Figure 2. Bioequivalence study: mean (SD) plasma concentration-time profiles for (A) DRV, (B) COBI, (C) FTC, and (D) TAF following administration of a single oral dose of D/C/F/TAF 800/150/200/10 mg or a single oral dose of the separate agents DRV 800 mg, FTC/TAF 200/10 mg FDC, and COBI 150 mg, under fed conditions (standardized regular breakfast). C or COBI indicates cobicistat; D or DRV, darunavir; FDC, fixed-dose combination; F or FTC, emtricitabine; TAF, tenofovir alafenamide.

Although the exposure (AUC_{inf}, AUC_{last}) to COBI administered as D/C/F/TAF was 16% to 29% lower in fasted conditions than in fed conditions, this observation is not considered to be clinically relevant. A previous study has shown that food had no significant effect on COBI pharmacokinetics, and the administration of COBI with food is driven by the recommendations for the drug of which the pharmacokinetics it is boosting. As expected, the FTC exposure (AUC_{last}) was unaffected by food. For TAF, the AUC_{inf} was 20% lower, and the AUC_{last} was comparable in fasted compared to fed conditions. Differences in exposure to FTC and TAF in fasted versus fed conditions are also not considered to be clinically relevant. The TAF results in the current food-effect bioavailability study are also consistent with a previous study (GS-US-292-0110), which assessed the pharmacokinetics of TAF after dosing of the single-tablet HIV-1 regimen elvitegravir 150 mg, COBI 150 mg, FTC 200 mg, and TAF 10 mg (E/C/F/TAF) under fasted and fed conditions and showed that there was a minimal effect of food on TAF exposure. The combined assessment for the D/C/F/TAF components is therefore driven by the impact of food on the DRV pharmacokinetics.

An intensive pharmacokinetic analysis in a subset of 32 participants from an exploratory phase 2 trial of 153 treatment-naive, HIV-1–infected adults showed the mean plasma exposure (AUC_{last}) for TAF to be 130.5 ng·h/mL, with a median plasma half-life of 0.45 hour. These values are consistent with those seen in the food-effect study under fed conditions (117 ng·h/mL and 0.5 hours, respectively). Plasma tenofovir levels were not measured in the current food-effect study, but participants in the TAF group of the phase 2 study had a greater than 90% lower mean systemic tenofovir exposure than those in the TDF group (339 versus 3737 ng·h/mL, respectively).

Given the recommended intake with food of DRV-based regimens, the bioequivalence study was conducted under fed conditions. This is also the recommended intake in the ongoing phase 3 studies with the D/C/F/TAF single-tablet regimen. Systemic
Table 3. Bioequivalence Study: Pharmacokinetic Parameters and Statistical Analysis of DRV, COBI, FTC, and TAF Following Administration of a Single Oral Dose of D/C/F/TAF 800/150/200/10 mg or Single Oral Doses of the Separate Agents Under Fed Conditions (Standardized Regular Breakfast)

| Parameter | Mean (SD) | Separate Agents DRV 800 mg, FTC/TAF 200/10 mg FDC, and COBI 150 mg | Geometric Mean Ratio (90.14%CI) |
|-----------|-----------|-------------------------------------------------------------------|---------------------------------|
|           | (Test) N = 94 | (Reference) N = 96 | |
| DRV       |            |                                                                  |                                |
| C_{max}, ng/mL | 7042 (1481)c | 6620 (1429)c | 106.73 (103.50–110.06)c |
| t_{max}, h | 4.00 (1.50–8.00)c | 4.00 (2.00–12.00)c | – |
| AUC_{last}, ng·h/mL | 87,200 (27,385)c | 84,406 (29,481)c | 104.84 (100.87–108.97)c |
| AUC_{inf}, ng·h/mL | 87,280 (28,097)d | 85,210 (29,561)d | 103.74 (99.62–108.02)d |
| t_{1/2}, h | 5.9 (2.1)j | 6.2 (2.7)l | – |
| COBI      |            |                                                                  |                                |
| C_{max}, ng/mL | 894 (254)c | 881 (207)c | 100.69 (96.80–104.73)c |
| t_{max}, h | 4.00 (1.50–6.00)c | 4.00 (1.50–5.05)c | – |
| AUC_{last}, ng·h/mL | 6681 (2486)d | 6763 (2436)d | 98.77 (95.14–102.52)d |
| AUC_{inf}, ng·h/mL | 6785 (2518)d | 6686 (2459)d | 98.76 (95.15–102.52)d |
| t_{1/2}, h | 3.7 (0.7)j | 3.7 (0.7)c | – |
| FTC       |            |                                                                  |                                |
| C_{max}, ng/mL | 2041 (481)e | 2053 (469)e | 99.32 (95.61–103.17)e |
| t_{max}, h | 2.00 (0.60–5.00)e | 2.00 (0.50–5.00)e | – |
| AUC_{last}, ng·h/mL | 11,722 (1959)f | 11,746 (1868)f | 100.04 (98.46–101.66)f |
| AUC_{inf}, ng·h/mL | 11,882 (2002)f | 11,927 (1935)f | 100.13 (98.36–101.93)f |
| t_{1/2}, h | 16.5 (3.3)f | 17.0 (3.4)f | – |
| TAF       |            |                                                                  |                                |
| C_{max}, ng/mL | 110 (54.1) | 120 (74.0) | 96.87 (88.95–105.50) |
| t_{max}, h | 1.50 (0.25–3.50) | 1.01 (0.25–4.00) | – |
| AUC_{last}, ng·h/mL | 123 (42.0) | 132 (58.1) | 96.59 (91.72–101.73) |
| AUC_{inf}, ng·h/mL | 127 (39.4) | 141 (59.7) | 95.42 (90.62–100.48) |
| t_{1/2}, h | 0.3 (0.1)g | 0.3 (0.1)g | – |

AUC_{inf} indicates area under the plasma concentration-time curve from time of administration to infinity; AUC_{last}, AUC from time of administration up to the last time point with a measurable concentration post-dose; C_{max}, maximum plasma concentration; C or COBI, cobicistat; D or DRV, darunavir; FDC, fixed-dose combination; F or FTC, emtricitabine; TAF, tenofovir alafenamide; t_{1/2}, terminal half-life; t_{max}, time to C_{max}. 

- Except t_{max} = median (range).
- As a result of a blinded (for treatment) sample size reestimation, to control the nominal type I error rate, an adjusted 90.14% CI was calculated as opposed to the traditional 90.00% CI. No additional participants were recruited beyond the originally planned number.
- c n = 93 test, n = 95 reference.
- d n = 87 test, n = 91 reference.
- e n = 93 test, n = 96 reference.
- f n = 85 test, n = 87 reference.
- g n = 79 test, n = 78 reference.

Exposures to all 4 components of D/C/F/TAF (DRV, COBI, FTC, TAF), as indicated by C_{max}, AUC_{last}, and AUC_{inf}, were comparable following administration of the D/C/F/TAF tablet or combined administration of the separate agents DRV, the FTC/TAF FDC, and COBI. Indeed, the 90.14% CIs of the GMRs for these pharmacokinetic parameters were within the 80.00% to 125.00% bioequivalence limits. This finding is noteworthy because a D/C/F/TAF 800/150/200/10-mg single-tablet HIV-1 regimen would reduce the pill burden for HIV-1–infected patients, and such regimens have previously been shown to improve treatment adherence and virologic outcomes compared with multitablet regimens.1-4

Even though these phase 1 studies investigated only a single dose of D/C/F/TAF, both studies also showed that administration of D/C/F/TAF was generally well tolerated under both fed and fasted conditions. No grade 3/4 or serious AEs, deaths, or discontinuations due to AEs occurred. The tolerability profile is consistent with earlier studies, with the most commonly reported AEs during the studies, headache.
Table 4. Summary of Safety and Tolerability

|                  | Food-Effect Relative Bioavailability Study | Bioequivalence Study |
|------------------|--------------------------------------------|---------------------|
|                  | D/C/F/TAF (Fasted) N = 24                  | D/C/F/TAF (Fed) N = 94 DRV, FTC/TAF, COBI (Fed) N = 96 Overall |
| AE, n (%)        |                                            |                     |
| Any AE           | 9 (38)                                     | 52 (55)             | 46 (48) | 66 (69) |
| Most common AEs (≥ 10% of volunteers) |                                            |                     |
| Headache         | 3 (13)                                     | 14 (15)             | 15 (16) | 25 (26) |
| Nausea           | 4 (17)                                     | 17 (18)             | 14 (15) | 21 (22) |
| Vomiting         | 2 (8)                                      | 6 (6)               | 6 (6)   | 10 (10) |

AE indicates adverse event; C or COBI, cobicistat; D or DRV, darunavir; F or FTC, emtricitabine; TAF, tenofovir alafenamide.

and nausea, reported previously for studies of DRV, COBI, FTC, and TAF5,10–13 and in the ongoing phase 3 studies.28,29

In conclusion, the once-daily D/C/F/TAF 800/150/200/10-mg single-tablet HIV-1 regimen was shown to be bioequivalent to the combined administration of the separate agents DRV, the FTC/TAF FDC, and COBI. When administered as D/C/F/TAF, DRV exposures were lower in fasted versus fed conditions, similar to other coformulations of DRV. The recommended intake of D/C/F/TAF is with food, which is also the recommendation in the ongoing phase 3 AMBER and EMERALD trials with D/C/F/TAF in HIV-1–infected adults.

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