COMBAT Candida

Combination Antifungal Therapy for *Candida* Bloodstream Infections

Combination Antifungal Therapy with Micafungin plus Flucytosine vs. Micafungin alone for adults with *Candida* Bloodstream Infections: an open-label phase III randomised controlled trial

Acronym: COMBAT Candida: COMBination **A**ntifungal **T**herapy for **Candida** bloodstream infections

Version:	**1.4**
Date:	**06 August 2026**
ISRCTN #:	ISRCTN56596656
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GENERAL INFORMATION

This document describes the COMBAT Candida trial and provides information about procedures for entering participants into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but sites entering patients for the first time are advised to contact the co-ordinating centre to confirm they have the most up-to-date version.

COMPLIANCE

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki 2008, the principles of Good Clinical Practice (GCP), and applicable national and local regulations.

SPONSOR

Wits Health Consortium (Pty) Ltd (WHC), a company wholly owned by the University of Witwatersrand

FUNDING

The trial is funded by the Wellcome Trust (316549/Z/24/Z)

TRIAL REGISTRATION

This trial is registered with the ISRCTN Clinical Trials Register (ISRCTN56596656)
Registration with SANCTR is pending (XXX)

Important notes

RANDOMISATION
To randomise: Upon confirmation of eligibility criteria and completion and entry of the informed consent details, the REDCap Electronic Data Capture tool will select the next available randomisation slot and present the details of the selection to the Study Doctor / Nurse.

SERIOUS ADVERSE EVENT (SAE) REPORTING
Within 24 hours of becoming aware of an SAE, please make a report on the REDCap Electronic Data Capture tool.

TRIAL ADMINISTRATION

Please direct all queries to the Trial Manager, Lauriane Fomete, at lauriane.fomete@witshealth.co.za in the first instance; clinical queries will be passed to the local site PIs, national PI or co-principal investigators via the Trial Manager.

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For full details of all trial committees, please see Section 13

SUMMARY OF TRIAL

Summary Information Type	Summary Details
Acronym (Short Title)	COMBAT Candida (COMBination Antifungal Therapy for Candida Bloodstream Infections)
Long Title	Combination Antifungal Therapy with Micafungin plus Flucytosine vs. Micafungin alone for adults with Candida Bloodstream Infections: an open-label phase III randomised controlled trial
Version	1.4
Date	06 August 2026
ISRCTN #	ISRCTN56596656
SAHPRA Reference Number	20260522
WITS HREC Reference #	260506 (pending)
Study Design	An open label dose-ranging adaptive phase III randomised controlled trial
Type of Participants to be Studied	Adults admitted to trial site hospitals with *Candida* spp. growing in blood culture and clinical evidence of a bloodstream infection
Setting	Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa Helen Joseph Hospital (HJH), Johannesburg, South Africa Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Johannesburg, South Africa Netcare Milpark Hospital (NMH), Johannesburg, South Africa Wits Donald Gordon Medical Centre (DGMC), Johannesburg, South Africa
Interventions to be Compared	Step 1 – Dose-selection 1. Micafungin 100 mg/d IV plus flucytosine 50 mg/kg PO/ nasogastric NG (split QDS) 2. Micafungin 100 mg mg/d IV plus flucytosine 100 mg/kg PO/NG (split QDS) 3. Micafungin 100 mg/d IV – INTERNATIONAL FIRST-LINE STANDARD Step 2 – Optimal dose 1. Micafungin 100 mg/d IV plus flucytosine *either* 50 mg/kg *or* 100 mg/kg PO/NG (split QDS) - INTERVENTION 2. Micafungin 100 mg/d IV – INTERNATIONAL FIRST-LINE STANDARD For each arm: - Oral switch to fluconazole 800 mg (or fluconazole plus flucytosine if in a combination arm) can be made if clinical improvement, bloodstream clearance of *Candida* and fluconazole susceptibility is confirmed after 7 days of treatment. - Antifungals to be continued for 2 weeks following blood culture sterility (defined as negative blood cultures for any *Candida* sp for ≥48 hours if at least one blood culture has been taken during the time period , throughout the protocol)

Study Hypothesis	Combination antifungal therapy will improve clinical outcomes, mycological clearance and reduce resistance emergence during and following treatment.
Primary Outcome Measure(s)	<u>Step 1 – Population pharmacokinetic modelling of flucytosine</u> Achievement of the target drug exposure of flucytosine in plasma. <u>Step 2 - Primary efficacy outcome (hierarchical composite at 30 days):</u> 1) time to death (all-cause mortality); 2) breakthrough Candida bloodstream infection (BSI) during treatment (binary); 3) Candida BSI relapse following treatment (binary); 4) time-to-blood culture-sterility; 5) time to emergence of micafungin resistance (defined as minimum inhibitory concentration [MIC] > European Committee for Antimicrobial Susceptibility Testing [EUCAST] resistance breakpoint) in commensal *Candida* spp.
Secondary Outcome Measure(s)	<u>Step 2 - Secondary outcomes:</u> 1. All-cause mortality at day 30 and 90; 2. Time to all-cause mortality within 30 and 90 days; 3. Proportion of participants who have a breakthrough of *Candida* BSI during treatment; 4. Proportion of participants who have a relapse of *Candida* BSI at day 14, end-of-treatment (EOT) +5 days, day 30; and EOT +30 days 5. Time to blood culture sterility; 6. Time to micafungin resistance in colonising or invasive *Candida* spp; 7. Proportion of participants who have any micafungin resistance detected in any colonising or invasive *Candida* spp up until day 14, EOT+5 days, day 30; 8. Duration of ICU admission and hospitalisation; 9. Rate of change in fungal biomarkers (serum beta-D-glucan (BDG), blood *Candida* qPCR) until day 30; 10. Proportion who develop grade 3 cytopenias, other grade 4 adverse events and other serious adverse events (SAEs) until EOT +5 days.
Exploratory outcome	Exploratory outcomes: Estimate of flucytosine % time>MIC and micafungin AUC/MIC of the infecting *Candida* spp to assess for any relationship between drug exposure and clinical/ mycological outcomes. Flucytosine resistance % in infecting/colonising *Candida* species isolated from participants on combination therapy vs. monotherapy. Incidence of *Candida* ocular complications detected through ophthalmic assessment during the second week of treatment.
Randomisation	Individual randomisation using REDCap Randomisation Module via a permuted-block randomisation method stratified by site. Block sizes will vary.
Number of Participants to be Studied	STEP 1: 60 participants (20 participants per each of 3 arms) STEP 2: 460 participants (230 participants per each of 2 arms) (Total: 520 participants; total evaluable in step 2 at the end of the trial: 500 participants, i.e. 20+20+460. This accounts for an estimated 10% loss to follow up.)

Duration	4 years of enrolment (STEP 1: 6 months, STEP 2: 42 months) plus 90 days of follow-up
Ancillary Studies/Sub studies	- Pharmacometrics of micafungin and flucytosine in patients with *Candida* bloodstream infections - Cost-effectiveness from the health provider and patient perspective of combination antifungal therapy for the treatment of *Candida* bloodstream infections - Rectal swab mycobiome - Pharmacokinetic/ pharmacodynamic (PK/PD) analysis linking antifungal PK with PD markers of mycological clearance and resistance emergence - Micafungin abdominal PK - Host immunogenetics and immune response markers
Sponsor	WITS Health Consortium (Pty) Ltd
Funder	Wellcome Trust
Co Principal Investigators	Prof Nelesh Govender Dr Rachel Wake
Trial Manager	Dr Lauriane Fomete
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Site PI (HJH)	Dr Jeremy Nel
Site PI (CMJAH)	Dr Lyle Murray
Site PI (DGMC)	Dr Monique Da Fonseca
Site PI (NMH)	Prof Ken Boffard and Dr Evan Shoul

TRIAL SCHEMA

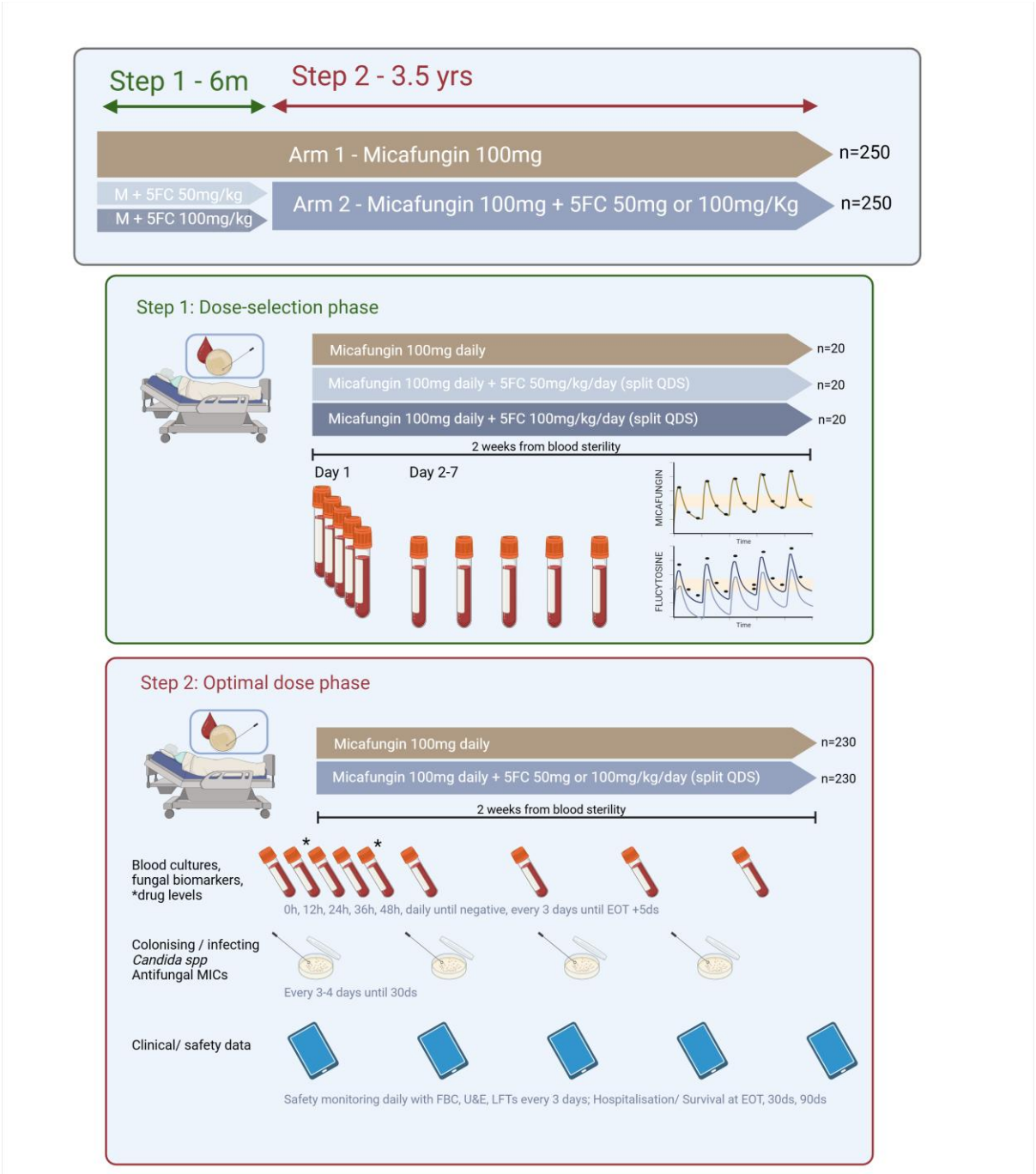

Figure 1 Trial randomisation and follow-up

TRIAL SUMMARY

LAY SUMMARY

Fungi are a common cause of serious illness, particularly in people with weak immune systems or those hospitalised for other conditions, causing an estimated ~2.5 million deaths/year globally. Like other 'superbugs' (antibiotic-resistant bacteria), fungi are becoming harder to treat because of increasing resistance to antifungal medicines. However, while antibiotic resistance is widely acknowledged as a global health problem, antifungal resistance is often neglected.

Fungi in the *Candida* group are the most common cause of serious fungal infections (an estimated ~1.5 million cases/year), mostly affecting critically ill patients in intensive care units (ICUs). *Candida* bloodstream infections (BSI) lead to prolonged hospitalisation and death in 30%-60% of cases. There are only three classes of drugs that are routinely used to treat *Candida* BSI (i.e. echinocandins, azoles, polyenes) and resistance is increasing. *Candida* species (some of which are recently renamed) have emerged that are resistant to two or even all antifungal drug classes, such as *Candida auris*, spreading rapidly worldwide and now deemed a critical and urgent global threat to health (World Health Organization). South Africa (SA) has the largest documented epidemic of antifungal resistant *Candida* infections. Most *Candida* causing BSIs in SA are now resistant to at least one class of antifungal drug, and more than a third are caused by *Candida auris*.

Candida BSIs are mostly caused by the six *Candida* species commonly carried on skin and/or within the gut, i.e. *Candida albicans, Candida parapsilosis, Candida auris, Nakaseomyces glabratus, Pichia kudriavzevii, Candida tropicalis* (all these species will be referred to as *Candida* throughout the protocol). Antifungal resistance can develop when patients are given a single antifungal drug causing adaptation and selection of drug-resistant *Candida,* that has the potential to spread to other people in the same hospital/ unit via shared equipment and the hands of healthcare workers. As with other infections e.g. TB, HIV, resistance in *Candida* might be prevented by giving two antifungal drugs together (combination therapy) for an infection. Since the drugs have different modes of action and are able to get into different sites in the body, this could improve clearance of fungi from the body, and prevent drug-resistance from developing.

The COMBAT *Candida* trial will investigate whether combination therapy with two licenced drugs (i.e. micafungin and flucytosine) is better than micafungin alone for the treatment of patients with *Candida* BSI at 5 hospitals in Johannesburg, South Africa. These drugs have been chosen because micafungin is internationally recommended as the first-line treatment of this infection, and the addition of flucytosine has been shown to improve fungal killing in laboratory experiments. Flucytosine is also used in combination therapy for another severe fungal infection, HIV-associated cryptococcal meningitis, where it has been shown to be safe and effective in randomised trials.

Patients who have *Candida* BSI at the trial sites will be invited to participate. If they do not have the capacity to make a decision about participation at the time of enrolment, informed consent will be obtained retrospectively from them at the earliest opportunity when they regain capacity and following provision of proxy consent. Proxy consent will be requested from their next of kin, a family member, or an independent clinician. If consent is provided, the participant will be randomly assigned to receive either micafungin alone (standard care) or micafungin plus flucytosine (the trial intervention) for two weeks after their blood is clear of *Candida*.

During the first phase of the trial, patients will be assessed for eligibility using specified inclusion and exclusion criteria. Participants will then be randomised to a control or one of two intervention arms.

Those in the intervention arm will be randomised to receive either a standard dose of flucytosine (100 mg/kg daily) or a half measure of this dose (50 mg/kg daily). The levels of flucytosine in the blood will then be measured and the lower dose will be selected to be used in the next phase of the trial providing that the first phase shows that sufficient blood levels are achieved with this dose to kill the *Candida*.

During the second phase of the trial, there will be two arms of the trial; micafungin alone (the internationally recommended treatment), or micafungin plus flucytosine at the selected dose from phase 1. Success of each treatment arm of the trial will be measured by assessing survival of participants during the 30 days after enrolment into the study, how effectively the blood is cleared of *Candida*, whether the *Candida* infection persists on treatment or relapses following treatment, and whether antifungal resistance emerges in any *Candida* identified growing on the body (by using swabs of nose, mouth, hands, armpits, groins, and skin between the buttocks).

ABSTRACT AND SUMMARY OF TRIAL DESIGN

Study design
An open label dose-ranging adaptive phase III randomised controlled trial to compare combined treatment of micafungin plus flucytosine to micafungin alone for the treatment of patients with *Candida* BSI.

Setting
Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa
Helen Joseph Hospital (HJH), Johannesburg, South Africa
Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Johannesburg, South Africa
Netcare Milpark Hospital (NMH), Johannesburg, South Africa
Wits Donald Gordon Medical Centre (DGMC), Johannesburg, South Africa

Disease/patients studied
Hospitalised adults (aged ≥18 years) with *Candida* BSI, defined as blood culture growth of any *Candida* spp. and clinical evidence of infection.

Trial interventions
Step 1 – Dose-selection
1. Micafungin 100 mg/d IV plus flucytosine 50 mg/kg PO/NG (split QDS)
2. Micafungin 100 mg/d IV plus flucytosine 100 mg/kg PO/NG (split QDS)
3. Micafungin 100 mg/d IV – INTERNATIONAL FIRST-LINE STANDARD

Step 2 – Optimal dose
1. Micafungin 100 mg/d IV plus flucytosine *either* 50 mg/kg *or* 100 mg/kg PO/NG (split QDS) - INTERVENTION
2. Micafungin 100 mg/d IV – INTERNATIONAL FIRST-LINE STANDARD

For each arm:
- Oral switch to fluconazole 800mg (or fluconazole plus flucytosine if in a combination arm) can be made if clinical improvement, bloodstream clearance of *Candida* and fluconazole susceptibility is confirmed after 7 days of treatment.
- Antifungals to be continued for 2 weeks following blood culture sterility

OUTCOME MEASURES

STEP 1 – Dose-selection

A target flucytosine exposure of >45% time/MIC derived from prior work will be used(1). A population PK model of flucytosine plasma concentrations will be developed. This model will be used to simulate probability of target attainment. The lowest dose achieving the PKPD target in >90% of patients will be taken forward to step 2.

STEP 2 – Optimal dose

Primary efficacy outcome (hierarchical composite at 30 days):

1) time to death (all-cause mortality, measured in ordinal blocks of 5 days);
2) breakthrough during treatment (binary – whether or not any blood cultures taken subsequent to blood culture sterility, become positive with *Candida* spp);
3) relapse within 30 days of end of treatment (binary - whether or not a blood culture taken following the end of treatment is positive with the same *Candida* spp as grown from the initial blood culture);
4) time to blood culture sterility (measured in ordinal blocks of 12 hour periods);
5) time to emergence of micafungin resistance (MIC > EUCAST breakpoint) in commensal *Candida* species.

Secondary outcomes:

1. All-cause mortality at day 30 and 90;
2. Time to all-cause mortality within 30 and 90 days;
3. Proportion of participants who have a breakthrough of *Candida* BSI during treatment;
4. Proportion of participants who have a relapse of *Candida* BSI at day 14, EOT +5 days,day 30 and EOT + 30 days;
5. Time to blood culture sterility;
6. Time to micafungin resistance in colonising or invasive *Candida* spp;
7. Proportion of participants who have any micafungin resistance detected in any colonising or invasive *Candida* spp up until day 14, EOT+5 days, day 30;
8. Duration of ICU admission and hospitalisation;
9. Rate of change in fungal biomarkers (serum beta-D-glucan (BDG), blood *Candida* qPCR) up until day 30;
10. Proportion who develop grade 3 cytopenias, other grade 4 adverse events, and other serious adverse events (SAEs) up until EOT +5 days.

Exploratory outcomes:

- Estimate of flucytosine % time >MIC and micafungin AUC/MIC of the infecting *Candida* spp to assess for any relationship between drug exposure and clinical/ mycological outcomes.
- Flucytosine resistance % in infecting/colonising *Candida* species isolated from participants on combination therapy vs. monotherapy.
- Incidence of ocular complications of candidaemia using ophthalmic assessment during the second week of treatment.

Analysis:

Step 1 - Population PK modelling of flucytosine drug exposure in participants of Step 1 taking flucytosine (with fewer than 4 missed doses).

Step 2 - The primary outcome will be compared between arms in those who receive at least one antifungal dose (modified intention to treat analysis), using Win Ratio(2), classifying pairs as wins, losses and ties, prioritizing time to all-cause mortality (figure 2).

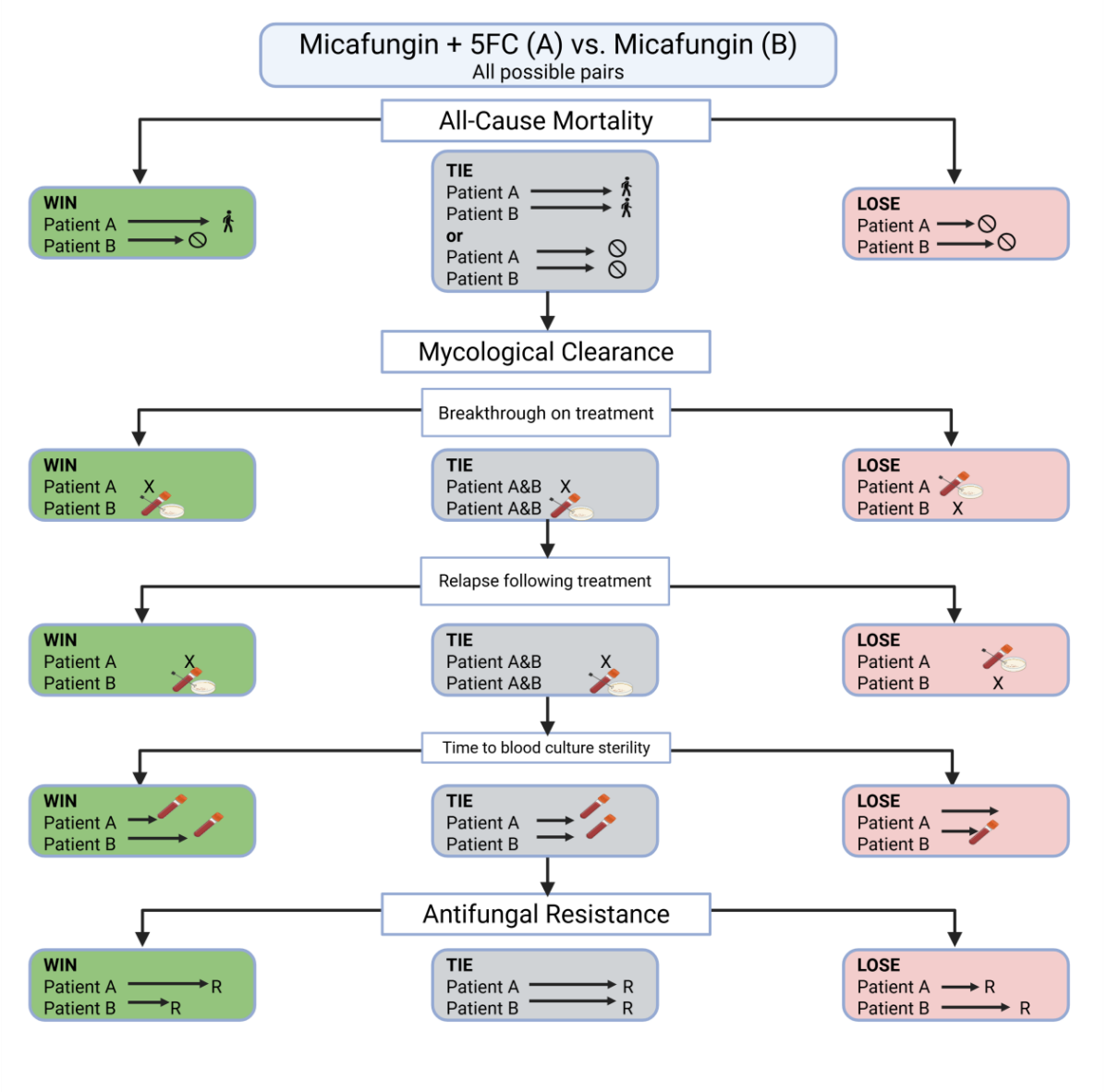

Figure 2 Representation of the Win Ratio method for primary statistical analysis. Classifying pairs as wins, losses and ties, prioritizing time to all-cause mortality before mycological clearance and resistance in the hierarchical composite endpoint. Participants will be paired (unmatched) and compared for time to death, blood culture sterility (referring to negative for Candida), relapse and resistance evolution. Participant A is depicted as winning in the green column, and losing in the red column. If both participants die, the 'winner' will be the participant who had a longer time between randomisation and death using ordinal blocks of 5 days. If both participants die within the same 5 day period, or remain alive beyond 30 days (tie), the pair will be compared on breakthrough (on treatment) and relapse (following treatment up until day 30) ('winner' = any breakthrough/ relapse, 'tie' = neither breakthrough/ relapse), then on time to blood culture sterility within 12 hour ordinal blocks ('winner' = shortest time, 'tie' = equal time to sterility or no further positive blood cultures) then emergence of micafungin resistance in infective or colonising isolates up until day 30 ('winner' = longest time to resistance).

Duration
The intervention will be given for 14 days following the first of all subsequent blood cultures which are negative for *Candida* spp if taken during treatment (clearance of the initial initial *+/-* any breakthrough *Candida* BSI).

Follow up – assessment of safety and efficacy
During Step 1 (dose-selection phase), plasma will be taken at 12 time points during the first week: 0.25, 0.5, 1, 2, 6, 24, 48, 72, 96, 120, 144, 168 hours, for the analysis of flucytosine and micafungin PK in participants of the intervention arms.

During both steps, blood will be drawn at baseline to check full blood count and differential (FBC), serum creatinine and alanine transaminase (ALT) and aspartate aminotransferase (AST) levels, then every 3-4 days for FBC and ALT/AST up until EOT +5 days. Blood cultures will be taken at baseline (0h), 12h, 24h, 36h, 48h (each timepoint within a window of +/- 6 hours, but at least 6 hours apart) after treatment is started and 12 hourly as per this schedule until 2 consecutive blood cultures are negative (for any bacterial or fungal growth) following ≥48 hours incubation (usually takes 1-2 days). Blood cultures will then be taken every day until day 7 and then every 3-4 days until 5 days after end of treatment (EOT), to check for breakthrough (during treatment) or relapse (after treatment) of infection. Composite superficial swabs will be taken from commonly colonised body sites (i.e. nares/ oral, hands/ axillae/ groins and perianal skin) twice weekly until discharge from hospital, and during a study visit at day 30 (or within the following 7 days) after start of treatment for *Candida* culture and resistance testing. Participants will be followed up at least every 3 days during treatment until EOT + 5 days to record survival, any newly identified complications, grade 3 cytopenias, other grade 4 AEs and SAEs, and at 30 days (or within the following 7 days) and 90 days (or within the following 30 days), by phone call, or visit if discharged from hospital, to determine hospitalisation and survival status. A further pre-dose plasma level of micafungin and flucytosine will be taken on day 1 and day 7.

Reimbursement will be provided for participants' time during admission and if required to travel back to study sites for their day 30 follow up, and to enable contact with study teams up until day 90. For participants discharged before the Day 30 and Day 90 follow-up visits, an allowable window of 1 week for Day 30 and 4 weeks for Day 90 has been incorporated. This provides flexibility and may align follow-up assessments with routine hospital visits, for example if a participant has a scheduled appointment around the Day 90 timepoint.

ANCILLARY STUDIES/SUB-STUDIES

Cost effectiveness will be assessed from the health provider perspective using a decision tree model and Incremental Cost Effectiveness Ratio. Additionally, a patient perspective economic evaluation will be carried out, assessing out-of-pocket and catastrophic healthcare expenditure incurred following *Candida* BSI.

A PK/PD analysis will apply derived models from Step 1 to sparse sample drug concentrations in plasma taken at day 1 and day 7 to assess the impact of combination drug exposures on PD endpoints including mycological clearance (time to culture conversion, BDG, qPCR) and resistance evolution. In selected patients with abdominal drains, we will take remnant samples from drains at the same time as plasma samples (pre-dose on day 1 and 7) to undertake an additional abdominal (compartmental) micafungin PK analysis to evaluate drug penetration to a common *Candida*

infection focus and explore its relationship with echinocandin resistance emergence at the abdominal site. Urine samples will be taken and stored on days 1 and 7 for similar analysis. Co-Is Tracy Kellerman; Eric Decloedt; Joe Standing

Gut mycobiome: a faecal sample (at least 1 g) will be taken from each participant on day 0 and 7 of treatment, stored and shipped to France as part of the Candi-NET consortium with the aim of examining the impact of antifungal therapy on the faecal diversity of fungal species using shotgun metagenomics. Collaborator: Alexandre Alanio, Institut Pasteur, Paris

Host immunogenetics and immune response will be assessed using human nucleic acids extracted from whole blood, and sequenced. Immunogenetic data will be shared for analysis of predictors of host response to antifungal and immune adjunctive therapy. Collaborator: Frank Van Der Veerdonk, Radboud University, The Netherlands.

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AE	Adverse event
ALT	Alanine aminotransferase
AR	Adverse reaction
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
BSI	Bloodstream Infection
Candida	This refers to any organism that has previously had the genus name '*Candida'.* For example, this now includes *Nakaseomyces glabratus* (formerly *Candida glabrata)* and *Candidozyma* (formerly *Candida) auris*
CI	Confidence interval
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standards Institute
DMC	Data Monitoring Committee
DMP	Data management plan
DPA	(UK) Data Protection Act
eCRF	Electronic case report Form
EOT	End of treatment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
REDCap	Research Electronic Data Capture
FBC	Full blood count
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus-1
HR	Hazard ratio
IB	Investigator brochure
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit

IMP	Investigational medicinal product
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
LSHTM	London School of Hygiene and Tropical Medicine, UK
MIC	Minimum Inhibitory Concentration
NG	Nasogastric
NICD	National Institute for Communicable Diseases, SA (will be merged into the National Public Health Institute of South Africa)
NOK	Next of kin
OD	Once daily
PI	Principal Investigator
PIS	Participant information sheet
PD	Pharmacodynamics
PK	Pharmacokinetics
PO	Per oral
QA	Quality assurance
QC	Quality control
QDS	Quarter die sumendum (four times daily)
R&D	Research and development
RCT	Randomised controlled trial
REC	Research Ethics Committee
SAE	Serious adverse event
SAR	Serious adverse reaction
SOP	Standard operating procedure
SPC	Summary of Product Characteristics
SSA	Sub-Saharan Africa

SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
WHO	World Health Organization
WMA	World Medical Association
5FC	Flucytosine

BACKGROUND

1.0 RATIONALE

Species in the genus *Candida* (and those recently renamed to other genera) are gut/ skin commensals or environmental organisms, which can cause severe life-threatening infections (invasive candidiasis, including *Candida* bloodstream infections), particularly in immunocompromised and critically ill patients. An estimated **1.5 million cases of invasive candidiasis** occur every year,(3) a rising incidence with an attributable mortality of ~30%(4) and diminishing treatment options. Only three drug classes are currently recommended for treatment (i.e. azoles, echinocandins, polyenes), with efficacy threatened by an epidemiological shift toward intrinsically-resistant species, rising levels of secondary or acquired resistance and emergence of frequently multi-drug resistant species, e.g. *Candida glabrata (*now *Nakaseomyces glabratus)* and *Candida auris*(5). Novel approaches (6)to optimise treatment and curb the rise in **antifungal-resistant *Candida*** are needed.

Observational clinical studies, and *in vitro* evidence show that currently recommended treatment using **echinocandin monotherapy can lead to on-treatment emergence of echinocandin resistance** in invasive and commensal *Candida* spp, including *C. auris*(6–13). Echinocandin monotherapy exerts selective pressure, induces stress responses, and leads to sub-optimal drug penetration at some body sites (e.g. abdominal cavities, urinary tract and central nervous system). Combination therapy, using a licensed, safe and accessible antifungal, flucytosine, might improve drug penetration to certain body compartments and improve *Candida* clearance, whilst reducing selective pressure for resistance evolution (14).

COMBAT *Candida* aims to determine whether **combination antifungal therapy** with flucytosine, in addition to the echinocandin, micafungin, is safe and effective in improving clinical and mycological outcomes, as well as combatting antifungal resistance emergence observed during echinocandin monotherapy.

1.1 POPULATION

Adults (>=18 years) who are hospitalised for any reason and have *Candida* BSI, defined as blood culture growth of *Candida* spp. and clinical evidence of a BSI, who fulfil the inclusion/exclusion criteria outlined in Section 3 – Selection of Participants.

1.2 OBJECTIVES

Primary Objective
To determine whether combination treatment of micafungin plus flucytosine for 2 weeks following blood sterility is **superior** to standard treatment of micafungin monotherapy in improving clinical, mycological and resistance outcomes.

Secondary Objectives
To determine the following:

1. All-cause mortality at day 30 and 90;
2. Time to all-cause mortality within 30 and 90 days;
3. Proportion of participants who have a breakthrough of *Candida* BSI during treatment;
4. Proportion of participants who have a relapse of *Candida* BSI at day 14, EOT +5 days day 30 and EOT + 30 days;

5. Time to blood culture sterility;
6. Time to micafungin resistance in colonising or invasive *Candida* spp;
7. Proportion of participants who have any micafungin resistance detected in any colonising or invasive *Candida* spp up until day 14, EOT+5 days, day 30;
8. Duration of ICU admission and hospitalisation;
9. Rate of change in fungal biomarkers (serum beta-D-glucan (BDG), blood *Candida* qPCR) up until day 30;
10. Proportion who develop grade 3 cytopaenias, other grade 4 adverse events and other serious adverse events (SAEs) up until EOT +5 days.

Exploratory outcomes:

- Estimate of flucytosine % time >MIC and micafungin AUC/MIC of the infecting *Candida* spp to assess for any relationship between drug exposure and clinical/ mycological outcomes.
- Flucytosine resistance % in infecting/colonising *Candida* species isolated from participants on combination therapy vs. monotherapy.Incidence of ocular complications of candidaemia through ophthalmic assessment during the second week of treatment.

Objectives relating to additional sub-studies will be described in Section 6.2 Other Assessments.

1.3 STUDY DESIGN

An open label dose-ranging adaptive phase III randomised controlled trial to compare combined treatment of micafungin plus flucytosine to micafungin alone for the treatment of patients with *Candida* BSI.

1.4 RELEVANT STUDIES/TRIALS

Issues with currently recommended first line treatment for Candida BSI – echinocandin monotherapy

Current first line treatment for *Candida* BSI with echinocandin monotherapy is associated with relatively poor clinical outcomes for patients with candidaemia and invasive candidiasis, with high mortality and prolonged hospitalisation(15). This regimen also exerts selective pressure on infecting and colonising *Candida* species(16) inducing on-treatment evolution of phenotypic and genotypic resistance(6, 8, 16, 17). Since a patient's colonising *Candida* can go on to cause invasive candidiasis, this is concerning for the patient themselves, as well as others in the hospital to whom drug-resistant *Candida* may be transmitted. Sub-inhibitory concentrations of drug in body sites poorly penetrated by echinocandins such as the urinary tract, abdominal cavities, and mucosal surfaces can lead to resistance compartmentalisation (i.e. emergence of resistance in the *Candida* sub-population in a sanctuary site), with potential for onward transmission of resistant *Candida* to other patients(7, 8, 18, 19). A prospective study of 193 patients treated for *Candida* BSI in Denmark found acquired echinocandin-resistance in 21.6% of *C. glabrata* from oral swabs following ≥7 days of anidulafungin[20]. *Candida* spp, including *C. auris* evolved with echinocandins *in vitro*(12, 20, 21), undergo rapid acquisition of resistance mutations, and the observed resistance stability following withdrawal of drug pressure is concerning for onward transmission potential(20).

Combination treatment with micafungin plus flucytosine

Combination antifungal therapy has potential to **mitigate resistance emergence** whilst **optimising treatment** of *Candida* BSI. Drug combinations have been tested for efficacy in *Candida* clearance using

i*n vitro* and animal models, finding largely additive or synergistic interactions(14). Three clinical trials have been conducted using combinations of antifungals. A randomised trial (n=219) conducted in 2003 compared fluconazole monotherapy to fluconazole and amphotericin B for the treatment of *Candida* BSI and found that combination treatment led to an increased proportion of blood culture sterility at 5 days (94% vs 83%, p=0.02) without any significant difference in the clinical endpoint of 'time to failure', defined as death, withdrawal, toxicity, or switch to an alternative antifungal therapy (there was limited power to detect survival benefit)(22). Two further small trials conducted in the 1990s randomised 40 and 72 patients respectively to amphotericin B plus flucytosine vs. fluconazole. There was insufficient power in either trial to compare clinical outcomes, though mycological clearance (defined as *Candida* no longer growing and/or identified histologically in affected tissue), was reported to be more rapid in particiants randomised to the combination arm(23, 24).

Echinocandins inhibit fungal cell wall beta-D-glucan synthesis and are first-line for *Candida* BSI due to fungicidal activity, superior efficacy in a patient-level meta-analysis of clinical trials and excellent safety profile(25). Flucytosine targets fungal DNA/RNA and has excellent oral (or nasogastric) bioavailability and penetration(26) to compartments in which echinocandin resistance is known to develop(7, 8, 27). Its intranuclear site of action may be potentiated by antifungals which act on the fungal cell wall, facilitating access(28). *Candida* spp are highly susceptible to flucytosine (Table 1), including *C. auris* (modal MIC 0.12 µg/ml in SA(29)) and combination therapy including flucytosine is already recommended for complex invasive *Candida* infections (e.g. endocarditis, meningitis)(25). Clinical experience reporting emergent resistance when flucytosine was added to a *failing* regimen for a multidrug-resistant *C. auris* infection emphasises the importance of investigating its use as upfront combination therapy, rather than salvage treatment(30).

Table 1 Flucytosine MIC range, mode and Environmental Cutoff Value (ECV) estimate in Candida spp tested by Clinical & Laboratory Standards Institute (CLSI) reference microdilution method in 16 different laboratories in Brazil, Canada, Europe, and the United States(31, 32). C. auris data are from South Africa(29).* Comparison with *C. neoformans* isolates tested in the UK*(32)*

Species	No. isolates	Range MIC µg/mL	Mode MIC µg/mL	ECV MIC µg/mL (%)
C. albicans	8007	≤0.006 - ≥64	0.06	0.5 (94.2)
C. glabrata	3387	≤0.006 - ≥64	0.12	0.5 (98.0)
C. parapsilosis	3165	≤0.006 - ≥64	0.12	0.5 (98.0)
C. tropicalis	2046	≤0.006 - ≥64	0.12	0.5 (93.0)
C. krusei	499	≤0.006 - ≥64	8	32 (99.4)
C. lusitaniae	85	≤0.006 - ≥64	0.12	0.5 (95.3)
C. dubliniensis	84	0.12 – 1	0.12	0.5 (98.8)
C. guilermondii	41	0.12 – 8	0.12	1 (92.7)
C. auris*	400	0.015 - 2	0.12	0.25 (97.5)
C. neoformans	509	≤0.125 - ≥64	4	16

Our preliminary data show that flucytosine is synergistic with echinocandins against *C. auris in vitro*(33) (Figure 3) and suppresses the expansion of anidulafungin-heteroresistant subpopulations during evolution experiments(34) (Figure 4). Combination with anidulafungin also demonstrated increased rates of fungal clearance and improved survival in a *Galleria* model of *C. auris* infection (Figure 5). We have demonstrated that flucytosine is a safe and essential component of combination regimens for cryptococcal meningitis (CM)(35), where it reduces mortality, increases fungal clearance rates and suppresses heteroresistance induced by fluconazole monotherapy(36). Grade 4 neutropenia was recorded in only 3.2% of the patients who were taking a regimen that included 2 weeks of flucytosine, compared to 1.3% in the flucytosine-free arm in the ACTA trial(37).

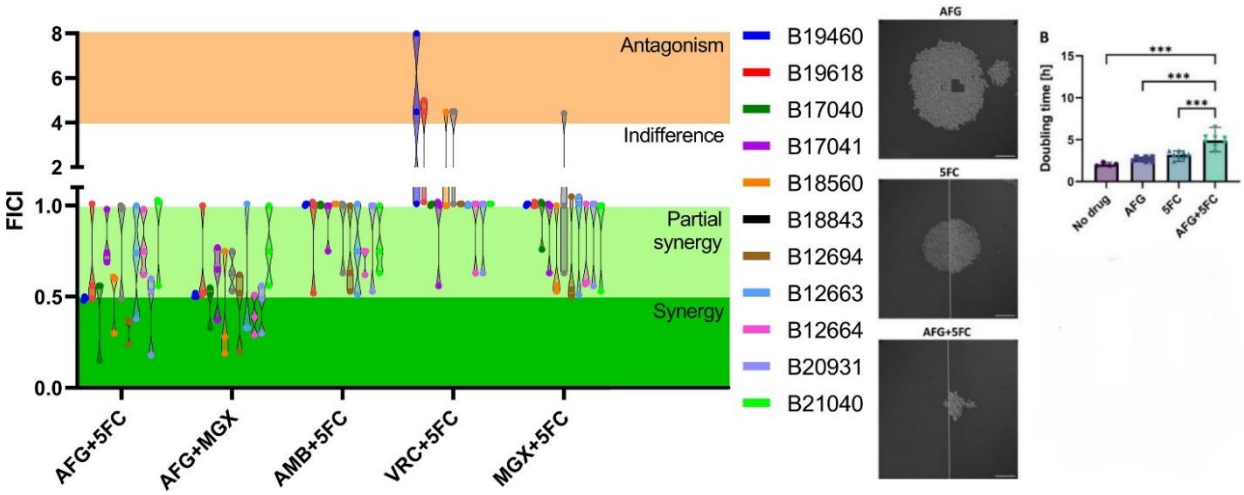

*Figure 3(33) Checkerboard experiments demonstrated i**n vitro synergy of anidulafungin and 5-FC (AFG+5FC) against 11 C. auris isolates** measured by fractional inhibitory concentration indices (FICI). Abbreviations: AFG, anidulafungin; 5FC, flucytosine; AMB, amphotericin B; MGX, manogepix, VRC, voriconazole.*
*On the right, microfluidics imaging and doubling times show **suppressed growth of C. auris in anidulafungin + flucytosine combined** than in either drug alone. C.auris cells were grown in the presence of anidulafungin (AFG), flucytosine (5FC) or AFG +5FC combined at their MIC concentrations for 16 hours. Doubling times were calculated via the two-dimensional colony area changes for several colonies from two independent experiments. Mean ± range. Scale bars: 100 µm. *, P ≤ 0.05; **, P ≤ 0.01; ***, P < 0.001 (one-way analysis of variance (ANOVA) with Bonferroni's correction).*

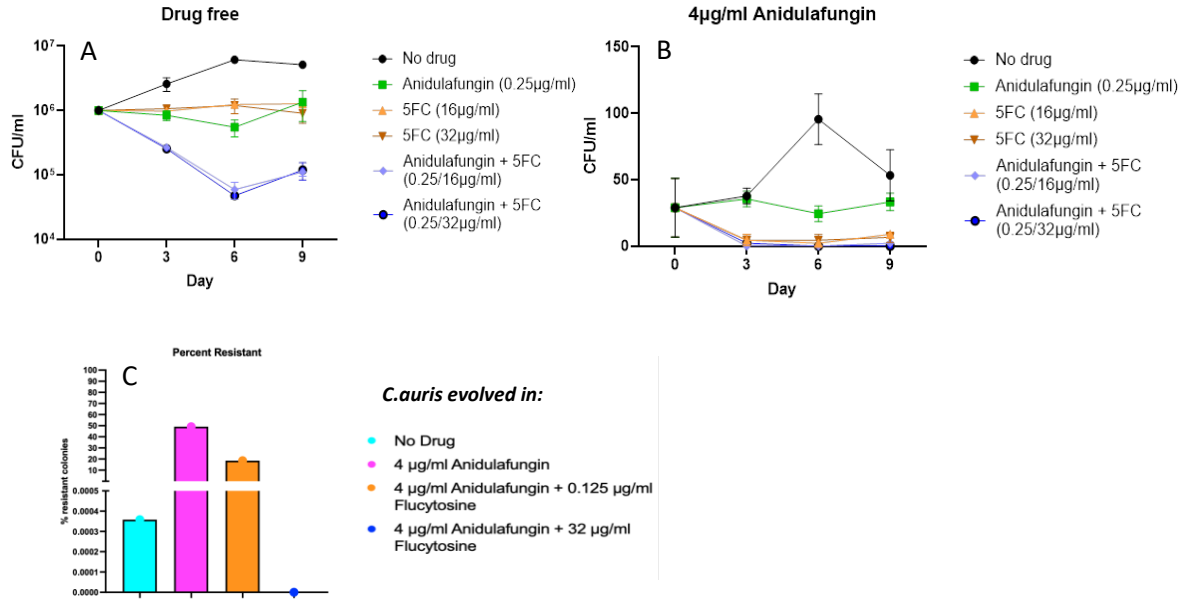

Figure 4 ***In vitro evolution of C. auris in anidulafungin + 5FC suppresses expansion of heteroresistance (hetR)****. C. auris observed to cause breakthrough BSI in a patient during anidulafungin monotherapy, was passaged every 3 days in broth containing different drug conditions. Colony forming units (CFU/mL) showed suppression of growth on drug-free agar (A) and also on agar supplemented with anidulafungin at the concentration of CDC tentative breakpoint (4µg/mL) (B), in the isolate evolved in flucytosine + anidulafungin compared to isolate evolved in no drug, or anidulafungin alone. The % resistant growth (C) represents the proportion of CFUs growing on anidulafungin-infused agar vs. growth on drug-free agar in a similar experiment after a single passage in different drug conditions. Abbreviation: 5FC, flucytosine.*

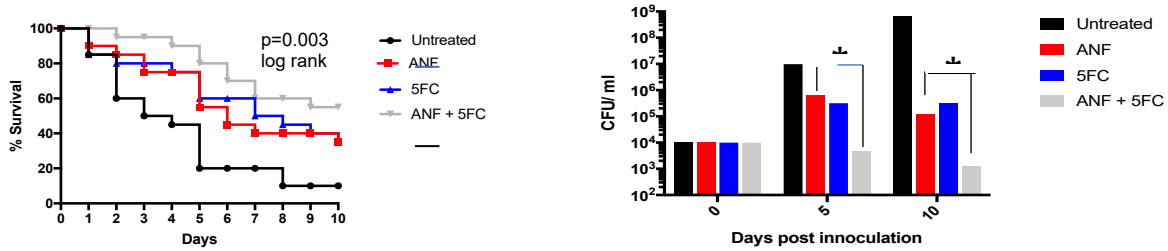

Figure 5 ***A Galleria model of C. auris infection showed improved survival and fungal clearance with anidulafungin plus flucytosine compared to each drug alone.*** *Abbreviations: ANF, anidulafungin; 5FC, flucytosine; CFU, colony forming units (unpublished, T Bicanic)*

Flucytosine combined with micafungin has greatest immediate potential as a candidate regimen for upfront first-line treatment of *Candida* BSI. As a result of our work in cryptococcal meningitis, generic flucytosine (on the WHO and SA Essential Medicine List) is now registered and becoming widely available in resource-constrained environments, including in SA. Micafungin has WHO and SA Essential Medicine List status for the treatment of *Candida* BSI and is available in both the public and private sectors in SA. While being accessible in resource-limited settings, this regimen is highly applicable globally including in high-income countries where first-line echinocandin use is well-established. Studying this novel approach in a high *Candida* resistance setting such as SA, will inform the global response to a rising health threat of antifungal resistant *Candida* infections.

Micafungin was selected as the echinocandin for this trial for several reasons. First, echinocandins are recommended as first-line treatment for invasive candidiasis in international guidelines, with a well-characterised safety and efficacy profile across age groups. Second, micafungin is listed on both the

WHO and South African Essential Medicines Lists and is routinely available in public and private sector hospitals at the trial sites, ensuring that the control arm is a readily available drug at these settings rather than an aspirational comparator. Third, selecting the agent already embedded in routine clinical practice internationally maximises the generalisability of trial findings: results will be directly applicable to the treatment decisions clinicians are already making, without the additional complexity of introducing an unfamiliar agent. Together, these features make micafungin the most appropriate and pragmatic echinocandin on which to evaluate the addition of flucytosine as combination therapy.

The **optimal dose of flucytosine** in combination regimens for *Candida* is undefined. The current dose (100 mg/kg/day) is extrapolated from treatment of invasive infections with *Cryptococcus* spp. (higher MICs, table 1), based on limited evidence balancing the risks of toxicity and resistance. However, murine pharmacokinetic and -dynamic (PK/PD) modelling, with bridging to humans and Monte Carlo simulation reveal that lower doses would allow maximal killing of *C. albicans* with MICs <32 µg/mL(38). Dose reduction would lower cost and may further reduce dose-dependent bone marrow toxicity observed in <5% patients with cryptococcal meningitis 44,45. Since limited human PK data exists, particularly in (often critically ill) patients with candidaemia, COMBAT Candida will use PK modelling during a dose-selection phase to identify the optimal flucytosine dose to carry forward.

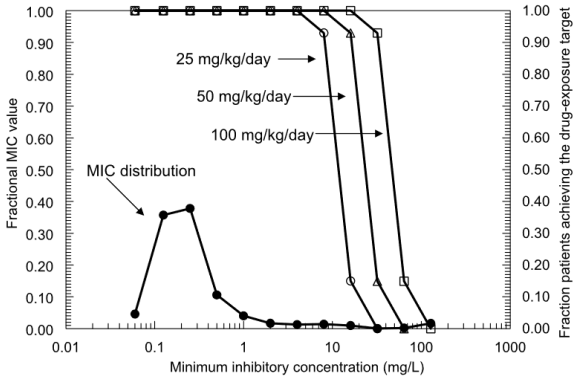

Figure 6(38) Monte Carlo Simulations of 9,999 patients receiving a range of 5FC doses were performed. The proportion who achieved the drug exposure target (i.e., serum 5FC concentrations greater than the MIC for at least 45% of the dosing interval) are shown following the administration of 25, 50 and 100 mg/kg/day, depicted by open circles, triangles, and squares, respectively. The distribution of the MICs for 1,941 C. albicans isolates, obtained using the EUCAST method, is shown by the solid circles. A dose of 25, 50, and 100 mg/kg/day resulted in fractional population target attainment of 98%, 98.2% and 98.6% respectively.

1.5 RISKS AND BENEFITS

The independent Data Monitoring Committee (DMC) will closely monitor the trial. Risks associated with the combination of micafungin and flucytosine are expected to be low and this combination is already recommended in international guidelines for complex invasive candidiasis(39), and for the treatment of cryptococcal meningitis(40, 41). Both drugs are included in the South African and WHO Essential Medicine List (EML)(42, 43)

Echinocandins are very well tolerated. In a randomised trial of echinocandins (micafungin or caspofungin) for treatment of *Candida* BSI, the most common treatment-emergent adverse events included abnormal results of liver function tests, nausea, constipation, hypokalemia, and rash. Of patients receiving micafungin 100 mg for 14 days, 5/191 (2.5%) were withdrawn from the study due to study drug-related adverse events, including liver function abnormalities as the most common cause(44). Patients with liver failure (ALT ≥10-fold normal) or chronic liver disease (Child Pugh Score ≥ 9) will be excluded from the COMBAT Candida trial, and serum liver enzyme levels will be monitored every 3-4 days until EOT + 5 days. Haematological abnormalities are rarely attributed to

echinocandins, occurring less frequently than in participants assigned to fluconazole or amphotericin B in trials of treatments for invasive candidiasis(45, 46).

Flucytosine can cause bone marrow suppression resulting in cytopenias, including anaemia, leukopenia (neutropenia) and thrombocytopenia; however very little toxicity was observed in a prior cryptococcal meningitis trial in African countries where participants were treated with flucytosine combination therapy dosed at 100 mg/kg/d, including in patients receiving 2 weeks of treatment (Grade 4 neutropenia in 3.2%, compared to 1.3% in the flucytosine-free arm)[4]. However, the risk-benefit for flucytosine in the population susceptible to *Candida* bloodstream infections, who are likely to be critically ill with multi-organ failure and non-HIV related immunosuppression, is not established. Patients will be excluded from participation on the basis of baseline cytopenias (Grade 4 neutropenia or Grade 3 thrombocytopenia), liver failure (ALT ≥10-fold normal) or chronic liver disease (Child Pugh score ≥ 9). Study participants will be monitored with full blood count and differential, creatinine and ALT levels every 3-4 days until EOT + 5 days. Renal function will be monitored more frequently if renal impairment is identified. Flucytosine dose will be adjusted to renal function (see 5.3.4), and reduced or held in cases of significant neutropenia according to standard protocols, as used in prior trials. As well as blood monitoring every 3-4 days, participants will be closely monitored by the on-site study team for any other toxicities or adverse events (AEs) until EOT plus 5 days (See Section 5 – Treatment of patients, Section 7 – Safety Assessments), and these data by treatment arm reviewed by the DMC as per the DMC Terms of Reference. The clinical team will be trained regarding the complications of *Candida* BSI and the clinical features to monitor.

There are very few drug-drug interactions reported for either trial drug. For micafungin, co-administration of certain drugs may increase risk of hepatotoxicity, and for flucytosine, co-administration may increase risk of haematological toxicity – both of which will be monitored closely. Patients will be excluded from participation if they are already taking cytarabine, which is the only contra-indicated medication with flucytosine (see Section 5 - Medications not permitted).

SELECTION OF SITES/CLINICIANS

The Trial Management Group (TMG) holds overall responsibility for site and investigator selection.

2.0 SITE/INVESTIGATOR INCLUSION CRITERIA

Trial recruitment will take place at 5 sites in Johannesburg, South Africa:

1. Chris Hani Baragwanath Academic Hospital (CHBAH)
2. Helen Joseph Hospital (HJH)
3. Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)
4. Netcare Milpark Hospital (NMH)
5. Wits Donald Gordon Medical Centre (DGMC)

The trial will be performed over 4 years, plus 90 days of follow up. Participant recruitment for Step 1 will take place over 6 months, and for Step 2, over 42 months. Based on SA surveillance data and site laboratory data, we estimate that during the trial period, there will be around 2000 episodes of *Candida* BSI in adults occurring at the five sites. Taking inclusion and exclusion criteria into account, and 10% losses to follow-up by 30 days, this will allow us to reach our target (refer to sample size section 9.3) within the trial period, providing that at least 500 (~25%) of eligible participants are enrolled in the trial. Enrolment will be "competitive" across sites, i.e. all sites will continue recruiting until the overall recruitment target is met.

To enrol participants in the COMBAT *Candida* trial, investigators and clinical trial sites must fulfil a set of basic criteria that have been agreed by the COMBAT *Candida* trial management group (TMG) and are defined below.

Once a site has been identified as being compliant with the site selection criteria, the trial team will provide the site with a copy of this protocol, a trial summary, the Summary of Product Characteristics (SPC) for micafungin and flucytosine, and the Investigators' Brochures (IB).

Sites where a previous serious protocol breach has occurred will be visited and thoroughly reviewed before allowing participants to enter the trial.

2.0.0 PIs' Qualifications & Agreements

The site investigators should be qualified by education, relevant training (GCP), and clinical trials experience to assume responsibility for the proper conduct of the trial at their site and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the research ethics committee (REC), the institutional review board (IRB), and/or the regulatory authority(ies).

The investigators should be thoroughly familiar with the appropriate use of the investigational medicinal product (IMP), as described in the protocol, the product information and in other information sources provided by the Sponsor.

The investigators should be aware of, and should comply with, the principles of ICH GCP and the applicable regulatory requirements. A record of GCP and ethics training should be accessible for all investigators.

The investigators/sites should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority(ies).

The investigators should maintain a delegation log of appropriately qualified persons to whom the investigator has delegated significant trial-related duties.

The investigators should sign an investigator statement, which verifies that the site is willing and able to comply with the requirements of the trial.

2.0.1 ADEQUATE RESOURCES

The investigator should be able to demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period (that is, the investigator regularly treats the target population).

The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational products, and their trial-related duties and functions.

The site should have sufficient data management resources to allow prompt data return to the co-ordinating centre (refer to the Data Management Plan (DMP) for timelines). Sites that have previously participated in trials coordinated by the same centre should have a proven track record of good data return.

2.0.2 SITE ASSESSMENT

Each selected clinical trial site must complete the Accession form and staff contact details. The Accession Form verifies that the site is willing, and able to comply with the requirements of the trial. In addition, and in compliance with the principles of ICH-GCP, all site staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the trial co-ordinating centre (i.e. Wits Mycology). The co-ordinating centre must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the site and at the co-ordinating centre.

2.1 SITE/INVESTIGATOR EXCLUSION CRITERIA

Failure to meet the above requirements.

2.2 APPROVAL AND ACTIVATION

The Trial Steering Committee (TSC) will activate the trial only once all relevant approvals are in place. The ethics committees/Institutional Review Boards of the participating institutions will be supplied with the names and addresses of the national principal investigator and all participating site principal investigators. Trial staff at the co-ordinating centre will perform this task; hence it is vital to receive full contact details for all investigators prior to their entering patients.

1. The site should conduct the trial in compliance with the protocol as agreed by the Sponsor and, if required, by the regulatory authority(ies), and which has been approved by the REC and/or IRB.

2. The site PI or delegate should document and explain any deviation from the approved protocol and communicate this with the trial team at the co-ordinating centre and the Data Monitoring Committee.
3. A list of activated sites may be obtained from the Trial Manager.

2.3 SITE MANAGEMENT

Prof Nelesh Govender will serve as national PI; he has been international PI and site sub-investigator on two prior mycology trials (EFFECT, 5FC PROTECT). At Chris Hani Baragwanath Academic Hospital, A/Prof Shahed Omar will lead the trial. A/Prof Omar has been site PI for several trials and is a Critical Care consultant at the hospital, and Adjunct Professor at the University of the Witwatersrand.

At Helen Joseph Hospital, Dr Jeremy Nel will lead the trial as site PI and Dr Lauren Richards will support as a co-investigator under Dr Nel's supervision. Dr Nel has been site PI for 6 clinical trials including currently as site PI for the phase III EFFECT trial (cryptococcal antigenaemia) and is the Academic head of the Infectious Diseases at the University of the Witwatersrand and at the hospital. Dr Richards is also a sub-investigator for the EFFECT trial, a lecturer at the University of the Witwatersrand and is a Consultant Physician and Infectious Diseases specialist at the hospital.

At Charlotte Maxeke Academic Hospital, Dr Lyle Murray (site PI) will lead the trial. Dr Murray has been site sub-investigator for 8 clinical trials, is a Lecturer at the University of the Witwatersrand and a Consultant Physician and Infectious Diseases specialist at the hospital.

At Netcare Milpark Hospital, Prof Ken Boffard will lead the trial as site PI with support of Dr Evan Shoul. Prof Boffard has been the site PI for several clinical trials and is the Trauma Director and Academic Head at the hospital and Professor Emeritus at the University of the Witwatersrand. Dr Shoul is a Consultant Physician and Infectious Diseases specialist at the hospital.

At Wits Donald Gordon Medical Centre, Dr Monique de Fonseca will lead the trial as site PI with support of Dr Dr Gitti Bertelsmann. Dr de Fonseca is a research medical officer and has been site PI for 1 clinical trial and sub-investigator on 7 trials (TB, HIV, gonorrhoea, HPV). Dr Bertelsman is a clinician at the hospital with research experience.

All sites have agreed to, and are fully committed to study participation.

SELECTION OF PARTICIPANTS

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate patients are considered for this study. This is for the safety of the participants, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases. There will be **no exceptions** to eligibility requirements at the time of randomisation. Questions about eligibility criteria should be addressed prior to attempting to randomise the participant.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

3.0 INCLUSION CRITERIA

1. Age ≥18 years
2. Evidence of microscopy-confirmed yeast bloodstream infection
3. ≥1 systemic signs of infection during a 24 hour period before or after blood culture draw.

Table 2 Systemic signs of infection

Systemic signs of Candida BSI	*Qualifying parameters*
Fever	Oral or axillary temperature ≥38°C (100.4°F) or a tympanic, temporal, rectal, or core body temperature ≥38.3°C (101°F)
Hypothermia	Tympanic, temporal, rectal, or core body temperature ≤35°C (95.2°F)
Hypotension	Systolic blood pressure <90 mm Hg or mean arterial pressure <70 mm Hg with normvolaemic or hypervolaemic status (or requiring inotropic support)
Tachycardia	Heart rate >100 beats per minute with a normovolaemic or hypervolaemic status (on 2 or more recordings within 24 hours of the blood culture being taken)
Tachypnoea	Respiratory rate >20 breaths per minute (on 2 or more recordings within 24 hours of the blood culture being taken)

3.1 EXCLUSION CRITERIA

1. Yeast growing in blood culture is confirmed not to be a *Candida* spp (late exclusion criterion - may take up to 3 days to identify the organism)
2. Invasive candidiasis with complex organ or prosthetic involvement (e.g. infection involving prosthetic material that cannot be removed within 5 days of randomisation, osteomyelitis, endocarditis or myocarditis, endophthalmitis, chorioretinitis or any central nervous system involvement) identified as likely to be present within 5 days of *Candida* BSI (late exclusion criterion)
3. Pregnancy or breastfeeding
4. Receipt of a systemic antifungal, at treatment-dose, to which the *Candida* spp causing bloodstream infection is susceptible (> 3 doses of a once daily or >5 doses of a twice daily drug within 4 days before randomisation) (late exclusion criterion once antifungal susceptibility testing results are available)
5. Micafungin or flucytosine resistant *Candida* spp (by EUCAST breakpoints) in initial bloodstream isolate (late exclusion criterion)

6. A known hypersensitivity to trial drugs
7. Co-administration of cytarabine
8. Known complete dihydropyrimidine dehydrogenase deficiency
9. Alanine transferase or aspartate aminotransferase level >10-fold upper limit of normal, or history of chronic cirrhosis (Child-Pugh score >9)
10. Neutrophil count <500 x 10^6/L or platelet count <50,000 x 10^6/L
11. Previous participation in this trial or in another trial for the same indication
12. The site principal investigator (PI) is of the opinion that the individual is not suitable for participation in the study due to any other factors
13. For Step 1 only, haemoglobin <8 g/dL, or participants without adequate intravenous access for regular blood draws during the first 24 hours of treatment.

3.2 NUMBER OF PATIENTS

520 participants

STEP 1: 60 participants (20 participants per each of 3 arms – micafungin alone, micafungin plus flucytosine 50 mg/kg/d, micafungin plus flucytosine 100 mg/kg/d)
STEP 2: 460 participants (230 participants per each of 2 arms – micafungin alone, micafungin plus flucytosine 50 mg or 100 mg/kg/d

(Total: 520 participants; total evaluable in step 2 at the end of the trial: 500 participants, i.e. 20+20+460)

3.3 CO-ENROLMENT GUIDELINES

Participants may not be enrolled in any other studies for the treatment of invasive fungal infections or which may impact on the management of their care as laid down in this protocol. Enrolment in other trials must be discussed with and approved by the TMG and will be at the discretion of the site PIs and under the direction of the site-specific Research Ethics Committee.

3.4 SCREENING PROCEDURES & PRE-RANDOMISATION INVESTIGATIONS

Notification of cases
Timely identification of potential participants is critical given the clinical urgency of initiating antifungal therapy as soon as possible on diagnosis of *Candida* BSI. Prior to trial activation, a formal notification arrangement will be established at each site between the site microbiology laboratory and the local study team under the provisions of GERMS-SA surveillance of *Candida* BSI (WIts HREC reference M230985). At each site, members of the study team hold dual roles as GERMS-SA surveillance officers. Candidaemia cases identified through the direct laboratory notification pathway will therefore be captured concurrently within routine GERMS-SA surveillance activity.

Upon detection of a yeast-positive blood culture, site microbiology laboratory staff will notify the local study team directly, by telephone or other pre-agreed method, within 12 hours of yeast detection. Notification should include the patient's name, hospital number, ward, and the date and time of blood culture collection. The study team will then access the patient's clinical record to assess potential eligibility and initiate the screening process as described in Section 3.4.2.
Each site must document the agreed notification arrangements, including responsible laboratory and study team contacts, prior to site initiation. Contact details must be kept up to date and included in the Site File. Any breakdown in the notification arrangement must be reported to the Trial Manager.

Patients with blood cultures confirmed as growing a yeast will be screened for participation in the trial and their details recorded in a screening eCRF. Participants will be enrolled into the study based on the criteria for eligibility outlined in Sections 3.1 and 3.2. For those found to be ineligible, the reason for non-inclusion will be recorded on the eCRF. The blood tests and clinical assessments required for assessment of eligibility will usually be available as part of routine care and thus will not be performed as study related tests/procedures. Results will be documented in the eCRF. If any these tests are not performed by the treating clinician by the time the patient is approached for enrolment or there is no clear documentation of these results, these tests will be performed as study-related screening procedures (refer to Section 5.1). Blood results from samples taken within the previous 48 hours will be used to assess eligibility for trial participation. The patient may be enrolled to the trial while the results of these screening tests are pending, and excluded if the results are consistent with late exclusion criteria.

3.5 INFORMED CONSENT

Due to the nature of the disease being studied, many potential participants of this trial will not have capacity to provide informed consent, and trial interventions must be initiated urgently. Informed consent must therefore be obtained either prospectively from the participant or retrospectively following proxy consent depending on if the participant has capacity at the time of randomisation. The approach to gaining informed consent for partcipation in this trial is discussed further in Section 10 - Ethical considerations.

3.5.0 IF THE PARTICIPANT HAS CAPACITY TO PROVIDE INFORMED CONSENT – PROSPECTIVE CONSENT

The participants will, before being enrolled into the study, have the conditions of the study, as set out in the Participant Information Sheet (PIS) explained to them. The information contained in the PIS will be translated into the appropriate local languages. Literate patients will be asked to read the PIS and literate and illiterate patients will have the contents explained to them by the Local Investigator or Research staff. The patient will have the opportunity to discuss the PIS. Once the person taking consent is satisfied that the patient has understood the content of the PIS and Consent Form, the patient will be asked to sign the Consent Form either on an electronic consent embedded within the REDCap database, or on a paper hard copy. For illiterate patients, or for those who are unable to provide a signature for any other reason, an impartial witness (which may be next of kin or a member of hospital staff who is not involved in the study) should write the patient's name and date, and the patient can then use a thumb print, or other mark to sign. The witness should then write their own name, sign and date (on the witness line of the form).

It must be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspects of the trial, at any time and for any reason, without incurring any penalty or affecting their access to the standard treatment available at the recruiting site.

3.5.1 IF THE PARTICIPANT DOES NOT HAVE CAPACITY TO PROVIDE INFORMED CONSENT – RETROSPECTIVE CONSENT FOLLOWING PROXY

Next-of-kin (NOK) will be approached in person or by discussion on the phone with the provision of written information (in person or electronically). Should the NOK be unavailable or lack capacity, consent will be sought from another relative. The following order serves as a guide and does not constitute a strict hierarchy: (1) spouse or partner, (2) parent, (3) grandparent, (4) adult child or sibling, in line with the National Health Act No. 61 of 2003 (SA). Other family members may also be approached and asked if they are willing to serve as the NOK for the purposes of the consenting

procedure. If the patient has no identifiable and contractable family members, an associate may be considered. An associate is defined as a person with a substantial or material interest in the patient's wellbeing, or a person who is in substantial contact with the patient. This approach is consistent with the National Department of Health (2024) South African Ethics in Health Research Guidelines: Principles, Processes and Structures(47). If NOK/relative consent is obtained over the phone, they will be able to sign a consent form electronically or an impartial witness (medical staff member who is not involved in the study) will be asked to sign the consent form following witnessed telephonic confirmation of consent.

If there is no NOK or relative available to give consent, an independent clinician) will be asked to give consent on the participant's behalf, confirming that they are suitable for the trial and fulfils eligibility criteria. An Independent Clinician (IC) is a registered medical practitioner who is independent of the COMBAT *Candida* study team and the Wits Mycology Division, and who has no treating relationship with the participant at the time of consent. The IC must have completed HPCSA-accredited GCP training and received COMBAT *Candida* protocol-specific training prior to obtaining any consent. A list of appointed ICs at each site, together with their GCP certificates, must be submitted to and acknowledged by the Wits HREC (Medical) before the IC may sign any consent form. Pooling of designated independent clinicians can occur across the five sites, with independent clinicians able to provide consent across different sites. Independent clinicians providing consent on a participant's behalf will be required to submit a signed declaration to the Wits HREC confirming their independence from the study and their assessment of the participant's eligibility and best interests.

If independent clinician consent is obtained over the phone, they will be able to sign a consent form electronically or an impartial witness (medical staff member who is not involved in the study) will be asked to sign the consent form following witnessed telephonic confirmation of consent. In all cases where telephonic consent is obtained, the method of consent (electronic signature or impartial witness signature) must be documented in the eCRF. All consent forms, whether signed electronically or on paper, will be stored in accordance with section 3.5.2

If a NOK or relative becomes available at any point during the study period, re-consent will be requested from this inidividual, as above, at the earliest opporunity.

If/when the participant regains capacity during the study period, retrospective consent will be requested from them at the earliest opportunity. The rights of the participant to refuse to participate in the study without giving a reason will be respected. Similarly, the participant must remain free to change their mind at any time about the study follow-up without giving a reason and without prejudicing his/her further treatment.

3.5.2 CONSENT FORM COPIES AND STORAGE

Original signed consent forms must be kept by the investigator (electronically, or if signed on paper, the signed paper form must be uploaded for electronic storage, and the original stored in a locked cabinet). A copy will be offered to the participant or family, and a copy placed in the participant's medical notes.

REGULATION & RANDOMISATION

Patients will be randomised individually using a computer-generated programme (REDCap Randomisation module). Randomisation will be stratified by site.

4.0 RANDOMISATION PRACTICALITIES

Further details on the process of randomisation can be found in Section 9.1 - Method of Randomisation.

RANDOMISATION

To randomise:
Upon confirmation of eligibility criteria and completion and entry of the informed consent details, the electronic REDCap data capture system will select the next available randomisation slot and present the details of the selection to the Study Doctor / Nurse on screen.

Note: Randomisation is stratified by site ONLY. The randomisation code will have 6-digit numbers: The first two digits for the site and the last three for participants within each site.

4.1 RANDOMISATION CODES & UNBLINDING

Randomisation codes are as detailed above. Randomisation is stratified by site. The trial is open label.

TREATMENT OF PATIENTS

5.0 SCREENING TESTS

Pre-randomisation:

- Pregnancy test – urine or serum - in women of reproductive age (18-60, or ≤2 years post menopause)
- Vital signs including temperature, blood pressure, heart rate and respiratory rate (within 24 hours pre- and post- time of blood culture draw)):
- Full blood count (for haemoglobin, leucocyte with differential and platelet counts)
- ALT and creatinine levels
- Calculation of Child-Pugh score for patients with history of cirrhosis

Pre- or post-randomisation (within 5 days)

- Review of medical history and any available radiology to exclude evidence of complicated candidiasis
- Retinal examination - for evidence of a Candida eye infection, including endophthalmitis or chorioretinitis. This should be performed within 3 days of enrolment if possible, but can occur up until day 5, and should be repeated during the second week of treatment and/or earlier if the individual develops visual symptoms at any point (as part of follow up, not screening). Retinal examination is standard of care for subjects with candidaemia and should be performed by an ophthalmologist when available, or if unavailable, by the study doctor using pupil dilatation and retinal photography (using a handheld battery-operated device), reviewed remotely by an ophthalmologist. Participants who have evidence of a Candida eye infection at initial examination but after initiating study drug/drugs must be withdrawn (late exclusion) from the trial since echinocandins do not adequately penetrate the posterior chamber of the eye. Alternative antifungal treatments can be discussed between the routine doctors, site PI and trial PIs as required.

5.1 BASELINE ASSESSMENT

i) Demographics

a. Age, sex, self-reported or documented ethnicity

ii) Clinical

a. Significant past medical history including HIV status and if living with HIV, the most recent CD4, viral load and antiretroviral treatment (ART) history
b. Risk factors for invasive candidiasis during past 4 weeks (e.g. central line, active malignancy, broad-spectrum antibiotic therapy, diabetes mellitus, immunodeficiency, immunosuppressive treatment, major surgery, total parenteral nutrition, solid organ or haemopoietic stem cell transplant, extracorporeal membrane oxygenation (ECMO) recipient, renal replacement therapy, trauma, dialysis, burns, pancreatitis, ICU admission and duration)
c. Antibiotic and antifungal history during the past 4 weeks
d. Presence of central line and dates of insertion and removal (recommended within 48 hours of diagnosis of *Candida* BSI)
e. Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores
f. Other relevant medical and surgical history

iii) Physical examination

a. Focused physical exam as clinically indicated or driven by participants' signs or symptoms, including cardiac auscultation and fundoscopy.

b. Anthropometry when not measured and documented by the routine clinical team

iv) Laboratory (pre-randomisation)

a. Bedside/laboratory-based pregnancy test on serum or urine taken at any time during admission

v) Laboratory (≤48 hours of randomisation)

a. FBC and differential count
b. Serum creatinine
c. Serum ALT

vi) **Mycology**

a. Species and results of antifungal susceptibility tests for any *Candida* spp growing on invasive samples (e.g. blood, surgical aspirates, CSF) within the prior 4 weeks leading up to randomisation and during the trial period

5.2 ANTIFUNGAL DRUG TREATMENT

5.2.0 DRUG REGIMENS

Patients will be randomised to one of three trial regimens in Step 1 and one of two trial regimens in Step 2:

Step 1 – Dose-selection

1. Micafungin 100 mg/d IV plus flucytosine 50 mg/kg PO/NG (split QDS)
2. Micafungin 100 mg mg/d IV plus flucytosine 100 mg/kg PO/NG (split QDS)
3. Micafungin 100mg/d IV – INTERNATIONAL FIRST-LINE STANDARD

Step 2 – Optimal dose

3. Micafungin 100mg/d IV plus flucytosine *either* 50 mg/kg *or* 100 mg/kg PO/NG (split QDS) - INTERVENTION (dose of flucytosine based on Step 1 findings)
4. Micafungin 100 mg/d IV – INTERNATIONAL FIRST-LINE STANDARD

For each arm:

- Oral switch to fluconazole 800 mg (or fluconazole plus flucytosine if in a combination arm) can be made if clinical improvement, bloodstream clearance of *Candida* and fluconazole susceptibility is confirmed after 7 days of treatment.
- Antifungals to be continued for 2 weeks following the first of subsequent sterile blood cultures taken during treatment

5.2.1 SOURCE OF DRUGS

Micafungin and flucytosine will be sourced from approved WHO-qualified manufacturers. Drugs will be shipped directly to a central research pharmacy, and then when required to the sites following approval from the relevant regulatory authority.

5.2.2 DISPENSING

Drugs will be stored in the pharmacy at each site, clearly labelled as trial medication, and segregated from other pharmacy stock. The trial staff will collect the amounts needed from the pharmacy. Detailed records of drugs dispensed and received will be maintained by both the study pharmacist and trial staff.

5.2.3 Dose Modifications, Interruptions & Discontinuations

See manufacturer's latest Summary of Product Characteristics (SPC) for flucytosine and micafungin for dose adjustments and management of expected adverse events. This information is contained in the Investigators' Brochure and detailed instructions for dose modification and management of toxicity may be found in the clinical Work Practice Documents (WPDs) for this trial.

- https://www.drugs.com/pro/flucytosine.html
- https://www.drugs.com/pro/micafungin.html

Table: Dose adjustments for flucytosine in renal impairment (adapted from the Sanford Guide)

Creatinine clearance (mL/min)	**Dose adjustment to flucytosine**	**Suggested renal function monitoring**
>40	No dose adjustment	As per study schedule
20-40	25 mg/kg 12 hourly	Alternating day monitoring
10-20	25 mg/kg 24 hourly	Daily monitoring
<10	25 mg/kg 48 hourly / at discretion of local trial principal investigator	Daily monitoring
Haemodialysis	25-50 mg/kg 48-72 hourly post-dialysis. Monitor trough level pre-dialysis, adjust post-dialysis dose accordingly.	Daily monitoring
Continuous ambulatory peritoneal dialysis	25 mg/kg 24 hourly	Daily monitoring

The above table is a guide to flucytosine dose adjustments and renal function monitoring. The blood draw schedule can be modified according to full clinical picture, input from site PI and managing routine care team.

Neutropenia

Flucytosine may cause bone marrow suppression resulting in neutropenia. Neutrophil counts should be graded according to the DAIDS toxicity table and monitored closely throughout treatment. Please note that a neutrophil count <0.5 x 109/L pre-enrolment means that the participant is not eligible for enrolment.

Absolute neutrophil count	**Dose adjustment to flucytosine**	**Suggested monitoring**
<0.4 x 10^9/L (DAIDS Grade IV)	Stop flucytosine immediately	Monitor daily
0.4- 0.599 x10^9/L (DAIDS Grade III)	If sustained (2 serial counts), 50% of dose if improves to Grade II or better, consider re-escalation to full dose at clinician discretion	Repeat white blood cell count and differential within 2 to 3 days until improves to >0.6 If sustained Grade III, daily FBC monitoring

≥ **0.6 x10^9**/L	No dose adjustment	Repeat white blood cell count and differential as clinically indicated

Thrombocytopenia

Platelet count	**Dose adjustment to flucytosine**	**Suggested monitoring**
<25 x 10^9/L (DAIDS Grade IV)	If sustained Grade IV or complications of thrombocytopenia – Stop flucytosine, until grade III level at which point resume at 50% dose	Monitor FBC daily.
25 - <50 x 10^9/L (DAIDS Grade III)	If sustained Grade III, halve dose of flucyotsine	Monitor FBC daily. If grade III is confirmed next day, halve dose of flucytosine (50%)
≥ 50 x 10^9/L	No dose adjustment required	

Stopping Drug Early

Discontinuation criteria are considered in Section 5.5 - Protocol Treatment Discontinuation

5.2.4 Accountability & Unused Drugs/Devices

Drug stocks will be regularly monitored, and the remaining stocks checked against the amount dispensed.

The investigator/pharmacist/investigational drug storage manager must maintain records of the drugs delivery to the trial site, the inventory at the site, the use by each participant.

These records will include dates, quantities, batch/serial numbers, expiry ('use by') dates, and participant trial numbers. The investigator/pharmacist/investigational drug storage manager will maintain records that document adequately that the participants were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

Please refer to the trial specific pharmacy SOPs and SPC document for advice on prescribing, IMP formulation, storage, labelling, temperature monitoring, administration, cautions, contra-indications, accountability and returns information.

5.2.5 Adherence

Adherence to study drugs will be documented in the eCRF by recording missed doses or incomplete infusions.

Missing 24 hours of drug (i.e. one dose of micafungin or >=4 doses of flucytosine) will be considered a protocol deviation and reported to the site PI, trial co-ordinating centre and the DMC.

4.3.7 Weights for drug dosing
Where possible, participants will be weighed using a standing scale. However, many participants may be critically ill at the time of enrolment and unable to stand.

If a standing weight cannot be obtained, the following methods are acceptable for estimating weight for drug dosing:

- Documented bed scale weight
- Estimated body weight, as assessed by the managing clinical team
- Estimated weight using ideal body weight formulae

5.3 OTHER MANAGEMENT/ TREATMENT

5.3.0 COMPLICATED CANDIDIASIS

Patients with complicated candidiasis will be excluded from this trial. However, patients with *Candida* BSI may develop complications during treatment, or have evidence of complex candidiasis following routine investigations performed after enrolment in the trial. These may include involvement of prosthetic devices, endocarditis, meningitis, abdominal candidiasis, bone and joint infections as well as chorioretinitis or endophthalmitis (discussed previously). It is recommended that patients with suspected or confirmed *Candida* BSI have imaging performed to investigate organ involvement including fundoscopy, abdominal ultrasound/ CT if intra-abdominal focus, echocardiography if endocarditis is clinically suspected and/or if there is persistent candidaemia or prosthetic cardiac device(39). Management of these complications will be discussed in liaison with the site PI and clinicians responsible for the participant's care. Management recommendations will be in line with FIDSSA(29 - specific to *C. auris*), IDSA(25) and ECMM Guidelines(39) and recent published guidance for the management of ocular candidiasis(49).

5.3.1 INTRAVENOUS LINES

It is recommended that central venous catheters are removed as early as possible and within 48-72 hours from diagnosis of *Candida* BSI. Removal time (hours) will be documented within the eCRF.

5.3.2 OTHER PROSTHETIC DEVICES

Candida spp can form biofilms on prosthetic materials, which are poorly penetrated by antifungal drugs including micafungin. If evidence emerges of involvement of prosthetic material in *Candida* infection, the material should be removed if possible at the earliest opportunity. Participants with *Candida* infections involving prosthetic devices which cannot be removed within 5 days of randomisation will be excluded from the trial (late exclusion).

PROTOCOL TREATMENT DISCONTINUATION/ WITHDRAWAL

In consenting to the trial, participants are consenting to trial treatment, trial follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

- Unacceptable toxicity or adverse event (for full details see the IB)
- Intercurrent illness that prevents further treatment
- Any change in the participant's condition that justifies the discontinuation of treatment in the clinician's (routine clinician responsible for care, site or trial PI) opinion
- Withdrawal of consent for treatment by the participant

As the participant's involvement in the trial is entirely voluntary, they may choose to discontinue the trial treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although the participant is not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting the participant's rights.

Participants should remain in the trial for the purpose of follow-up and data analysis (unless the participant withdraws their consent from all stages of the trial). If a participant is withdrawn from follow-up, refer to Section 6.6 - Early Stopping of Follow-up.

Data will be kept and included for participants who stop follow-up early as long as consent for this is not withdrawn.

5.4 NON-TRIAL TREATMENT

All non-trial treatment taken by the patient will be recorded at enrolment, at follow-up while receiving the study intervention, and in the event of an SAE occurring.

5.4.0 MEDICATIONS PERMITTED

Drugs not known to be contraindicated with the trial drugs will be permitted. Drugs which might also lead to hepatotoxicity or bone marrow suppression are permitted considering the careful monitoring of liver function (ALT) and FBC during the treatment period; however, may be avoided at the discretion of the routine clinical team or site PI, or stopped if these toxicities arise.

5.4.1 MEDICATIONS NOT PERMITTED

There are no medications, which are absolutely contraindicated with micafungin in the context of monitoring. Cytarabine arabinoside, a cytostatic agent, has been reported to inactivate the antifungal activity of flucytosine by competitive inhibition, and so should not be given to any participants of the intervention arm.

5.4.2 TREATMENT AFTER TRIAL EVENT

Treatment will be at the discretion of the responsible physician.

ASSESSMENTS & FOLLOW-UP

Participants will be reviewed at least every 3-4 days until the end of treatment (EOT). Clinical and laboratory assessments will be conducted at these reviews, and then at EOT + 5 days. Survival status will be ascertained on day 30 and 90 (by phone call if discharged from hospital). Colonising flora skin/ mucosal swabs will be taken twice weekly up until date of hospital discharge, death or day 30 (whichever comes first), and if discharged during this period, on day 30 by arranged site or home visit.

6.0 TRIAL ASSESSMENT SCHEDULE

i. **Pharmacometric assessments:**
- Step 1 - Plasma samples (<2 mLs) for flucytosine and micafungin concentrations will be taken from participants of Step 1 in the intervention arms (50 mg and 100 mg flucytosine) at 0.25, 0.5, 1, 2, 6, 24, 48, 72, 96, 120, 144,) 168 hours post first dose of flucytosine.
- Step 2 – Plasma samples (<2 mLs) for micafungin and flucytosine concentrations will be taken from all participants of each arm on day 1 and day 7, within an hour before flucytosine and micafungin dose is due, with accurate recording of dosing and sampling times (for the PKPD sub-study).

ii. **Clinical and laboratory assessments:**
- Day ≤0 (screening): FBC and differential; serum creatinine; serum ALT; clinical review; medication review; Child-Pugh scoring if history of cirrhosis; pregnancy test if woman of childbearing potential; fundoscopy (up until day 5).
- Day 0: demographics; clinical data; severity scoring
- Every 3-4 days until end of treatment: FBC and differential; creatinine; ALT (by routine care, or study team if not available routinely)
- Every 3-4 days until EOT, EOT + 5 days, day 30 and day 90: clinical review

iii. **Mycological clearance and to monitor for breakthrough infection or relapse:**
- Baseline (0h), then twelve hourly (+/- 6 hours, at least 6 hours apart) until two consecutive blood cultures are sterile for *Candida* and any other organism following incubation for >48 hours, then every 3-4 days until EOT+5, weekly until discharge, death or day 30.

iv. **Antifungal resistance of colonising *Candida* flora:**
Twice weekly composite swabs of 1) nares/oral, 2) hands/axillae/groins, 3) perianal skin sites until whichever occurs first of discharge from hospital, death or day 30, and if discharged during this period, on day 30.

An additional <5 mL of serum and whole blood collected at day 1, day 6-7, day 13-14, EOT, EOT+5 days, day 30 will be stored for retrospective analysis of biomarkers and urine/ faecal sample at baseline/ day 7 will be taken for other sub-studies (See Section 6.5). Separate consent will be sought for storage of whole blood (EDTA) for human genetic testing. Abdominal drain fluid collected during treatment (if a drain is placed by routine care) will also be stored. *Candida* spp isolates cultured from any routine samples sent to laboratories during 2 weeks prior to enrolment and the trial follow up period will also be obtained and stored for future analysis.

Any blood samples that can be taken together within the allowable time window, will be taken with a single venepuncture or from an intravenous line already in position to reduce discomfort to the participant. Where routine blood results are available, these will be used instead of taking additional study blood samples.

Table 3 Event Schedule

	Screen & randomise	Week 1							Week 2							EOT	EOT +5	Day 30	Day 90
Study Day	≤D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14				
Consent forms																			
PIS and signed consent	X																		
Screening																			
Blood tests (<48 hours): FBC + differential, creatinine, ALT	X																		
Pregnancy test (urine/ serum) pre-enrolment)	X																		
Child Pugh score if cirrhosis history	X																		
Fundoscopy	X (<5days)								X (once during week 2)										
Clinical / laboratory																			
Clinical review	X			X			X			X			X			X	X	X	X
Safety bloods (FBC + differential, creatinine, ALT)				X			X			X			X			X	X		
PK bloods		X*	X	X	X	X	X	X											
Mycological clearance																			
Blood cultures		X√	X	X			X			X			X			X	X (weekly)		
Colonising *Candida* spp – antifungal susceptibility																			
Composite swabs of colonising sites	X			X			X			X			X			X	X$^{\phi}$ (every 3 days		
Additional samples for substudies																			
Serum and whole blood for biomarkers		X					X						X			X	X	X	
Stool and urine samples		X						X											
Abdominal drain fluid and matched plasma samples									X$^{\Upsilon}$										
Routine samples growing *Candida* spp	X								X										

*PK bloods at 0.25, 0.5, 1, 2, 6, 24, 48, 72, 96, 120, 144, 168 hours for participants of Step 1, and day 1 and 7 only (pre-dose) for participants of Step 2; ϕ composite swabs will only be taken twice per week up to day 30 if the participant remains in hospital and on day 30 if discharged; ϒ abdominal drain fluid will only be collected from participants who have abdominal drains inserted as part of routine care, and will only involve collection of remnant samples (which would otherwise be discarded) from the drain without any additional intervention to the participant. The samples will be taken with the matched plasma samples taken in Step 1 or with additional PK samples at 2, 6, 24, 48, 72 and 168 hours post micafungin dose if within Step 2.√Blood cultures will be taken every 12 hours (+/- 6 hours) until 2 consecutive samples (taken >6hrs apart) are negative for 48 hours incubation, and then every day until day 7, then every 3-4 days until EOT.

6.0.0 PROCEDURES FOR ASSESSING OUTCOMES

6.0.0.A Step 1 - Plasma Pharmacokinetics of Flucytosine at Standard vs Low Dose

Population PK modelling of flucytosine exposure will be performed using rich sampling from participants of Step 1 (in the intervention arms - See Table 1). The model will provide a precise estimate of the proportion of patients who achieve the target flucytosine exposure (45% time > MIC)(38). If modelling shows that >90% of patients are estimated to achieve target using the lower dose of flucytosine, and following discussion of all available data with the Trial Steering Committee (TSC), this dose will be continued in the intervention arm of Step 2.

6.0.0.B Step 2 - Primary Efficacy Outcome

Efficacy will be assessed using a 'Win Ratio' analysis of a hierarchical composite endpoint, among participants who receive at least one dose of trial treatment (modified intention to treat (mITT)), with the composite consisting of the following endpoints (in priority order):

1) Time to death up to 30 days (measured as an ordinal variable using blocks of 5 days);
2) Breakthrough during treatment (measured as a binary variable – whether or not any blood culture taken after blood culture sterility and during treatment are positive for any *Candida* spp. (when blood culture sterility is defined as negative blood cultures for any *Candida* sp for ≥48 hours if at least one blood culture has been taken during the time period)
3) Relapse within 30 days after end of treatment (measured as a binary variable – whether or not a blood culture taken following the end of treatment (weekly during hospitalisation) is positive with the same *Candida* spp as grown in the initial blood culture)

4) Time to blood culture sterility (measured as an ordinal variable using blocks of 12 hour periods from time that trial medication was started, to time of first blood culture that is negative for *Candida* spp., if subsequent blood cultures taken during the following ≥48 hours remain negative for *Candida* spp.));
5) Time to emergence of micafungin resistance (MIC > EUCAST breakpoint) in any *Candida* of the same species as *Candida* growth in blood during treatment (measured as an ordinal variable using twice weekly swabbing episode)

6.0.0.C Step 2 - Secondary outcomes

1) All-cause mortality at day 30 and 90
2) Time to all-cause mortality within 90 days
3) Proportion of participants who have a breakthrough and/or relapse of *Candida* BSI at day 14, EOT +5 days, day 30, EOT +30 days
4) Time to blood culture sterility
5) Time to micafungin resistance in colonising/invasive *Candida* spp
6) Proportion of participants who have micafungin resistance detected at any point in any colonising *Candida* spp up until day 14, EOT+5 days, day 30
7) Duration of ICU admission and hospitalisation
8) Rate of change in fungal biomarkers (serum beta-D-glucan (BDG), blood qPCR) until day 30
9) Proportion who develop grade 3 cytopaenias, other grade 4 adverse events and other SAEs until EOT +5.

6.0.0.D Exploratory outcome

- Estimate of flucytosine % time >MIC and micafungin AUC/MIC of the infecting *Candida* spp to assess for any relationship between drug exposure and clinical/ mycological outcomes.
- Flucytosine resistance % in infecting/colonising *Candida* species isolated from participants on combination therapy vs. monotherapy.
- Incidence of ocular complications of candidaemia through ophthalmic assessment during the second week of treatment.

6.0.1 Procedures for Assessing Safety

Participants will be monitored for signs and symptoms of drug toxicity (including liver toxicity for micafungin, and cytopenias for flucytosine) during study visits with blood tests at least every 3-4 days from day 0 to EOT + 5 days. The clinical team will be trained and made aware of the complications in patients with *Candida* BSI, drug toxicities and the clinical features to monitor. Adverse events will be carefully monitored. All toxicities leading to temporary or permanent discontinuation of the study therapy, and all new Grade 3 cytopenias and other Grade 4 toxicity effects will require thorough investigation with relevant clinical and laboratory tests as clinically indicated. These should be repeated until final resolution or stabilization of the toxicity.

Safety will be evaluated by presenting summaries of any AE leading to discontinuation of drug, and serious adverse events (SAEs) tabulated for the Safety population (all participants who received any amount of drug) by treatment group.

6.1 OTHER ASSESSMENTS FOR ANCILLARY/ SUB-STUDIES

6.1.0 Biomarkers as Prognostic and Treatment Response Indicators

This work falls within work packages with a separate but related, JPIAMR-funded research consortium Candi-NET- *Integrating Candidaemia trial samples, Data and Infrastructure to define*

Novel clinical trial Endpoints and Treatment strategies (project lead Bicanic City-St George's; project partners: Alanio, France; Van der Veerdonk, Netherlands; and Wake/ Govender, SA). Data and sample sharing will be governed by a dedicated consortium agreement as well as MTAs at each Institution. All data and *Candida* samples taken from participants will be stored for up to 10 years, for future ethically approved studies, if consent is provided.

Mycological treatment response biomarkers: Serial measurements of serum BDG and plasma Candida qPCR will be performed. The rate of biomarker decline will be calculated using linear mixed-effects models.

Host genetics and immune response biomarkers: Measurement of baseline and serial immune/inflammatory response parameters (serum) and host immunogenetics (human DNA from whole blood) to establish immuno(genetic) predictors of host response to antifungal and immune adjunctive therapy (e.g. IFN-γ).

Resistance: Serial *Candida* isolate MIC data will be merged with other candidaemia clinical trial datasets in the Candi-NET consortium and fitted using continuous-time Markov models to allow for time-varying antifungal exposures to be linked to resistance emergence.

6.1.1 Pharmacokinetic/Pharmacodynamic (PKPD) Modelling of Micafungin and Micafungin in Combination with Flucytosine for the Treatment of Candida BSI

PKPD modelling using clinical (survival), mycological clearance (time to blood culture sterility, breakthrough and relapse) and resistance (MIC change in invasive/colonising *Candida* spp) endpoints.

6.1.2 Abdominal Pharmacokinetics of Micafungin and Flucytosine

For any participants who has an abdominal drain inserted (as part of routine care) during study drug administration, abdominal drain fluid and matched plasma samples will be collected at 0.25, 0.5, 1, 2, 6, 12, 24 (day 1), 36, 48 (day 2), 72 (day 3) and 168 (day 7) hours post-micafungin dose and centrifuged. The pellet will be cultured and any *Candida* isolated stored for identification and susceptibility testing. The supernatant will be stored for future analysis of abdominal cavity compartmental pharmacokinetics in relation to micafungin plasma PK. Samples will be taken from the drain with no additional intervention to the participant. Exploratory analyses will be undertaken linking PK with Candida resistance emergence in the abdominal compartment.

6.1.3 Gastrointestinal mycobiome

Faecal samples on day 0, 7 will be collected and stored at -80c and analysed at Institut Pasteur, exploring the impact of antifungal therapy on broader diversity of fungal species using shotgun metagenomics.

6.1.4 Economic analyses

A health economics sub-study will be embedded within the COMBAT Candida trial to assess the value for money of trial interventions from multiple perspectives. This includes:

1. Cost-Effectiveness Analysis (CEA) from the South African public healthcare provider perspective,
2. Patient Perspective Cost Analysis, and
3. Cost-Utility Analysis (CUA) from a limited societal perspective.

1. Provider Perspective: Cost-Effectiveness Analysis

The CEA will compare micafungin 100 mg versus micafungin 100 mg plus flucytosine (5FC) at optimal daily dose, based on in-trial data collected at 30 and 90 days post-treatment initiation. Clinical outcomes will include mortality, time to negative blood culture, resistance development, and predefined treatment success. A decision tree model will be used given the short time horizon. Life-years saved will be estimated using mortality differences and local life expectancy data. Direct medical costs (e.g. drugs, hospitalisation) will be collected prospectively using an ingredients-based costing tool. Costs will be adjusted using South African CPI and reported in USD. ICERs will be calculated and interpreted against local cost-effectiveness thresholds. Probabilistic and deterministic sensitivity analyses will assess parameter uncertainty.

2. Patient Perspective: Cost and Financial Burden
Data on out-of-pocket expenses (OOPE), lost income, and household coping strategies will be collected via structured questionnaires at baseline, follow-up visits, and end of study. Catastrophic healthcare expenditure (CHE) will be defined as health-related costs (OOPE + lost income) exceeding 20% of household income. Descriptive statistics and comparisons between treatment arms will be presented.

3. Societal Perspective: Cost-Utility Analysis
The CUA will combine provider and patient costs. Outcomes will be expressed in quality-adjusted life years (QALYs) gained using utility weights sourced from the Tufts Cost-Effectiveness Analysis Registry. The decision model will generate ICERs in cost per QALY gained, with uncertainty explored through sensitivity analyses and cost-effectiveness acceptability curves.

6.2 EARLY STOPPING OF FOLLOW-UP

If a participant chooses to discontinue their trial treatment, they should always be followed up providing they are willing, that is, they should be encouraged to not leave the whole trial and agree to day 30 and day 90 in person/telephonic follow-up. If they do not wish to remain on trial follow-up, however, their decision must be respected, and the participant will be withdrawn from the trial completely. The TMG should be informed of this by the site in writing using the appropriate documentation. Participants stopping early have a negative impact on a trial's data. We will ensure participants have all relevant information about the trial so that they can make an informed decision to stop or continue, as they wish.

The medical data collected during the patient's participation in the trial will be kept safe for research and analysis purposes and will be fully anonymised as with all participant data. Consent for future use of stored samples already collected can be refused when leaving the trial early and this will be discussed fully with the participant and appropriately documented.

Participants may change their minds about stopping trial follow-up at any time and re-consent to participation in the trial. Participants who stop trial follow-up early will not be replaced.

6.3 PATIENT TRANSFERS

If a participant is transferred to another hospital, every effort should be made for the participant to be followed up in-person/telephonically and for appropriate medical follow-up and treatment to be arranged.

6.4 LOSS TO FOLLOW-UP

Every effort will be made (for example with mobile telephone calls and financial help with travelling expenses) to obtain accurate and complete follow-up data. Follow-up will be performed according to this protocol and will be identical in both trial arms. If unable to contact the participant following discharge from hospital, survival status will be ascertained by speaking to NOK/ relative/ other contacts, if permission and contact details have been provided by the participant, by visiting home or workplace (also with consent), or by accessing clinical, laboratory or national death register data. Follow-up for end of study (day 90) will continue until a definitive outcome can be assessed. Thus, study teams may continue to attempt contact participants and or their next-of-kin beyond day 90 until an outcome can be captured.

ASSESSMENTS AT TRIAL CLOSURE

The trial will be considered closed when the last participant has completed day 90 follow-up in the study and all follow-up and laboratory reports have been received.

SAFETY REPORTING

The principles of ICH-GCP require that both investigators and sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. Section 7.1 - Definitions lists definitions, Section 7.3 - Investigator Responsibilities gives details of the investigator responsibilities and Section 7.4 - Sponsor Responsibilities provides information on sponsor responsibilities.

7.0 DEFINITIONS

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial protocol. These definitions are given in Table 4.

Table 4 Definitions

TABLE	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening* ▪ Requires hospitalisation or prolongation of existing hospitalisation** ▪ Results in persistent or significant disability or incapacity ▪ Consists of a congenital anomaly or birth defect ▪ Is another important medical condition***

*The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, that has not worsened or for an elective procedure do not constitute an SAE.

*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency. Presence of malaria is not an SAE unless the current infection is life threatening.

7.0.0 MEDICINAL PRODUCTS

An investigational medicinal product (IMP) is defined as the tested investigational medicinal product and the comparators used in the study. (EU guidance ENTR/CT 3, April 2006 revision).

The IMP being tested is flucytosine in combination with micafungin and the comparator is micafungin alone.

Adverse reactions include any untoward or unintended response to drugs. Reactions to the IMP should be reported appropriately.

7.0.1 ADVERSE EVENTS

Adverse Events include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment

7.0.2 EXEMPTED ADVERSE EVENTS

Adverse Events do not include:

- Medical or surgical procedures; the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisations where no untoward or unintended response has occurred, e.g. elective cosmetic surgery, social admissions
- Overdose of medication without signs or symptoms

7.0.3 DISEASE-RELATED EVENTS

Candida BSIs have high crude mortality, with an expected acute mortality of 30-60%. All deaths will be reported as serious adverse events (SAEs).

7.1 OTHER NOTABLE EVENTS

Complications may arise in patients with *Candida* BSI, including disseminated candidiasis, abdominal candidiasis, infected prosthetic devices, endocarditis, chorioretinitis or endophthalmitis, bone and joint infections.

7.2 INVESTIGATOR RESPONSIBILITIES

SAEs and ARs will be monitored from day 1 to EOT + 5 days and recorded in medical notes and the eCRF SAE Form and sent to the co-ordinating centre within the agreed timescale (as part of the monthly reports from each site). SAEs which are Grade 3 cytopenias or Grade 4 AEs and SARs should

be notified to the co-ordinating centre within 24 hours of the investigator becoming aware of the event and reported to relevant regulatory authorities within the specified timeline.

7.2.0 INVESTIGATOR ASSESSMENT

Medically qualified members of the trial management group (TMG), excluding the co-PIs, will evaluate all SAEs received for seriousness, expectedness and causality. Investigator reports of SUSARs will be reviewed and those that are SUSARs identified and reported to the regulatory authorities. The causality assessment given by the site investigator cannot be overruled in the case of disagreement, but both opinions will be provided in subsequent reports.

7.2.0.A Seriousness

When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether the event is serious using the definition given in Table 4.

7.2.0.B Severity or Grading of Adverse Events

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the CTCAE grading (Version 5.0, 2017). Common Terminology Criteria for Adverse Events (CTCAE).

7.2.0.C Causality

The investigator must assess the causality of all serious events or reactions in relation to the trial therapy using the definitions in Table 5. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR.

Table 5 Assigning type of SAE through causality

RELATIONSHIP	DESCRIPTION	SAE TYPE
Unrelated	There is no evidence of any causal relationship	Unrelated SAE
Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient's clinical condition, other concomitant treatment).	Unrelated SAE
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient's clinical condition, other concomitant treatments).	SAR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	SAR
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

If an SAE is considered to be related to trial treatment and drug is stopped or the dose modified, refer to Section 5.3.4 - Dose Modifications, Interruptions & Discontinuations.

7.2.0.D Expectedness

If there is at least a possible involvement of the trial treatment (or comparator), the investigator must assess the expectedness of the event. An unexpected adverse reaction is one not previously reported in the current Summary of Product Characteristics (SPC) or one that is more frequent or more severe than previously reported. The definition of an unexpected adverse reaction (UAR) is given in Table 4. Please refer to latest SPC for flucytosine and micafungin for a list of expected toxicities associated with the drugs being used in this trial. If a SAR is assessed as being unexpected, it becomes a SUSAR.

- https://www.drugs.com/pro/flucytosine.html
- https://www.drugs.com/pro/micafungin.html

7.2.0.E Notification

The co-ordinating centre should be notified of all SARs and only the SAEs mentioned above (Grade 3 cytopaenias and Grade 4 AEs) within 24 hours of the site investigator becoming aware of the event.

Investigators should notify the co-ordinating centre of all SAEs occurring from the time of randomisation until EOT + 5 days. SARs and SUSARs must be notified to the co-ordinating centre until trial closure.

7.2.1 Notification Procedure

1. The SAE eCRF must be completed by the site investigator (the delegated clinician named on the Signature List and Delegation of Responsibilities Log who is responsible for the patient's care), with due care being paid to the grading, causality and expectedness of the event as outlined above. In the absence of the responsible investigator, the form should be completed by a member of the site trial team. The responsible investigator should subsequently check the SAE eCRF and make changes as appropriate. The initial report must be followed by detailed, follow-up and final reports as appropriate.

The minimum criteria required for reporting an SAE are the trial number and date of birth, name of investigator reporting and why the event is considered serious.

The SAE eCRF must be completed on the trial database. Once submitted, the co-ordinating centre is alerted of the submission by an automated email to its dedicated pharmacovigilance email address: XXX@witshealth.ac.za (TBC).

Follow-up: patients must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of protocol treatment, if necessary. A further SAE eCRF, indicated as 'Follow-up' should be completed as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The participant must be identified by trial number, date of birth and initials only. The participant's name should not be used on any correspondence and should be deleted from any test results.

Staff should follow their institution's procedure for local notification requirements.

7.3 SPONSOR RESPONSIBILITIES

The co-ordinating centre (Wits Mycology) will undertake a number of duties as delegated by the trial sponsor and is responsible for the reporting of SAEs, SUSARs, and other SARs to the regulatory authority (i.e. South African Health Products Regulatory Authority (SAHPRA)) and the research ethics committee/s, as appropriate. Medically qualified staff in the TMG will review all SAE reports received.

The causality assessment given by the site investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports.

Fatal and life-threatening SAEs and SUSARs must be reported to the competent authorities (Regulatory Authority and Ethics Committee) within 7 days of the co-ordinating centre becoming aware of the event; other SUSARs must be reported within 15 days.

The co-ordinating centre will also keep all investigators informed of any safety issues that arise during the course of the trial.

The co-ordinating centre, as delegated by the Sponsor, will submit Annual Safety Reports in the required format to Competent Authorities (Regulatory Authority and Ethics Committee).

QUALITY ASSURANCE & CONTROL

8.0 RISK ASSESSMENT

The quality assurance (QA) and quality control (QC) considerations have been based on a formal risk assessment, which acknowledges the risks associated with the conduct of the trial and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of ICH GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled. This risk assessment has led to the development of a monitoring plan, standard operating procedures and a tailored training schedule for all parties involved in the trial.

8.1 CENTRAL MONITORING AT THE CO-ORDINATING CENTRE

Co-ordinating centre staff will review electronic Case Report Form (eCRF) data for errors and missing data points.

Other essential trial issues, events and outputs will be detailed in the Monitoring Plan that is based on the trial-specific Risk Assessment.

8.2 ON-SITE MONITORING

The frequency, type and intensity for routine monitoring and the requirements for triggered monitoring will be detailed in the Monitoring Plan. This plan will also detail the procedures for review and sign-off. The study may be subject to internal audit by Wits Health Consortium under their remit as sponsor, and external audit by independent and/or regulatory bodies to ensure adherence to GCP.

8.2.0 DIRECT ACCESS TO PATIENT RECORDS

Participating site investigators agree to allow trial-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Patients' consent for this must be obtained and is specifically included in the ICF.

8.2.1 CONFIDENTIALITY

We will follow the principles of the SA Protection of Personal Information Act (Act No. 4 of 2013), UK Data Protection Act (DPA) and the General Data Protection Regulation (EU) 2016/679 (GDPR). Paper consent forms will be stored under the supervision of the site PI in a secured office and accessible to trial staff only. Electronic consent forms will be stored on the REDCap database, which will only be accessible to delegated study staff at the relevant site, and the data management team, through password control and accessed through password-protected electronic devices. Data are stored in a secure server at Wits University. Two separate databases will be maintained: one containing identifiable participant information (e.g., names, hospital file numbers, and consent forms), and the other containing de-identified trial data. Both databases will use the same system-generated Participant Identification Number (PID), created using REDCap's Auto Record Generation external module at the time of enrolment, ensuring consistent linkage without the need for a separate

linkage file All other data within the database, and any trial samples or results will be stored using participant identification numbers, with no individual identification information.

STATISTICAL CONSIDERATIONS

9.0 METHOD OF RANDOMISATION

Participants will be randomised individually and randomisation codes will be generated using REDCap via permuted-block randomisation method, stratified by site. During step 1 (where there are 3 arms) block sizes will vary between size 3 and 6. During step 2 (where there are 2 arms), block sizes will vary at size 2, 4 and 6. Randomisation number will have 6 digit numbers with the first digit for step, next 2-digits for site and last 3-digits for participants within each site. For example, “101001” means the first patient in the step 1 at site “1”.

9.1 BLINDING

This is an open-label trial for several reasons. Adding 4 placebos of flucytosine (given 6 hourly) to the control arm will involve substantial logistical and methodological challenges including weight-based dosing, adjustments for renal function and neutrophil level, nasogastric administration, and the need for multiple matched placebos. These factors would significantly increase trial complexity, cost, and the risk of unblinding due to tolerability differences. In addition, there is no perfect way of measuring adherence to antifungal drugs and so if the intervention arm is made inferior because of the complexity of the trial design, use of a placebo would obscure this result.

The potential limitations of an open-label design will be mitigated using objective endpoints assessed by the study team (i.e. mortality, fungal clearance, fungal resistance) rather than treating clinicians. We will adhere to a strict assessment schedule, in which blood cultures and swabs for assessment of microbiological outcomes will be taken by the study team and not by the routine clinical team. This will remove potential bias introduced by sampling practices.

The trial statistician will be blinded regarding the treatment code when developing the statistical analysis plan and writing the statistical analysis programmes, which will be validated and completed using dummy randomisation codes.

9.2 SAMPLE SIZE

9.2.0 STEP 1

For the dose-selection stage (STEP 1) of the clinical trial we have calculated a sample size and sampling regimen on the basis of a stochastic and simulation estimation (SSE) study, to yield precise estimates of drug (flucytosine) exposure in the two intervention arms. For the SSE, we used an interim model (unpublished, C. Thomas, J. Standing) based on flucytosine plasma concentrations from participants of the AMBITION-cm study(50) and a published flucytosine PK model derived from healthy volunteers(51). The sample size (20 per arm) and plasma sampling regimen precisely estimates clearance and volume of distribution and absorption rate constant (>90% power to precisely estimate clearance and volume, and >80% power to precisely estimate absorption rate, with "precise" defined as % relative standard error <20). This is a feasible sample size and regimen to achieve within 6 months at the 5 study sites without significantly delaying the 2-arm trial or increasing risk of Type 1 error.

9.2.1 STEP 2

An evaluable sample size of 225 participants in each arm will provide 91% power at a significance level of 0.05, to find a win ratio of 1.47, calculated by performing simulations assuming a mortality of

40%(52, 53) in the control arm at day 30 (reduced to 30% in the intervention arm), a breakthrough rate of 8%(45, 46, 54, 55)(reduced to 4%), a relapse rate of 3%(45, 46, 54) (reduced to 1.5%), a median time to blood culture sterility of 48 hours (46, 54, 56) (reduced to 36 hours), and an emergent micafungin resistance rate of 15% (8, 57)(reduced to 7.5%) (Table 6). Estimates are based on clinical trial, *in vitro* and animal model evidence. The impact of the two most influential elements (mortality and time to blood culture sterility) on the power was also estimated using more conservative assumptions for each, and this is shown in Table 7. The number of simulations used was 1000 for each scenario tested. Time-to-event endpoints were simulated assuming an exponential distribution.

Allowing 10% 30-day loss-to-follow-up, this sample size is feasible if at minimum just 500/2000 (25%) patients with *Candida* BSI enrol in the trial, based on current site rates of *Candida* BSI.

Table 6

Rank	Parameters	A	B	Win
	Sample size	n_A=225	n_B=225	
1	Cumulative proportion died at 30 days			
(rate of death)π_A =0.4 (rate=0.0169)	π_B=0.3 (rate=0.0118)	Fewer deaths/ Smaller rate		
2	Proportion of breakthrough during treatment	π_A=.08	π_B=.04	Smaller
3	Proportion of relapse following treatment	π_A=.03	π_B=0.015	Smaller
4	Median time to blood culture sterility in days (rate of blood culture sterility)	M_A =48 hours (rate=0.0144)	M_B=36 hours (rate=0.0193)	Shorter time/ larger rate
5	Cumulative resistance rate at 30 days	π_{1A} =0.15	π_B=0.075	fewer resistant

Table 7

Sample size	Mortality difference	Breakthrough	Relapse	Time to sterility	Resistance difference	Power
225 per arm	40%: 30%	8%:4%	3%:1.5%	48h:36h	15%:7.5%	91%
225 per arm	40%:32%	8%:4%	3%:1.5%	48h:36h	15%:7.5%	85%
225 per arm	40%:30%	8%:4%	3%:1.5%	48h:42h	15%:7.5%	81%
225 per arm	40%:32%	8%:4%	3%:1.5%	48h:42h	15%:7.5%	67%

9.3 SAFETY MONITORING

The trial will be monitored for safety by an independent DMC. A DMC Charter will be drawn up that describes the membership of the DMC, relationships with other committees, terms of reference, and decision-making processes. Regular data and safety monitoring will be done by the DMC.

9.4 ANALYSIS PLAN (BRIEF)

Statistical analyses will be detailed in the statistical analysis plan.

Briefly:
Pharmacokinetic analysis in Step 1:
Flucytosine plasma concentrations from rich sampling will be used to develop a population PK model. From this model we will establish if >90% of patients achieve the target drug exposure of (45% time > MIC)(38). If >90% of patients achieve this target with the lower dose in Step 1, and after discussion with the TSC, taking account of all available data, this will be continued as the dose for intervention arm in Step 2. There will be a pause of recruitment betwee Step 1 and Step 2 to allow for this analysis, discussion and conclusion on the optimal dose to be used in Step 2.

Analyses populations: 1) Modified intention-to-treat (mITT) population: all randomised subjects who receive ≥1 dose of study drug and are not withdrawn due to late exclusion criteria; 2) Per-protocol (PP) population: a subset of the ITT population - subjects with major protocol deviations will be excluded from this PP population. 3) Safety population - a subset of the ITT population, consisting of all randomised subjects who receive at least one dose of study drug. This will additionally include those excluded on late exclusion criteria. Primary analyses of efficacy will be based on the mITT population with further supportive analyses on the PP and safety populations.

Primary endpoint analysis: The primary analysis will compare the two treatment groups using the Win Ratio method (described in more detail in section 9.7 below) which compares each individual in the control arm with each individual in the intervention arm and counts up the number of times the intervention “wins”, “loses” or “ties” based on the criteria shown in Figure 2. The win ratio is the total number of times the intervention “wins” divided by the total number of times the intervention “loses” (ties are excluded) and is estimated along with its 95% confidence interval. The win ratio can be interpreted as the estimated relative odds of having a better outcome (as defined by the hierarchical composite outcome) if receiving the intervention compared to receiving the control. In accordance with best practice for using the win ratio in randomized clinical trials, we will additionally report the win difference, and also the estimated effect of the intervention on each element of the composite outcome separately (as secondary outcomes)(58).

The primary analysis will be unadjusted (the win ratio is a non-parametric method that cannot accommodate covariate adjustment). Given the fact the study is randomized and the relatively large sample size, we expect reasonable balance between the two arms in terms of potential confounders, so an unadjusted analysis is appropriate. Also, as the intervention effect on each of the five elements of the compposite endpoint will be analysed separately in a secondary analysis, we will be able to adjust for any potential imbalances that occurred between arms

Another potential consideration is the fact that although hierarchically ordered, a statistically significant Win Ratio could be driven by the least meaningful component. However, all endpoints chosen in the COMBAT Candida trial are clinically meaningful “hard” endpoints, such that, if the results were driven by the lowest ranking endpoint, time to emergence of resistance, this remains an

important finding. And because of the secondary analyses described above we will be able to unpick which of the outcome elements drove any observed differences between trial arms, ensuring full transparency of our results.

Secondary endpoint analyses: Secondary endpoints will be analysed using generalised linear models (GLMs) with the treatment arm as the sole predictor. The point estimate of the treatment effect with 95% CI will be derived via specification of a GLM model for each secondary outcome depending on it's distribution. For a binary endpoint, the GLM model with binomial distribution and log-link function will be estimated and the treatment effects, measured as a Risk Ratio, will be generated. For a continuous outcome, a Gaussian distribution will be assumed and identity link function will be used. Correspondingly the mean difference and its 95% CI between two arms will be calculated. For a count outcome, Poisson distribution and log link function will be used, from which incidence rate ratio (IRR) and its 95% CI will be computed.

Analyses of survival data will be conducted using unadjusted Cox regression analysis to calculate the hazard ratio (HR) and 95% CI between the treatment groups. Kaplan-Meier survival curves will also be plotted by treatment group. A log-rank test will be performed to compare the survival curves between the treatment groups. Additional analyses with adjustment for confounding variables, and of subgroups including by BSI causative *Candida* spp, and duration of intravenous catheters is planned.

Safety analysis: The safety analysis will be descriptive and the frequency and proportions of patients suffering clinical and laboratory-defined SAEs (CTCAE grade 3 cytopaenias and other grade 4 adverse events) will be generated by treatment arm.

A full definition and explanation of all primary endpoint, secondary endpoint and safety data analyses will be included in the statistical analysis plan. The trial results will be reported following the CONSORT guidelines for randomised clinical trials.

9.5 OUTCOME MEASURES

9.5.0 PRIMARY OUTCOME

A hierarchical composite outcome made up of the following five elements in priority order

1. Time to death, within 30 days (measured as an ordinal variable using blocks of 5 days)
2. Breakthrough during treatment (measured as a binary variable – whether or not any blood cultures taken subsequent to blood sterility for ≥48 hours, become positive with any *Candida* spp)
3. Relapse within 30 days after end of treatment (measured as a binary variable – whether or not a blood culture taken following the end of treatment (weekly during hospitalisation) is positive with the same *Candida* spp as was growing in the initial blood culture);
4. Time to blood culture sterility (measured as an ordinal variable using blocks of 12 hour periods from first blood culture positive, to first blood culture negative, when two consecutive blood cultures remain negative during twice daily testing)
5. Time to emergence of micafungin resistance (MIC > EUCAST breakpoint) in any commensal Candida spp (measured as an ordinal variable using twice weekly swabbing episode)

9.5.1 SECONDARY OUTCOMES

Each of the separate elements of the composite endpoint, treated separately and additionally:

1. All-cause mortality at day 30 and 90

2. Time to all-cause mortality within 90 days
3. Proportion of participants who have a breakthrough and/or relapse of *Candida* BSI at day 14, EOT +5 days, day 30, EOT +30 days
4. Time to blood culture sterility
5. Time to micafungin resistance in colonising/invasive *Candida* spp
6. Proportion of participants who have micafungin resistance detected at any point in any colonising *Candida* spp up until day 14, EOT+5 days, day 30
7. Duration of ICU admission and hospitalisation
8. Rate of change in fungal biomarkers (serum beta-D-glucan (BDG), blood qPCR) until day 30
9. Proportion who develop grade 3 cytopaenias, other grade 4 adverse events and other SAEs until EOT +5 days, and serious adverse reactions (SARs), suspected unexpected serious adverse reactions (SUSARs); and deaths occurring after randomisation to day 30.

9.5.2 EXPLORATORY OUTCOMES

Sparse plasma sampling, together with prior PK models will enable an estimate of flucytosine % time >MIC and micafungin AUC/MIC of the infecting *Candida* spp to assess for any relationship between drug exposure and the clinical/ mycological outcomes.

Flucytosine MICs will be performed on all *Candida* sp. to assess for the emergence of flucytosine resistance in infecting/colonising *Candida* species isolated from participants on combination therapy.

Ophthalmological examination will conducted for every participant in week 2, allowing assessment of the incidence of ocular candidiasis as a complication of *Candida* BSI.

9.6 WIN RATIO ANALYSIS

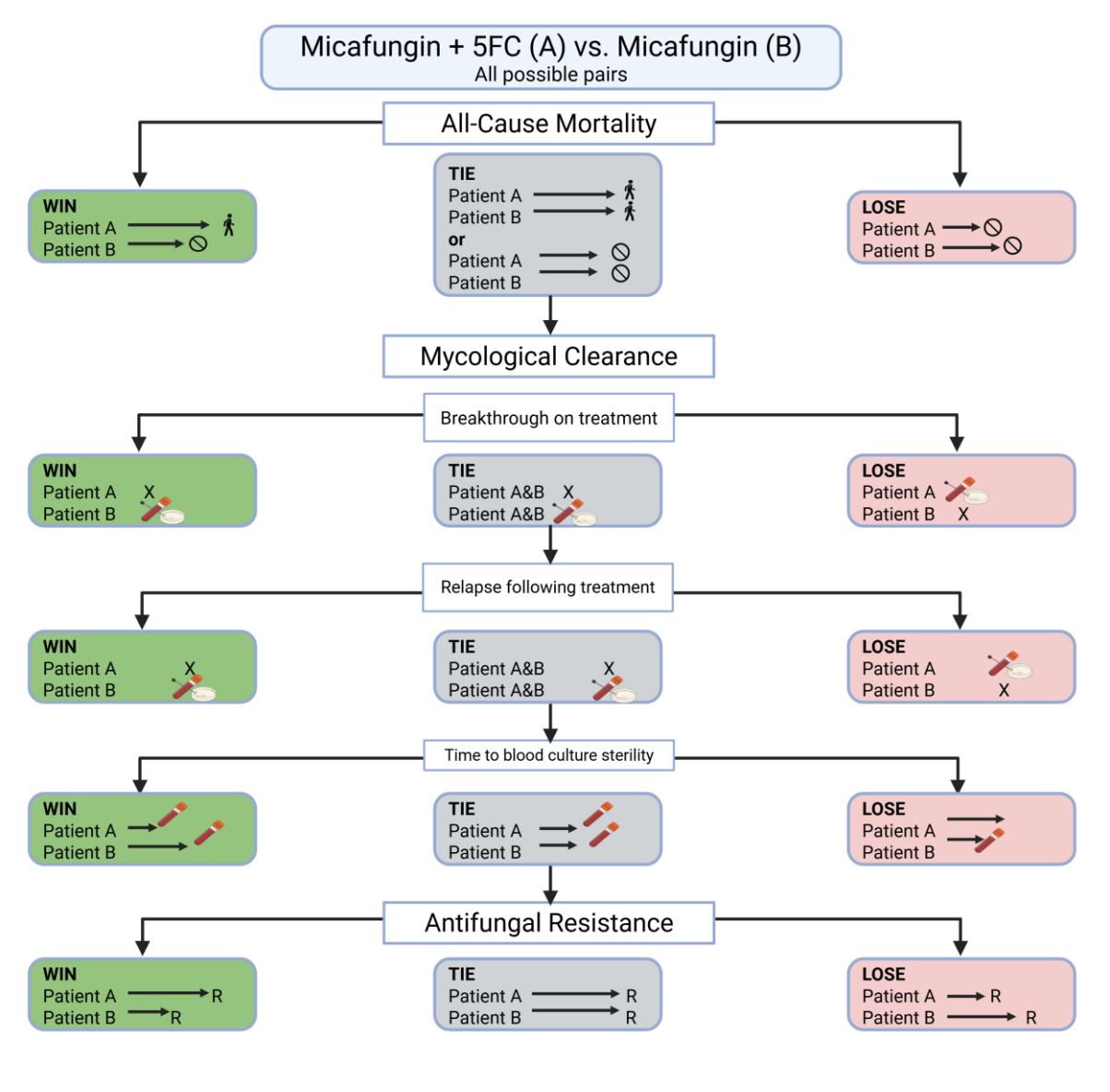

Figure 2 Representation of the Win Ratio method for primary efficacy outcomes

Our method prioritizes time to all-cause mortality before measures of mycological clearance and resistance in a hierarchical composite endpoint. Participants will be paired (unmatched) and compared for time to death, time to blood culture sterility, breakthrough and relapse rate, and time to resistance evolution. Figure 2 depicts 'Wins', 'Ties' and 'Losses' if Participant A is given the intervention treatment (micafungin + flucytosine) and Participant B is given the control treatment (micafungin alone). Participant A is depicted as winning in the green column, and losing in the red column. **1) Time-to-death:** If Participant A dies, vs. Participant B survives during 30 days, Participant A is considered the 'winner', but if both participants die, the 'winner' will be the participant who had a longer time between randomisation and death. If both participants die within the same 5 day block, or remain alive beyond 30 days (considered a 'tie'), the pair will be compared on **2) breakthrough** of *Candida* BSI during treatment ('winner' = Participant A no breakthrough vs Participant B has breakthrough, 'tie' = neither or both have breakthrough, loser = Participant A has

breakthrough vs Participant B no breakthrough). If a 'tie', then comparison on **3) relapse** of *Candida* BSI during 30 days following treatment ('winner' = Participant A no relapse vs Participant B has relapse, 'tie' = neither or both have relapse, loser = Participant A has relapse vs Participant B no relapse). Finally, if all endpoints are 'ties', comparison on **4) time to blood culture sterility** ('winner' = shortest time to sterile blood culture, 'tie' = equal time to sterility or no further positive blood cultures, 'loser' = longest time to sterile blood culture). If there is a tie, then the pair will be compared on **5) time-to-emergence of micafungin resistance** in infective or colonising isolates up until day 30 ('winner' = longest time to resistance, 'tie' if emergence within the same twice weekly swabbing episode, or no emergence, 'lose' = shortest time to resistance.

REGULATORY & ETHICAL ISSUES

10.0 COMPLIANCE

10.0.0 REGULATORY COMPLIANCE

The trial complies with the principles of the Declaration of Helsinki: as amended by *59th WMA General Assembly, Seoul, Korea, October 2008*.

It will also be conducted in compliance with the approved protocol, the principles of Good Clinical Practice (GCP) and applicable local laws and guidelines.

10.0.1 SITE COMPLIANCE

The site will comply with the above and international sites will comply with the principles of GCP as laid down by the ICH topic E6 (R2) (Note for Guidance on GCP) and applicable national regulations. An agreement will be in place between the site and the co-ordinating centre, setting out respective roles and responsibilities (see Section 13 - Finance).

The site will inform the co-ordinating centre as soon as they are aware of a possible serious breach of compliance, so that the co-ordinating centre can report this breach if necessary within 7 days as per the SA regulatory requirements. For the purposes of this regulation, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects in the trial, or
- The scientific value of the trial.

10.0.2 DATA COLLECTION & RETENTION

eCRF data collected and validated using REDCap will be stored in an electronic database that is protected using a scheme of authentication and encryption (see below). Paper documents, such as clinical notes and administrative documentation will be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for 15 years after the end of the trial. During this period, all data should be accessible to the competent or equivalent authorities, the Sponsor, (and other relevant parties) with suitable notice. The data may be subject to an audit by the competent authorities.

11.1.4 PROTECTION OF ELECTRONICALLY STORED SUBJECT DATA

We will follow the principles of the SA Protection of Personal Information Act (Act No. 4 of 2013), UK Data Protection Act (DPA), and the General Data Protection Regulation (EU) 2016/679 (GDPR). Paper consent forms will be stored under the supervision of the site PI in a secured office and accessible to trial staff only. Electronic consent forms will be stored on the REDCap database, which will only be accessible to delegated study staff at the relevant site, and the data management team, through password control and accessed through password-protected electronic devices. Data are stored in a secure server at Wits University. Two separate databases will be maintained: one containing identifiable participant information (e.g. names, hospital file numbers, and consent forms), and the other containing de-identified trial data. Both databases will use the same system-generated Participant Identification Number (PID), created using REDCap's Auto Record Generation external module at the time of enrolment, ensuring consistent linkage without the need for a separate linkage file. All other data within the database, and any trial samples or results, will be stored using participant identification numbers, with no individual identification information.

Security of electronic records and data is a significant concern. All components of the REDCap electronic data capture system will use authentication and encryption to render electronically stored

subject identity and personal health information unusable, unreadable, or indecipherable to unauthorized individuals for “data in use” (e.g. data being analysed by study investigators), “data in motion” (e.g. data being transferred between data entry points and the Wits REDCap server), and “data at rest” (e.g. data stored on the Wits REDCap server and other forms of data backups for redundancy).

All data transmissions to and from REDCap will be encrypted using industry-standard protocols (e.g. HTTPS/TLS). User authentication will be enforced through unique usernames and passwords with role-based access controls to restrict data access to authorized study staff. User profiles in REDCap will control access to specific forms, data fields, and reports, ensuring that users only see data appropriate to their roles.

Data stored on the REDCap server will be encrypted at rest according to institutional IT policies. Regular backups of the REDCap database will also be encrypted and securely stored. Access to the REDCap server environment will be limited to authorized system administrators, who will use secure authentication methods. Mobile devices, tablets, or remote data entry will be conducted via secure, authenticated REDCap web sessions. Tablets, devices, and laptops have been set up by WHC IT with security and passwords. Staff will not store personal health information locally on unencrypted devices. Where necessary, REDCap’s data export controls will be used to limit or de-identify exported data fields, protecting subject confidentiality.

10.1 ETHICAL CONDUCT OF THE STUDY

The Co-Principal Investigators have overall responsibility for the ethical conduct of the trial.

10.1.0 ETHICAL CONSIDERATIONS

10.1.0.A Informed consent

We expect that a majority of participants with *Candida* BSI will not have capacity to provide informed consent due to critical illness, and receipt of sedative medications. The interventions within this trial must be initiated urgently, due to the immediate time critical imperative to provide treatment for *Candida* BSI and to facilitate valid evaluation of the interventions. It will therefore be important to allow participants to provide informed consent both prospectively (if they have capacity to do so at the time of enrolment), and retrospectively (at the earliest opportunity when they regain capacity) following the provision of proxy consent from a NOK, family member or an independent clinician. If informed consent is provided by an independent clinician, and a NOK or family member becomes available during trial enrolment and while the participant lacks capacity, re-consent will be requested from the NOK or family member. The rights of the participant, or a proxy to refuse to participate in the study without giving a reason will be respected. Similarly, the participant or proxy must remain free to change their mind at any time about the study follow-up without giving a reason and without prejudicing the participant’s further treatment.

Proxy consent from a next-of-kin (NOK), family member, or independent clinician may need to be obtained remotely. Where NOK or relative consent is obtained by telephone, this will be formalised either by the NOK or relative signing the consent form electronically, or by an impartial witness — defined as a member of medical staff who has no involvement in the study — signing the consent form following witnessed telephonic confirmation of consent. The method by which consent was obtained must be documented in the eCRF. Where consent is provided by an independent clinician (i.e. a physician with no involvement in the study), that clinician will be required to submit a signed declaration to the Wits Human Research Ethics Committee (Wits HREC) confirming their independence from the study and their assessment that participation is in the participant's best interests and that the participant fulfils the eligibility criteria. If a NOK or relative becomes available

at any point after independent clinician consent has been provided, re-consent will be requested from that individual at the earliest opportunity. If and when the participant regains capacity, retrospective consent will be sought from them directly at the earliest opportunity, in accordance with section 3.5.1.

This follows a similar approach to previous trials conducted with critically unwell individuals, for example REMAP-CAP (Wits HREC Ref: 231117).

10.1.0.B Additional blood samples

Step 1 of the trial will involve taking additional blood samples for PK analysis. The volume of blood required for this analysis is <24 mLs. Enrolment will be limited to patients who have a baseline haemoglobin level of ≥80 g/L, and who have viable intravenous access (e.g. long intravenous catheter) for ease of frequent sampling. For all trial participants, additional blood cultures will be taken during the first few days of treatment to measure the time to blood sterility. International guidelines for the management of *Candida* BSI recommend daily blood cultures until blood culture sterility(25, 39). The blood cultures that will be taken as part of the trial, therefore represent an additional 5-10 mLs of blood per day. From prior research studies(59), we would expect that blood culture sterility will be achieved during the first 24-36 hours during this trial. And so the expected additional volume of blood will be <20 mLs in most cases. The total volume of blood required from each trial participant will comply with the South African Department of Health Ethics in Health Research (2015) guideline of ≤450 mLs over 8 weeks.

10.1.1 Ethical Approvals

Before initiation of the trial, the protocol, all informed consent forms, and information materials to be given to the prospective participant will be submitted to Wits Human Research Ethics Committee (Wits HREC), which will have direct oversight of trial sites, including investigators/ work at City St George's University of London, and Stellenbosch University. Any further amendments will be submitted and require approval to this committee.

10.2 COMPETENT AUTHORITY APPROVALS

This protocol will be reviewed by/submitted to the national competent authority (SAHPRA). Regulatory approvals/CTA will be sought from the regulatory authorities for the import of study drugs.

The progress of the trial and safety issues will be reported to the competent authority, regulatory agency or equivalent in accordance with local requirements and practices in a timely manner.

Safety reports, including expedited reporting and SUSARS will be submitted to the competent authority in accordance with each authority's requirements in a timely manner.

10.3 OTHER APPROVALS

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site or to other local departments for approval. A copy of the local R&D approval (or other relevant approval as above) and of the PIS and Consent Form on local headed paper should be forwarded to the co-ordinating centre before patients are enrolled.

10.4 COMMUNITY ENGAGEMENT

We will establish a community advisory board (CAB) for the COMBAT Candida programme with members of
the public and particularly individuals in SA who have lived experience of AMR/ fungal infections and critical
illness (ICU survivors). We will renumerate these individuals for the time they engage with study materials, provide advice and input to design and patient-facing materials using UK NIHR recommended rates. We will also provide CAB meetings for networking and planning of advocacy and awareness-raising activities around antifungalresistance and for the dissemination of findings from the COMBAT studies. CAB members will be funded to attend TMG meetings to provide input to discussions of study progress and planning.

10.5 INDEMNITY

The sponsor of the trial is Wits Health Consortium and as such provides indemnity and insurance for the trial. All personnel involved in the trial will be expected to be indemnified by their employing authority.

10.6 FINANCE

The study is funded by the Wellcome Trust.

OVERSIGHT & TRIAL COMMITTEES

There are a number of committees involved with the oversight of the trial. These committees are detailed below.

11.0 TRIAL MANAGEMENT GROUP (TMG)

A TMG will be formed comprising the Co-Principal Investigators, supported by site PIs and co-investigators (clinical and non-clinical) as needed, and members of the co-ordinating centre. The TMG will be responsible for the day-to-day running and management of the trial. It will meet approximately once weekly on-line, with yearly in-person meetings for all investigators during the trial, if feasible and appropriate. The full details can be found in the TMG Charter.

11.1 INDEPENDENT TRIAL STEERING COMMITTEE (TSC)

The TSC has membership from five independent voting members: Emeritus Prof Guy Richards (Chair); Prof Fathima Paruk; A/Prof Jantjie Taljaard; Prof Andrew Whitelaw; Ms Vanessa Carter (Patient Advocate), as well as representative from the Wellcome Trust and members of the TMG (Prof Nelesh Govender, Dr Rae Wake, Prof Tom Harrison, A/Prof Síle Molloy, Prof Tihana Bicanic, Mr Jack Adams, Dr Raphaela Berghammer-Bohmer, Dr Lauriane Fomete) as observers. The role of the independent TSC is to provide overall supervision for the trial and provide advice through its independent Chair. The ultimate decision for the initiation and continuation of the trial lies with the TSC. Further details of TSC functioning are presented in the TSC Charter. All independent TSC members will have opportunity to comment on the protocol as early as possible. Wellcome will appoint a TSC member with observer status. The lead clinician, trial manager and site PIs can also attend as observers. This steering committee will meet at the beginning and then annually and will be responsible for the overall direction of the research.

11.2 INDEPENDENT DATA MONITORING COMMITTEE (DMC)

An independent Data Monitoring Committee (DMC) will be formed. The DMC will be the only group which sees the confidential, accumulating data for the trial. Reports to the DMC will be produced by the trial statistician. The DMC will meet within 6 months of the trial opening; the frequency of meetings will be dictated in the DMC charter. The DMC will consider data using the statistical analysis plan (see Section 9.5 - Analysis Plan (Brief)) and will report to the TSC. The DMC can recommend premature closure or reporting of the trial, or that recruitment to any research arm be discontinued. Further details of DMC functioning, and the procedures for analysis and monitoring, are provided in the DMC Charter. The committee will comprise Prof Shabbar Jaffar (Chair), Dr Francesca Conradie, A/Prof John Gregson, Dr Halima Dawood, and A/Prof Sean Wasserman.

11.3 COMMUNITY ADVISORY BOARD

A CAB may be established to provide community perspectives on aspects of the study, including participant information materials, acceptability of study procedures, and approaches to community engagement. The CAB will serve in an advisory capacity and will not be involved in trial management or decision making (except if represented on the TSC). Where convened, input from the CAB may be considered by the study team when refining participant facing materials and engagement strategies.

DISSEMINATION

12.0 DISSEMINATION PLAN

Trial findings will be disseminated promptly involving the following steps: Firstly, peer-reviewed publications and presentations at international meetings. Secondly, engagement, from the onset of the trial, and advocacy for international policy and guideline change facilitated by co-applicants' direct links to research and policy stakeholders. Findings with relevance to national policy e.g. micafungin accessibility in the public sector, will be discussed with the South African Department of Health (DoH). Thirdly, public engagement will be achieved through the COMBAT Candida community advisory board in South Africa, communications divisions of applicant institutions, and advocacy/education groups.

12.1 PUBLICATIONS

The results from the different centres will be analysed together and published as soon as possible as described in the Publication Plan. Individual clinicians must not publish data concerning their patients that are directly relevant to questions posed by the study until the TMG has published its report.

The TMG will form the basis of the Writing Committee and will advise on the nature of the publication. The names of all investigators who have contributed will be included in the authorship of any publication. The Publication Plan will be agreed before any publication and will be inclusive and decided in a fair transparent manner. The members of the TSC and DMC will be listed with their affiliations in the Acknowledgements/Appendix of the main publication. The funders will have no role in the decision to publish or the content of the publication.

PROTOCOL AMENDMENTS

Version number	Date	List of amendments
1.1	12 June 2026	Dr June Fabian listed as site PI at WDGMC Case identification updated under section 2.4 (page 38) Rationale & justification for choice of micafungin (page 33)
1.2	13 July 2026	Dr Lauren Richards removed as a sub-investigator at HJH (page 5) Clarification of definition of independent clinician added (page 42)
1.3	24 July 2026	Dr Evan Shoul role updated to be co-PI of Netcare Milpark, together with Prof Boffard (pages 5-6, 10) Additional comment about pooling of independent clinicians added following Wits HREC feedback (page 40) Additional comment about consenting process updated following Wits HREC feedback (page 74-75).
1.4	06 August 2026	Dr Monique Da Fonseca role updated to be PI of WDGMC (page 6). Dr June Fabian role updated to be sub-investigator at WDGMC (page 6). SAHPRA and HREC reference numbers added (page 8)

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