Clinical Trial Protocol Synopsis

A 10 week, open-label randomized controlled parallel-group study to evaluate the comparative bioavailability, efficacy, and safety of sustained release flucytosine versus immediate release flucytosine in adults with cryptococcal meningitis

Name of product(s)	Sustained Release Flucytosine pellets (SR 5FC) – experimental Ancotil tablets (IR 5FC) – control Liposomal Amphotericin B (L-AmB, Ambisome) Fluconazole
Drug Class	5FC: Pyrimidine: fluorinated cytosine analogue L-AmB: Polyene antibiotic Fluconazole: Triazole
Phase	Phase II
Indication	HIV-associated Cryptococcal Meningitis
Protocol Number	DNDi-5FC-03-CM
Sponsor	DNDi, 15 Chemin Camille-Vidart, 1202 GENEVA, Switzerland Phone: +41 22 906 9230
Global/National Coordinating Investigator/Principal Investigator	Global coordinating investigator: National Malawi Investigator: International Disease Expert for Investigators:
SAC approval	*17 February 2023*
Clinical Trial Protocol Synopsis Version / Date	Synopsis V3.0 02 June 2023

SYNOPSIS

Background Information and Trial Rationale	HIV-associated cryptococcal meningitis (CM) remains a significant driver of AIDS-related mortality. There are an estimated 152 000 cases and 112 000 deaths annually and CM is the cause of 19% of all AIDS-related mortality[1,2]. The greatest burden is in sub-Saharan Africa, driven by the persistent burden of advanced HIV disease despite widespread access to antiretroviral therapy. Based on recent large trials (ACTA and AMBITION[3,4]), flucytosine is now recommended as an essential component of induction antifungal treatment. World Health Organisation (WHO) guidance[5,6] recommends a single dose of liposomal amphotericin B (L-AmB, Ambisome) at 10 mg/kg/d iv on day-one, combined with 14 days of flucytosine (5FC, 100 mg/kg/d in four divided doses) and fluconazole (1200 mg/d). If L-AmB is not available, one week of amphotericin B deoxycholate (AmBd) plus 5FC, as described above, followed by one week of fluconazole 1200 mg/d is recommended; and where amphotericin B is not available, two weeks of an oral combination of 5FC and fluconazole is recommended. The current immediate-release (IR) 5FC formulation has a short half-life necessitating dosing four times per day. To achieve clinically effective concentrations, the recommended daily dose is high (100 mg/kg/d) and the dosing regimen is sub-optimal: every 6 hours i.e., 4 times per day. Data show that the fourth dose in particular is often missed. In addition, this current formulation has not been developed or approved for nasogastric (NG) administration, necessary for participants with a reduced level of consciousness. A convenient and patient-adapted formulation, such as a sustained released (SR) multi-particulate, pellet formulation, will greatly facilitate clinical practice by reducing the number of administrations per day, standardization of therapy, and potentially improving the concentration and exposure levels of 5FC over time. A SR-5FC formulation, which could be adapted to administration via NG tube and optimized for twice daily administration is considered an urgent priority to attain significant reductions in mortality and change the practice of medicine for CM. In addition, screening for cryptococcal antigen (CrAg) in asymptomatic HIV participants with low CD4 cell counts (below 100 or 200 cells/μL), and pre-emptive treatment for those tested CrAg positive, are currently recommended by the WHO and being adopted as a policy in increasing number of countries in order to prevent the development of symptomatic infection. Currently, pre-emptive treatment is with fluconazole, 800-1200mg/d for the first two weeks[5,6]. However, this may be

sub-optimal for some participants and trials are underway to test alternative treatment, including the EFFECT study (ISRCTN30579828), evaluating combination 5FC (25 mg/kg four times daily) plus fluconazole (1200 mg/day) for the first two weeks in this participant group.

Study purpose and rationale:

Flucytosine (5FC) is a key component of antifungal therapy for cryptococcal meningitis, associated with more rapid sterilization of the cerebrospinal fluid (CSF) and a significant reduction in mortality as compared to fluconazole monotherapy or fluconazole plus Amphotericin B[3]. However, administration of 5FC is difficult due to the fact that existing formulations require dosing four times daily. Such dosing is problematic in resource-limited settings where hospitals are overcrowded and staff overburdened. In addition, many patients with CM have reduced consciousness and are unable to tolerate medicines by mouth. In this case, current standard 5FC formulations are crushed and given by naso-gastric tube, 4 times a day, although such administration is challenging, and the dose effectively administered has not been evaluated.

Adherence to a 4 times daily dosing schedule for patients completing therapy as outpatients is even more difficult. Recent changes to recommended treatment have reduced the duration of intravenous therapy (from 2 weeks of AmBd, to 1 week in ACTA[3], and now to a single i/v dose of L-AmB in AMBITION[4]). This may allow earlier discharge from hospital for some patients but increases the importance of out-patient adherence to complete 2 weeks of oral 5FC and fluconazole. In addition, it is likely that the oral combination of 5FC and fluconazole[3,7] may be used in future for the pre-emptive outpatient treatment of some or all patients identified by screening as having a positive CrAg test in blood, further increasing the demand for 5FC and the need for alternative formulations.

The development of a sustained release (SR) 5FC formulation, allowing twice daily dosing, and that could facilitate easy and effective administration via oral or NG tube, is therefore an urgent priority in order to translate and maximize the impact of recent trial findings and support reduction cryptococcal related mortality. This SR formulation is developed as a substitute for the current IR formulation, as part of first line combination treatment regimen.

<u>Justification of dose:</u>

The recommended dosage of 5-FC ranges from 100 to 200

	mg/kg per day divided into 3 to 4 doses, depending on the nature of the infection, its site and sensitivity of the causative agent. For the treatment of cryptococcal meningitis, the WHO recommended first line induction regimen includes 5-FC at 100 mg/kg per day divided into 4 doses. There is limited pharmacokinetic-pharmacodynamic information available on this compound. In different studies with isolates from different geographies, the minimum inhibitory concentration (MIC) of 5-FC capable of inhibiting Cryptococcus spp. fungal growth by ≥ 50% and by ≥ 90% was determined at 2-4.0 and 4-8.0 µg/mL, respectively[8,9]. The target flucytosine drug levels are estimated at > 20 mg/mL and < 100 mg/mL. This target range is proposed for SR dose regimen in patients in order to avoid too low plasma concentrations to minimize resistance and disease deterioration (debilitating and life-threatening disease) (< 20 mg/mL) and not too high concentrations to avoid concerning safety issues mainly observed with C_{max} greater than 100 mg/mL for several days. An initial Phase I (1A) study under fasting conditions, evaluated three test pellet formulations (treatments B, C , and D) of SR 5FC (3 g) versus the reference IR 5FC (treatment A) under fasted conditions, to determine the comparative bioavailability and safety. The prototypes were formulated for release *in vitro* at the following rates, as suggested by previous/legacy PBPK modelling: • Formulation-B: Not more than 20% at 1 hour, 20-50% at 3 hours, and not less than 70% at 9 hours, • Formulation-C: Not more than 35% at 1 hour, 35-65% at 3 hours, and not less than 80% at 9 hours, • Formulation-D: Not more than 45% at 1 hour, 50-80% at 3 hours, and not less than 85% at 9 hours. Based on PK modelling and data obtained, the SR 5FC pellet prototype with most rapid absorption (treatment D) was selected as the product to utilize for further development and testing. Using linear mixed modeling, the relative bioavailability was the highest for treatment D, with 49.0% (90% CI: 43.5-55.1%) for C_{max} and 53.6% (90% CI: 49.2-58.3%) for $AUC_{(0,t)}$, respectively. Following single dose administration of IR and SR flucytosine, 5-FC $AUC_{0\text{-}inf}$ (mg*h/L) of 524±322 and 327±242 were observed, respectively. Peak plasma concentrations (C_{max}) were 39.2± 6.80 mg/L (after second dose) and 16.9±3.81 mg/L, respectively. Relative bioavailability of selected prototype was calculated at 0.62. PBPK modelling results of DNDi-5FC-01-CM suggested a higher dose of selected prototype of SR pellets was needed under fasting and fed conditions to meet target therapeutic 5-FC exposure (20-100mg/mL).

	Based on dissolution profile and PK modelling data, peak 5-FC concentrations after administration of a dose of 6000 mg SR pellets were estimated to be below 100.0 mg/mL for healthy participants. Toxicity is more likely when peak serum 5-FC concentrations are greater than 100.0 mg/mL and with prolonged treatment courses. No new safety signals were observed at dose of 3000 mg in DNDi-5FC-01-CM fasting study with all tested prototypes, including the one selected for this study. In addition, the PBPK modelling indicated that time to maximum concentration with 5FC SR formulation was longer by 24 minutes to the one of 5FC IR formulation: IR and SR 5FC formulations reach C_{max} (26mg/l) at 1.6h and 2.0h, respectively. IR and SR 5FC time to reached MIC90 concentration (8 mg/L) was 0.4 h (0.3, 0.6) and 0.7 h (0.5, 0.9), respectively. Expert consultation indicates that it is unlikely that the small difference in achieving maximum concentration in combination with L-AMB + Fluconazole would have an impact on disease outcomes for SR 5FC to substitute the current tablet formulation. In line with these results, the Data Monitoring Committee recommended that the SR-5FC dose be increased to 6 g in the second Phase I single dose study (DNDi-5FC-02-CM) in fed conditions. DNDi-5FC-02-CM showed that a single dose of 6 g of SR 5FC was safe in healthy volunteers, without any treatment-related study interruption. In addition, fed status of the participants receiving a standard high fat high calorie breakfast seemed to have an impact on the treatment absorption. A higher peak concentration and a higher exposure were observed with the SR formulation compared to the IR: 48.12 vs 36.03 ug/mL for the mean C_{max}, respectively, and 628.06 vs 451.05 ug.h/mL for the mean exposure, respectively. The highest individual C_{max} for the SR formulation was 75.90 ug/mL and the highest AUC_{0-t} 944.44 ug.h/mL. With the modelled high peak concentrations exceeding target of 100 mg/L in fed conditions in healthy volunteers and the good overlap seen in fasting conditions (in Figure 1), the recommended regimen of SR 5FC in participants with HIV associated CM is 6000mg BID in fasting conditions. The unknown feeding status at the time of admission of unconscious patients to the hospital should not pose any risk of exceeding the therapeutic interval as the Cmax of the first dose is below the upper therapeutic limit.

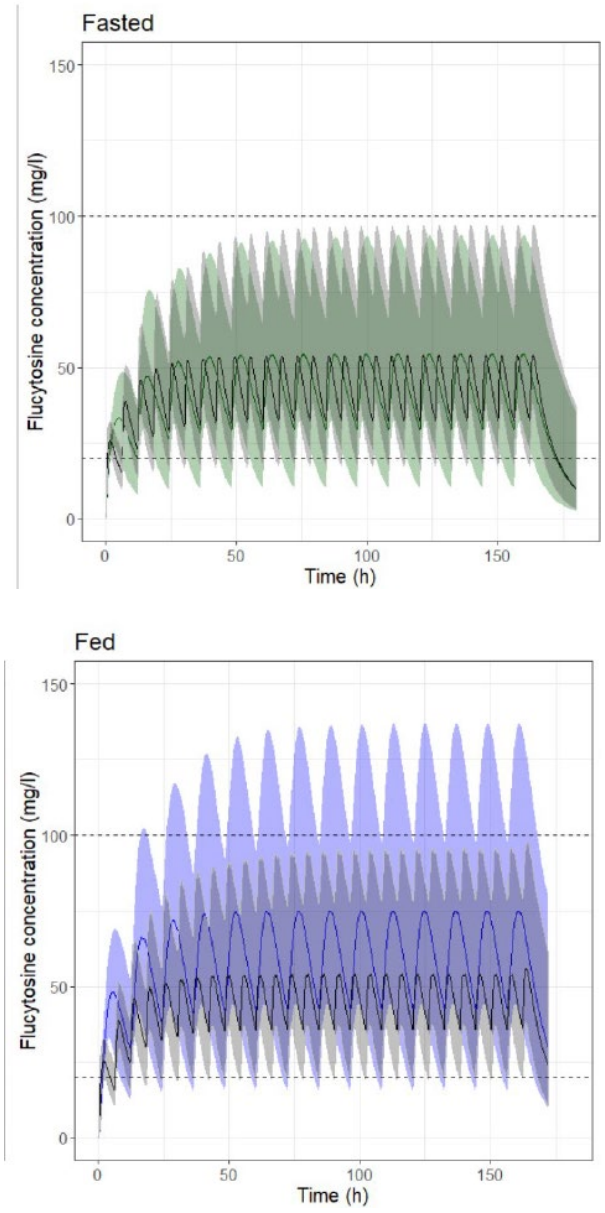


Figure 1 PBPK remodeling in fasted and fed state SR (6000mg BID) and IR (1500mg 4 times per day) 5FC formulations in healthy volunteers.

IR (black) and SR 5FC 6000 mg (colored) - dots represent observed full data, and line (median) and shaded area (5-95% range) are simulations after update to the PBPK model.

This Phase II trial, led by NIMR and carried out in Tanzania and Malawi, forms part of a clinical development programme led by DNDi ... to develop and test such an SR formulation. In addition, given the Glasgow Coma Score (GCS) of the trial participants at enrolment (above 10 for all participants at study entry), most will have treatment administered orally and a formal comparison of oral and NG administration will not be possible in this trial. In this Phase II study, the selected SR formulation, given twice daily, will be compared to the current immediate release (IR) 5FC

	formulation, given 4 times daily. Both formulations will be administered with L-AmB and fluconazole according to latest WHO recommendations[5]. The bioavailability of SR 5FC and IR 5FC will be compared as well as early fungicidal activity (EFA), now a well-established pharmacodynamic endpoint and potential surrogate endpoint for cryptococcal meningitis survival[10–13]. Data will also be collected on safety and clinical outcomes in both study arms.

Trial Objectives, endpoints and estimands

Primary Objective	Primary Endpoint	Primary Estimands
Primary PK objective: To assess the primary bioavailability of SR 5FC as compared to IR 5FC, administered in combination with L-AmB and fluconazole.	Primary PK parameter for IR and SR 5FC: Area-under-curve for 5FC plasma concentration versus time, from time zero to t, where t is the time of the last quantifiable concentration ($AUC_{(0-t)}$)	Geometric mean ratio of the 5FC $AUC_{(0,t)}$ between reference (IR) and SR 5FC through linear mixed models PK Population Treatment effect regardless of intercurrent event
Primary Efficacy/PD objective: To evaluate early fungicidal activity of SR 5FC, as compared to IR 5FC, administered in combination with L-AmB and fluconazole.	Early fungicidal activity (EFA) defined by cryptococcus clearance rate of decrease in the cerebrospinal fluid (CSF)	Difference in mean rates of decrease in log10 CFU/ml/day between reference (IR) and SR 5FC groups through linear mixed models PD population Treatment effect regardless of intercurrent event
Secondary objectives	**Secondary Endpoints**	**Secondary Estimands**
Secondary PK objective: To assess the secondary bioavailability parameters (C_{max}, C_{min}, C_{av}, C_{max} C_{min} fluctuation, T_{max}) of SR 5FC as compared to IR 5FC, administered in combination with L-AmB and fluconazole.	Secondary PK parameters for IR and SR 5FC: - Maximum observed plasma concentration (C_{max}) and C_{max} at steady-state ($C_{max,ss}$); - Time to maximum observed plasma concentration (t_{max}); - Minimum observed plasma concentration (C_{min}) and C_{min} at steady-state ($C_{min,ss}$); - Area under the plasma concentration versus time curve, with extrapolation to infinity ($AUC_{(0-\infty)}$); - Average concentration during a dosing interval ($AUC_{(0-t)}$ / t)(C_{av}) - Fluctuation ([(C_{max}-C_{min})/C_{av}]) - Terminal elimination rate constant (λz); - Apparent terminal elimination half-life (t½);	Geometric mean ratios of the 5FC C_{max},C_{max} at steady-state ($C_{max,ss}$); C_{min} and C_{min} at steady-state ($C_{min,ss}$), and C_{av}, between reference (IR) and SR 5FC through linear mixed models Difference of medians between reference (IR) and SR 5FC via Wilcoxon test for tmax, fluctuation, t1/2, λz and time over MIC90. PK population Treatment effect regardless of intercurrent event

	Time over antibiotic concentration that would inhibit the growth of 90% of *Cryptococcus neoformans* (MIC90)	
To assess the safety of SR 5FC, as compared to IR 5FC, both administered in combination with L-AmB and fluconazole.	- Treatment Emergent Adverse Events (TEAEs) (clinical, laboratory and ECG) - Treatment Emergent Serious Adverse Events (TESAEs) (clinical, laboratory and ECG) - Grade III, IV and V TEAEs and TESAEs - Treatment discontinuation due to Aes.	Frequency (n(%)) and cumulative incidence of the safety endpoints by treatment arm and for each of the treatment drug administered and overall Safety population. Treatment effect regardless of intercurrent event
Exploratory objectives	**Exploratory Endpoints**	**Exploratory estimands**
To assess the efficacy of 5FC + L-AMB + fluconazole induction regimen in each of the study treatment arms.	All-cause mortality at 2, 4 and 10 weeks	Overall occurrence (n(%)) of death from any cause by treatment arm and overall. Mortality at day 14, 28 and 70 will also be analysed as time-to-event outcomes (e.g. time from randomisation to the occurrence of death from any cause) through Kaplan Meier curves by treatment arm and overall. Hazard ratio using an unadjusted Cox regression analysis. ITT population. Treatment effect regardless of intercurrent event
	Disability at 10 weeks determined by simple two-question assessment and modified Rankin Score	Difference in mean rates of disability at 10 weeks by treatment arm ITT population. Treatment effect regardless of intercurrent event
To assess the acceptability and palatability of IR and SR 5FC formulations, both from the participants and from the healthcare workers point of view.	Acceptability and palatability of IR and SR 5FC will be assessed using questionnaires – an Investigator questionnaire to assess acceptability of oral and nasogastric route of administration (ease of administration, time, volume) and a participant questionnaire to assess acceptability and palatability (taste, texture, flavour, size).	Frequency (n(%)) for categorical items and descriptive statistics (mean, SD, median, range) for quantitative variables by treatment arm Safety population Treatment effect regardless of intercurrent event
To monitor resistance to treatment among non-responders	Resistance development will be assessed via antifungal drug susceptibility testing, MIC	Difference in MIC90 (mean, SD, median, range) and presence of key gene mutations from

	determination, DNA sequencing and variant calling (loss of function mutations in FCY1, FCY2, and FUR1 genes), among treatment non-responders (EFA<0.20 log10 CFU/mL/day)	baseline among treatment non-responders, by treatment arm Treatment non-responders population
To perform cost-minimisation analyses of SR 5FC in comparison to IR 5FC both administered in combination with L-AmB and fluconazole.	Direct costs relating to IR and SR 5FC combination treatment, hospital stay, laboratory tests and management of adverse events will be measured in local currency and converted to USD.	Total direct costs, by treatment arm Safety population.
To explore comparative bioavailability of IR and SR 5FC administered orally and via nasogastric tube	All described primary and secondary PK parameters for IR and 5FC	Geometric mean ratio of the 5FC C_{max} and C_{max} at steady-state ($C_{max,ss}$); C_{min} and C_{min} at steady-state ($C_{min,ss}$) , C_{av} at steady state ($C_{av\ ss}$) and $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ between reference (IR) and SR 5FC and through linear mixed models, and between oral and nasogastric administration Medians of reference (IR) and SR 5FC for tmax fluctuation, t1/2 and λz, and oral and nasogastric administration PK population Treatment effect regardless of intercurrent event

Trial Design	Open-label multi-centre phase II randomised parallel-group controlled trial, bioanalytical/microbiology (EFA only) laboratory blinded. Participants will be recruited from two district hospitals in Dar es Salaam, Tanzania (Mwananymala and Amana District Hospitals) and from Kamuzu Central Hospital in Lilongwe, Malawi. Participant recruitment is expected to take place over a 12-month period. Participants will be randomised individually with a 1:1 allocation ratio using a computer-generated list. The first 7-days of the two-week induction treatment will be given in a directly observed hospital setting with study medications administered directly by the study team, ensuring compliance to the trial intervention. During the second phase of treatment, if the participant is stable, he/she could be discharged, on condition that they return for blood tests and LP on day 15. Daily telephone calls will be implemented to check for compliance and how the participant is feeling. Follow up will be conducted in clinic at 2, 3, 4, 6, and 10 weeks. Telephone numbers and/or locator information will be collected from participants and relatives and financial

	assistance will be provided to cover travelling expenses. Home visits will be undertaken, if needed. To avoid administering the study drug in severely ill participants (in case the exposure is not reached), only participants with a Glasgow Coma Score (GCS) of 15 will initially be included. After six participants have completed induction treatment with the SR 5FC formulation, bioavailability, clinical and safety data will be reviewed in a safety review. Once it is known that the exposure is reached quickly enough and that there is no risk for the participant, the inclusion criteria may be modified to include participants with a GCS ≥10. The expected recruitment rate being low, recruitment will not be paused during the safety review. Data management will be overseen by Luxembourg Institute of Health using an electronic data capture system.
Main Entry Criteria Inclusion Exclusion	Participants who fulfill all inclusion criteria and do not present any of the exclusion criteria will be eligible to be enrolled in this study. Inclusion Criteria: • Male and female ≥ 18 yrs with a first episode of cryptococcal meningitis (CSF India ink or CSF positive CrAg test) • Serologically confirmed HIV infection at the point of study screening or at any time prior • Willing to participate in the study. Consent will be obtained from family/guardians/person with legal responsibility for participants who lack capacity to consent, and from the participants themselves after recovery. • Glasgow Coma Scale (GCS) assessed as 15/15. Following the safety review and DMC recommendation, participants allowed with GCS ≥10/15. Exclusion Criteria: • Pregnant (confirmed by a highly sensitiveurinary pregnancy test) or breastfeeding. • Women of childbearing potential who do not agree to use contraception during study period. • Male participants (or their female partners of childbearing potential) who do not agree to use effective contraception during study period. • Known dihydropyridine dehydrogenase (DPD) deficiency • Already taking systemic antifungal treatment for > 48 hours • Participants <40kgs or BMI<16 (with severe signs of malnutrition)

	• History or known hypersensitivity or allergy or contraindication to the study drugs (5FC, L-AmB and fluconazole) or its excipients or any related medication • Concomitant medication that is contraindicated with study drugs (5FC, L-AmB and fluconazole) or its excipients or any related medication (including anti-viral anti-herpetic agents (brivudine, sorivudine or their analogues), Uracil); products known to prolong the QT interval and which are metabolised via the cytochrome P450 (CYP) 3A4 (such as cisapride, astemizole, pimozide, quinidine and erythromycin, azithromycin). • Use of any medication, prescribed or over-the-counter or herbal remedies, within 2 weeks before the first administration of IMP except if this will not affect the outcome of the study in the opinion of the investigator. • History of radiotherapy • Additional serious or life-threatening disease or HIV related complications or co-morbidities (notably, diseases affecting gastrointestinal tract and participants likely to die within 14 days from conditions other than cryptococcal meningitis) based on the opinion of the investigator • Absolute neutrophil count of <500 x 10^6/L on baseline blood testing • Platelets <50,000x10^6/L on baseline blood testing • Creatinine clearance; eGFR < 60 ml/min on baseline blood testing (calculation method Cockroft/Gault) • Hepatic impairment (transaminases >3x upper limit of normal) on baseline blood testing • Participants should be excluded in case of any severe medical or psychiatric condition that may increase the risk associated with study participation or may interfere with the interpretation of study results.
Study Duration	The treatment lasts for 14 days and is followed by a period of 8 weeks follow-up (for a total of 10 weeks). The recruitment period is expected to be 12 months and the follow-up period will be 10 weeks. Therefore, FPI to LPO is expected to be 14.5 months.
Study treatments	Arm 1 (control): Liposomal amphotericin B (Ambisome, Gilead Sciences[14]) at 10 mg/kg/d iv on day-one, combined with 14 days of both **flucytosine ((**Ancotil**, Immediate-release, MEDA Pharma.), 100 mg/kg/d, orally, in four divided doses**[15]**)** and fluconazole (Capsule, Medochemie Ltd.,1200 mg/d[16]). Arm 2 (experimental): Liposomal amphotericin B (Ambisome, Gilead Sciences) at 10

	mg/kg/d iv on day-one, combined with 14 days of both **flucytosine (Sustained release*, Mylan, 6g twice per day, orally**[17]) and fluconazole (1200 mg/d). ***note: both 5FC formulations will de administered in fasting conditions, first dose before breakfast and second dose before diner (4 hour after lunch)** After the first two weeks, all participants will receive standard consolidation (fluconazole 800 mg daily for eight weeks) and subsequently maintenance treatment with fluconazole 200 mg daily until immune reconstitution (CD4> 200 cells/mm3) and suppression of viral loads on antiretroviral therapy. Antiretroviral therapy will be started or restarted at 4-6 weeks after initiation of antifungal therapy, in line with international and country-specific guidance and policies.
Statistics Sample size Randomisation Summary of analysis	<u>Sample size calculation:</u> Seventy-two (72) participants (36 per arm) will be recruited in this clinical study. Sample size considerations are driven by the two primary endpoints (PK and EFA parameters) and correct for multiplicity by controlling the overall Type I error rate at 5%. For the primary objective of bioavailability, 33 participants evaluable for PK in each arm (66 participants evaluable for PK in total) will have 80% power to reject both null hypotheses that the ratio of the 5FC SR mean to the reference mean is below 0.8 and that it is above 1.25; i.e., that the test and reference are not equivalent, in favor of the alternative hypothesis that the means of the two groups are equivalent, assuming that the expected ratio of means is 1, the coefficient of variation for the standard is 0.28 (observed in the fasting Phase I study), that data will be analyzed on the log-scale using t-tests for differences in means, and that each t-test is made at the 2.5% level. Assuming that 3 participants per arm will not be evaluable for PK, a total of 72 participants will be entered into the study to complete the study with at least 33 participants evaluable for PK in each arm. For the primary objective of evaluation of EFA, when the sample size of participants evaluable for PD is 33 in each arm (66 participants evaluable for PD in total), a two-group one-sided 1.25% significance level t-test will have 82% power to reject the null hypothesis that the test 5FC SR and reference are not non-inferior (the difference in means between the test and the reference arm is 0.2 or farther from zero in the same direction) in favor of the alternative hypothesis that the means of the two groups are non-inferior, assuming that the expected difference in means is 0 and the common standard deviation is 0.25 (based on the AMBITION phase 2 trial[18]). Assuming that 3 participants per arm will not be evaluable for PD, a total of 72 participants will be

	entered into the study to complete the study with at least 33 participants evaluable for PD in each arm. The non-inferiority margin of 0.20 is the same as in the AMBITION phase 2 trial[18]. It is based on an EFA (i.e. rate of decrease in CSF) of -0.50 log_{10}CFU/d in the reference arm, together with data showing that increases in mortality are not seen unless the EFA is drops below -0.30 log_{10}CFU/d[11,13], resulting in a non-inferiority limit difference of -0.30-(-0.50)=0.20. An association between EFA and mortality is much less clear with EFA above -0.30[11,13]. ***Randomization:*** A randomization schedule will be generated utilizing the PROC PLAN procedure of SAS software or appropriate equivalent. ***Multiplicity:*** The overall type I error for the 2 primary endpoints in the final analysis will be controlled by the Holm procedure. No multiplicity adjustment will be required for safety review, as no formal statistical test will be performed. ***Analyses populations*:** 1) Intention-to-treat (ITT) population: all randomised participants; 2) Safety population - a subset of the ITT population, consisting of all randomised participants who receive at least one dose of study drug. 3) Pharmacokinetic (PK) population - a subset of the ITT population, consisting of all randomized participants who receive at least one dose of study drug, who have a PK profile and who have no major protocol deviations thought to impact the analyses of the PK data. 4) Pharmacodynamic (PD) population - a subset of the ITT population, consisting of all randomized participants who receive at least one dose of study drug and who have a PD profile (baseline and at least one post baseline on day 3 measurement of log_{10}CFU/mL). ***Safety review:*** After six (6) participants have completed induction treatment with SR-5FC formulation, PK samples from both IR and SR groups will be shipped to FARMOVS for immediate analysis in order to ensure that target concentrations have been achieved, notably that mean $C_{min,ss}$ concentrations above the required MIC_{90} are observed and the toxicity threshold mean $C_{max,ss}$ < 100 mg/L has not been exceeded. Clinical and laboratory safety information will also be reviewed by the DMC. Recruitment will only be paused during the review of the AEs and SAEs, that

	occurred during the 2 weeks treatment of the 6 impacted SR participants and the control IR participants No formal statistical test will be performed and therefore there will be no impact on the overall risk alpha level of the study. ***PK analysis*** The primary endpoint analysis will be based on the PK population. Relative bioavailability of the test and reference products will be assessed on the basis of the 95% confidence intervals (CIs) for estimates of the geometric mean ratio between the primary PK parameter of the test and reference products for 5FC via linear mixed models. PK results for 5-fluorouracil (5FU), a metabolite of 5FC will serve as supportive analysis only. ***Early fungicidal activity PD analysis*** The primary PD endpoint analysis of EFA data will be be based on the PD population. Linear mixed models will be used to compare the mean rate of decrease in $\log_{10}$ CSF cryptococcal CFU per day (EFA), over 14 days, giving the summary difference with its 97.5% CI. ***Safety analysis*** Safety data will be reported in the safety population. The analysis will be of descriptive nature: Safety data will be listed and summarized by treatment arm as indicated in the SAP. AE participant incidence will be summarized by System Organ Class (SOC) and preferred term. Data will be tabulated by severity, physician assessment of relationship to each investigational products, SAEs and AEs leading to treatment discontinuation. The frequency and proportions of participants suffering from clinical and laboratory-defined AEs, SAEs and AEs leading to treatment discontinuation, overall and by SOC, will be generated by treatment arm and overall.participant. ***Exploratory analyses:*** Efficacy Analysis of clinical efficacy - all-cause mortality and disability score – will be based on the ITT population and presented as indicated in the SAP.

	Acceptability: Acceptability and palatability analyses will be performed in the safety population who have received either the IR 5FC or the test product SR 5FC. Results (scores) obtained from the Investigator and participant questionnaires, respectively, will be presented descriptively as indicated in the SAP. Economic Full costing analysis of the two treatment regimens over the 10-week trial period will be obtained from a health care provider perspective. The trial will pay for all costs related to medical care during the trial, including travel, so out-of-pocket expenses will not be included in this analysis. The study will conform to the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) guidelines. The data on individual resource use will be collected from all trial participants.

Table 1- Schedule of events

PK

Blood samples will be collected for 5FC and 5FU PK estimation following dosing at pre-dose, on Day 1, at 2 h, 4 h, 6 h, 7.5 h, 12 h, 24h (Day 2) and Day 6 at the following time points: 2 h, 4 h, 6 h, 7.5 h, 12 h, and 24 h (Day 7) post-dose . On Day 15 an additional PK sample will be collected at the time the safety laboratory samples are collected (sparse PK). Times of 5FC administration and of the venipuncture will be registered. 5FC and 5FU concentrations will be determined using high-performance liquid chromatography tandem mass spectrometry at FARMOVS, South Africa, after completion of the study. 5FC CSF concentrations will be determined at Day 7. There will be a safety review after 6 participants have completed induction SR 5FC treatment to ensure that adequate and safe 5FC concentrations have been achieved. PK samples collected on Day 1 12h and 24h post-dose, will only be determined if required for complementary analysis.
PK collection date/time and Food intake timing will be recorded for consideration in the analyses.

In parallel, few drops of the blood samples will be immediately applied to Whatman paper, dried and stored at room temperature protected from light, until analysis. Based on PK data, dried blood spots (DBS) will be analysed at selected timepoints to evaluate PK DBS, as a potential method to measure flucytosine levels in the future.

CSF PD and PK

Lumbar punctures will be done before dosing, 3, 7, and 15 and CSF plated for quantitative fungal cultures, as in prior studies[3,4,7,10–13,18,19]. Usually no more than 30mL CSF will be drained at any one therapeutic lumbar puncture. The CSF pressure should be rechecked after removal of every 10mL CSF.
CSF PD: QCC (Quantitative Cryptococcal Culture), glucose, protein, lymphocytes count; sample storage for resistance testing for treatment non-responders (Drug susceptibility testing (fluconazole, amB, flucytosine or only flucytosine) and DNA sequencing).
Treatment non-responders are defined as EFA < 0.20 log, based on analyses of pooled individual-level CSF data from cryptococcal meningitis clinical trials conducted during 2010–2017, mortality through 18 weeks was 50% for those with EFA < 0.20 CFU/mL/day[13].

CSF PK: PK samples will be analysed at FARMOVS , Bloemfontein, South Africa.

5FC CSF concentrations will be determined at Day 7. CSF PK samples collected at other timepoints, will only be determined if required for complementary analysis.

Safety

Participants will be monitored daily during the 14 days of induction treatment (as in-participants or through follow-up calls at home after they have been discharged) for the development of new clinical signs and symptoms and safety of the administered medications. Peripheral blood samples will be taken at admission and on Day 3, 7 and 14 for creatinine and electrolytes, and for full blood count and liver function tests. Participant management will follow standard operating procedures (SOPs) for cryptococcal meningitis developed over prior studies by SGUL. Safety will be assessed through routine monitoring of adverse events (AEs). AEs will be collected by study personnel at the time-points indicated in the Schedule of Events. AEs may also be directly observed by study personnel or spontaneously reported by participants and should be reported by the investigator using concise medical terminology. The severity of AEs will be graded using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events.. The terms are defined as follows:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death

Efficacy

The study is not powered for clinical endpoints, however, data on 2-4 and 10-weeks all-cause mortality, as well as on 10-week disability (by two-question assessment and modified Rankin Score) will be collected.

Table 2- Schedule of events

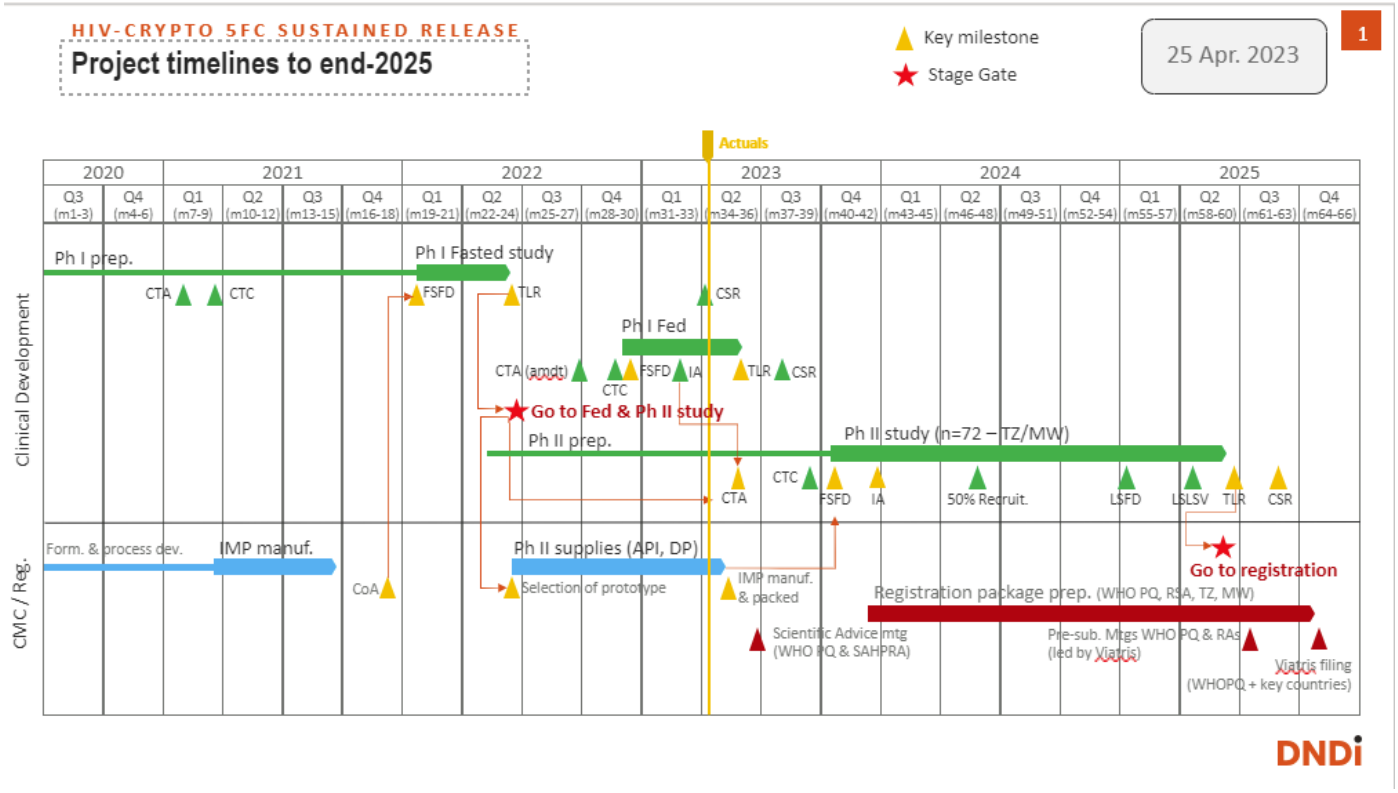
SCHEDULE OF EVENTS														
Protocol Activities and Forms to Be Completed	**Screening[b]**	**Pre-trial feasibility/ Randomisation[b]**	**Treatment**									**Follow-up Period**			
	(day)		**(day)**								**End of treatment (day)**	**(day)**	**(weeks)**		
			1[b]	*2*	*3*	*4*	*5*	*6*	*7*	*8-14*	*15 (±1 day)*	*21 (±1 day)*	**4** *(±2 day)*	**6** *(±2 day)*	**10** *(±3 day)*
Informed Consent	X														
Demographic Data and Medical History	X														
Concomitant Treatments	X		X	X	X	X	X	X	X	X	X				
Vital signs and Physical Examination	X		X	X	X			X	X		X		X	X	X
Height, Weight, BMI	X														
Intracranial Pressure[h]		X			X				X		X				
*12-lead ECG**	X[a]	X[a]													
Inclusion and exclusion criteria	X														
CSF india ink or CSF CrAg test	X														
Serum HIV[L]	X[L]														
COVID-19 test[i]	X														
*Haematology**	X				X				X		X				
*Liver tests**	X				X				X		X				
*Creatinine & electrolytes**	X				X				X		X				
Random Blood Sugar[M]	X														
Urianalysis	X										X				
Urine Pregnancy tests for women with childbearing potential	X								X			X		X	X

SCHEDULE OF EVENTS															
Protocol Activities and Forms to Be Completed	**Screening[b]**	**Pre-trial feasibility/ Randomisation[b]**	**Treatment**									**Follow-up Period**			
	(day)		**(day)**								**End of treatment (day)**	**(day)**	**(weeks)**		
			1[b]	*2*	*3*	*4*	*5*	*6*	*7*	*8-14*	*15 (±1 day)*	*21 (±1 day)*	**4** *(±2 day)*	**6** *(±2 day)*	**10** *(±3 day)*
Blood PK[e] Whatman DBS PK samples		X	5 timepoints (+2, 4, 6, 7.5, 12h)	X (D1+24h)				6 timepoints (pre-dose, +2, 4, 6, 7.5, 12h)	X (D6+24h)		X				
CSF for PharmacoDynamics[c,d]		X			X				X		X				
CSF for PharmacoKinetics [e]		X			X				X		X				
Randomisation		X													
Safety assessment	X	----------SAEs and AEs monitoring----------											-------SAEs monitoring------		
Vital status assessment											X		X		X
Health care worker/Participant's acceptability/palatability questionnaire			----------								----------				
Disability questionnaire	X														X
Health economic questionnaire															X
Trial treatment - Flucytosine			----------								*X[g]*				
Trial treatment - AmBisome			X												
Trial Treatment - Fluconazole			----------1200 mg/d----------								1200 or 800 mg/d[k]---	----------800 mg/d[f]----------			
Hospitalization			Hospitalization (possible discharge at D7 and counselling participants on contraceptive use)									----------Home----------			
ART initiation and/or switch[j]													X		

* May be tested at other timepoints as needed.

[a]: ECG could be done on Screening or Baseline Visit
[b]: all 3 visits could be performed on the same day, however assessment done on Screening and Pre-Dose baseline should be performed before the IMP administration.
[c]: if LP is performed for therapeutic needs, CSF samples will be stored for additional analyses
[d] : CSF Pharmacodynamics: QCC (Quantitative Cryptococcal Culture), glucose, protein, lymphocytes count; sample storage for resistance testing for treatment non-responders (Drug susceptibility testing (fluconazole, amB, flucytosine or only flucytosine) and DNA sequencing)
[e] : PK samples will be analysed at FARMOVS , Bloemfontein, South Africa. First batch once 6 participants have been enrolled in total for the safety review. Second shipment will be done at the end of trial. . In addition to plasma samples, dried blood spots will be collected in Whatman paper (selection of samples for DBS analyses will be informed based on plasma PK samples analyses).
[f]: maintenance treatment with fluconazole 200 mg daily until immune reconstitution (CD4> 200 cells/mm^3, as per site practice) and suppression of viral loads on antiretroviral therapy
[g]: optional if participant has only received partial dosing on day 1
[h]: Intracranial pressure might be needed more frequently depending on symptoms and signs, and in line with accepted guidance
[i]: COVID-19 screening test if applicable per hospital procedure
[j]: ART initiation and/or switch to start on Week 4
[k]: If last 5FC dose is given on Day 15 morning then Fluconazole 1200 mg/d will be administered. If 5FC treatment has been completed on Day 14, then Fluconazole 800mg/d will start on Day 15.
[L]: Serum HIV will only be assessed if no previous serologically confirmed HIV infection data are available
[M]: Random blood sugar: prior to Ambisome dosing, and then thereafter as needed (for example during seizure management)

Planning Information



Study Timelines

Final protocol available	30 May 2023
Study treatment supply available	Q2 2023
FSFV	Q4 2023
Duration of recruitment period	12 months
Duration of follow-up period (if applicable)	10 weeks = 2.5 months
LSLV	Q1 2025
Safety Review	After six (6) participants have completed induction treatment with SR-5FC formulation, PK samples will be shipped to FARMOVS for immediate analysis in order to ensure that target concentrations have been achieved, notably that mean $C_{min,ss}$ concentrations above the required MIC_{90} are observed and the toxicity threshold mean $C_{max,ss}$ < 100 mg/L has not been exceeded. Clinical and laboratory safety information will also be reviewed by the DMC. Recruitment will only be paused during the review of the AEs and SAEs, that occurred during the 2 weeks treatment of the 6 impacted participants.
Final study report	Q3 2025

STUDY SCOPE

Target countries	Malawi Tanzania
Enrollment target	72 participants will be enrolled in this study
Number of sites	There will be 3 study sites: 2 sites in Tanzania and 1 site in Malawi.
Number of subjects per site	Expected enrolment is 2 participants/month in Malawi and 2-4 in Tanzania (total for the 2 sites), in total 6 participants per month
DMC involvement	A DMC committee will be constituted of the following members: Prof Graeme Meintjes Prof Helen McIlleron Prof Stephen Senn One member from Malawi One member from Tanzania
Partners involvement	This study is a consortium between the following parties: **DNDi:** • sponsor of the clinical trials • organise the provision of the clinical trial supplies • responsible for monitoring the clinical trials, including medical supervision and site monitoring. • preparation of the clinical study report (in collaboration with consortium partners) **NIMR (National Institute of Medical Research, Tanzania):** • Lead of the clinical trial • Conduct of the clinical trial and engagement of the PI for the 2 sites in Tanzania • Clinical study activities as per clinical study protocol **UNC project - Malawi:** • Conduct of the clinical trial and engagement of the PI for 1 site in Malawi • Clinical study activities as per clinical study protocol **SGUL (St George's University of London):** • Trial management support through the provision of an international PI and co-investigators, trial coordinator • Oversee trial implementation on site • Support statistical analysis **Farmovs:** • Develop and validate the bioanalytical method for cerebral spinal fluid sample analysis • Perform all PK sample analysis and corresponding report **LIH (Luxemburg Institute of Health):** • Data management and biostatistics

Study Treatments Supply

Study treatments	Recruitment will be competitive. Study IPs will be ordered in 2 batches: the first batch will be shipped to the sites for initiation of the study. After the recruitment rates in each site have been better assessed, the second batch will be shipped to the sites according to expected recruitment. A buffer of 50% extra IMP will be shipped for the experimental IMP, SR 5FC, and a buffer of 50% extra will be shipped for manufactured IPs. Based on the fed study Phase I results, the clinical batch of SR 5FC will be manufactured and packaged according to the dosing, by Viatris. 1512 doses will be needed for the trial (this includes the 50% buffer). 9072 tablets of 500mg 5FC will be needed for the trial (this includes 50% buffer). AmBisome 50mg vials is marketed by Gilead. 1296 vials will be needed (this includes 50% buffer). 65'664 capsules Fluconazole 200 mg capsules will be needed.
Labeling instructions	All study drugs will be labelled (SR 5FC) or relabeled (IR 5FC, L-AMB and Fluconazole) for study use only.

References

1 R. Rajasingham, *Physiol Behav*.

2 R. Rajasingham, N. P. Govender, A. Jordan, A. Loyse, A. Shroufi, D. W. Denning, D. B. Meya, T. M. Chiller and D. R. Boulware, *Lancet Infect Dis*, , DOI:10.1016/S1473-3099(22)00499-6.

3 S. F. Molloy, C. Kanyama, R. S. Heyderman, A. Loyse, C. Kouanfack, D. Chanda, S. Mfinanga, E. Temfack, S. Lakhi, S. Lesikari, A. K. Chan, N. Stone, N. Kalata, N. Karunaharan, K. Gaskell, M. Peirse, J. Ellis, C. Chawinga, S. Lontsi, J.-G. Ndong, P. Bright, D. Lupiya, T. Chen, J. Bradley, J. Adams, C. van der Horst, J. J. van Oosterhout, V. Sini, Y. N. Mapoure, P. Mwaba, T. Bicanic, D. G. Lalloo, D. Wang, M. C. Hosseinipour, O. Lortholary, S. Jaffar and T. S. Harrison, *New England Journal of Medicine*, , DOI:10.1056/nejmoa1710922.

4 J. N. Jarvis, D. S. Lawrence, D. B. Meya, E. Kagimu, J. Kasibante, E. Mpoza, M. K. Rutakingirwa, K. Ssebambulidde, L. Tugume, J. Rhein, D. R. Boulware, H. C. Mwandumba, M. Moyo, H. Mzinganjira, C. Kanyama, M. C. Hosseinipour, C. Chawinga, G. Meintjes, C. Schutz, K. Comins, A. Singh, C. Muzoora, S. Jjunju, E. Nuwagira, M. Mosepele, T. Leeme, K. Siamisang, C. E. Ndhlovu, A. Hlupeni, C. Mutata, E. van Widenfelt, T. Chen, D. Wang, W. Hope, T. Boyer-Chammard, A. Loyse, S. F. Molloy, N. Youssouf, O. Lortholary, D. G. Lalloo, S. Jaffar and T. S. Harrison, *New England Journal of Medicine*, , DOI:10.1056/nejmoa2111904.

5 World Health Organization, New guidelines from WHO recommend a simpler, safer treatment for cryptococcal disease in people living with HIV.

6 World Health Organization, Guidelines for the diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children.

7 J. C. Nussbaum, A. Jackson, D. Namarika, J. Phulusa, J. Kenala, C. Kanyemba, J. N. Jarvis, S. Jaffar, M. C. Hosseinipour, D. Kamwendo, C. M. Van Der Horst and T. S. Harrison, *Clinical Infectious Diseases*, , DOI:10.1086/649861.

8 L. K. Archibald, M. J. Tuohy, D. A. Wilson, O. Nwanyanwu, P. N. Kazembe, S. Tansuphasawadikul, B. Eampokalap, A. Chaovavanich, L. B. Reller, W. R. Jarvis, G. S. Hall and G. W. Procop, *Emerg Infect Dis*, , DOI:10.3201/eid1001.020779.

9 M. Li, Y. Liao, M. Chen, W. Pan and L. Weng, *Brazilian Journal of Infectious Diseases*, , DOI:10.1016/S1413-8670(12)70301-X.

10 A. E. Brouwer, A. Rajanuwong, W. Chierakul, G. E. Griffin, R. A. Larsen, N. J. White and T. S. Harrison, *Lancet*, , DOI:10.1016/S0140-6736(04)16301-0.

11 T. Bicanic, C. Muzoora, A. E. Brouwer, G. Meintjes, N. Longley, K. Taseera, K. Rebe, A. Loyse, J. Jarvis, L.-G. Bekker, R. Wood, D. Limmathurotsakul, W. Chierakul, K. Stepniewska, N. J. White, S. Jaffar and T. S. Harrison, *Clin Infect Dis*.

12 J. N. Jarvis, T. Bicanic, A. Loyse, D. Namarika, A. Jackson, J. C. Nussbaum, N. Longley, C. Muzoora, J. Phulusa, K. Taseera, C. Kanyembe, D. Wilson, M. C. Hosseinipour, A. E. Brouwer, D. Limmathurotsakul, N. White, C. Van Der Horst, R. Wood, G. Meintjes, J. Bradley, S. Jaffar and T. Harrison, *Clinical Infectious Diseases*, , DOI:10.1093/cid/cit794.

13 M. F. Pullen, K. H. Hullsiek, J. Rhein, A. K. Musubire, L. Tugume, E. Nuwagira, M. Abassi, K. Ssebambulidde, E. Mpoza, R. Kiggundu, A. Akampurira, H. W. Nabeta, C. Schutz, E. E. Evans, R. Rajasingham, C. P. Skipper, K. A. Pastick, D. A. Williams, B. M. Morawski, A. S. Bangdiwala, G. Meintjes, C. Muzoora, D. B. Meya and D. R. Boulware, *Clinical Infectious Diseases*, , DOI:10.1093/cid/ciaa016.

14 GILEAD, 2020.

15 MEDA Pharma HA693, 2022.

16 Medochemie Ltd, 2022.

17 Drug for Neglected Diseases initiative, 2023.

18 J. N. Jarvis, T. B. Leeme, M. Molefi, A. A. Chofle, G. Bidwell, K. Tsholo, N. Tlhako, N. Mawoko, R. K. K. Patel, M. W. Tenforde, C. Muthoga, G. P. Bisson, J. Kidola, J. Changalucha, D. Lawrence, S. Jaffar, W. Hope, S. F. Molloy and T. S. Harrison, *Clinical Infectious Diseases*, , DOI:10.1093/cid/ciy515.

19 A. E. Brouwer, H. J. M. Van Kan, E. Johnson, A. Rajanuwong, P. Teparrukkul, V. Wuthiekanun, W. Chierakul, N. Day and T. S. Harrison, *Antimicrob Agents Chemother*, , DOI:10.1128/AAC.01188-06.