

Short Communication: Bioequivalence of Tenofovir Component of Tenofovir/Rilpivirine/Emtricitabine in Digital Pills

Peter R. Chai,¹⁻³ Luis M. Pereira,⁴ Guruprasad D. Jambaulikar,² Adam W. Carrico,⁵
Conall O’Cleirigh,^{1,3,6} Kenneth H. Mayer,^{1,3,7} and Edward W. Boyer¹⁻³

Abstract

Digital pills, gelatin capsules with radiofrequency transmitters activated by stomach chloride ions, directly measure antiretroviral therapy adherence. In individuals with substance use disorders and HIV, real-time nonadherence detected by digital pills creates a platform to deliver substance use and adherence interventions. In this study, we determined the bioequivalence of tenofovir (TFV), administered as tenofovir disoproxil fumarate (TDF) in healthy human volunteers administered a commercial drug product and a digital pill formulation. We adhered generally to the US FDA Analytical Procedures and Methods for Validation for Drugs and Biologics guidelines. Ten HIV-uninfected adults without reported allergy to TFV, emtricitabine, or rilpivirine were enrolled. Participants ingested a digital pill containing TDF/emtricitabine/rilpivirine. Peripheral venous blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h postingestion. After a 14-day washout period, the same participants ingested Complera™. Serial venous blood samples were collected using the same protocol as the digital pill. Liquid chromatography/mass spectrometry was used to determine a maximum concentration (C_{max}), area under curve from time zero to last measured concentration (AUC_{0-t}), and area under curve from time zero to infinity (AUC_∞) of TFV. Ten participants with an average age of 27 and body mass index of 25.4 successfully completed the study. Predose TFV was undetectable before the second administration of Complera confirming adequate washout period after ingestion of the digital pill. The geometric means of AUC_{0-t}, AUC_∞, and C_{max} for test and reference products were within the 95% confidence intervals and, therefore, bioequivalent. TFV overencapsulated in digital pills are bioequivalent to TFV in commercial formulations.

Keywords: medication adherence, digital pills, PrEP, HIV prevention

THE ID CAP SYSTEM (eTectRx, Newberry, FL) is a digital pill comprising a commercial gelatin capsule overencapsulating a tiny battery, radiofrequency emitter, and antiretroviral therapy (ART) medication (Fig. 1). Ingestion of the ID Cap activates the integrated transmitter, producing a signal that is captured by a wearable device (e.g., the reader). The reader then stores medication ingestion data while simultaneously transmitting it to the cloud, allowing patients and clinicians to view and respond to real-time adherence data.¹ Direct monitoring of antiretroviral pre-exposure prophylaxis (PrEP) allows for real-time detection of non-

adherence and enables interventions to support adherence in individuals at risk for HIV.^{2,3} Recent investigations have demonstrated bioequivalence of tenofovir (TFV) and emtricitabine overencapsulated by other digital pill systems.⁴ In this study, we studied in healthy human volunteers, the bioequivalence of TFV using Complera (TFV/emtricitabine/rilpivirine) with and without digital pills.

This longitudinal crossover design generally adhered to the United States Food and Drug Administration (US FDA) Analytical Procedures and Methods for Validation for Drugs and Biologics guidelines.⁵ We enrolled participants >18

¹Harvard Medical School, Boston, Massachusetts.

²Division of Medical Toxicology, Department of Emergency Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts.

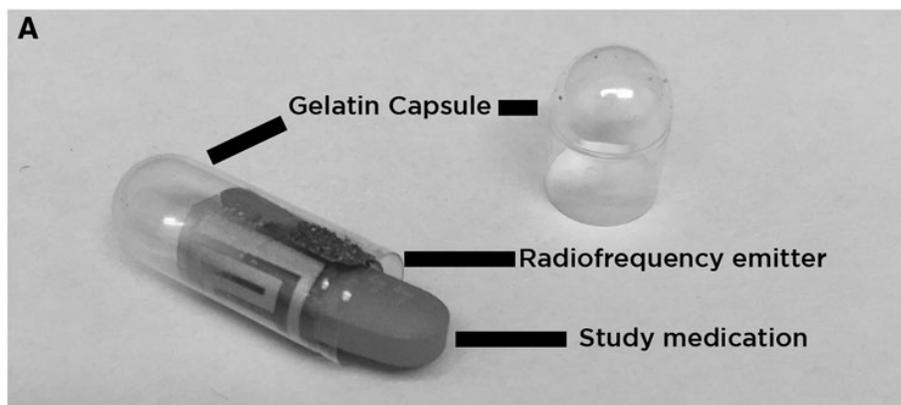
³The Fenway Institute, Fenway Health, Boston, Massachusetts.

⁴Pharmacometrics Research Core, Department of Anesthesia, Boston Children’s Hospital, Boston, Massachusetts.

⁵University of Miami School of Medicine, Miami, Florida.

⁶Massachusetts General Hospital, Boston, Massachusetts.

⁷Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts.

**B**

Pharmacokinetics and bioequivalence results

Parameter	Product	LSM	GM	GMR(Test/Ref.) %	GMR 90% CI	AH-p	Power
AUC _{0-t} (ng.h/mL)	Digital Pill	2.993	985.1	100.4	91.7-110.0	0.00018	0.988
	Ref.	2.995	989.5	-	-	-	-
AUC _∞ (ng.h/mL)	Digital Pill	7.146	1269	99.5	86.8-114.0	0.00176	0.863
	Ref.	7.152	1127	-	-	-	-
C _{max} (ng/mL)	Digital Pill	4.78	119.3	107.4	97.9-117.9	0.00747	0.985
	Ref.	4.71	111.1	-	-	-	-

Ref: COMPLERA® (Gilead Sciences Inc., USA), LSM=Least Squares Mean, GM=geometric mean, GMR=geometric mean ratio, CI=confidence interval, AH-p=Andersen-Hauck p-value, AUC_{0-t} area under the curve from time zero to the time of the last measured concentration, AUC_∞ area under the curve from time zero to infinity, C_{max} highest measured plasma concentration

FIG. 1. (A) The ID Cap digital pill comprising a gelatin capsule with integrated radio frequency emitter and circuit that over encapsulates medication (sample pill sized to dimensions of Complera™ pictured). (B) Bioequivalence data of tenofovir.

years, nonpregnant, HIV seronegative, and without reported allergy to TFV, emtricitabine, or rilpivirine. We excluded individuals on medications known to interact with TFV. Participants fasted overnight, then received a meal of at least 400 calories and 10 g fat, and ingested a digital pill containing TFV/emtricitabine/rilpivirine (Complera). Peripheral venous blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h postingestion. After a 14 days washout period, the same 10 participants repeated the procedure with Complera alone (i.e., without the digital pill). Serial venous blood samples were collected using the identical protocol to that used with the digital pill.

A tandem liquid chromatography mass spectrometry (MS) was validated according to FDA guidance on Good Laboratory Practices (GLP).⁵ The ultrahigh-performance liquid chromatography separation (UPLC) was carried out on an Acquity H-Class System (Waters Co., Milford, MA) equipped with an Acquity UPLC BEH C18 column (1.7 μm 2.1 × 100 mm). The mobile phase consisted of a gradient of MilliQ water with 0.1% formic acid (component A) and acetonitrile (component B). From 0.3 to 2.5 min the gradient changed from 10% A:90% B to 90% A:10% B. This mixture was held until minute 4.5, after which it reverted to 10% A–90% B until the end of the run at 5.5 min. The flow rate was a

constant 0.3 mL/min under a column temperature of 35°C. The sample vials were thermostatic at 4°C and the injection volume was 3 μL.

Detection by MS was performed on a tandem Xevo TQ system (Waters Co, Milford MA) running multiple reaction monitoring in electrospray ionization (ESI) positive mode. The lower limit of quantitation of this assay was 1 ng/mL and the precision and accuracy were 5.3% and 99.3%, respectively.

Plasma was separated from blood samples and 100 μL of either spiked or study plasma was transferred to a 1.5 mL tube, and added 10 μL of a 1 μg/mL stock solution of lamivudine as internal standard. Next, 600 μL acetonitrile and 10 μL of 5% trichloroacetic acid were added to each vial and vortexed. We centrifuged all vials at 10,000 rpm for 10 min to complete the extraction. About 700 μL of each sample were then transferred to a new tube and evaporated to dryness and reconstituted with 100 μL aqueous 0.1% formic acid:acetonitrile (95:5). These concentrates were transferred into a Waters amber screw cap conic vial and loaded onto the autosampler tray for injection. At least three injections were made from each vial to generate triplicate results.

A noncompartmental analysis of the concentration versus time data was conducted to estimate parameters such as the

maximum observed concentration (C_{max}), the area under the curve from time zero to time of the last measured concentration (AUC_{0-t}), the AUC from time zero extrapolated to infinity ($AUC_{0\infty}$), the time to C_{max} (T_{max}), the apparent first-order elimination rate constant (k_e), and the apparent elimination half-life ($t_{1/2}$). According to FDA guidance, in the absence of documented estimates of intrasubject variability, an adaptive two-stage design is advisable.⁶ We conducted an interim analysis after a first-stage study conducted with a number of subjects based on a true geometric mean ratio of 0.95 and $V = 0.05$. Based on observed intrasubject variability, a new power calculation is made, which if $<90\%$ triggers a second stage of bioequivalence assessment now with $V = 0.0294$. If at least 90% then the study is concluded after the first stage.

Bioequivalence criteria were based on log-transformed $AUC_{0\infty}$ and C_{max} data from which a geometric mean ratio is calculated. The 90% confidence intervals for its point estimate must be within 0.80 and 1.25 to validate bioequivalence between the formulations.

We enrolled a total of 10 participants who successfully completed the study. Mean age was 27, 20% ($N = 2$) men, and 80% ($N = 8$) women. Participants had a mean body mass index of 25.4.

At the interim analysis of the data collected from 10 subjects sequentially administered with both test and reference formulations, as per FDA guidance, the calculated power for AUC_{0-t} , $AUC_{0\infty}$, and C_{max} ratios was 98.8%, 86.3%, and 98.5%, respectively. Since the calculated power was $>90\%$ for both AUC_{0-t} and C_{max} , we determined that a second stage was not deemed necessary and concluded the bioequivalence study. Analysis of standardized residuals identified no outliers. Predose concentrations were not observed upon the second administration to each subject, which validated the washout period established.

The geometric means of AUC_{0-t} , $AUC_{0\infty}$, and C_{max} for test and reference products were 985.1 and 989.5 ng.h/mL; 1269 and 1127 ng.h/mL; and 119.3 and 111.1 ng/mL, respectively. Their ratios were 1.00, 0.995, and 1.07, respectively, all falling within 90% confidence intervals of 0.8–1.25 (Fig. 1).

We confirmed that digital pills have comparable C_{max} of TFV, used for PrEP and incorporated into multiple regimens for ART. Our results support the findings of a recent study demonstrating that digital pills have no effect on the dissolution of Complera.⁴ The significance of our findings is that digital pills containing TFV can be used to measure adherence without concern for altered drug levels.

The efficacy of ART and PrEP is closely tied to adherence, yet “adherence” is often determined as the number of missed doses for a specified time period, often weeks or even months.^{7,8} Existing methods to measure adherence include electronic adherence monitoring (EAM) and dried blood spots detecting specific drug concentrations. These methods measure adherence in aggregate but may miss nascent periods of nonadherence. Digital pills such as the ID Cap System offer the ability to detect suboptimal adherence and deliver interventions that may serve as corrective feedback and return individuals to adherence, a significant difference from other EAMs such as Wisepill that only indirectly infers

medication ingestion. Similar to other EAMs, digital pills may suffer from technological shortcomings (e.g., reader out of range or out of battery), and their real-world acceptance remains to be described.

Limitations are few. First, we utilized a small sample size. Second, our study design enrolled a sample of HIV-uninfected individuals. We were unable to enroll HIV-infected individuals because it would have been unethical to ask participants who were HIV-infected to stop taking their ARV for a 2-week washout.

Acknowledgments

The authors thank Jung Lee and the Brigham and Women’s Hospital Center for Clinical Investigation for the support of this study. Funding: The study was funded by K24DA037109 (Boyer) and Dr. Chai is supported by K23DA044874.

Author Disclosure Statement

No competing financial interests exist.

References

1. Chai PR, Castillo-Mancilla J, Buffkin E, *et al.*: Utilizing an ingestible biosensor to assess real-time medication adherence. *J. Med. Toxicol.* 2015;11:439–444.
2. Mayer KH, Ramjee G: The current status of the use of oral medication to prevent HIV transmission. *Curr Opin HIV AIDS* 2015;10:226–232.
3. Chai P, Rosen RK, Boyer EW: *Ingestible Biosensors for Real-Time Medication Adherence Monitoring: MyTMed*. IEEE & Computer Society Press, Hawaii, 2016.
4. Ibrahim ME, Brooks KM, Castillo-Mancilla JR, *et al.*: Bioequivalence of tenofovir and emtricitabine after coencapsulation with the proteus ingestible sensor. *AIDS Res Hum Retroviruses* 2018;34:835–837.
5. Food and Drug Administration: *Analytical Procedures and Methods Validation for Drugs and Biologics*. Center for Drug Evaluation and Research, Food and Drug Administration. Silver Spring, MD 2015, pp. 1–18.
6. Food and Drug Administration: *Guidance for Industry: Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA* Center for Drug Evaluation and Research, Food and Drug Administration. Silver Spring, MD 2013, pp. 1–24.
7. Nance RM, Delaney JAC, Golin CE, *et al.*: Co-calibration of two self-reported measures of adherence to antiretroviral therapy. *AIDS Care* 2017;29:464–468.
8. Robbins RN, Spector AY, Mellins CA, Remien RH: Optimizing ART adherence: update for HIV treatment and prevention. *Curr HIV/AIDS Rep* 2014;11:423–433.

Address correspondence to:

Peter R. Chai
Division of Medical Toxicology
Department of Emergency Medicine
Brigham and Women’s Hospital
75 Francis Street
Boston, MA 02115

E-mail: pchai@bwh.harvard.edu

Bioequivalence of Tenofovir in Digital Pills

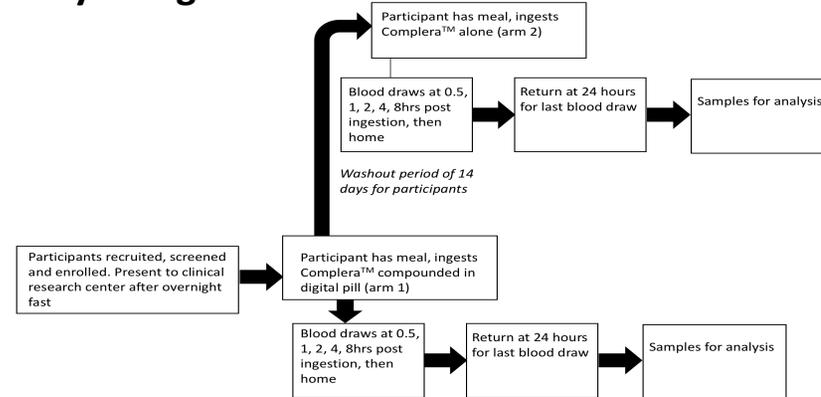
Peter R Chai MD MMS^{1,4,5}, Luis M Pereira PhD², Adam W Carrico PhD³, Conall O’Cleirigh PhD^{4,5}, Kenneth Mayer MD^{4,5}, Edward W Boyer MD PhD^{1,4,5}

1: Department of Emergency Medicine, Brigham and Women’s Hospital; 2: Pharmacometrics Core, Boston Children’s Hospital; 3: University of Miami; 4: The Fenway Institute; 5: Harvard Medical School

Introduction:

- Digital pills (a standard off the shelf gelatin capsule with an integrated radiofrequency emitter) directly measure medication ingestion events
- Recognizing real-time medication ingestion allows for delivery of behavioral interventions that reinforce adherence prior to the onset of nonadherence
- In this study, we demonstrate bioequivalence of Tenofovir in digital pills compared to a tenofovir containing formulation alone.

Study Design:



HOW THE DIGITAL PILL WORKS



- 1 The digital pill is ingested.
- 2 The pill is activated in the stomach and transmits a radio-frequency signal to a hip-worn device.
- 3 The device collects and transmits ingestion data to a cloud-based server driving an interface.
- 4 The interface then displays ingestion data to clinicians and patients.

Methods:

- We used US FDA Administration Analytical Procedures and Methods for Validation for Drugs and Biologics guidelines
- We enrolled individuals >18yo, HIV negative, non-pregnant, with no allergy to tenofovir, emtricitabine or rilyvirine
- Participants ingested a digital pill combination tenofovir/emtricitabine/rilyvirine (Complera digital pill) or Complera alone in a crossover format, and serial venous blood sample was drawn for tenofovir

Data analysis:

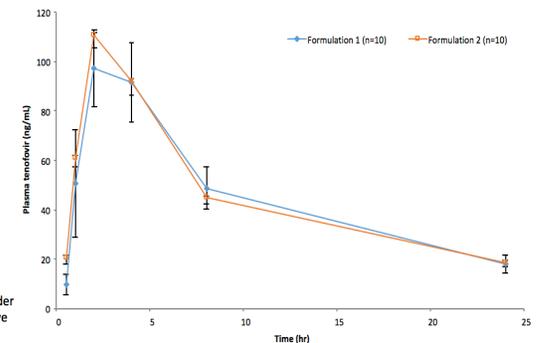
- Tandem LC/MS to assess tenofovir in venous blood samples
- Calculated C_{max} and AUC for digital pill Complera vs Complera alone

Results:

Table 3: Pharmacokinetics and bioequivalence results

Parameter	Product	LSM	GM	GMR[Test/Ref.] %	GMR 90% CI	AH-p	Power
AUC _{0-t} (ng.h/mL)	Digital Pill	2,993	985.1	100.4	91.7-110.0	0.00018	0.988
	Ref.	2,995	989.5	-	-	-	-
AUC _∞ (ng.h/mL)	Digital Pill	7,146	1269	99.5	86.8-114.0	0.00176	0.863
	Ref.	7,152	1127	-	-	-	-
C _{max} (ng/mL)	Digital Pill	4.78	119.3	107.4	97.9-117.9	0.00747	0.985
	Ref.	4.71	111.1	-	-	-	-

Ref: COMPLERA® (Gilead Sciences Inc., USA), LSM=Least Squares Mean, GM=geometric mean, GMR=geometric mean ratio, CI=confidence interval, AH-p=Andersen-Hauck p-value, AUC_{0-t} area under the curve from time zero to the time of the last measured concentration, AUC_∞ area under the curve from time zero to infinity, C_{max} highest measured plasma concentration



Discussion/Conclusion:

- Digital pills do not alter bioequivalence of tenofovir in healthy human volunteers