Received: 28 November 2023 | Revised: 31 January 2024 | Accepted: 16 February 2024

DOI: 10.1111/cts.13756

ARTICLE

Bioavailability of three novel oral, sustained-release pellets, relative to an immediate-release tablet containing 500 mg flucytosine: A randomized, open-label, crossover study in healthy volunteers

Vishal Goyal[1] | **Edrich Krantz**[2] | **Francois Simon**[3] | **Anouk Neven**[4] | **Johanna Eriksson**[5] | **Amaria Saayman**[2] | **Nabila Ibnou Zekri Lassout**[3] | **Mathieu Louis**[3] | **Stephen Robinson**[3] | **Abhijit Deshmukh**[6] | **Amit Antarkar**[6] | **Carol Ruffell**[7] | **Sarika Victor**[2] | **Marylore Chenel**[5] | **Aljosa Celebic**[4] | **Henri Caplain**[3] | **Jean-Yves Gillon**[3] | **Isabela Ribeiro**[3]

[1]Drugs for Neglected Diseases Initiative, New York, New York, USA

[2]FARMOVS, Bloemfontein, South Africa

[3]Drugs for Neglected Diseases Initiative, Geneva, Switzerland

[4]Luxembourg Institute of Health, Strassen, Luxembourg

[5]Pharmetheus, Uppsala, Sweden

[6]Viatris, Hyderabad, India

[7]DNDi GARDP Southern Africa, Cape Town, South Africa

Correspondence
Vishal Goyal, Drugs for Neglected Diseases Initiative, 40 Rector Street, 16th Floor, New York, NY 10006, USA.
Email: vgoyal@dndi.org

Funding information
European and Developing Countries Clinical Trials Partnership Association (EDCTP2) programme supported by the European Union, Grant/Award Number: RIA2018CO-2516; Médecins Sans Frontières International; Swiss Agency for Development and Cooperation (SDC), Switzerland

Abstract

The opportunistic fungal infection cryptococcal meningoencephalitis is a major cause of death among people living with HIV in sub-Saharan Africa. We report pharmacokinetic (PK) and safety data from a randomized, four-period crossover phase I trial of three sustained-release (SR) oral pellet formulations of 5-flucytosine conducted in South Africa. These formulations were developed to require less frequent administration, to provide a convenient alternative to the current immediate release (IR) formulation, A. Formulations B, C, and D were designed to release 5-flucytosine as a percentage of the nominal dose in vitro. We assessed their safety and PK profiles in a single dose (1 × 3000 mg at 0 h), relative to commercial IR tablets (Ancotil 500 mg tablets; 3 × 500 mg at 0 h and 3 × 500 mg at 6 h) in healthy, fasted participants. Forty-two healthy participants were included. All treatments were well-tolerated. The primary PK parameters, maximum observed plasma concentration (C_{max}) and area under the concentration-time profiles, were significantly lower for the SR formulations than for the IR tablets, and the geometric mean ratios fell outside the conventional bioequivalence limits. The median maximum time to C_{max} was delayed for the SR pellets. Physiologically-based PK modeling indicated a twice-daily 6400 mg dose of SR formulation D in fasted condition would be optimal for further clinical development. This regimen is predicted to result in a rapid steady-state plasma exposure with effective and safe trough plasma concentration and C_{max} values, within the therapeutic boundaries relative to plasma exposure after four times per day administration of IR tablets (PACTR202201760181404).

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The 5-flucytosine is a key, brain-permeable antifungal for the treatment of cryptococcal meningoencephalitis (CM), however, it has a limited plasma half-life and needs to be administered every 6 h, posing major practical challenges that may result in treatment failures and death.

WHAT QUESTION DID THIS STUDY ADDRESS?

This phase I study compared the bioavailability of three sustained-release (SR) formulations (B, C, and D) of 5-flucytosine after single dose oral administration, relative to an immediate-release (IR) formulation in healthy, fasting participants using a randomized four-period crossover study. The treatments were evaluated for safety and their pharmacokinetic (PK) profiles.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

All treatments were well-tolerated and had a comparable safety profile. The primary PK parameters maximum observed plasma concentration (C_{max}) and area under the concentration-time profiles from the SR formulations were significantly lower than from the IR tablets, and the geometric mean ratios fell below the conventional bioequivalence limits. The median time to C_{max} was delayed for the SR pellets. The 5-fluorouracil (5-flucytosine metabolite) exposure was too low for proper PK assessment.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our data suggests that the sustained 5-flucytosine formulation D has the highest potential to be explored in future studies in non-fasting healthy volunteers and in patients. Our physiologically-based pharmacokinetic modeling predicts that a 4700 mg dose of that formulation, given twice a day in fed state, has the potential to produce trough plasma concentration and C_{max} values that are effective and safe. Such sustained 5FC formulation has the potential to reduce treatment failure in CM and to improve treatment adherence.

INTRODUCTION

Cryptococcal meningoencephalitis (CM) is caused by an opportunistic, cerebrospinal infection of *Cryptococcus neoformans* or, less frequently, *C. gatti* fungus. The disease is fatal when left untreated, killing an estimated 181,100 people annually in 2014,[1] with revised estimates of 112,000 deaths in 2020, mostly in low- and middle-income countries.[2] Despite the roll-out of antiretroviral therapies in previous decades, many people living with human immunodeficiency virus worldwide are left undiagnosed or untreated,[3] with dropping $CD4^+$ T-cell counts resulting in high risk of life-threatening opportunistic infections. South Africa is the most affected country, based on the incidence of cryptococcal antigenemia.[2]

The standard of care for CM, per 2022 World Health Organization (WHO) guidelines, includes induction treatment with a single dose of liposomal amphotericin B (10 mg/kg), 14 days of 5-flucytosine (100 mg/kg per day divided into 4 doses per day), and fluconazole (1200 mg/daily for adults).[4] Previously, a combination regimen of amphotericin B deoxycholate with 5-flucytosine followed by fluconazole was effective but challenging to implement with the potential for failed treatments and emergence of 5-flucytosine resistance.[5]

The use of 5-flucytosine is an old, antifungal agent that efficiently crosses the blood–brain barrier.[6] To treat patients with CM, it must be combined with fluconazole and amphotericin B to prevent development of 5-flucytosine-resistant fungi and treatment failure. This balance requires judicious control of drug exposure in patients, which is problematic because of 5-flucytosine's very short (3–4 h) plasma half-life.[7] Presently, the drug is administered as immediate release (IR) tablets every 6 h, frequently resulting in missed doses.[8,9] Moreover, many patients with CM have reduced consciousness, and thus standard solid IR 5-flucytosine formulations are crushed and given by nasogastric tube four times a day, even though this formulation has not been specifically

approved for such administration. Continued adherence to this strict dosing schedule in outpatients is challenging. Finally, current 5-flucytosine dosing regimens may not be optimal, even when properly administered.[10]

The availability of a sustained-release (SR) pellet formulation of 5-flucytosine would simplify 5-flucytosine administration by reducing the number of administrations per day, and would facilitate administration both in outpatients and in hospital settings, including by nasogastric tube (as required). A consortium of European and African partners, led by Drugs for Neglected Diseases initiative and funded by the European and Developing Countries Clinical Trials Partnership, was set up to develop the three new SR 5-flucytosine formulations evaluated in this phase I study. Its ultimate goal is to deliver a new, widely accessible, safe, and affordable 5-flucytosine SR treatment to reduce CM-related mortality that is easy to use and improves compliance.

This single-dose study in healthy volunteers under fasting condition aimed to compare the 5-flucytosine release profile of three SR formulations with a commercial 5-flucytosine IR tablet, to allow selection of one SR formulation for further clinical development.

A model-informed drug development (MIDD) approach (i.e., physiologically-based pharmacokinetics [PBPKs]), was used to support 5-flucytosine SR tablet development, from guiding initial formulation development to the final pharmacokinetic (PK) predictions in patient populations. MIDD has been proven to be a valuable tool in the development of new treatments, and there are numerous examples of its successful implementation in formulation and dose selection, optimizing dosing regimens and predictions of PK in patient populations based on PK in healthy volunteers.[11]

METHODS

Objectives, participants, and oversight

This open-label, bioanalytical laboratory-blind, randomized, four-period crossover study with orally administered 5-flucytosine IR tablets and three types of SR pellets, was conducted under fasting condition in healthy men and women between January and April 2022 at a single study center (FARMOVS Clinical Research Organization, Pharmacology Building, University of the Free State, Bloemfontein, South Africa). The primary objective was to compare the relative bioavailability of each of the three 5-flucytosine SR pellet products (B-D: single dose: 1 × 3000 mg at 0 h) with the reference product A, IR flucytosine (5-flucytosine) Ancotil 500 mg tablets (3 × 500 mg at 0 h and 3 × 500 mg at 6 h after first dosing). The secondary objective was to evaluate the safety and tolerability of these formulations (see Supplement for details).

The main inclusion criteria (see Text S1 for complete lists) were: both sexes, aged 18–55 years, a body mass index greater than or equal to 18.5 and less than or equal to 30 kg/m^2, and weighing greater than or equal to 50 (men) or greater than or equal to 51 (women) kg. The main exclusion criteria were any major illness, dihydropyrimidine dehydrogenase deficiency (defined as a plasma uracil level of >16 ng/mL), and severe acute respiratory syndrome-coronavirus 2 vaccination received within 2 weeks before the first administration of the Investigational Medicinal Product (IMP).

Details on the screening period, washout periods, and follow-up are provided in Figure 1. The schedule of assessments is given in Text S1.

Ethical considerations

This study was conducted in compliance with the Declaration of Helsinki, the International Council for Harmonization Good Clinical Practice guidelines, and the South African Good Clinical Practice guidelines. The clinical study protocol, including participant information sheets, informed consent forms, and protocol amendment No. 1, were reviewed by an independent ethics committee and by the South African Health Products Regulatory Authority.

This study was registered at the Pan African Clinical Trial Registry (Trial ID: PACTR202201760181404) before enrolling the first participant.

Randomization and study treatment

A William design[12] was used to randomly assign volunteers in equal numbers to one of the four treatment sequences (1:1:1:1 ratio) before the first administration of an IMP. The randomization schedule was provided by the Luxembourg Institute of Health, Competence Center for Methodology and Statistics, and was generated utilizing the SAS software PROC PLAN procedure (SAS Institute).

Three prototypes of the 5FC SR formulation (formulation B, formulation C, and formulation D) were developed by Mylan Laboratories Limited (now a Viatris company), R&D Center, Hyderabad, India, as pellets containing 5-flucytosine and the following excipients and release-controlling agents: microcrystalline cellulose, hypromellose, ammonio methacrylate copolymer type A and B, talc, triethyl citrate, and colloidal silicon dioxide.

All three prototypes contained the same excipients and release-controlling agents, however, the proportions of

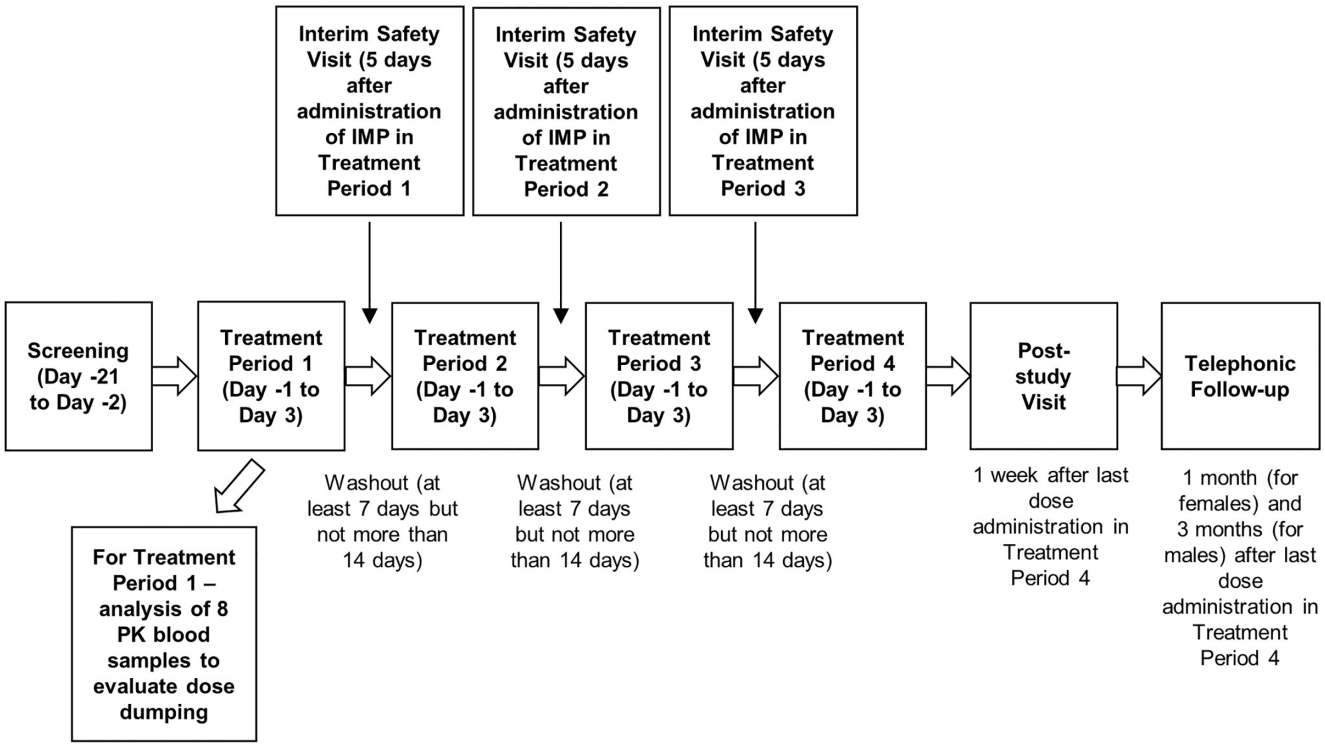

FIGURE 1 Study flow chart. Each participant received the four treatments, with wash-out periods (cross-over design), in randomized order. IMP, investigational medicinal product, PK, pharmacokinetic.

the release controlling agents that formed the sustained-release coating differed, in order to achieve different rates of 5FC release. A higher proportion of the release-controlling agents in the SR coating resulted in a slower release of 5FC from the pellets.

Volunteers received each of the following treatments once, with a different treatment in each of the four treatment periods:

- Treatment A (reference; Ancotil 500 mg 5-flucytosine IR tablets, Viatris Santé, France): twice daily (b.i.d.) dose: 3 × 500 mg (0 h) and 3 × 500 mg (6 h).
- Treatment B (formulation B 5-flucytosine SR pellets): single dose: 1 × 3000 mg (0 h).
- Treatment C (formulation C 5-flucytosine SR pellets): single dose: 1 × 3000 mg (0 h).
- Treatment D (formulation D 5-flucytosine SR pellets): single dose: 1 × 3000 mg (0 h).

The IR reference dosage was below that recommended for patients with CM (1500–4000 mg, 3–4 times a day for a 60 kg body weight). This choice of a lower dosage was guided by potential safety issues with using a high dose of 5-flucytosine in healthy volunteers, in accordance with international guidelines for bioavailability studies. Two IR doses were administered to allow for better comparison with the single SR dosing. The IMP was administered with the participant in the standing position, as a recent study suggests that changes in posture after medicinal intake is a confounding factor in PK outcomes.[13]

Safety and tolerability

An independent safety and data monitoring committee (DMC) was established before study start to review the safety data on an ongoing basis.

An interim assessment of 5-flucytosine plasma concentrations (at 8 timepoints) and emerging safety data was performed after treatment period 1. The DMC had no substantive safety concerns and concluded that none of the treatment allocations should be stopped for safety reasons, and that the study could continue as planned. The safety results were evaluated by the investigator prior to dosing in the next treatment period, to confirm the volunteer's health status and whether it was safe for the volunteer to receive the next dosage. AEs were coded using the Medical Dictionary for Regulatory Activities version 23.1 and summarized by System Organ Class and preferred term and product. Prior and concomitant medication were coded using the September 2019 WHO Drug Dictionary.

The safety population included all volunteers who received at least one dose of the IMP.

Bioanalytical methods

Venous blood samples (4 mL each) for the determination of plasma 5-flucytosine and metabolite 5-fluoruracil concentrations were collected into labeled, EDTA-containing plastic tubes and kept on ice. Within 1 h of collection, the samples were centrifuged for 10 min at ~2700 g at 0–8°C. The supernatant of each sample was divided into two aliquots (of at least 0.8 mL plasma each), transferred to labeled, plastic tubes, and immediately placed in a freezer. Plasma samples were stored at −20°C until transfer to the FARMOVS Bioanalytical Services Division. Quantitative analysis of 5-flucytosine and 5-fluoruracil in the plasma samples was performed using liquid chromatography (using an Agilent Poroshell 120 EC-C_{18}, 50 × 4.6 mm, 2.7 µm analytical column) with tandem mass spectrometry.

Pharmacokinetic evaluations

The PK population included all volunteers who completed the PK sampling in all periods, for whom primary PK parameters could be calculated for all treatment periods and who had no major protocol deviations thought to impact the PK analysis.

PK parameters were processed using Analyst version 1.6.3 for the acquisition of raw data, and Watson LIMS version 7.4.2 for the management and processing of laboratory information.

The software packages have been validated according to the requirements of Part 11 of Title 21 of the Code of Federal Regulations of the United States.[14,15]

Calculation of the PK parameters was performed using noncompartmental analyses with Phoenix WinNonlin 8.3 and the actual sampling time intervals relative to IMP administration. The area under the concentration-time profiles ($AUC_{(0–t)}$) and AUC from zero to infinity ($AUC_{(0–inf)}$) were calculated using the linear up/log down method. The terminal half-life ($t_{1/2}$) was calculated as 0.693/lλz. Concentration values reported as "below the limit of quantification" (BLOQ), that is, less than 0.4 µg/mL, were set to 0 at predose and before the first quantifiable concentration, and to *missing* after the last quantifiable concentration. Not reportable concentrations were set to missing.

Statistical analysis

Trial sample size considerations were driven by the primary PK evaluation and planned pairwise comparison between the SR treatments and the IR reference. Assuming the intra-participant coefficient of variation (CV) was no more than 17%, with 32 evaluable volunteers in total (i.e., 8 participant completers per sequence), a crossover design has 80% power to reject both that the ratio of the SR mean to the IR mean is below 0.8 and that the ratio of SR mean to the IR mean is above 1.25, assuming that the expected ratio of means was 1, the crossover analysis of variance, √MSE (in scale) was 0.17, data were analyzed in the natural log scale using *t*-tests for differences in means, and that each *t*-test was made at the 1.7% level. To account for possible dropouts and withdrawals, 36 volunteers were planned to be recruited.

After treatment period 4, only 30 of the 36 randomized volunteers had completed the study. As per protocol, six replacement volunteers were enrolled and received the same treatment sequence as those withdrawn, in order to reach the required number of 32 evaluable volunteers (completers).

The plasma concentrations of 5-flucytosine and the PK parameters were summarized in the PK population using descriptive statistics (n, arithmetic mean, standard deviation [SD], CV% of arithmetic mean, median, minimum, maximum, and geometric mean).

The PK outcomes maximum observed plasma concentration (C_{max}), $AUC_{(0–t)}$, and $AUC_{(0–inf)}$ were log-transformed and analyzed based on a linear mixed effect model with sequence, product, and period as fixed effects and the participant as a random effect. The residual variance from the model was used to construct 90% confidence intervals (CIs) for least-squares mean differences between treatments. These differences were then back-transformed to obtain point estimates (ratios) of geometric means and corresponding 90% CIs. The T_{max}, $t_{1/2}$, and lambda_z were analyzed nonparametrically using the Wilcoxon signed rank test based on the median of differences between treatments across volunteers.

All statistical analyses were performed using the SAS System, version 9.4 (SAS Institute).

Physiologically-based pharmacokinetic modeling

PBPK modeling was performed in PK-Sim version 9.1. Previously, a legacy model for 5-flucytosine had been developed based on literature data.[10] The legacy model included a description of oral absorption of an IR formulation (Ancobon) that immediately dissolved and subsequently permeated across the intestinal mucosa. The unbound fraction was set to 97% according to literature[16] and elimination was completely attributed to glomerular filtration.[17] The PK data obtained from this study were then used to further update and refine the legacy model by including a Weibull function to

account for slower dissolution of the IR formulation (as informed by slower T_{max} for administration of IR compared to solution[17]), the distribution parameter LogP was re-estimated and the intestinal permeability was increased to account for the fast T_{max} seen for the solution. The model was evaluated by visual inspection of the concentration-time profile and comparison of simulated versus observed PK parameters. To simulate the 5-flucytosine PK after intake of food, the gastric emptying time was prolonged, in accordance with the available implementation in PK-Sim.

RESULTS

Participants

Participant disposition is summarized in Table S1; 35 volunteers completed the study. The demographic summary of all 42 participants is shown in Table S2. Although the number is small, they are representative of the South African population, including both sexes (28 men), the country's three major racial groups (30 were Black, 9 White, and 3 were Colored), and were aged 18–55 years.

Six volunteers were withdrawn from the study by the investigator, and one withdrew for personal reasons. Of the six who were withdrawn, three had intercurrent illness requiring medication and two tested positive for drug or alcohol abuse. One had (>3-fold) elevated aspartate aminotransferase (AST) reported as an AE possibly related to treatment (see below), although bilirubin and alkaline phosphatase (ALP) were within normal limits. Consequently, it was decided to discontinue the participant. Details of protocol deviations can be found in Text S1.

Safety

All 42 volunteers enrolled were included in the safety analysis. Twenty of the 42 volunteers (48%) reported a total of 36 treatment-emergent adverse events (TEAEs) during the study. The safety profile was similar for all treatment formulations, with rates of TEAEs varying between 13% and 22%. Twenty-eight of the reported events were of mild intensity and eight were of moderate intensity. Nervous system disorders were the most common category of TEAE, with 10 volunteers reporting headache and one somnolence. Thirteen volunteers were administered concomitant medications for the reported TEAEs. Eight of the 36 TEAEs (22%) were considered as possibly related to the IMP, and one event (3%) as probably related to the IMP (2 events related to the reference product and 7 related to the test products; see also Tables S3 and S4). During clinical chemistry evaluations at 30 h postdose in treatment period 2, one participant had an isolated and transient elevated AST concentration of 156 U/L (3.4 times the upper limit of normal [ULN]), ALT 67 U/L (1.2 × ULN) without other associated liver abnormalities that reversed to normal values 5 days post-dosing of IMP. This was evaluated thoroughly by a series of liver chemistry investigations and medical history that did not reveal any other cause of transient transaminase elevation. This was reported as an AE of moderate intensity (considered as possibly related to the IMP, seen with treatment C) and the participant was subsequently withdrawn from the study. The most common IMP-related TEAEs were headaches (5 events). The remaining IMP-attributed TEAEs were single events of dyspepsia, nausea, and palpitations. No serious AEs (SAEs) or deaths were reported during this study and none of the AEs were of severe intensity.

There were no clinically significant electrocardiogram abnormalities, changes in vital signs, or physical findings after administration of the IMPs. No cases of leukopenia or thrombocytopenia were observed.

5-flucytosine pharmacokinetic profiles

All 35 completers (83% of randomized volunteers) were included in the PK analysis. No participant was excluded from the PK population due to major protocol deviations. A total of 1.2% of the PK samples were taken outside the prespecified tolerance windows and were reported as protocol deviations. Only values at predose (100%), 30 min (18.6%), and 48 h (34.3%) postdose were BLOQ.

The 5-flucytosine PK profiles for the individual volunteers are plotted in Figure 2 and the arithmetic mean graphs for each treatment formulation are represented in Figure 3. The IR plasma profile was characterized by the presence of two relatively sharp peaks (explained by the two dosings for the IR treatment), followed by a steep decline. However, the plasma concentration-time profile of the SR pellets was characterized by a gradual increase to a single peak, followed by a gradual decline. Table 1 represents the summary statistics for the PK parameters and further illustrates the differences between the IR and SR formulations.

Table S5 shows the results obtained from linear mixed modeling. The 90% CIs for the primary PK parameters (C_{max} and $AUC_{(0-t)}$) for 5-flucytosine for each of the SR formulations fell below the conventional acceptance range (i.e., 80%–125%) for establishing comparable bioavailability with the IR reference product. In the comparison of SR pellets with IR tablets, the geometric mean ratio was the highest for treatment D, with 49.0% (90% CI: 43.5–55.1%)

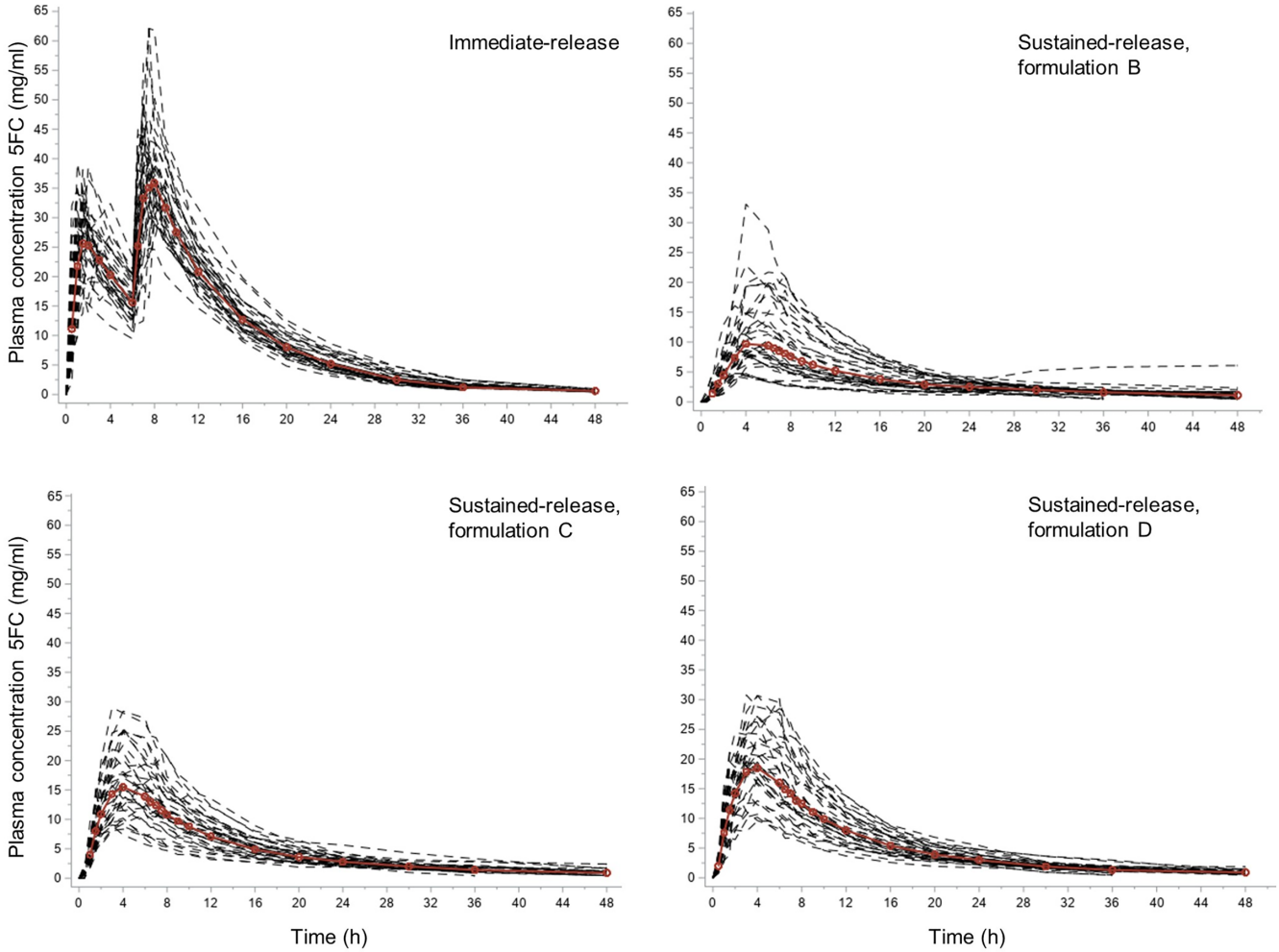

FIGURE 2 Flucytosine plasma concentrations for the IR reference tablets (2 doses, 1500 mg each, 6 h apart) and three SR formulations (B, C, D; 3000 mg) in individual volunteers. The red line represents the geometric mean. PK sampling was conducted over 48 h. The IR plasma profile was characterized by the presence of two relatively sharp peaks (explained by the 2 dosings for the IR treatment), followed by a steep decline. On the other hand, the plasma concentration-time profile of the SR pellets was characterized by a gradual increase to a single peak, followed by a gradual decline. IR, immediate-release; PK, pharmacokinetic; SR, sustained-release.

for C_{max} and 53.6% (90% CI: 49.2–58.3%) for $AUC_{(0,t)}$. Geometric mean ratios also fell below the conventional acceptance range for the secondary PK end point $AUC_{(0,inf)}$. The median T_{max} and lambda_z were significantly higher, and $t_{1/2}$ was significantly lower, with the IR product than with either of the SR formulations (p value < 0.001).

PK data for 5-fluoruracil were used as supportive data. As over 90% of the plasma 5-fluoruracil values were BLOQ, no summary statistics have been provided for this 5-flucytosine metabolite. The maximum concentration detected was 27 ng/mL.

Physiologically-based pharmacokinetic modeling

The legacy PBPK model, which was used to inform the formulation development, was updated based on the PK profiles obtained in this study, in order to guide further development and the design of subsequent studies in fed state and in patients with CM. The final drug dependent parameter values for the 5FC model in fasted and fed state for treatment A and treatment D are presented in Table S6.

A middle-out approach was applied, where some of the parameters were informed by clinical data. The final model was selected on the basis of (1) the model's ability to capture reference data (total residual error and profile resemblance), (2) parameter identifiability, and (3) parameter value plausibility given available data and current knowledge.

The final model in fasted state included optimization of tissue distribution (via logP), intestinal permeability, and dissolution (described as Weibull functions) for each formulation. The final model in fed state included optimization of meal energy content and meal fraction solid (determines the gastric emptying time in PK-Sim) as well

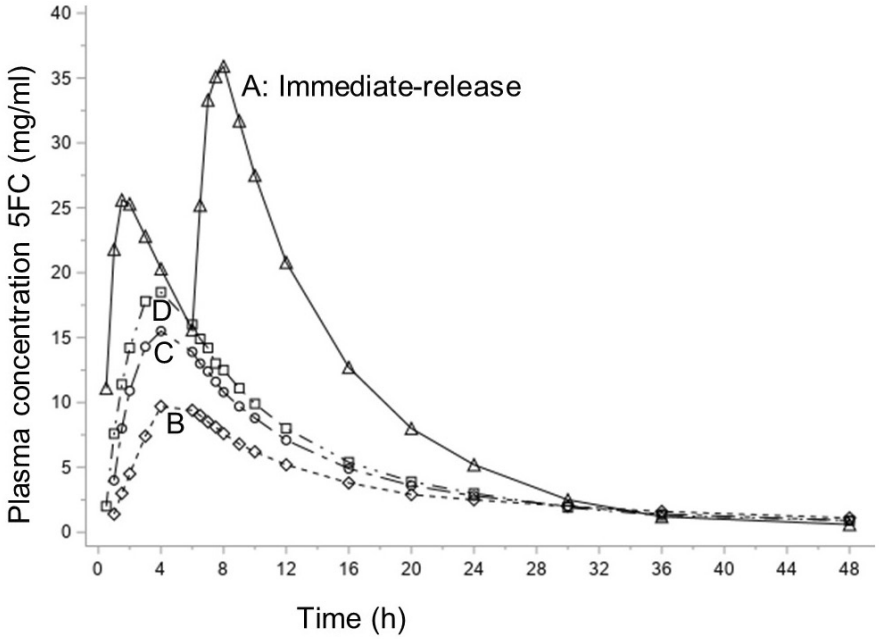

FIGURE 3 Arithmetic mean flucytosine plasma concentrations on linear scales for the IR reference tablets (two doses, 1500 mg each, 6 h apart) and three sustained-release formulations (B, C, D; 3000 mg). PK sampling was conducted over 48 h. The IR plasma profile was characterized by the presence of two relatively sharp peaks (explained by the two dosings for the IR treatment) followed by a steep decline, whereas the plasma concentration-time profile of the SR pellets was characterized by a gradual increase to a single peak followed by a gradual decline. IR, immediate-release; PK, pharmacokinetic; SR, sustained-release.

TABLE 1 Summary of plasma flucytosine PK parameters (PK population).

	Arithmetic mean ± SD							
	IR reference		SR-B		SR-C		SR-D	
Parameter (unit)	*n*[a]		*n*[a]		*n*[a]		*n*[a]	
C_{max} (μg/mL)	35	40.9 ± 8.30	35	12.1 ± 6.53	35	17.3 ± 5.75	35	20.5 ± 5.91
$AUC_{(0-t)}$ (h μg/mL)	35	471.8 ± 72.15	35	179.4 ± 68.64	35	227.8 ± 61.21	35	258.3 ± 64.59
$AUC_{(0-\infty)}$ (h μg/mL)	35	478.0 ± 72.34	34	236.3 ± 103.43	35	257.9 ± 74.05	35	276.6 ± 63.39
λ_z (/h)	35	0.11 ± 0.02	34	0.05 ± 0.03	35	0.06 ± 0.03	35	0.07 ± 0.03
$t_{½}$ (h)	35	6.3 ± 1.17	34	28.2 ± 35.66	35	17.0 ± 13.00	35	12.7 ± 6.00
T_{max} (h) (median and range)	35	7.5 (1.5–9.0)	35	6.0 (2.0–48.0)	35	4.0 (2.0–7.0)	35	4.0 (1.5–6.0)

Note: Reference product is Ancotil 500 mg IR tablets, SR-B-D are three sustained-release formulations of 5-flucytosine.

Abbreviations: $AUC_{(0-\infty)}$, area under the plasma concentration versus time curve, with extrapolation to infinity; $AUC_{(0-t)}$, area under the plasma concentration versus time curve, from time zero to *t*, where *t* is the time of the last quantifiable plasma concentration; C_{max}, maximum observed plasma concentration; IR, immediate-release; PK, pharmacokinetic; SD, standard deviation; SR, sustained-release; $t_{½}$, apparent terminal elimination half-life; T_{max}, time to maximum observed plasma concentration; λz, terminal elimination rate constant.

[a]Number of volunteers in the PK population is 35; *n* = number of volunteers assessed. Six volunteers were withdrawn from the study by the investigator, and one withdrew for personal reasons.

as a fed-state dissolution (described as a Weibull function) for treatment D.

Best model performance, that is, ability to describe observed data, was achieved applying the PK-Sim standard model for 5FC tissue distribution. The updated PBPK model described the observed data well, as seen from the comparison of predicted and observed plasma concentrations versus time profiles and PK parameters, in fasted and fed state (Table S7).

The final model assumes a dose-linearity for drug release and, because of limited 5-flucytosine absorption in the colon (informed by the clinical data), the dose-PK is subproportional. This analysis also shows that a steady-state is reached within about 24 h, obviating the need for a loading dose. In addition, treatment D only took an additional 20 min to reach the C_{max} of the IR, which also shows that a loading dose is not required for the SR regimen.

The bioavailabilities calculated for the three SR formulations are listed in Table 2.

By applying a change to gastric emptying time as a result of food intake, 5-flucytosine plasma exposure was

TABLE 2 PK parameters obtained by physiologically-based PK modeling, in fasting volunteers.

Treatment	[IR reference, (fasted)]	B (fasted)	C (fasted)	D (fasted)
AUC_{inf} (mg h/L)	524 ± 322	231 ± 196	297 ± 226	327 ± 242
C_{max} (mg/L)	39.2 ± 6.80 (second dose)	9.09 ± 2.89	14.2 ± 3.60	16.9 ± 3.81
T_{max} (h)	7.4 ± 0.30 (second dose)	6.3 ± 1.82	5.3 ± 1.48	4.7 ± 1.30
Relative bioavailability (%)	–	44	57	62

Note: Reference product is Ancotil 500 mg IR tablets, SR-B-D are three sustained-release formulations of 5-flucytosine. IR tablet given twice 6 h apart to cover the 12 h period.

Abbreviations: AUC_{inf}, area under the plasma concentration versus time curve, with extrapolation to infinity; C_{max}, maximum observed plasma concentration; IR, immediate-release; PK, pharmacokinetic; SR, sustained-release; T_{max}, time to maximum observed plasma concentration.

TABLE 3 PK parameters obtained by physiologically-based PK modeling, in fed volunteers for the 3 SR formulations B, C, and D, compared to the IR.

Treatment	B (fed)	C (fed)	D (fed)
AUC_{inf} (mg h/L)	317 ± 208	384 ± 243	417 ± 260
C_{max} (mg/L)	12.7 ± 3.26	17.6 ± 3.94	19.9 ± 4.23
T_{max} (h)	9.75 ± 2.7	8.10 ± 2.2	7.2 ± 2.07
Relative bioavailability (%)	60	73	80

Note: The simulations predicted a delayed and increased C_{max}, with an increase in relative bioavailability reaching 80% for treatment D in fed state.

Abbreviations: AUC_{inf}, area under the plasma concentration versus time curve, with extrapolation to infinity; C_{max}, maximum observed plasma concentration; IR, immediate-release; PK, pharmacokinetic; SR, sustained-release; T_{max}, time to maximum observed plasma concentration.

B–D are three sustained-release formulations of 5-flucytosine.

predicted for treatments B-D in fed volunteers. The simulations predicted a delayed and increased C_{max}, with an increase in relative bioavailability reaching 80% for treatment D (Table 3). This is because the formulation is retained in the stomach and thus there will be a longer time period for absorption to occur in the small intestine. The therapeutic target for the repeat dosing of a SR 5-flucytosine is a C_{max} less than 100 mg/L and a trough plasma concentration (C_{trough}) between 20 and 70 mg/L to achieve plasma levels constantly above minimum inhibitory concentration (MIC_{90}) estimated at 16 mg/L.[9] Figure 4 shows simulations with the predicted dosing needed for each treatment to produce an acceptable C_{trough} and safe C_{max} when administered twice daily in fed state, namely 6400, 5100, and 4700 mg for treatments B, C, and D, respectively. Treatment D requires the lowest dose of the SR formulation and reaches T_{max} at 4 h, again suggesting this formulation could be selected for future clinical trials. However, if the (lower) bioavailability is assumed from the fasting state analysis, significantly higher doses are required, namely 10,800, 7500, and 6400 mg, respectively (data not shown). According to additional simulations, a first dose of 6400 mg of treatment D could be given to patients regardless of their prandial state and still be within the therapeutic interval (a C_{max} well below 100 mg/L for 6400 mg in the fed state), if the next doses take into consideration the prandial state (i.e., shifting to 4700 mg if in a fed state). This approach might be considered when simulating the first study in patients with CM.

DISCUSSION

We describe the results of a phase I study in healthy participants that was conducted to assess the relative bioavailability of three 5-flucytosine SR formulations, compared to a 5-flucytosine IR formulation, under fasting condition. In general, the IMPs were well-tolerated by the 42 healthy participants in this study. No deaths or SAEs were reported, and all AEs were of mild or moderate intensity. Although one participant presented with a transient elevation of AST and ALT (considered as possibly treatment related, increase in transaminase is expected as per safety profile of 5FC), there were no other associated liver abnormalities and no signal of drug-induced liver toxicity or bone marrow depression.

Conversion of 5-flucytosine to 5-fluoruracil has been proposed as one key mechanism for the development of 5-flucytosine-associated toxicity.[18] In this study, 5-fluoruracil concentrations following treatment with 5-flucytosine were BLOQ levels for the vast majority of samples, with the highest concentrations at 27 ng/mL (orders of magnitude below 5-fluoruracil chemotherapeutic levels >1000 ng/mL).

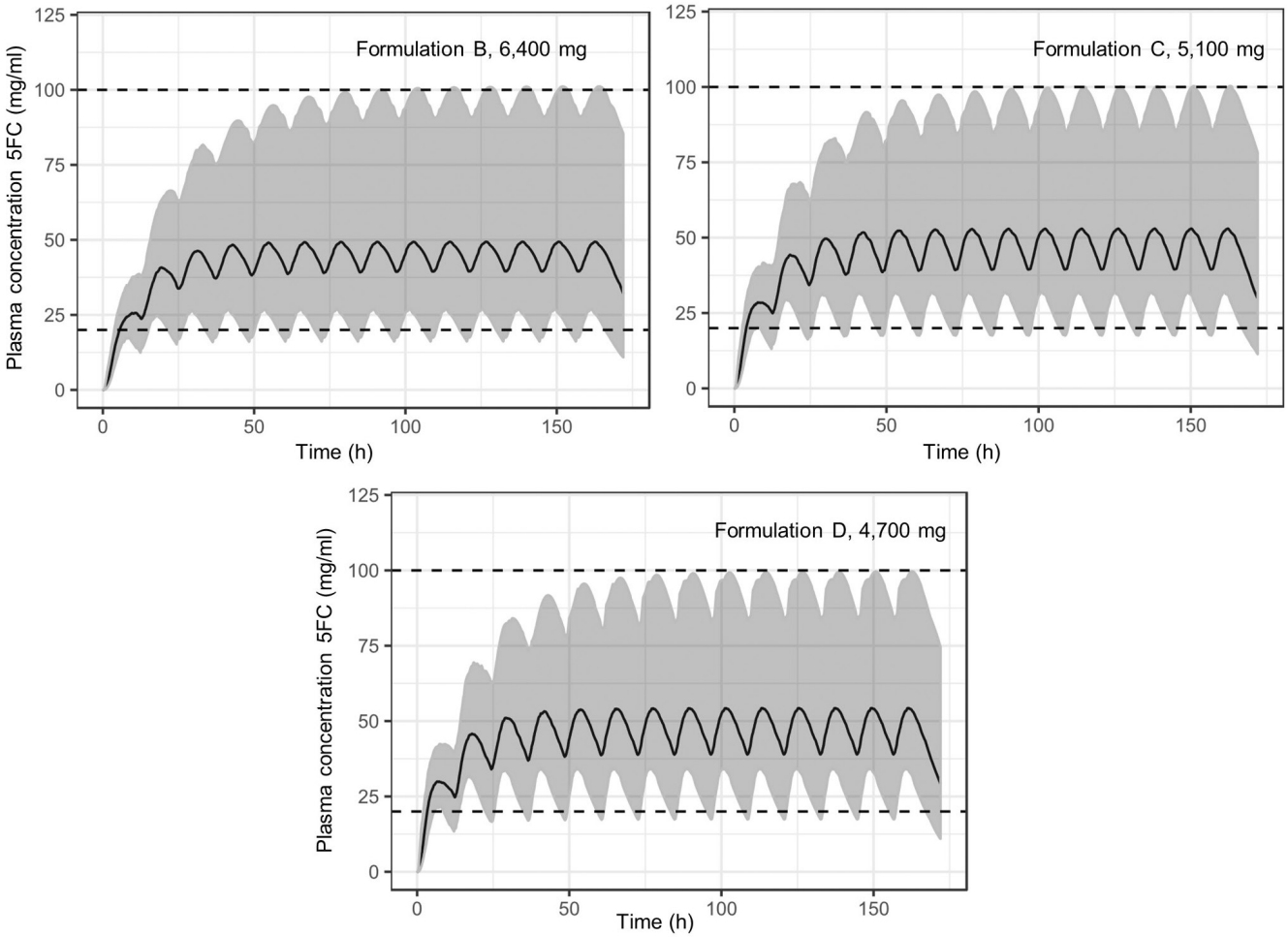

FIGURE 4 Physiologically-based pharmacokinetic modeling for plasma exposure of 5-flucytosine for twice-daily treatments B, C, or D in fed volunteers. Predicted dosing needed for each treatment to produce an acceptable C_{trough} between 20 and 70 mg/L and safe $C_{max} < 100$ mg/L when administered twice daily in fed state. C_{max}, maximum observed plasma concentration; C_{trough}, trough plasma concentration.

Data from the 35 evaluable volunteers who completed the study were available for PK analysis. Based on the results of the study and the PBPK modeling data, treatment D showed the most potential for further development and testing, as its 90% CIs were the closest to establishing comparable bioavailability with the reference product (Ancotil given twice, 6 h apart), while delaying T_{max}. For further development of treatment D, a safety profile comparable with the IR formulation is needed, which seems to have been shown in this study. However, exposure to 5-flucytosine was lower for treatment D than for the twice-dosed reference treatment, so further studies are needed to confirm a similar safety profile at equivalent exposure.

PBPK modeling confirmed that treatment D is the most promising for further study, with an estimated 62% bioavailability in fasting condition, and a predicted 80% bioavailability in fed volunteers. Further modeling suggests that, in fasting conditions, a twice-daily dose of 6400 mg of treatment D would be required. In fed conditions, twice-daily dosing with 4700 mg would result in a similar onset of action as the IR formulation, and rapid (within 24 h) attainment of steady-state with acceptable C_{trough} and C_{max} values. MIDD was successfully used to support the design of the second study in healthy volunteers. Questions arising during development, such as the need for a loading dose and the effect of prandial state, can be investigated and decision making can be supported by the model, emphasizing the importance of including MIDD in the strategic plan for drug development. A phase I single-dose study assessing the relative bioavailability of selected prototype SR treatment D versus an IR formulation under fed conditions could then be conducted. If conclusive, a phase II study could then assess the safety, efficacy, and PK of the new SR formulation in patients with CM in the African region.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. E.K., S.F., A.N., A.C., A.S., H.C., J.-Y.G., and I.R. designed the research. V.G., E.K., F.S., A.N., A.S., M.L., S.R., A.C., J.-Y.G., and I.R. performed the research. V.G., E.K., F.S., N.L.Z.L., A.N., J.E., A.S., S.R., M.C., A.C., H.C., J.-Y.G., and I.R. analyzed the data. A.D., A.A., A.S., M.L., and S.R. contributed new reagents/analytical tools.

ACKNOWLEDGMENTS

The authors thank Dr Rob Hooft van Huijsduijnen for writing the first draft of the manuscript and Dr Louise Burrows (Drugs for Neglected Diseases initiative) for help with editing and formatting.

FUNDING INFORMATION

The study was funded through Drugs for Neglected Diseases initiative and supported by grants from the European and Developing Countries Clinical Trials Partnership Association (EDCTP2) programme supported by the European Union (grant RIA2018CO-2516); Médecins Sans Frontières International; Swiss Agency for Development and Cooperation (SDC), Switzerland; and other private foundations and individuals.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

The data underlying the results of this study are available upon request because they contain potentially sensitive information. Interested researchers may contact the Drugs for Neglected Diseases initiative (DNDi), commissioner of this study, for data access requests via email at ctdata@dndi.org. Researchers may also request data by completing the form available at https://www.dndi.org/category/clinical-trials/. In this, they confirm that they will share data and results with DNDi and will publish any results open access.

ORCID

Vishal Goyal https://orcid.org/0000-0003-3564-129X
Francois Simon https://orcid.org/0009-0003-6068-9874
Anouk Neven https://orcid.org/0000-0003-2253-0427
Johanna Eriksson https://orcid.org/0000-0002-9921-0969
Stephen Robinson https://orcid.org/0009-0002-4150-4099
Carol Ruffell https://orcid.org/0000-0002-3180-7574
Aljosa Celebic https://orcid.org/0009-0001-0614-6874
Henri Caplain https://orcid.org/0009-0008-6977-326X
Jean-Yves Gillon https://orcid.org/0000-0003-1467-0403
Isabela Ribeiro https://orcid.org/0000-0001-7402-4628

REFERENCES

1. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017;17:873-881.
2. Rajasingham R, Govender NP, Jordan A, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. *Lancet Infect Dis*. 2022;22:1748-1755.
3. Kadia BM, Dimala CA, Fongwen NT, Smith AD. Barriers to and enablers of uptake of antiretroviral therapy in integrated HIV and tuberculosis treatment programmes in sub-Saharan Africa: a systematic review and meta-analysis. *AIDS Res Ther*. 2021;18:85.
4. World Health Organization. Guidelines for diagnosing, preventing and managing cryptococcal disease among adults, adolescents and children living with HIV. https://www.who.int/publications/i/item/9789240052178. Accessed March 21, 2023.
5. Iyer KR, Revie NM, Fu C, Robbins N, Cowen LE. Treatment strategies for cryptococcal infection: challenges, advances and future outlook. *Nat Rev Microbiol*. 2021;19:454-466.
6. Block ER, Bennett JE. Pharmacological studies with 5-fluorocytosine. *Antimicrob Agents Chemother*. 1972;1:476-482.
7. Vermes A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother*. 2000;46:171-179.
8. Lopez-Jodra O, Torres-Rodriguez JM, Mendez-Vasquez R, et al. In vitro susceptibility of *Cryptococcus neoformans* isolates to five antifungal drugs using a colorimetric system and the reference microbroth method. *J Antimicrob Chemother*. 2000;45:645-649.
9. Archibald LK, Tuohy MJ, Wilson DA, et al. Antifungal susceptibilities of *Cryptococcus neoformans*. *Emerg Infect Dis*. 2004;10:143-145.
10. Pai MP, Bruce H, Felton LA. Clinical pharmacokinetics of oral controlled-release 5-fluorocytosine. *Antimicrob Agents Chemother*. 2010;54:1237-1241.
11. Workgroup EM, Marshall SF, Burghaus R, et al. Good practices in model-informed drug discovery and development: practice, application, and documentation. *CPT Pharmacometrics Syst Pharmacol*. 2016;5:93-122.
12. Wang B-S, Wang X-J, Gong L-K. The construction of a Williams design and randomization in cross-over clinical trials using SAS. *J Stat Softw*. 2009;29:1-10.
13. Lee JH, Kuhar S, Seo JH, Pasricha PJ, Mittal R. Computational modeling of drug dissolution in the human stomach: effects of posture and gastroparesis on drug bioavailability. *Phys Fluids (1994)*. 2022;34:081904.
14. US Food and Drug Administration. Guidance for industry – computerized systems used in clinical trials. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/fda-bioresearch-monitoring-information/guidance-industry-computerized-systems-used-clinical-trials. Accessed March 21, 2023.
15. US Food and Drug Administration. Food and Drugs Part 11—electronic records; electronic signatures. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-11. Accessed March 21, 2023.
16. American Society of Hospital Pharmacists. Flucytosine. In: McEvoy G, ed. *AHFS drug information 2010*. American Society of Health-System Pharmacists; 2010:567-570.

17. Cutler RE, Blair AD, Kelly MR. Flucytosine kinetics in subjects with normal and impaired renal function. *Clin Pharmacol Ther*. 1978;24:333-342.
18. Diasio RB, Lakings DE, Bennett JE. Evidence for conversion of 5-fluorocytosine to 5-fluorouracil in humans: possible factor in 5-fluorocytosine clinical toxicity. *Antimicrob Agents Chemother*. 1978;14:903-908.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Goyal V, Krantz E, Simon F, et al. Bioavailability of three novel oral, sustained-release pellets, relative to an immediate-release tablet containing 500 mg flucytosine: A randomized, open-label, crossover study in healthy volunteers. *Clin Transl Sci*. 2024;17:e13756. doi:10.1111/cts.13756