CLINICAL TRIAL PROTOCOL

A RANDOMIZED, DOUBLE BLIND PHASE II PROOF-OF-CONCEPT SUPERIORITY TRIAL OF FOSRAVUCONAZOLE 200 MG OR 300 MG WEEKLY DOSE VERSUS ITRACONAZOLE 400 MG DAILY, ALL THREE ARMS IN COMBINATION WITH SURGERY, IN PATIENTS WITH EUMYCETOMA IN SUDAN

Short title	A study to assess whether in combination with surgery, fosravuconazole (fos) is superior to itraconazole (itra) in the treatment of mycetoma in Sudan
Name of product(s)	Fosravuconazole (former code name E1224 ; Eisai Ltd, Japan) Itraconazole (Sporonox®)
Drug Class	Both fosravuconazole and itraconazole are azoles
Phase	Phase II
Indication	Patients with eumycetoma in Sudan
Clinical Trial Protocol Number	DNDi-FOSR-04-MYC
EudraCT	*N/A*
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Clinical Trial Protocol Version / Date	Version 4.0_05th Mar 2019
Protocol Amendment Number / Date	3.0_05th Mar 2019

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ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
ACTH	Adrenocorticotropic Hormone
ADR	Adverse Drug Reaction
AIDS	Acquired Immune Deficiency Syndrome
AE	Adverse Event
AESI	Adverse Events of Special Interest
ALT	Alanine aminotransferase (SGPT)
AR	Adverse Reaction
ARV	Antiretroviral Drugs
AP	Alkaline Phosphatase
AST	Aspartate aminotransferase (SGOT)
AUC	Area under the curve
BID	Twice Daily
BIL	Bilirubin
BMI	Body Mass Index
BP	Blood Pressure
CE	Clinically Evaluable
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum concentration
CMH	Ceramide Monohexosides
CRF	Case report form (paper CRF)
CTCAE	Common Terminology Criteria for Adverse Events
CRE	Creatinine
DNA	Deoxyribonucleic Acid
DNDi	Drugs for Neglected Diseases initiative
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EOS	End of Study
EOT	End of Treatment
FDA	Food and Drug Administration
FNA	Fine Needle Aspiration
FPI	First Patient In
GCP	Good clinical practice
HIV	Human Immunodeficiency Virus
HR	Heart rate
ICF	Informed Consent Form
ICH	International Conferences on Harmonization
ID	Identification
IEC	Independent Ethics Committee

IFN	Interferon
IL	Interleukin
i.m.	Intramuscular
IMP	Investigational Medicinal Product
ITS	Internal transcribed spacer
ITT	Intent to Treat
i.v.	Intravenous
K	Potassium
LC-MS	Liquid chromatography–mass spectrometry
LFTs	Liver function tests
LPO	Last Patient Out
MCV	Mean Corpuscular Volume
MedRA	Medical Dictionary for Regulatory Activities
MESI	Medical Event on Special Interest
mITT	Modified Intention to Treat
MIC	Minimum Inhibitory Concentration
MRI	Magnetic Resonance Imaging
MRC	Mycetoma Research Centre
Na	Sodium
NOAEL	No observable adverse effect level
NONMEM	Non-linear Mixed Effects Modelling
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics
PI	Principal investigator
RR	Respiratory Rate
PK	Pharmacokinetics
QC	Quality Control
QD	Daily
qRT	Quantitative Real Time
qPCR	Quantitative PCR
SAC	Scientific Advisory Committee
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOE	Schedule of Events
Th	T Helper
TNF	Tumour Necrosis Factor
ULN	Upper limit of normal
U/S	Ultrasound
VNTR	Variable Number Tandem Repeat
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

PROTOCOL SUMMARY

Eumycetoma is a fungal disease commonly caused by *Madurella mycetomatis.* The disease is chronic, granulomatous and inflammatory. It usually involves subcutaneous tissues and leads to masses and sinuses from which fungal grains are discharged. It is most probably introduced post trauma e.g. thorn prick. It is associated with major morbidity and can be disabling, disfiguring and highly stigmatizing. In advanced cases it may be fatal. Eumycetoma is most prevalent in what is known as the mycetoma belt.

Current treatment modalities for eumycetoma are disappointing. The response is characterized by low cure rates, high amputation rates, high drop out from follow up and high recurrence rates. The available drugs for the treatment of eumycetoma are expensive, potentially toxic and require a long treatment period up to 12 months. By that time the mass is well encapsulated and is removed surgically. Despite prolonged medical treatment, the causative organisms are commonly found to still be viable and can be cultured from the surgical specimen.

Study Design

This study will be a single-centre, comparative, randomized, double-blind, parallel-group, active-controlled, clinical superiority trial in subjects with eumycetoma requiring surgery.

There will be three arms in this study; all treatments are given for 12 months.

- Arm 1: The first arm will have fosravuconazole 300 mg weekly for 12 months.
- Arm 2: The second arm will have fosravuconazole 200 mg weekly for 12 months.
- Arm 3: The third arm will have itraconazole 400 mg daily for 12 months (as the standard treatment control arm).

After End of Treatment visit at 12 months, one more follow-up visit will be done at 15 months (End of Study visit).

Arms 1 and 2 will be evaluated at 3 months. At this time-point, one of the study arms will be dropped according to the drop-the-loser design, based on efficacy or toxicity.

An interim analysis is planned after data has been accumulated for sample size of 28 per arm for the 3-month's end point.

This study will be done at the Mycetoma Research Centre, Sudan. It is hoped that it will support the identification of a new, safer and more efficacious eumycetoma treatment.

Sample Size

A total of 138 subjects will be included in the study, 28 subjects per arm at the interim analysis. The number of females will be restricted to 20 initially and adjusted accordingly after interim analysis.

Participants

Subjects will be 15 years old or older with primary moderate eumycetoma (size 2 cm up to < 16 cm in diameter) caused by *Madurella mycetomatis* confirmed by molecular identification using Polymerase Chain Reaction (PCR).

Study Duration

Each subject's participation in the study will be approximately 15.5 months from screening to end of study visit. The recruitment period shall last 18 to 24 months based

on an estimated recruitment rate of 6 -10 patients per month. Therefore, the overall study duration is expected to be 36 to 42 months from first patient in (FPI) to last patient out (LPO). Considering the analysis and reporting period, the study shall last around 45 months.

Study Objectives

Primary Objectives

To determine whether, in addition to surgery, the selected fosravuconazole monotherapy of either 200 mg or 300 mg weekly is more effective (defined as complete cure at the End-of-Treatment [EOT; 52-week] visit) than the standard-of-care 12-month regimen of itraconazole monotherapy.

Secondary Objectives

- Assess the comparative efficacy of fosravuconazole 200 mg or 300 mg monotherapy versus that of itraconazole monotherapy. Determine whether each of the three regimens is compatible with the targeted efficacy rate of >70% of “cured” subject at EOT.
- Assess the comparative efficacy and toxicity of fosravuconazole 200 mg and 300 mg weekly doses at 3 and 15 months.
- Assess the comparative pathogen eradication rates for each of the three regimens at 6 months and at EOT or EOS using culture and qPCR.
- Assess the comparative efficacy of each arm by etiologic pathogen (subtype of fungus) and correlate outcome with in vitro susceptibility (pre-treatment, pre-surgery and post-treatment for failures).
- Evaluate the comparative rates of change in initial mycetoma lesion size over time and the rate of resolution of any pre-therapy clinical signs and/or symptoms over time for each regimen.
- Assess the comparative safety and tolerability profile for each regimen based on the incidence of adverse events and laboratory test abnormalities. Evaluate the comparative durability of response for each treatment regimen at the End-of-Study (EOS) visit.
- Evaluate the pharmacokinetics (PK) of ravuconazole and itraconazole in subjects with eumycetoma using population PK modeling.
- Assess relationships between PK of ravuconazole and itraconazole using pharmacogenetics studies.
- Measure the immune response before, at 3, 6, 9 and 12 months after treatment.
- Explore potential relationships between exposures to ravuconazole and itraconazole and pharmacodynamic (PD) parameters including, but not limited to, reduction in lesion size, reduction in viable pathogens, and the probability of achieving clinical endpoints (>50% reduction in lesion size with closure of >50% of sinuses (if present at t=0) at 6 months, and cure at 12 months), using population pharmacokinetic/pharmacodynamic (PK/PD) modeling.

1. Background and Study Rationale

Mycetoma is a specific, chronic, granulomatous, progressively morbid inflammatory disease. It usually involves the subcutaneous tissue, most probably after a traumatic inoculation of the causative organism. (1) Mycetoma may be caused by true fungi or by higher bacteria and hence it is usually classified as either eumycetoma or actinomycetoma, respectively. (2) The characteristic triad of a painless subcutaneous mass, multiple sinuses, and a discharge containing grains is pathognomonic of mycetoma. It usually spreads to involve the skin and the deep skin structures resulting in destruction, deformity, and loss of function, and occasionally it can be fatal. (3,4)

Mycetoma has a worldwide distribution, but its incidence is extremely uneven. It is endemic in tropical and subtropical regions; however, it has been reported from nations all over the globe. (1,4) It prevails in what is known as the mycetoma belt, stretching between the latitudes 15^0 South and 30^0 North. (5)

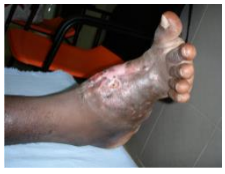
Figure 1: The Mycetoma Belt

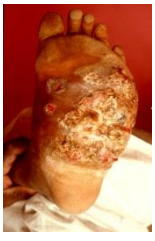
The geographic distribution of the individual mycetoma pathogens shows considerable variations, which can be explained on an environmental basis. These organisms are usually present in the soil. The infecting agent is most probably implanted into the host tissue through a breach of the skin as the result of trauma. (5)

No age is exempted, but mycetoma most commonly affects young adults between 15-30 years of age who are the wage-earning members of their society especially in developing countries. Mycetoma is seen most commonly in cultivators, field labourers, and herdsmen. In endemic areas, people of other occupations are affected as well. (6,7)

The clinical presentation of mycetoma is almost identical irrespective of the causal organism. It presents as a slowly progressive, painless, subcutaneous swelling at the site of previous trauma. Multiple secondary nodules then evolve; they may suppurate and drain through multiple sinus tracts. These sinuses may transiently close after discharge during the active phase of the disease. The discharge is usually serous, serosanguinous, or purulent. The sinuses discharge grains, the colour of which depends on the causative organism. (8)

Figure 2: Massive foot mycetoma





As the mycetoma granuloma increases in size, the overlying skin becomes attached and stretched, and increased local hyperhidrosis confined only to the mycetoma lesion is commonly observed. Mycetoma eventually invades the subcutaneous tissue, fat, muscles, and bone leading to massive deformities and destruction. Mycetoma is usually painless in nature. It has been suggested that the mycetoma produces substances that have a local anaesthetic action, and that may be one of the causes of the subjects' late presentation. (1,2,6,8)

In most subjects, the regional lymph nodes are small and spotty. An enlarged regional lymph node is common, and this may be due to a secondary bacterial infection, genuine lymphatic spread of the mycetoma organisms, or it may be due to immune complex deposition as part of a local immunological response to mycetoma infection. (1,2,3,5)

The most common site of mycetoma is the foot (79.2%). The hand ranks as the second most common site (6.6%). In endemic areas, other parts of the body may be involved, but less frequently, and these include the knee, arm, leg, head and neck, thigh, and the perineum. Rare sites such as the chest and abdominal walls, facial bones, mandible, paranasal sinuses, eyelid, vulva, orbit, scrotum, tongue, and oral cavity may be affected. (9-14)

A large variety of microorganisms are capable of producing mycetoma. (1,2,15) They can be identified by their textural description, morphology, and biological activities in pure culture. The grains are the source of the culture, and they should be alive and free of contaminants. Many culture media are in use. The culture technique is cumbersome as well as time consuming, and chance contamination may give a false-positive result. Therefore, other diagnostic methods, such as sequencing of the rDNA internal transcribed spacer region, fine needle aspiration (FNA) and histopathological examination, ultrasound. and serology such as the enzyme linked immunosorbent assay are also used in the diagnosis of mycetoma. However, in endemic areas, most of these techniques are not available. (16-20)

The treatment of mycetoma depends mainly on its aetiological agent and the extent of the disease. No case of self-cure has ever been reported in the medical literature. (21) To date, no proper clinical trial has been performed in eumycetoma. All the reports on mycetoma treatment are based on case reports, cases series, or personal experiences. In many centres, surgery is the most acceptable form of treatment for eumycetoma cases. Usually it is in the form of aggressive surgical excisions, debulking surgery, or amputation in advanced disease. (22) Unfortunately, surgery alone does not cure the disease, and pre- and post-operative antifungal treatment is always necessary to prevent recurrence of infection.

Reports of successful medical treatment of eumycetoma are few. A number of studies showed that itraconazole could cure eumycetoma subjects but only after prolonged treatment duration. The standard itraconazole dose used is 400-800 mg daily. The cure rate seems to be dose dependent and the drug's side effects are dose dependent too. Treatment of these subjects may continue for periods ranging from a few months to many years. (23) Itraconazole has numerous side effects with hepatic failure being the most serious one.

Recently, itraconazole was tried in the treatment of subjects with eumycetoma. It had a reasonable success rate, but the recurrence rate was high and the treatment was for at least one year in duration. Subjects showed good clinical responses to 200-400 mg itraconazole daily. Surgical exploration revealed that, in all subjects, the lesions

became well localized, were encapsulated with a thick capsule, and were then easily removed surgically. However, in all these lesions, grains were found and they grew viable fungi upon culture. This suggests that Itraconazole may only limit the extent of the infection instead of curing the disease. [24] Medical treatment should still be given pre- and post-operatively as it facilitates surgery, accelerates healing, and reduces the chance of relapse. Clinical observations showed that recurrence is more common after an incomplete course of medical treatment, and there is also a good chance for the organism to develop drug resistance. In an unpublished retrospective interim analysis of itraconazole administered for one year in a cohort of > 100 patient, the results showed an apparently high improvement rate at 6 months followed by a very low cure rate at 12 months.

In summary, the treatment of mycetoma is unsatisfactory. The treatment outcome is disappointing, characterized by a low cure rate and a high amputation rate. Subjects often aren't seen for follow-up, drop out of treatment, and have recurrence rates. [25]

Newer azole-drugs seem promising [26,27] and one of the most promising is ravuconazole. It is a potent investigational triazole compound that exhibits broad spectrum antifungal activity, has a remarkably long half-life in humans with a large volume of distribution, and has a good safety profile. [28]

Fosravuconazole is a parenterally and orally active monolysine prodrug salt of ravuconazole that is rapidly, enzymatically dephosphorylated in animals and humans, thereby generating ravuconazole. To be able to effectively treat mycetoma and onychomycosis, a fosravuconazole dosing regimen is needed that is safe, well tolerated, and effective but also readily complied with. Because of the prolonged half-life of ravuconazole, a loading dose strategy was considered necessary to rapidly reach target steady state levels. In Phase 1 studies, a variety of loading and maintenance doses were evaluated. These studies demonstrated that steady state ravuconazole concentrations were achievable in 7 days with a 2-stage dosing regimen (3 days BID, then 4 days QD). Further simulations suggest that following such a loading dose, short additional courses of daily or less frequent intermittent doses of fosravuconazole should provide drug levels comparable to those achieved with sustained daily (or more frequent) dosing with other azoles such as voriconazole and posaconazole, thus providing opportunities for treatment courses shorter than those currently in use. Fosravuconazole dosing regimens were chosen based on doses associated with a low probability for causing liver enzyme elevations in Phase 1 studies. Also, these doses were expected to rapidly provide and maintain plasma and tissues levels many fold greater than the MIC of *Madurella mycetomatis* and common dermatophytes throughout the dosing interval and were intended to safely maximize an optimal AUC/MIC ratio which is expected to pharmacodynamically correlate with efficacy.

In preclinical rat and monkey studies of labelled fosravuconazole, skin levels of resultant ravuconazole were at least 1.2 times the blood concentration of ravuconazole (in monkey) and skin levels were 1.2 times the blood level at day 1 post dosing. Skin levels were maintained up until day 7 after a single dose with the skin concentration decreasing to 0.16 times the day 1 skin concentration.

Pharmacokinetic modelling of steady state PK levels using NONMEM showed that plasma concentrations of resultant ravuconazole after dosing with fosravuconazole 200 mg on days 1, 2, and followed weekly thereafter with 200 mg weekly yielded steady concentrations that were several folds the MIC_{90} of ravuconazole against *Madurella*

mycetomatis [36].

Ravuconazole has *in vivo* activity that was determined in many animal models of infection which showed that it is effective in the treatment of mucosal candidiasis in mice, intra-abdominal abscesses due to *C. albicans* in rats, disseminated aspergillosis in guinea pigs, and systemic murine histoplasmosis. [29,30]

Ravuconazole proved to be highly active *in vitro* against *Aspergillus* spp., often more so than amphotericin B or itraconazole. Its activities were found to be comparable to those of voriconazole. [31] This azole agent also has inhibitory effects on some other species of filamentous fungi, such as *Scedosporium, Fusarium,* and *Chaetomium* as well as *Candida spp., Cryptococcus neoformans*, and other yeast species. [32,33]

The combination of ravuconazole and micafungin showed synergistic effects for the treatment of experimental pulmonary aspergillosis in neutropenic rabbits. This combination led to significant reductions in rates of mortality and fungal burdens compared with those achieved with mono-therapies. [34,35]

In a recent study, 25 *Madurella mycetomatis* strains were tested *in vitro* for susceptibility to ravuconazole. They were found to be significantly more susceptible towards ravuconazole than towards the currently used azoles, ketoconazole and itraconazole. [36]

Toxicity data from animal studies:

The results of a pre- and postnatal development study in rats, a 26-week rat toxicity (tox) study, and a 39-week toxicology study in monkeys have shown the following results:

- In the pre- and postnatal development study in rats, the no-observable-adverse-effect level (NOAEL) for F0 maternal general toxicity and reproductive function was 27.4 mg/kg. The NOAEL for F1 development was 8.2 mg/kg. The NOAEL for F1 reproductive function and F2 embryo development was 109.4 mg/kg.
- Results from the 26-week rat tox study (dosed at 0, 10, 30, and 60 mg/kg/day) revealed no death or moribundity related to the test article. There were no changes in clinical signs, body weight, food consumption, ophthalmoscopy, or urinalysis. There were increased adrenal weights in females at ≥30 mg/kg, increased adrenal vacuolation of cortical cells in both sexes at ≥30 mg/kg, and decreased triglycerides in males at 60 mg/kg. Based on these results, the NOAEL was considered to be 10 mg/kg.
- Results from the 39-week toxicity study in monkeys with ravuconazole doses of 1 to 30 mg/kg revealed no death or moribundity related to the test article. No changes were observed in clinical signs or food consumption, though one male monkey in the 30 mg/kg dose group demonstrated gradual body weight decrease throughout the dosing period (-11%, vs pre-dosing). There were no changes in ophthalmoscopy, ECG, urinalysis, or haematology measurements. Gross findings showed enlarged liver and adrenals. The histopathological changes in liver appeared to be related to adaptive hepatocellular hypertrophy. These findings were comparable to those observed in the previous 4- and 13-week toxicity studies at doses of 5 and 30 mg/kg. These were adaptive changes related to hepatic enzyme induction, and no evidence of progression or increased severity was observed after 39 weeks of administration. In the adrenal glands, increased vacuolation of the cortical cells in males at 30 mg/kg and females at 5 and 30 mg/kg; this was an expected class effect of azoles. A 3- to 4-fold increase in triglycerides was noted in the 30 mg/kg dose male

monkeys compared to control. Based on these results, NOAEL was considered to be 5 mg/kg for males and 1 mg/kg for females.

Table 1: Relationship between dose and expected average concentration of ravuconazole (Cav, total and unbound) in humans and expected ratio of Cav to MIC50 and MIC90.

Dose mg	Cav mg/L	Cav (unbound) mg/L (assumes 98% ppb)	Cav/MIC_{50} (0.004 mg/L)	Cav/MIC_{90} (0.016 mg/L)
200	1.4	0.028	7	1.7
300	2.1	0.042	10.5	2.6
400	2.9	0.058	14.5	3.6

The values presented in the table are based on steady state values obtained with weekly dosing.

Justification of dose selection of fosravuconazole in the study design

In terms of toxicity:
Based on the Table, the Cav obtained by fosravuconazole dosing of 200 and 300 mg (the latter being 5 mg/kg in a 60kg person) remain below the concentrations at which the NOAEL were found in the animal toxicity studies. Although for females the NOAEL was lower at 1 mg/kg, this could be random effect. Nevertheless, intensive monitoring for safety will be incorporated in the study and the number of females will be restricted to 20 initially. After this an interim analysis will be done and the restriction will be adjusted accordingly.

In terms of efficacy:
The 200mg regimen will give concentrations above MIC50 for all patients and the 300mg regimen will also achieve concentrations above MIC90, but not in all patients. To achieve concentrations above MIC90 in all patients, a 400mg regimen would be required, but this would be at the expense of considerable increased risk of toxicity and will therefore not be pursued.

Study Rationale

1. Current treatment modalities for eumycetoma are disappointing.
2. Response is characterized by low cure rates, high amputation rates, and subjects who drop out of follow-up with resultant high recurrence rates.
3. The available drugs for the treatment of eumycetoma require a long treatment period to affect partial cure and have serious side effects.

4. Despite prolonged medical treatment, the causative organisms are commonly found viable within the well-encapsulated lesions during surgery, after 12 months of treatment.
5. The prolonged use of the available drugs proved to be not cost effective by both the health authorities and patients.
6. A recently conducted *in vitro* study showed *M. mycetomatis* was highly susceptible to ravuconazole.
7. Recently, FDA and EMA recommended against the use of ketoconazole (the prior drug of choice for eumycetoma treatment) for any fungal infection except for life saving situations.
8. Fosravuconazole is an orally available prodrug of ravuconazole which is rapidly converted to ravuconazole and provides substantial pharmacokinetic benefits over ravuconazole itself.

2. Study Objectives and Endpoints

To determine the comparative efficacy, safety, and tolerability of fosravuconazole versus itraconazole as first-line treatment for subjects with eumycetoma caused by *Madurella mycetomatis.*

2.1. Objectives

2.1.1. Primary Objective

This superiority study aims to determine whether, in addition to surgery the selected fosravuconazole monotherapy of either 200 mg or 300 mg weekly is more effective (defined as complete cure at the End-of-Treatment [EOT; 52-week] visit) than the standard-of-care 12-month regimen of itraconazole monotherapy.

The selection of the dose to be tested for the primary objective will be based on a “drop the loser” design (see section on Trial design).

2.1.2. Secondary Objectives

- Assess the comparative efficacy of fosravuconazole 200 mg or 300 mg monotherapy versus that of itraconazole monotherapy. Determine whether each of the three regimens is compatible with the targeted efficacy rate of >70% of “cured” subject at EOT.
- Assess the comparative efficacy and toxicity of fosravuconazole 200 mg and 300 mg weekly doses at 3 and 15 months.
- Assess the comparative pathogen eradication rates for each of the three regimens at 6 months and at EOT or EOS as applicable.
- Assess the comparative efficacy of each arm by etiologic pathogen (subtype of fungus) and correlate outcome with in vitro susceptibility (pre-treatment, pre-surgery and post-treatment for failures).
- Evaluate the comparative rates of change in initial eumycetoma lesion size over time and the rate of resolution of any pre-therapy clinical signs and/or symptoms over time for each regimen.
- Assess the comparative safety and tolerability profile for each regimen based on the incidence of adverse events and laboratory test abnormalities. Evaluate the comparative durability of response for each treatment regimen at the End-of-Study (EOS) visit.
- Evaluate the pharmacokinetics (PK) of ravuconazole and itraconazole in subjects with eumycetoma using population PK modeling.

- Assess relationships between PK of ravuconazole and itraconazole and host genetics.
- Measure the immune response before, at 3, 6, 9 and 12 months after treatment.
- Explore potential relationships between exposures to ravuconazole and itraconazole and pharmacodynamic (PD) parameters including, but not limited to, reduction in lesion size, reduction in viable pathogens, and the probability of achieving clinical endpoints (>50% reduction in lesion size with closure of >50% of sinuses at 6 months, and cure at 12 months), using population pharmacokinetic/pharmacodynamic (PK/PD) modeling.

2.2. Study Endpoints

2.2.1. Primary Endpoint

Primary end-point will be complete cure at the 12-month End-of-Therapy Visit as evidenced by clinical assessment showing absence of eumycetoma mass, sinuses and discharge, normal MRI examination of the eumycetoma site, and a negative fungal culture from a surgical biopsy from the former eumycetoma site.

If there are no serious signals of lack of efficacy or safety, it is envisaged to conduct a 12-month follow-up study to assess the stability of the response.

2.2.2. Secondary Endpoint(s)

- The outcome (complete cure or not) at the 6 and 15month visit based on the same criteria as defined at 12-month EOT visit (see above) based on the clinical assessment showing absence of mass, sinuses or discharge, normal MRI examination, and a negative culture from a surgical biopsy of the former eumycetoma site.
- Treatment-related adverse events at the 3 and 15-month visits.
- Effective treatment (combination of mycological eradication and clinical improvement in the eumycetoma lesion) at EOT and EOS visits.
- Overall improvement (mycological eradication and ≤10% of the eumycetoma mass remaining) at EOT and EOS visits.
- Complete cure, effective treatment, and overall improvement categorized by etiologic fungal genus and species at EOT and EOS visits.
- Eradication of beta-glucan from peripheral blood.
- Immunological parameters indicating a switch from a Th2 to a Th1 response by EOT.
- Time to complete cure, time to effective treatment, and time to failure.

3. Study design and study design rationale

3.1. Study design

This is a single-centre, comparative, randomized, double-blind, parallel-group, active-controlled, clinical superiority trial in subjects with eumycetoma requiring surgery.

There will be three arms in this study:

- Two with the study drug fosravuconazole

- Arm 1: The first arm will have fosravuconazole 300 mg weekly,
- Arm 2: The second arm will have fosravuconazole 200 mg weekly,

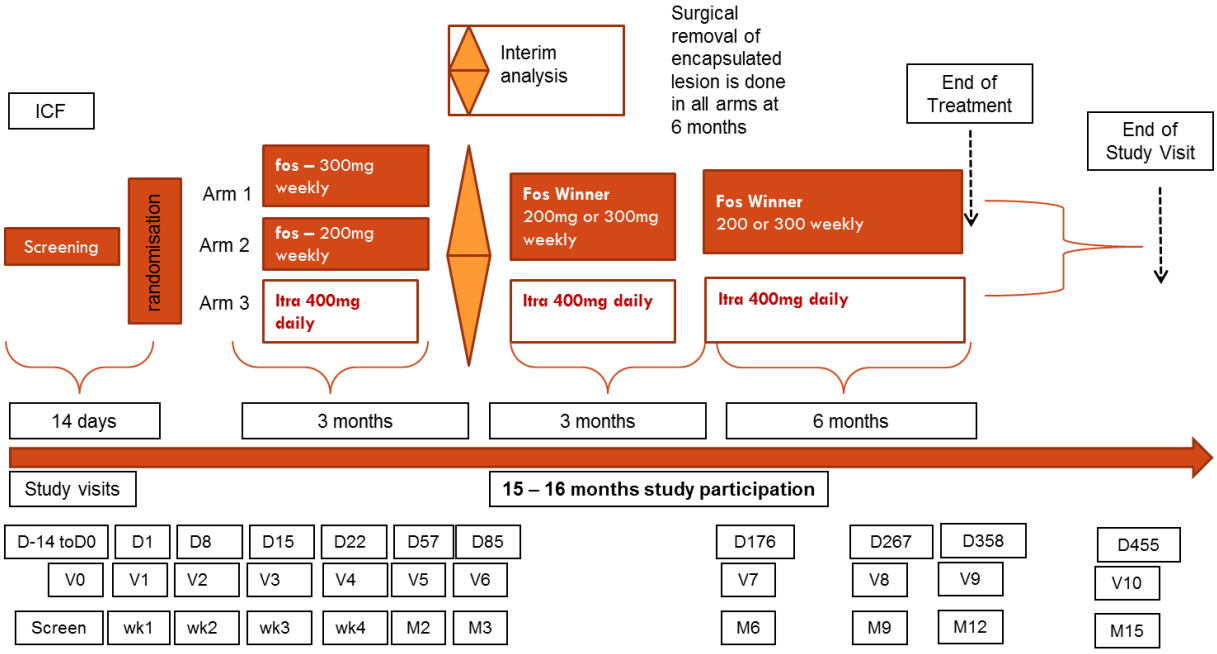
Both arms 1 and 2 will be evaluated at 3 months. At this 3-month time-point, one of the study arms will be dropped according to the drop-the-loser design, based on efficacy

or toxicity (see section 3.4 for drop the loser design at 3 months).

- Arm 3: The third arm will have itraconazole 400mg daily throughout the study for 12 months as the comparator arm.

Below is the summary of the drop the loser study trial design indicating the screening and treatment phase and the study visits.

Figure 3: Summary of the drop the loser study trial design



Fos= Fosravuconazole, Itra=itraconazole, D = Day, V= Visit, Wk= week M=month. Note: individual subjects cannot switch treatment

After the interim analysis, the Fosravuconazole loser arm will not recruit new subjects. The Fosravuconazole loser arm subjects will continue treatment until 12 months unless there is an indication for rescue treatment.

3.2. Randomisation

Subjects will be randomized to treatments utilizing a 1:1:1 allocation ratio up to the interim analysis and 1:1 once the loser is dropped.

3.3. Assumptions

At 3 Months

- fosravuconazole: >30% of subjects will have >50% reduction in size with closure of >50% of sinuses, if present at baseline.
- Itraconazole: ≥ 20% of subjects will have >50% reduction in size with closure of >50% of sinuses, if present at baseline.

At 12 Months

- Fosravuconazole efficacy: ≥70% of subjects will be cured;
- Itraconazole: ≥ 40% of subjects will be cured.

3.4. Drop-the-loser design at 3 Months

Interim analysis will be done when all arms have randomize 28 patients and they have reached 3 months follow-up (*see Sample size determination section 10.1 under Data Analysis and Statistical Methods*). It will be conducted by an independent statistician. Results will be communicated to the person in charge of the randomization to prepare the second randomization scheme which is the same as the first one but without the loser.

It will be based on responders as described above: N subjects with >50% reduction in size with closure of >50% of sinuses, if present at recruitment.

For the purpose of the interim analysis recruitment will be temporarily stopped and will restart with the selected arm.

In case of non-superiority of either study arm over itraconazole at 3 months, the DSMB may advise on continuation of the study based on efficacy and safety data of each arm at the interim analysis.

Table 2: Decision tree for drop the loser at 3 months

	Efficacy	**Safety**	**Decision**
Scenario 1			
fosravuconazole 300mg	>9/28 (>32.2%) **and** >10% to 200mg (excess cure rate)	Not worse than 200mg	
fosravuconazole 200mg	>9/28 (>32.2%)		Dropped
Itraconazole	≤ 9/28 (≤ 32.2%) or at least 10% less than 300mg		
Scenario 2			
fosravuconazole 300mg	> 9/28 (>32.2%) but not > 10% to 200mg		Dropped
fosravuconazole 200mg	> 9/28 (>32.2%)		
Itraconazole	≤ 9/28 (≤ 32.2%) or at least 10% less than 200 mg		
Scenario 3			
fosravuconazole 300mg	> 9/28 (>32.2%)		
fosravuconazole 200mg	≤ 9/28 (≤ 32.2%)		Dropped
Itraconazole	≤ 9/28 (≤ 32.2%) or at least 10% less than 300mg		

3.5. Study duration and duration of subject participation

The recruitment period shall last 18 to 24 months have based on recruitment rates under selection of subjects in section 4. Each subject's participation in the study will be approximately 12.5 months from screening to end of treatment outcomes. Therefore, the overall study duration is expected to be 30 to 36 months from first patient in (FPI) to last patient out (LPO). Considering the analysis and reporting period, the study shall last 39 months.

At three months of therapy and prior to surgery at 6 months of therapy, the response will be evaluated (assessing the attached efficacy parameters). With clinical improvement, treatment will be continued for a total of 12 months. If there is no evidence of improvement in the signs/symptoms or lesion size in any of the arms, the subject will be discontinued from the study, and rescue therapy initiated. After interim analysis, there will be no new recruitment in the Fosravuconazole loser arm and subjects on treatment will continue participation and treatment to the end of study unless there is an indication for rescue therapy. Recommended rescue therapy will be voriconazole (if available) or itraconazole at investigator discretion.

The end of the trial will be the date of the last patient last visit of the trial.

3.6. Rationale of the study design

This study will be a single-center, comparative, randomized, double-blind, parallel-group, active-controlled, clinical superiority trial in subjects with eumycetoma requiring surgery.

Drop-the-loser, an adaptive clinical trial design, allows two stages of the trial separated by a data based decision. In the first stage a decision will be taken on which arm to drop either the Fosravuconazole 300 or the Fosravuconazole 200 arm. The best treatment will be compared against the perceived best option of treatment. itraconazole. At the end, data can be pooled if the study continues to Phase III. Available study data could allow speedier and timelier study conclusions to be reached.

4. Selection of Subjects

This study will be conducted at the Mycetoma Research Centre (MRC) Soba University Hospital, in Khartoum, Sudan The Mycetoma Research Centre was established in 1991 and is a globally recognized world leader and an authoritative advisor in mycetoma research and management.

The MRC is a specialized centre in mycetoma diagnosis and treatment in Sudan. It is the main referral centre for cases seen in Sudan, Chad, eastern Africa, Yemen and Saudi Arabia. The centre has treated over 7,500 mycetoma cases. Each year approximately 300 new cases of mycetoma are seen which translates to 20-30 new cases per month. New cases come as referrals, walk-ins and through community outreach campaigns. Recruitment activities and routine laboratory work for the diagnosis of Mycetoma will be conducted at the MRC satellite sites in endemic areas for referral to the MRC for treatment.

Among new cases, the male to female ratio is approximately 3:1 with the majority being in the age group 15-30. Approximately 20% of new cases are under 18 years and the vast majority (99%) have a single lesion at diagnosis. Due to delay in diagnosis and referrals, a significant proportion of patients present late with advanced disease: 18% have lesions < 5 cm; 37% have lesions 5 -10 cm and 32% have lesions > 10 cm in diameter.

Subjects 15 years or older with eumycetoma caused by *Madurella mycetomatis* confirmed by molecular identification using PCR and meeting eligibility criteria will be selected to participate in the study. If the subject is a female of child bearing potential, they should be using adequate contraception for the period of the trial plus 2 months after they end their treatment in the trial. A total of 138 subjects will be enrolled, all at the MRC and MRC satellite sites in endemic areas.

Pre-screening of patients will be done in order to increase the chances of enrolling participants presenting for treatment at the MRC. Investigators should utilize active community screening to identify early cases in order to ensure the shortest possible recruitment period. Local health authorities will be informed of the study and the community screening methods. Whilst active pre-screening can support the identification of potential mycetoma patients, diagnosis still requires microbiological confirmation that can only be done at the MRC. For this purpose, potential mycetoma patients will be proposed to be referred to MRC and travel costs for them and accompanying person, covered by the Sponsor, irrespective of their further inclusion into the study.

Considering 20-30 patients seen per month, an estimated 60-70% who meet the amended age and lesion size criteria, and depending on recruitment methods and exclusions, there will be 6 patients enrolled/month. Thus, complete recruitment of 138 patients will be feasible over a period of 36 months.

Eligibility criteria must not be waived by the investigator. Any questions regarding a subject's eligibility should be discussed with DNDi Medical Coordinator prior to subject's enrolment.

4.1. Inclusion criteria

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Subjects with eumycetoma caused by *Madurella mycetomatis* confirmed by PCR.
2. Subjects with eumycetoma requiring surgery
3. Eumycetoma lesion ≥ 2 cm and < 16 cm in diameter
    - A lesion will be defined as either one single lesion on one anatomical area or multiple lesions on one anatomical area provided that the total affected area remains within the given limits
4. Age ≥ 15 years.
5. Able to comply with protocol procedures and available for follow-up.
6. Written informed consent from the subject. If the subject is less than 18 years old, there must be a signed consent from a parent or legal guardian AND a written assent signed by the subject.
7. Female specific inclusion criteria
    - Negative pregnancy test
    - If female of child bearing potential, using adequate contraception for the period of the trial to 2 months after completing study treatment.

4.2. Exclusion criteria

The presence of any of the following will exclude a subject from study enrolment:

1. Previous surgical or medical treatment for eumycetoma which includes with any previous antifungal treatment

2. Presence of loco-regional lymphatic extension, osteomyelitis, other bone involvement based on radiology or any pre- or co-existing condition that would preclude evaluation of the eumycetoma

3. Pregnancy or lactation at screening, intent to become pregnant

4. Concomitant or severe diseases that may compromise the subject follow-up or evaluation (psychiatric condition, chronic hepatitis, neutropenia, or HIV/AIDS, diabetes mellitus, adrenocortical insufficiency for example)

5. Severe malnutrition as defined by a Body Mass index (BMI) < 16

6. Contraindication to the use of itraconazole including congestive heart failure, ventricular dysfunction, ventricular arrhythmia, negative inotropic state. For a comprehensive list of contraindications and contraindicated concomitant medication refer to the package insert for itraconazole (Sporanox®)

7. Contraindication to the use of fosravuconazole

8. Pre-existing liver disease, transaminase levels >2 x the laboratory's ULN, or elevated levels of alkaline phosphatase or bilirubin

9. Receiving or likely to require drugs that are either a substrate for CYP3A4 and/or metabolized by CYP3A4 (cisapride, oral midazolam, nisoldipine, felodipine, pimozide, quinidine, dofetilide, triazolam, methodone, and levacetylmethadol [levomethadyl] are contraindicated)

10. QTcF >450 msec on any ECG known about or taken prior to entry

11. Subjects with Familial Short QT syndrome or QTc prolongation

12. History of hypersensitivity to any azole antifungal drug

13. Participation in other clinical trials within a six-month period.

5. Schedule of events

DNDi-FOSR-04 MYC (Schedule of Events)	Screening	Treatment Period									Follow up		Early withdrawal	
Visit(V) -Assessment day (D)	**V0: D(-14 - 0)**	**V1: D1**	**V2:D8**	**V3: D15**	**V4: D22**	**V5: D 57**	**V6: D85**	**V7: D176**	**V8: D267**	**V9: D358**	**V10: D455**		**VZ: D29-D469-**	
Week / month	**Week 0**	**week 1**	**week 2**	**week 3**	**week 4**	**month 2**	**month 3**	**month 6**	**month 9**	**month 12**	**month 15**		**Withdrawal**	
Alternative preferred visit day (recommended visit windows are shown in Section 8; Table 2)	None	None	D5-11	D12-18	D19-25	D50/D64	D78/D92	D169/D183	D253/D260 /D274/D281s	D351/D365	D441/D469		**Reason for withdrawal:-**	
													O-other	**P-patient choice**
Medical History, Physical Exam & eumycetoma exam	X	X	X	X	X	X	X	X	X	X	X		X **(o),**	X **(P)**
Vital signs (Temperature, BP, HR)	X	X	X	X	X	X	X	X	X	X	X		X **(o),**	X **(P)**
Obtain Informed consent & assent(if<18yrs/medical consent	X							**X**[a]						
Adverse Event reporting and concomitant medications	**X**[b]	X	X	X	X	X	X	X	X	X	X		X **(o)**	X **(p)**
Eligibility assessment and confirmation	X													
Enrolment (assign study ID and Randomize)		X[a]												
Drug adherence assessment and/ or Drug Dispensing	**X**[c∞]	X∞	**X**[c]	**X**[c]	X	X	X	X	X	X			**X (o)**[c]	**X (p)**[c]
Eumycetoma Surgery (hospitalization)								X						
Photography of eumycetoma lesion (2D & 3D - scan)	X	X	X	X	X	X	X	X	X	X	X		X **(o),**	
Ultrasound and Magnetic Resonance Imaging (MRI)	X							X		**X**[d]	**X**[d]		**X**[d]	
X-Ray	X									X				
12 lead ECG at rest	X				X		X	X		X	X		X **(o)**	
Biopsy –mycology, histopathology	X							X		‡X	‡X		*X **(o)**	
Culture sequence based identification (isolation of viable grains via trucut or surgical biopsy material)	X							X		‡X	‡X		*X **(o)**	
Full hemogram - purple top 4ml	X				X		X	X	X	X	X		X **(o),**	X **(P)**
Chemistry (Total Protein, Albumin, Cr, Urea, Na, K,, ALT, AST, ALP, TBil, DBil) & beta glucan – Red top 4ml	X				X		X	X	X	X	X		X **(o)**	X **(P)**
Morning serum cortisol yellow top 1x4ml ± Short ACTH stimulation test (t=0 & t=30min) 2x4ml	X				X		X	X	X	X	X		X **(o)**	X **(P)**
Urinalysis and *Pregnancy test – urine test	X				X	X	X	X	X	X	X		X **(o**	X **(P)**
HIV testing & counselling, Random blood glucose	X									X			X**(o)**	
Immunology (Immunoglobulins, Cytokines & Cells) 8ml		X					X	X	X	X			X **(o)**	
Pharmacokinetics (PK) – green top 3 ml		‡X[4]	X[1]	X[1]	*X[1]		X[3]	X[4]	X[1]	X[1]	X[1]		X **(o)**	
Pharmacogenetics – purple top 4ml and buccal swab		X												
Planned blood draw volume for the visit (ml)	16ml	24ml	3ml	3ml	19ml		29ml	32ml	23ml	23ml	15ml		15ml	(12ml)

For all follow up visits, ensure as much as possible that subject visits are timed on the same day of the week as Day 1 of treatment.

If reason for withdrawal is patient choice, invasive or research tests should not be done as study procedure. At early withdrawal visit (VZ), ***X**(o) *means medical decision needed for invasive test*

Notes for the Schedule of Events

- **Medical history, physical exam and eumycetoma lesion exam**: Perform detailed screening (V0) history and exam, A targeted history and exam can be done thereafter. Examine eumycetoma lesion at each visit to record evolution of the lesion and features. Measure the size of the lesion by applying tape measure or caliper at the site of maximum diameter and width from a fixed point. Assess for active, non-active and healed sinuses Specify the fixed point in the source notes.
- **Vital signs**: Perform vital signs at each scheduled study visit before any invasive tests are carried out e.g. biopsy, phlebotomy, PK sampling including imaging etc. For consistency, use the same methods and equipment throughout the study. [Height (cm) will be measured at screening (V0) only.; Weight (kg) in light clothing without shoes shall be recorded at each study visit. BMI calculation should be calculated once using screening (V0) Height and Weight, Axillary temperature (^{o}C) should be recorded at each study visit; Blood pressure (mmHg): Subjects should rest for at least 5 minutes prior to measurement. Measure diastolic and systolic BP at each study visit. BP cuff at heart level].
- **Informed consent:** Obtain informed consent for all subjects and assent if under 18 years – all assent forms must have a complementary consent form from the parent /legal guardian. Study specific procedures done after informed consent are 3D photos, ECG, MRI, immunology, PK, morning serum cortisol, Short ACTH stimulation test, β-glucan, pharmacogenetics, study specific questionnaires. At Day 176 visit (V7) **(X^{a})**, obtain medical consent for surgery per national guidelines.
- **Adverse Event Reporting:** See adverse event reporting section 8 for details. During **(X^{b})** i.e. after informed consent to screen out or enrolment report all SAEs and / or study related AEs.
- **Concomitant medications:** Record all concomitant medications for pre-existing disease and Adverse Events and check for contraindicated medications at every visit throughout the study.
- **Eligibility and enrolment:** Carefully review each eligibility criteria. Only assign Study ID (PID) and randomize after confirming eligibility. A subject is considered enrolled when randomized even if study drug is not taken. Day 1 is the first day the subject starts study treatment and must be preferably the same day (X^{a}) or within 24 hours of assigning Study ID and randomization.
- **Drug dispensing and adherence assessment:** During V0 & V1 ($X\infty$) only adherence training to be conducted. During **(X^{c})** and **($X(o)^{c}$ or $X(p)^{c}$)** fill questionnaire only (no study drug dispensed). Dispense drug only after randomization. Give instruction for proper drug use and storage. Recommend to the patient to take the drug at approximately the same times each day in the morning and evening after a full meal. No repeat dose even if vomiting occurs or drug accidentally falls to the ground. First doses and doses during PK days must be directly observed at the clinic and time administered recorded. Dispense an adequate amount of study medication with extra (buffer) doses. Refill and review drugs preferably monthly. Complete standard questionnaire(s) for drug accountability, dispensing and adherence assessment. Make progress notes at each visit. Record any new or ongoing treatment problems or concerns. Review pregnancy results for females and record findings each time prior to dispensing the next dose.
- **Hospitalization:** Pre-plan hospitalization for surgery at Day 176 (Visit 7). Subjects may also be hospitalized for PK assessments or for enrolment procedures if it is more convenient for them.
- **Photography:** At every study visit take two and three dimensional (2D photographs and 3D imaging of the eumycetoma lesion. Take 2D photographs with and without a tape measure or caliper next to the lesion. Use the same fixed point as above for the tape measure or caliper for reference. Take 3D imaging for serial measurement for size and volume of lesion.
- **X-ray, Ultrasound & MRI:** take **two x-ray views** of mycetoma (lateral and AP). Do US&MRI **(X^{d})** on Day 358 (V9), Day 455 (V10) or Early Withdrawal visit (VZ)
- **ECG:** At screening visit (V0) perform a single ECG after 5 min rest. Do not repeat ECG at screening visit (V0) if result significantly deviates from exclusion criteria. At V1-V10 ECGs will be performed once if the report is assessed as normal or abnormal not clinically significant. If ECG is abnormal and clinically significant, repeat the ECG after at least 20 min rest, for confirmation.
- **Biopsy:** Tru cut needle biopsies will be done to obtain grains and tissue for histopathological diagnosis and culture. Several grains will be transferred to sabouraud plates and cultured. ‡ at Day 358 (V9) or Day 455 (V10) a biopsy will be done after ultrasound.
- **Blood sampling and immunology:** Follow protocol blood collection schedule. Adhere to blood volumes and tubes. Always record actual date and time of blood draw, not theoretical time. Lab safeties will be performed at the local lab e.g. hematology or clinical chemistry, morning serum cortisol ± short ACTH. Recall the subject for review if at discharge the labs are abnormal and clinically significant. See immune responses section of protocol for volumes and tubes for blood draw.
- **Urinalysis and *Pregnancy test:** Do urinalysis on clean catch specimen on scheduled visits. Do Urine Pregnancy test monthly and before every drug refill. Time the drug refills visits with scheduled visits where possible. Record Last Menstrual Period. Menstrual history must be consistent with negative pregnancy results before drug can be dispensed or refilled.
- **HIV testing and Random Blood Sugar:** Follow national HIV pre- and posttest counseling and use national HIV testing algorithm. Perform Random blood sugar and HIV test at V0 and V9.
- **Early withdrawal visit (VZ):** Procedures depend on reason for withdrawal. **X** (P) = patient reason, **X** (O)= other reason (specify). Document subject agreement for **X** (P) tests.

Blood sampling for PK analysis will be done as shown below: Record exact date(s)/time(s) of all drug administration and PK sampling times.

- **On, Week 1**, Day 1 (Visit 1), subjects will take their first dose of either fosravuconazole or itraconazole in the morning, Blood will be drawn at **four** time points (‡X^{4}) for determination of blood concentrations of ravuconazole and itraconazole; one at each of the following collection time windows: ‡pre-dose , 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-dose..- **On Week 2**, Day 8 (Visit 2) and week 3 Day 15 (Visit 3), Month 9, Day 267 (Visit 8), Month 12, Day 358 (Visit 9) at the (EOT) and Day 455 (Visit 10) at End of Study visit (EOS) and at early withdrawal visit (VZ); blood will be drawn at a **single** time point (X^{1}) prior to the morning dose (**pre**-dose sample).- **On, Week 4** Day 22 (Visit 4), subjects will be instructed to take the morning dose under observation at the site; blood will be drawn at **single** time point (*X^{1}) within 2 to 6 hours **post**-dose.- **On Month 3** Day 85 (Visit 6), subjects will be instructed to take the morning dose under observation at the site; blood will be drawn at **three** time points (X^{3}), one at each of the following collection time windows: 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours, post-dose. **On Month 6,** Day 176 (Visit 7), following surgery, blood will be drawn at **four**-time points (X^{4}), each one at the following collection time windows: prior to the morning dose (pre-dose sample), 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-dose.

6. Enrolment procedures

As noted, patients come to the Mycetoma Research Centre passively or through referral from health professionals. The referrals are usually based on clinical signs and symptoms and high index of suspicion. The MRC also has an outreach clinic periodically. The clinic is performed by mobile teams who regularly visit highly endemic communities. Outreach clinics identify early cases and will boost enrolment to the clinical trial as patients with clinically severe diseases seen at referral clinic are excluded from study participation.

Subjects who present to MRC either passively or through referral, equal to or greater than 15 years who fulfill the inclusion, do not have any exclusion criteria, and from whom informed consent/assent has been obtained, will be enrolled in the study.

Patients who enter screening assessments for this trial will be documented in a Patient Screening/Enrolment Log. The patients included in the screening log will not be limited to patients who have undergone consent process. Each patient who meets eligibility criteria will be assigned a unique study number after randomization. The study number may be the same as randomization number and will be recorded in the Patient Screening/ Enrolment Log. A note describing the Informed consent process and enrolment in the trial will also be made in the patient's file.

Treatment should be started within 14 days after the screening assessment. Otherwise compliance with eligibility criteria must be reassessed (blood tests) except if screening is awaiting PCR from culture or subculture.

An update on the enrolment status of subjects will be sent to the study monitor on a weekly basis. Recruitment will stop for interim analysis once 84 subjects are enrolled in the study. The number of female subjects will initially be restricted to 20 after which an interim analysis will be done to assess toxicity and safety in comparison with male subjects. After interim analysis the study enrolment will continue based on written DSMB recommendation.

7. Study treatments

7.1. DNDi study treatment

Name: Fosravuconazole
Class: Azole
Mechanism of action: The parent compound, ravuconazole, has broad spectrum antimicrobial activity through inhibition of ergosterol biosynthesis similar to other azole antifungals
Commercial source: Eisai
Product appearance: Fosravuconazole (E1224) capsules are size #3 capsules containing 100 mg of E1224 drug substance. The colors of the cap and body are red and yellow, respectively, and each of them has the word “Eisai” printed on them. The capsule content is prepared by blending Fosravuconazole (E1224) drug substance and excipients.
Administration: Orally administered

Name: Placebo
Class: N/A
Mechanism of action: N/A
Product appearance: Similar to study drugs

Administration: To be administered as Fosravuconazole to mask drug administration regime
Drug interactions: N/A

There is no placebo arm in this study. The placebo is for purposes of maintaining study blind so that each subject will take the same number of capsules daily which appear the same irrespective of the study arm.

7.2. Comparator study treatment

Name: Itraconazole
Class: Azole
Mechanism of action: In vitro studies have demonstrated that itraconazole inhibits the cytochrome P450 dependent synthesis of ergosterol, which is a vital component of fungal cell membranes.
Commercial source: Janssen
Product appearance: It is a white to slightly yellowish powder, insoluble in water at pH 1-12, very slightly soluble in alcohol and freely soluble in dichloromethane. SPORANOX capsules contain itraconazole 100 mg, sucrose, starch, hypromellose and macrogol 20,000 in a hard gelatin capsule.
Administration: It is essential that SPORANOX capsules are taken immediately after a meal for maximal absorption. The capsules must be swallowed whole.

7.3. Doses and treatment regimens

Subjects will be treated with fosravuconazole 200 mg or 300 mg weekly dose versus itraconazole 400 mg daily, all three arms in combination with surgery after 6 months.

Itraconazole is the standard-of-care treatment in Sudan for eumycetoma - itraconazole 400 mg will be administered for a maximum duration of 12 months in its monotherapy arm.

Fosravuconazole will be given in a dose of 200 mg or 300 mg weekly for a total of 12 months; one of the arms will be dropped after evaluation at 3 months according to the drop-the-loser design (see under Trial design).

Fosravuconazole 200 mg - loading dose of 200 mg on Days 1, 2, and 3 followed by weekly doses of 200 mg for a total duration of 12 months.

Fosravuconazole 300 mg - loading dose of 300 mg on Days 1, 2, and 3 followed by weekly doses of 300 mg for a total duration of 12 months.

Subjects will not be aware of the loading dose. All doses are to be taken after a meal (breakfast or evening meal).

Blinding will be done by over encapsulating the itraconazole using the same size capsule. Subjects should not be re-dosed if for example they vomit, miss a dose, medicines fall to the ground or any other reason as the study is blinded and it will not be apparent which medicine is the placebo or active drug.

Treatments will be administered twice a day to conform with the standard administration of itraconazole and maximize. The drugs should be taken immediately after a full meal to maximize bioavailability of itraconazole.

7.4. Study treatments labelling, packaging

Study medications are packaged as wallets which contains capsules. There are two types of wallets a) Week 1 wallet which contains 37 capsules (to accommodate loading

dose) and b) Week 2-52 wallets which contains 31 capsules per wallet. Subjects will receive a week 1 wallet at initial dispensing and subsequently boxes of 4 x week 2-52 wallets. Drugs will be dispensed to provide approximately one-month dose per dispensing (see images in annex 2). The study medications are prepared per subject and sufficient to last the entire duration for the subject's participation. The sequence of medication has been defined to ensure the active or placebo medications are taken in the correct order without potentially unblinding the study or affecting PK assessments. There is consideration for extra or buffer capsules in case of scheduled visit delay. Extra or buffer capsules shall not be given during the last drug dispensing visit.

Treatment is dependent on the randomization arm. Each wallet bag will be labeled in a way that maintains double blinding. Appropriate translations will be included. The pharmacist will also be blinded but will keep a copy of the blinded Subject identification code list and Master randomization list. The pharmacist must agree in writing to maintain confidentiality and the blind except for circumstances provided under procedures for unblinding.

At each refill visit, all the wallets with both unopened and empty blisters that remain from the previous dispensing visit shall be returned to the pharmacy. The returned drugs will be kept separately and will not be dispensed again but kept for accountability purposes (except for buffer). Any reasons for non-return of the wallet or remaining medications should be noted before dispensing the next drugs.

Package labeling will be in accordance with the administration schedule and local regulatory requirements. Local translations will be done for all patient instructions and labels.

7.5. Drug Accountability

Study medication must be kept in a locked room that can be accessed only by the pharmacist or personnel designated by the investigator. The study medications must not be used for other purposes other than this protocol. Under no circumstances may the investigator or site staff supply study medications to other investigators or sites, or allow the medications to be used other than as directed by this protocol without prior authorization from DNDi.

Specific study forms for drug accountability will be designed to allow for adequate records on receipt, use, return, loss, or other disposition of medication. These accountability forms must be maintained in a locked cabinet by the study pharmacist or designated personnel. Study treatments should be brought back by the patients so that the accountability is done by the site staff. The compliance is assessed and reported in the Case Report Form (CRF). The returned study medications must not be disposed or destroyed without sponsor authorization.

7.6. Storage

Study drug must be kept in a locked cabinet and/or in a room with restricted access, under the control of the study pharmacist. The investigational drugs should not be stored above 30ºC in accordance with Sporanox® patient information leaflet. Do not freeze. Study drugs will be kept in a restricted access, temperature-controlled pharmacy at MRC. Daily temperature logs will be kept and monitored. Patients will be instructed to keep the medicines away from direct sunlight in a cool place at home.

7.7. Blinding and procedures for unblinding

This is a randomized double-blind study. The subject or any caregiver, investigator and outcomes assessors will be blinded to treatment assignments. Blinding will be maintained by using the same study procedures for all subjects including PK sampling, similar appearing study medication, similar drug administration regime and similar labelling. Study medication blinding will be done by over encapsulating the itraconazole using the same size capsule and colour. Fosravuconazole placebo capsules have been provided with the actives from Eisai. The placebo capsules will only be used for purposes of masking the frequency of administration. There is no placebo arm in this study.

Each subject will receive the same number of capsules. Each treatment kit will be labeled with a Subject number and instructions for use, but the treatment assignment will not be accessible to the Investigators or the subjects. To maintain blinding, labeling of the drugs and subject instructions will be the identical. Therefore, some usual information in labels e.g. expiry dates will be excluded for identical labeling of the study drugs to be done. Labels will be submitted for approval.

To permit easy identification of the subject for the predetermined unblinded study / sponsor staff, a subject identification log will be kept. The log cannot be accessed by unauthorized persons except when knowledge of the identity of the medication is required for treating the subject. If possible, DNDi should be contacted before breaking the code. If the blind is broken, the Investigator should document the date, time of day and reason for code breaking. Subjects will continue to be followed up to the End of Study even when the blind has been broken unless there is a reason to terminate their participation in the study.

Breaking the code of any trial patient should only be performed by the investigator or by an authorized person. The breaking of the code can ONLY be performed when the result of a life threatening (SAE) medical emergency may depend on the knowledge of the treatment allocation of a given study subject, which may be useful to treat him/her.

The DNDi clinical trial manager or the trial monitor must be immediately informed about the need of unblinding.

A Serious Adverse Event form should also be filled out including with the treatment allocation & if the reason for code-breaking meets the criteria.

Unblinding may not necessarily justify patient withdrawal from the trial; this will be discussed by the project team and Investigator on a case by case basis, and should be documented. In such cases, these patients should also continue with trial visits and assessments as planned.

7.8. Concomitant treatments

All concomitant conditions must be documented. Fluids, blood, herbal medications and other non-conventional treatment must be reported. Common conditions such as malaria and respiratory tract infections should be treated prior to starting treatment. The concomitant medications will be recorded in the CRF.

The prescribing information for all co-administered medications should be carefully reviewed and listed warnings or contra-indications must be respected. Contraindication to the use of itraconazole include congestive heart failure, ventricular dysfunction, ventricular arrhythmia, negative inotropic state. For a comprehensive list of precautions, contraindications and contraindicated concomitant medication refer to the package insert or summary of product characteristics (Sporonox®).

Fosravuconazole (E1224) is contraindicated in patients with a prior hypersensitivity reaction to E1224 or other azole drugs. Co-administration with Rifampin is also contraindicated. For a comprehensive list of precautions, contraindications and contraindicated concomitant medication refer to the investigator brochure for Fosravuconazole (E1224).

In general, drugs that are either a substrate for CYP3A4 and/or metabolized by CYP3A4 (cisapride, oral midazolam, nisoldipine, felodipine, pimozide, quinidine, dofetilide, triazolam, methodone, and levacetylmethadol [levomethadyl] are contraindicated from 14 days prior to baseline assessments to the End of Treatment

Subjects with Familial Short QT syndrome or QTc prolongation may not participate in the study.

7.9. Rescue treatments

In case of treatment failure or intolerability (treatment must be permanently interrupted) and rescue treatment will be provided to the subject, as the decision of the study physician. It is expected that the rescue treatment will be voriconazole (if available) or itraconazole and / or surgery as appropriate. Subject participation in the study will end when rescue treatment is initiated.

Subject withdrawal will be classified as a) patient choice e.g. consent withdrawal or b) other (reason must be specified).

Subjects will therefore be offered rescue treatment out of study for the following reasons;

- Patient choice: Withdrawal of study participation consent
- Other reasons which can include:
  - Occurrence of pregnancy or other protocol exclusions during treatment that may jeopardize patient safety
  - Lack of efficacy or inadequate response to treatment including recurrence of symptoms, signs of presence of *Madurella mycetomatis*
  - Occurrence of adverse event(s) during receipt of test drugs that requires permanent treatment interruption including failure to tolerate trial medication
  - Rescue treatment is indicated based on investigator judgment (see section 9)

Rescue medications *are medication given to the patients in case of failure of study treatment.*

7.10. Medical care after trial has ended

At the end of study participation or the trial all patients will continue follow up at the MRC which is routine for all mycetoma patients.

8. Study Assessments

8.1. Timing of Assessments

Every subject considered for screening will be registered into a screening log and the Case Report Form (CRF). Subjects will be enrolled into the trial database until recruitment to that arm is stopped. For example, once the interim analysis has been done, the fosravuconazole loser arm will be closed to new recruitment and only subjects already enrolled into that arm will continue assigned treatment and follow up.

Subject Assessments will be carried out during the screening period week 0 (day -14 to 0, Visit 0). The treatment period will last from Day 1 to Day 358 with respective schedule days named by convention as follows; Week 1 (Day 1, Visit 1), Week 2 (Day 8, Visit 2), Week 3 (Day 15, Visit 3), Week 4 (Day 22, Visit 4), Month 2 (Day 57, Visit 5), Month 3 (Day 85, Visit 6), Month 6 (Day 176, Visit 7), Month 9 (Day 267, Visit 8) and Month 12 (Day 358, Visit 9). An early withdrawal (Vz) will be conducted for subjects who end study participation prematurely. Hereafter within the protocol, except for screening and early withdrawal, scheduled visits are referred by specific visit day and abbreviation of the visit number e.g. Day 176 (V7) instead of month 6 visit to avoid ambiguity of the exact evaluation day. However, for consent purposes and ease of understanding, the natural way the language is spoken will be used e.g. ‘the seventh study visit will occur on month 6 and will involve the following procedures....’. A hypothetical calendar will be used as a study tool to aide understanding (see appendix 1).

The study procedures in this protocol include clinical assessments (history, physical exam, mycetoma lesion exam, ECG and vital signs), study specific procedures (consent, adverse event reporting, full eligibility assessment, ID assignment and randomization), lab assessments (haematology, clinical chemistry, urinalysis, pregnancy test, HIV, biopsy for PCR and histopathology), medical imaging (ultrasound, x-ray, MRI and photography), research tests (pharmacokinetics, immune responses, pharmacogenetics), adherence questionnaires, hospitalization and surgery. Most of these assessments are routine at the MRC the exceptions being the study specific procedures and research specific assessments. The exact timing of the assessments is presented in a table with the schedule of events listed under section 5.

There will be two steps before a subject can qualify to participate in the study. In the first step, subjects who present as potential subjects will be pre-screened using a checklist that combines diagnosis and study eligibility requirements. The initial assessments will be done to confirm eumycetoma diagnosis. These will include ultrasound, and routine Tru-cut needle biopsy for histopathology from deep tissue. From this material, grains culture will be done and a sample will be used for PCR to confirm the diagnosis. Subjects who fulfill the partial protocol eligibility criteria listed in a pre-screen checklist and have highly suspect or confirmed eumycetoma will be requested for consent. The pre-screen checklist will include rapidly assessable protocol eligibility criteria e.g. age, lactation, pregnancy, previous treatment of mycetoma etc. This first step is done to minimize inconvenience for patients who do not obviously qualify and ensure efficient study recruitment. In the second step, the potential subjects with confirmed eumycetoma who satisfy the pre-screening criteria and have consented to study participation will have the remaining and complete eligibility schedule of event procedures conducted to verify if they meet the full eligibility criteria to join the study. Assessments done in the screening period or prior to treatment

start (whichever is the worst) will be recorded in the CRF and used as baseline or reference for subsequent assessments in the subsequent scheduled study visits.

All screening (V0) procedures will be done on outpatient basis except when hospitalization is considered necessary by the clinician. Only after obtaining informed consent can the investigator conduct other study specific procedures, and research specific assessments. Research specific assessments in this protocol are 3D imaging, ECG, immunology, PK, serum cortisol (if needed, the short ACTH stimulation test), β-glucan, pharmacogenetics, study specific questionnaires.

After enrolment all subject will follow visits according to the schedule of events and should be willing to adhere to protocol requirements including keeping the follow up visits. Allowable windows and preferred alternative visit days are presented in the Table 2. These windows and preferred alternative visit days consider drug sequencing, timing of PK and timings of the next study visit. Protocol deviations should be recorded for missed or out of window schedule visits.

Unscheduled visits shall be planned to assess, confirm and /or follow-up on clinically significant / relevant AEs or laboratory abnormalities.

Table 3: Schedule of Events: Allowable windows and preferred alternative visit days

Visit number	Week	Visit day (Target Day)	Allowed Window	Other Preferred Day if visit is not done on target day
Visit 0	Screen	Screening	None	N/A
Visit 1	Week 1	Day 1	None	N/A
Visit 2	Week 2	Day 8	± 3 days	N/A
Visit 3	Week 3	Day 15	± 3 days	N/A
Visit 4	Week 4	Day 22	± 3 days	N/A
Visit 5	Month 2	Day 57	± 7 days	D50 or D64
Visit 6	Month 3	Day 85	± 7 days	D78 or D92
Visit 7	Month 6	Day 176	± 7 days	D169 or D183
Visit 8	Month 9	Day 267	± 14 days	D253 or D260 or D274 or D281
Visit 9	Month 12	Day 358	± 7 days	D351 or D365
Visit 10	Month 15	Day 455	±14 days	N/A

Note: If preferred date i.e. ‘target day’ is not possible, then other ‘Preferred Day’ would be better to synchronize PK studies. If both are not achievable then use any day of the window allowed and perform all scheduled PK on that visit date.

Subjects who have early withdrawal (Vz) or complete treatment period Day 358 (visit 9) will be referred to continue routine medical follow up in the MRC clinic. Follow up is routine practice for all patients registered and treated at the MRC. The routine follows up is done to assess for patient wellness, recurrence or any new problems.

8.2. Screening V0 Assessments.

The following screening procedures or assessments will be done during screening (V0) to confirm eumycetoma diagnosis and verify full eligibility by evaluating inclusion and exclusion criteria per patient. Since screening evaluations or evaluations prior to drug administration will be used as the baseline reference values for the subject, all screening (V0) must be done before the first intake of study drug. It is recommended that the equipment and methods used for baseline assessments should be consistent or the same ones used in the subsequent scheduled visit for clinical assessments e.g. vital sign measurements, lab and imaging equipment and procedures with appropriate

quality control.

- Informed Consent Process (must be prior to 3D imaging, ECG, MRI, PK, immunology, pharmacogenetics and study specific questionnaires)
- Demographic data namely age and gender
- Medical history: The screening (V0) history should be detailed. History to be documented includes mycetoma symptoms onset and duration, recurrence, family history, trauma, presence of pain and discharge, course of illness. Relevant history, previous medical or surgical treatment, concomitant medications and check on contra-indicated medications and any other pertinent history. The last Menstrual Period date will be recorded for females.
    - Clinical examination: The screening (V0) physical exam should be detailed. Physical examination will include clinical signs of mycetoma, body weight, height, BMI, vital signs (temperature, heart rate and blood pressure), whether skin is normal or abnormal, discharge colour, The examination of mycetoma lesion will involve recording site, size (by using a tape measure or calliper that is applied at the site of maximum diameter and width from a fixed point), consistency, sinuses, status of healing (i.e. active, non-active and healed sinuses), grains, discharge, whether enlargement of affected lymph nodes, presence of varicose veins, sweating. Nutritional status will be assessed using WHO BMI for age charts.
- Clinical laboratory evaluations and blood samples: Screening (V0) labs include
    - Haematology: Haemoglobin, total WBC with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelets, Peripheral Blood Film comment will be included.
    - Biochemistry: Total protein, Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL) Creatinine (CRE), Urea, Alkaline Phosphatase (ALP), Sodium (Na) and Potassium (K) and
        - Serum cortisol: a morning blood sample will be taken for cortisol levels. A short ACTH stimulation test may be done to assess adreno-corticol function. The short ACTH stimulation test will be done if the morning serum cortisol is below the clinical threshold or cut off. The short ACTH will be done by taking the first blood sample (t=0min) for morning cortisol level then a 250 ug dose of ACTH (tetracosactide) will be administered by either intravenous (i.v.) or intramuscular (i.m.) injection. The second blood sample for cortisol will be taken at 30 minutes post injection (cut-off for a normal response: cortisol >500 nmol/L at t=30 min). The date and time of the pre- and post-injection sample will be recorded with time of ACTH injection. A cortisol rise of > 500 nmol/l is considered an adequate response
    - Beta glucan – this is a β-D-glucose polysaccharide naturally occurring in the cell walls of cereals, yeast, bacteria, and a biomarker for fungal infections with regard to the response to treatment.
    - Random blood glucose

  - Urinalysis and Urine pregnancy tests
  - HIV test
- ECG: At screening (V0) before the first intake of trial medication, a 12 lead ECG will be done in supine position after at least 5 minutes rest. A repeat ECG must not be done at screening visit if it meets exclusion except for technical reasons. Twelve-lead ECGs will be recorded at a paper speed of 25 mm/s so that the different ECG intervals (RR, PR, QRS, QT) will be measured. The QT-intervals will be corrected for heart rate according to Bazett's (QTcB) and Fridericia's (QTcF) QT correction. Preferably, all ECGs will be read and interpreted by one and the same qualified site person throughout the study.
- Medical Imaging evaluations - The recommended technical specifications will be provided in the study manual where appropriate for imaging or photography. It is recommended that the same equipment and technique be used throughout the study.
  - Ultrasound and Magnetic Resonance Imaging (MRI) evaluations: At screening (V0) Ultrasound and Magnetic Resonance Imaging (MRI) will be used as part of the diagnostic workup to assess for evidence of mycetoma and extension of disease. The mycetoma size (3 dimensions) and extent, number of sinuses echogenicity of the grains, acoustic enhancement will be recorded. Each ultrasound and Magnetic Resonance Imaging (MRI) will be appropriately labelled and printed in hard copy and read locally. If available a copy of the digital image will be kept at the centre.
  - X-ray: two views of the affected anatomical site will be done at screening (V0). The main purpose is to exclude bone involvement. Each x-ray will be appropriately labelled and hard copy kept in file. If available a copy of digital x-ray will be kept at the centre.
  - Photography: Two-Dimensional (2D) and Three-Dimensional (3D) imaging will be taken at screening (V0) using strictly standardized conditions. The 2D photograph will be taken with and without a tape measure or calliper next to the lesion. 3D imaging will be done using a scanner and the images will be uploaded onto a computer with appropriate software; local staff will be trained, and the interpretation of the images will be done by the 3 D innovation lab, Free University, Amsterdam.
- Mycology, and histopathology: A routine Tru-cut biopsy to obtain deep tissue will be taken. (Part of the biopsy will be used for culture and DNA extraction; the other part will be embedded into paraffin. Care should be taken as grains will not be viable if they are only preserved in formalin. PBS or normal saline are needed for viable grains and DNA isolation. From the grains taken for culture, a sample will be analysed by PCR. Superficial tissue is considered inadequate for histopathology for this study and thus Fine Needle Aspiration is not recommended and should not be done. From this a culture will be taken and a sample will be analysed by PCR. The details of the procedures will be provided in standard operating procedures.
- Adherence assessment questionnaire - Adherence to the trial medications will be assessed via the Study Adherence Questionnaire. The Study Adherence Questionnaire will be completed by a study nurse or pharmacist. Adherence to trial

medication will also be assessed by pill counts at each visit.

8.3. Early withdrawal (VZ) Assessments

If an enrolled subject is prematurely terminating study participation (outside scheduled visits), an early withdrawal visit (VZ) shall be conducted. If the premature termination is occurring during a scheduled visit, the early termination procedures will take precedence. The early withdrawal visit (VZ) reason must be documented. Early withdrawal visit (VZ) procedures will depend on the reason for withdrawal e.g. consent withdrawn. The reasons may be either a) subject choice or b) other reason (specify reason). Appropriate exit procedures and referral for continuity of care must be given in all instances. Medical judgment and decision to perform invasive tests at exit will be needed as well as prior to Day 29.

When the reason for early withdrawal is 'Other', the reason for early termination must be documented e.g. safety issue, lack of efficacy or others accordingly. The assessments at this early withdrawal visit (VZ) shall seek to document safety and conduct research procedures for outcomes assessment.

When the reason for early termination is subject choice, assessments should be limited to safety after documenting patient agreement for any such assessment.

8.4. Assessment of Efficacy

8.4.1. Clinical assessments of eumycetoma

The medical review (since the last visit), physical exam and eumycetoma lesion examinations will be done at every scheduled study visit. An assessment will be made at each visit as to whether the lesion is improving, is static, worse or cured based on the predefined criteria or has recurred after apparent cure. Changes in eumycetoma lesions including recurrence are part of efficacy analysis and should not be reported as (S)AE.

8.4.2. Clinical Laboratory Safety assessments

Hematology parameters after screening (V0) will be done on Day 22 (V4), Day 85 (V6), Day 176 (V7), Day 267 (V8), Day 358 (V9) and Day 455 (V10). Hematocrit, hemoglobin, mean corpuscular volume (MCV), white blood cell (WBC) count with differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils) and platelet count will be done. Where manual WBC differential count is done, the absolute differential count recorded in the CRF will be obtained by multiplying the relative differential count (%) by total WBC count.

Biochemistry and beta glucan: - Total protein, Albumin, Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL) Creatinine (CRE), Urea, Alkaline Phosphatase, Sodium and Potassium, after screening (V0), will be done on Day 22 (V4), Day 85 (V6), Day 176 (V7), Day 267 (V8), Day 358 (V9) and Day 455 (V10). Beta glucan will be scheduled with clinical biochemistry.

Serum cortisol (morning) ± Short ACTH stimulation test, serum cortisol after screening (V0), will be done on Day 22 (V4), Day 85 (V6), pre-surgery Day 176 (V7) and Day 358 (V9). The short ACTH stimulation test will be done if the morning cortisol level is below the predefined threshold.

Random blood sugar, after screening (V0), will be tested at Day 358 (V9).

Urinalysis after screening (V0), will be done on Day 22 (V4), Day 57 (V5), Day 85 (V6), Day 176 (V7), Day 267 (V8), Day 358 (V9) and Day 455 (V10) whereas the urine pregnancy test will be done before each drug refill visit which will be monthly. Drug refill visits should be done with scheduled visits where possible. The menstrual history and Last Menstrual Period shall be recorded and it must be consistent with pregnancy results prior to drug dispensing.

HIV testing after screening (V0), will be done on Day 358 (V9). HIV test and Random blood sugar can be done from the single finger prick. The national guidelines will be used for HIV pre- and post-test counseling.

Hematology samples will be taken in 2ml EDTA tubes. Biochemistry samples will require 2ml of serum/plasma processed from whole blood collected in 4ml plain tube. Random blood sugar and HIV test will be done using a drop of blood. Lab safety samples will be analyzed at the MRC lab. Local reference lab values will be used for normal ranges. For detailed blood draw volumes see table under ethical considerations and schedule of events. Any test may be repeated within the study when clinically indicated.

8.4.3. Adherence assessments

Study drug adherence will be assessed at each study visit by a Study Adherence Questionnaire. Adherence counselling should begin at screening (V0). The Study Adherence Questionnaire will be completed by a study nurse or pharmacist which will include questions on general experience with the treatment, timing of doses, which if any are easier to take, relation to meals, subject reminder system, use of peers with the pharmacist's assessment of compliance and documentation of any problems and interventions with regards to adherence(V2-V9). Adherence to trial medication will also be assessed by pill counts at each visit and recorded together with any difficulties or new issues. The nurse or pharmacist must communicate with the investigator the findings of the assessment and any issues that need to be addressed. Drug dispensing will not be done at screening (V0) and at the end of treatment visit Day 358 (V9).

8.4.4. Medical Imaging

8.4.4.1. Photography

Photography with both 2D and 3D cameras will be done at every scheduled study visit using strictly standardized conditions. The 2D photographs will be taken with and without a tape measure or caliper next to the lesion from fixed point, preferably done by a medical photographer or trained health worker. Digital and print copies of the lesions will be stored. Photographs will be taken using the same device in the same conditions for standardization. The fixed point will be recorded.

3D images will be taken as indicated in the schedule of events using a scanner; sequential images will be able to estimate the reduction in size at each time point compared with baseline and previous time-points. This will be done by computer – assisted analysis using specially designed software. The results will not be used in the criteria for cure or reduction of size of the lesion, but will allow validation of 3D imaging in comparison with standard assessment (clinical, ultrasound, MRI).

8.4.4.2. Ultrasonography and Magnetic Resonance Imaging (MRI)

Ultrasound and Magnetic Resonance Imaging (MRI) assessment of the eumycetoma lesion after screening (V0) will be done on Day 176 (V7). This procedure will also be repeated at Day 358 (V9), Day 455 (V10) or at an Early Withdrawal visit (VZ) if a

mycetoma mass is present. The serial ultrasound and Magnetic Resonance Imaging (MRI) evaluations will be done to diagnose presence of eumycetoma and the size and volume of the lesion will be recorded. Using MRI, the volume of the lesion will be quantified and used to assess reduction in size at various time-points.

8.4.4.3. Radiology –x-ray

A radiological assessment using a plain x-ray of the affected anatomical site will be done in two views, lateral and anteroposterior. After screening (V0) x-ray will be done at the End of Treatment Day 358 (V9). This is to minimize exposure to radiation. The purpose of X-ray is to detect any new bone involvement. Each x-ray assessment will record findings pertaining to the presence or absence of a soft tissue mass, periosteal reaction or bone destruction.

8.4.5. ECG

A 12 lead ECG will be done in a supine position after at least 5 minutes rest. After enrolment, a repeat ECG is allowed and if requested it must be separated by at least 20 minutes after adequate rest. When an ECG is repeated to control for an abnormal reading, both records should be entered the CRF.

After screening (V0), ECG will be done on Day 22 (V4), Day 85 (V6), Day 176 (V7), Day 358 (V9) and Day 455 (V10).

The ECG will be read locally by the investigator who may consult a local cardiologist when needed. All study related ECGs will be sent to a central reader for purposes of standardization which in this study will be Cardiabase.

8.4.6. Mycology and Histopathology assessment

8.4.6.1. Mycology

Mycology assessment will be done at Screening (V0), Day 176 (V7), and Day 358 (V9) or Day 455 (V10). Grains culture and PCR identification will be done in any case for day 358/EOT. A tru-cut biopsy will be done to obtain the grains. PCR identification will be done directly on grains and on cultures. Direct identification on grains will be done with the *M. mycetomatis* specific PCR in the laboratory of the Mycetoma Research Centre. This identification will be confirmed by sequencing the ITS region at the ErasmusMC as this is currently considered the gold standard. Furthermore, for each strain also the ERG11 gene (target of the azoles) will be sequenced to determine if under therapeutic pressure mutations in the target gene occur and strains will be typed with the VNTR (variable number tandem repeat) typing system. In vitro susceptibility will be performed before start of treatment, during treatment (at the time of the surgery) and after treatment, if viable isolates are still obtained.

All strains will be kept in a culture collection at 37^{o}C. Strains will be shipped to the ErasmusMC, the Netherlands on culture medium at room temperature.

Furthermore, at these timepoints serum samples will be taken which can be used to measure antigens and beta-glucan levels over time. Beta-glucan can be found in fungal cell walls in patients with eumycetoma lesions.

8.4.6.2. Histopathology

Histopathology assessment will be done at pre/screening (V0), Day 176 (V7), and if a mass is present on Day 358 (V9) or Day 455 (V10). If there is a residual or recurrent lesion, tissue reaction type and any other pertinent findings shall be recorded. Grains and tissue reaction shall be recorded as Type 1 (Neutrophils only), Type 2 (Neutrophils and multinucleated giant cells) and, Type 3 (Neutrophils, multinucleated giant cells and granulomatous reaction). A tru-cut biopsy will be done and a paraffin block prepared at screening and at the end of treatment (Day 358). On Day 176 (V7) the surgical material obtained from surgery will be used for histopathology and paraffin block prepared.

8.5. Pharmacokinetics Assessments

8.5.1. Pharmacokinetic Assessments

PK assessments will be performed as follows

- On Week 1, Day 1 (Visit 1), subjects will take their first dose of either fosravuconazole or itraconazole in the morning. Blood will be drawn at four-time points for determination of blood concentrations of ravuconazole and itraconazole; one at each of the following collection time windows: pre-dose, 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-dose.

- On Week 2, Day 8 (Visit 2) and week 3 Day 15 (Visit 3), Month 9, Day 267 (Visit 8), Month 12, Day 358 (Visit 9) at the end of treatment (EOT) and Day 455 (Visit 10) and at early withdrawal visit (VZ); blood will be drawn at a single time point (X1) prior to the morning dose (pre-dose sample).

- On, Week 4 Day 22 (Visit 4), subjects will be instructed to take the morning dose under observation at the site; blood will be drawn at single time point within 2 to 6 hours post-dose.

- On Month 3 Day 85 (Visit 6), subjects will be instructed to take the morning dose under observation at the site; blood will be drawn at three-time points one at each of the following collection time windows: 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours, post-dose.

- On Month 6, Day 176 (Visit 7), following surgery, blood will be drawn at four-time points (X4), each one at the following collection time windows: prior to the morning dose (pre-dose sample), 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-doseThe table below provides a guide on the PK sampling schedule Table 3. It is reflected in the Schedule of Events notes.

Table 4: PK sampling schedule

Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Day	D0	D1	D8	D15	D22	D57	D85	D176	D267	D358	D455
Week/month	Wk0	Wk1	Wk2	Wk3	Wk4	Mo2	Mo3	Mo6	Mo9	Mo12	Mo15
Pre-dose		X	X	X				X	X	X	X
Hour 0 (reference time)											
2-4hrs post-dose		X			X		X	X			
6-12hrs post-dose		X					X	X			
24-96hrs post-dose		X					X	X			

Hour 0 for each visit will be the single reference point based for timing of all PK assessments of that visit. It is based on actual time a drug was administered. All the drug administration dates and time will be recorded where PK sampling span over more than one dose. At month 12 & 15, hour 0 is not applicable and PK sample will be collected at any time during the visit.

In case of accidental drug overdose outside schedule PK days, one 3ml sample will be collected stating the date and time of sample collection with exact or best estimate of drug time and date of drug overdose administration.

The exact time of drug administration and time of PK samples collection will be recorded. Where sample collection for a specific visit is done across more than one drug administration time point, all drug administration times shall be recorded. This particularly applies to visits with multiple PK sampling time points.

PK analysis will be done using plasma samples. At each PK time point, 3 ml whole blood will be collected into a heparin-lithium tube. The whole blood will be centrifuged for 5 minutes at 1900 g (room temperature) to obtain plasma which will be stored in polypropylene cryotubes preferably at -40°C until shipment.

Stored PK samples will be shipped for analysis of ravuconazole or itraconazole content at the Radboud University Medical Center, Division of Pharmacy, 6525 GA Nijmegen, The Netherlands following a protein precipitation method using Ultraperformance Liquid Chromatography with fluorescence detection (UPLC-UV). The assay for ravuconazole has been validated over the range of 0.02mg/L- 20 mg/L according to the bioanalytical method validation procedure as published by the European Medicines Agency white paper. [38] .

The development and validation of the assay for itraconazole (and metabolite) has previously been published.

8.5.2. Pharmacogenetics analysis

Pharmacogenetics will be done on Day 1 V1, from 2ml plasma and / or buccal swabs. The buccal sample and four ml whole blood will be collected, processed and stored at screening visit (V1) only. The samples will be kept frozen and preferably -40C to -80C until analysis. Samples will be shipped to the Erasmus MC, the Netherlands. Polymorphisms in CYP3A4 phenotype expression will be the target. The analysis might resolve unanticipated or significant issues related to inter-individual variation in efficacy or safety. Both samples will allow comparability of the results and stability.

8.6. Culture sequence based identification

PCR identification assessment will be done at screening (V0), Day 176 (V7) and if a mass is present on Day 358 (V9) or Day 455 (V10). A tru-cut biopsy will preferably be done at screening (V0) and end of treatment Day 358 (V9) or Day 455 (V10). At 6 months the material obtained from surgery will be used. PCR can be done from biopsy, culture or subcultures. Screening can be extended for PCR from culture.

The material will be split. Grains will be washed in normal saline and split. Part of it will be used for direct DNA isolation, part of it will be inoculated on sabouraud agar. The part used for direct DNA isolation will be immediately diluted with DNA stabilizing buffer solution (L6 buffer) and can then be stored up to 2 years at frozen temperature. DNA directly from grains will be isolated with the DNeasy extraction kit after bead-beating with chrome steel balls. The *Madurella mycetomatis* specific PCR will be used for identification. Cultured isolates will be sent regularly to Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands for molecular identification based on ITS sequencing and sequencing of the ERG11 genes. VNTR typing will be performed.

8.7. Immune responses

Immune responses will be assessed on Day 1 (V1) before drug administration, Day 85 (V6), Day 176 (V7), Day 267 (V8) and Day 358 (V9).

Immunoglobins will be measured in whole blood by ELISA. A blood sample of 3 ml in plain tube will be collected for IgG (total and IgG 1-4 subtypes), IgM, IgA, IgD and IgE levels.

Cytokines will be measured in whole blood by ELISA. A 3ml Lithium heparin tubes blood sample will be collected for measurement of Cytokines. IL-2, IFNɣ, TNFα, IL-10, IL-4, IL-5, TGFβ, and Granzyme B will be measured in the stimulated whole blood supernatant by ELISA.

Lymphocyte subsets will be identified from 2.0 ml of EDTA blood collected for flowcytometry; The subsets include CD4, CD8, CD45, CD3, CD19, NK cells and T regulatory cells at the same time points for immunoglobulin and cytokines.

Serum samples and supernatant will be stored at -20°C and analysed as a batch at the Mycetoma Research Centre or the Institute of Endemic Diseases Khartoum, Sudan.

8.8. Surgery

Subjects will be hospitalized at Day 176 (V7) visit and surgery will be done. The purpose is to completely remove any remaining mycetoma mass. Surgery assessment will record all details of surgery performed including the extent of excision, and bone involvement.

Anaesthesia will be standardized as much as possible, recorded and reported as concomitant medication. Unnecessary cross checks between anesthesia used against Adverse Events will not be done except if non-standard anesthesia is used.

Any complications during or after surgery will be reported and recorded as adverse events. Unplanned surgery or hospitalization will be recorded as (S)AE as appropriate. Hospitalization for surgery is not an SAE.

8.9. Assessment of Safety

Safety of the treatments will be assessed through routine monitoring of adverse events (AE). At each study visit, the subjects will be asked about current adverse events or any events observed during the period before the visit. In addition, regular measurement of vital signs and physical examinations, evaluation of hematology and blood chemistry parameters, urinalysis, ECG will be made at scheduled follow-up visits. See section 8.10 for AE definition and reporting.

Also, patients will be advised to return to the clinic on any day during the follow-up period if they present any medical occurrence, as to allow for AE assessments at any unscheduled visits.

The adverse event will be reported as verbatim, preferably with a diagnosis (if available) or signs and symptoms. In addition, all relevant details e.g. AE start and end date, relationship to study drug, outcome, action taken on study drug, any concomitant medications given, and seriousness of the AE. AEs or SAEs that lead to treatment discontinuation will be recorded. Follow up of cardiac and liver safety is detailed below.

<u>Cardiac Safety:</u>

ECG recordings will be performed at screening (V0), Day 22 (V4), Day 85 (V6), Day 176 (V7), Day 358 (V9) and Day 455 (V10). ECG should be done to assess for cardiac adverse events. To identify possible electrocardiographic abnormalities. If clinically significant abnormalities are judged by the investigators at these visits, unscheduled ECG should be performed until normalization or stabilization.

- Any patient with a QTc, post dosing <310 msec or with a QTc, post dosing <350 msec accompanied of cardiac symptoms, shall be withdrawn (Specific criteria for treatment discontinuation are described in sections 9.1 and 9.2). In those cases, a full cardiac assessment should be performed, including ECG, Echocardiogram and Holter.
- QTc> 500 msec at any time during treatment
- Any patient with a delta QTc >60 msec post dosing will have the trial medication permanently interrupted and will follow the standard trial schedule. For the cases where in addition to the delta QTc >60 msec post-dosing, cardiac symptoms are present, an ECG should be performed at unscheduled visits.
- As needed a cardiologist should be consulted based medical judgment of the investigator.

- Cardiac safety experts will periodically review all ECGs and present information to the DSMB. Central reading of the ECGs will constitute the ECG database. In addition, the cardiac safety experts from Cardiabase will review all Cardiac System Organ Class (SOC) AEs; and all non-Cardiac SOC that have the potential to be related to Cardiac AEs.

Liver Safety:

Liver function tests will be performed at screening (V0), Day 22 (V4), Day 85 (V6), Day 176 (V7), Day 267 (V8), Day 358 (V9) and Day 455 (V10).

As part of the liver risk assessment plan, treatment discontinuation should be considered if:

- ALT or AST >8xULN (to be reported as Adverse Event of Special Interest see section 8.10.3)
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN and (Total Bilirubin >2x ULN or prothrombin time INR >1.5) (Hy Law's criteria- seriousness criteria; see section 8.10.2)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

Liver enzymes will be repeated as soon as possible after the initial abnormality is identified. After that, the patient should be followed every 96 hours (4 days), or the closest possible interval, until the serum ALT and/or AST falls below 3 X ULN or stabilizes. If a patient develops symptoms of liver injury (fatigue, nausea, right upper quadrant pain, dark urine, or jaundice), s/he will be instructed to immediately contact the trial site for evaluation.

If the serum ALT exceeds 8 X ULN at any time or elevation of serum bilirubin >2 X ULN, the patient should be immediately withdrawn from the study.

All data on liver safety will be presented to the DSMB for review.

8.10. Adverse event definitions and reporting

Study safety regulatory reporting will be done in accordance with the applicable regulatory requirements.

8.10.1. Adverse Event definition

An adverse event will be defined as "*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment".*

Abnormal laboratory (hematology and biochemistry) results will be reported as adverse events if the abnormality occurs or worsens after institution of the study treatment, and assessed by the investigator as "clinically significant", e.g. if they require clinical intervention or further investigation, unless they are associated with an already reported clinical event.

An AE therefore can be any unfavourable and unintended sign (e.g. an abnormal laboratory finding, ECG), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Definition of an AE includes worsening (in severity and frequency) of pre-existing

conditions (“Medical history”) before first Investigational Medicinal Product (IMP) administration and abnormalities of procedures (i.e. ECG, X-ray…) or laboratory results which are assessed as “clinically significant”.

What is not an AE?

- Medical conditions present at the initial study visit that do not worsen in severity or frequency during the study are not considered as AE.
- Symptoms, exacerbation or worsening of the studied disease will NOT to be considered as AE nor captured on the AE page of the CRF if consistent with the anticipated natural progression of the disease (overall and for this given subject).
- Lack of efficacy of the IMP is not considered as AE.

8.10.2. Serious Adverse Event

An adverse event will be defined as serious if it is

- **Fatal**

  i.e. causes or contributes to the death.

- **life-threatening**

  In this context refers to an Adverse Event / adverse Reaction (AE/AR) in which the subject was at risk of death at the time of the AE/AR; it does not refer to an AE/AR that hypothetically might have caused death if more severe.

- **requires or prolongs hospitalization**

  i.e. the AE requires at least an overnight admission or prolongs a hospitalization beyond the expected length of stay.

  Standard management for eumycetoma anticipates hospitalization. Such planned admission does not qualify for an SAE.

  Hospital admissions for surgery planned before study entry, for social reasons, for any elective surgery (i.e. plastic surgery) or for standard management of eumycetoma (including treatment adjustment, planned hospital admission or if a patient is lodged for convenience for PK assessments to be done) are NOT to be considered as SAE according to this criterion (i.e. because the protocol or the standard management of the disease under study requires planned hospitalization).

  If a subject presents with an AE that requires hospitalization for more than 24hs beyond the anticipated discharge after planned procedures, the event will be considered an SAE except if the subject remains hospitalized for social reasons.

- **results in persistent or significant disability**

  i.e. the AE resulted in a substantial disruption of the subject’s ability to conduct normal activities.

- **is a congenital anomaly/birth defect**

  i.e. an adverse event outcome in a child or foetus of a subject exposed to the Investigational Medicinal Product (or marketed medicinal product) before conception or during pregnancy.

- **Liver function abnormalities reaching any Hy Law criteria**

  Liver function abnormalities reaching any Hy Law criteria (**ALT or AST > 3 x ULN in combination with total bilirubin elevation >2 x ULN or prothrombin time INR > 1.5)** will be considered as serious AEs if there is no evidence of obstructive disease and no other reason can be found to explain the combination of

aminotransferase and total bilirubin elevation. Subjects who experience these liver abnormalities should permanently be discontinued from treatment and be withdrawn from the trial. Liver enzymes should be followed until resolution or until return to baseline levels. Other possible causes of similar observed injury include viral hepatitis A, B, or C, preexisting or acute liver disease, or other drugs and should be investigated in case of such occurrence.

- results in an **important medical event** that may not be immediately life threatening or does not directly result in death or hospitalization, but which may jeopardize the patient or may require intervention to prevent the other outcomes listed above

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room, or at home for allergic bronchospasm, blood dyscrasias, blood transfusions, or convulsions that do not result in hospitalization or development of dependency or abuse or overdose.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event/reaction.

8.10.3. Adverse Event of Special Interest (AESI) or Medical Event of Special Interest (MESI):

AESI or MESI is defined as a non-serious adverse event of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the Sponsor may be appropriate. Such an event may require further investigation to characterize and understand it.

-**Short ACTH test abnormalities.**

Confirmed abnormal low morning cortisol AND abnormal short ACTH test results will be reported as an AESI; confirmation of an abnormal result will be done by repeating the short ACTH stimulation test after 2-7 days of an initial abnormal result or as specified in detail in the study manual. If repeat testing for confirmation is not done or feasible, the initial abnormal value will be considered confirmed and reported as an AESI. Subjects with an abnormal short ACTH test i.e. abnormal cortisol levels after stimulation with ACTH may continue treatment with close monitoring or treatment which can include supplementation with hydrocortisone and continuation in the trial or discontinuation based on medical judgment e.g. if the subject is symptomatic early soon after starting study drugs.

-**Liver function abnormalities > 8*ULN**

Liver enzymes will be repeated as soon as possible after the initial abnormality is identified and then every 96 hours (4 days), or the closest possible interval, until the serum ALT and/or AST falls below 3 X ULN or stabilizes. If patients develop symptoms of liver injury (fatigue, nausea, right upper quadrant pain, dark urine, or jaundice), they will be told to immediately contact the study site for evaluation. If the serum ALT exceeds 8 X ULN at any time or elevation of serum bilirubin >2 X ULN, the patient should be immediately withdrawn from the study.

AESI /MESI, are not SAEs per se (from a regulatory point of view) but the Sponsor shall be informed in an expedited manner within same timelines (irrespective of the grade of the AESI / MESI) as required for SAEs but using the AESI form. Shall they meet any seriousness criteria (8.10.2) they should then comply for associated safety reporting requirements and be reported on BOTH the AE CRF and SAE form.

8.10.4. Eliciting Adverse Event information

The investigator is required to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject. The investigator should use concise medical terminology to describe the adverse events. Trial subjects will be questioned about the occurrence of adverse events at each study visit (scheduled and non-scheduled) from the time of the previous study visit, as well as follow-up on the evolution from previously recorded adverse event. Generic questions such as *"Since the last visit have you had any health problem?"* will be used.

8.10.5. Adverse Event reporting period

The adverse events reporting period begins upon subject enrolment in the trial (after signature of informed consent) and ends at the predefined period specified below.

The adverse events reporting period for this trial will be as follows:

- Serious adverse events: **All SAEs** must be reported upon subject enrolment in the trial (**after signature of informed consent** during *screening period*) **until the end of the subject participation in the trial**.
- Non-serious adverse events: **All non-serious AEs** will be reported upon administration of the first dose of trial medication **until the end of subject participation in the trial**.
- Non-serious AEs that occur in the *screening period* AND are judged as study-related (e.g. hematoma due to venipuncture) will be reported in the AE CRF.
- Screening failure: beyond the date of screening failure (to be recorded), only serious study-related events will be followed-up.

All adverse events that occur during the adverse event reporting period specified in the protocol must be reported to DNDi, whether the event is considered related to study medication or not.

In addition, any serious adverse event that occurs subsequent to the adverse event reporting period that the investigator assesses as related (with at least a reasonable possibility) to the investigational medication should also be reported as an adverse event.

Reporting period for exposure in utero events are described in section 8.10.11.

8.10.6. Adverse Event reporting requirements

Information on adverse events must be evaluated by a physician. Each adverse event is to be classified by the investigator as serious or non-serious (includes AESI). This classification will determine the reporting procedure for the event.

All serious adverse events (SAE) and protocol pre-defined (AESI) events are **to be reported immediately (within 24 hours of awareness of SAE by the investigato**r) by using the SAE report form or the AESI form (respectively) to a pre-specified DNDi Mycetoma study safety team group email (SAEMycetoma@dndi.org and stated in the study manual or SOPs). SAEs must also be reported to Ethics or Regulatory

Authorities in accordance to current regulations. Where reporting is initially done by telephone this must be followed as soon as possible in writing through email or other document such as fax as evidence of communication. The SAE or AESI report will include, patient ID, a description of the event, onset date and type, duration, severity, relationship to study drug, outcome, measures taken and all other relevant clinical and laboratory data with the reporter's name.

The initial report is to be followed by submission of additional information (follow-up SAE form or follow-up AESI form) as it becomes available.

Any follow-up reports should be submitted as soon as possible and if possible within 5 working days from knowledge of new information.

As applicable, relevant forms will be used for reporting of pregnancies or their outcome to the Sponsor (as required by protocol section 8.10.11). The DNDi clinical team will be responsible for forwarding all SAEs, pregnancy and pregnancy outcomes reports to DNDi PV (pharmacovigilace@dndi.org) within the timelines agreed with Eisai.

Table 5: Investigator adverse event reporting timelines and to whom

Safety event	Reporting timeline	To whom*
SAE	24 hours	1. DNDi safety (SAEMycetoma@dndi.org) 2. EC / NRAs (as applicable)
AESI / MESI	24 hours	DNDi safety (SAEMycetoma@dndi.org)
Pregnancy/ pregnancy outcome/child reports	24 hours	DNDi safety (SAEMycetoma@dndi.org)
Other Non-Serious AE (i.e. not in above categories)	Fill the CRF	Entered to DNDi Clinical database

***Note:** All SAEs, AESI, Pregnancy and its outcome must also be reported in the CRF

Serious adverse events should also be reported on the clinical trial adverse event CRF. It should be noted that the form for reporting of SAE (i.e. SAE form) is not the same as the adverse event section of the CRF which must also be filled. The data collected in the two forms must be completed in a consistent manner, and the same medical terminology and ultimately reconciled.

Non-serious adverse events (including AESI) are to be reported on the CRF in addition to the AESI form, which is to be submitted to DNDi as specified in the Study Documentation section of this protocol. In the CRF, a given adverse event will be recorded only one time per patient, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF.

8.10.7. Grading of Adverse Event severity

Severity grading is used to describe the medical intensity of an AE. The severity for an AE should be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) and adapted for specific criteria e.g. neutrophils and haemoglobin or as specified in the study manual.

In case of AEs that are not described in the CTCAE v 4.03, the investigator will use the terminology MILD, MODERATE, SEVERE or LIFE-THREATENING to describe the maximum severity of the adverse event as follows:

This information will be entered in the adverse event case report forms. For purposes of consistency, these severity grades are defined as follows:

MILD	Does not interfere with subject's usual functions
MODERATE	Interferes to some extent with subject's usual functions
SEVERE	Interferes significantly with subject's usual functions

It is to be noted the distinction between severity and seriousness of adverse events. A severe adverse event is not necessarily a serious event neither is serious adverse event necessarily a severe event.

8.10.8. Adverse Event causality assessment For both serious and non-serious adverse events, the investigator is required to assess the relationship between the adverse event and the study drug, i.e. to determine whether there exists a reasonable possibility that the study drug caused or contributed to the adverse event. To help investigators with the decision binary tree in the evaluation of causality, the CIOMS VI group recommends that investigators be asked to consider the following before reaching a decision:

- Medical history
- Lack of efficacy/worsening of existing condition
- Study medications
- Other medications (concomitant or previous)
- Withdrawal of study medication, especially following trial discontinuation / end of study medication
- Erroneous treatment with study medication (or concomitant)
- Protocol related procedure

The decision to suspend, and resume treatment or to permanently interrupt treatment due to an adverse event will be made by the clinician in charge who should accordingly follow the protocol and consult in taking the decision.

The causality assessment of an AE to the investigational medicinal product will be rated as follows:

Not related:	There is no reasonable possibility of causal relationship.
Related:	There is at least a reasonable possibility of a causal relationship between an adverse event and an investigational medicinal product. This means that there are facts (evidence) or arguments to suggest a causal relationship.

8.10.9. Assessment of laboratory, ECG, Ultrasound, **Magnetic Resonance Imaging (MRI) and X-ray abnormalities**

The investigator will report the **laboratory test and ECG and Imaging results** as normal or abnormal and if abnormal, the investigator will be required to report if the abnormality is clinically significant or not. Comparisons of the change from baseline in

laboratory parameters, ECG and vital sign parameters shall be made.

If a laboratory parameter/ECG result is abnormal and “clinically significant” it should be reported as an adverse event (AE) and graded for severity.

Laboratory and ECG abnormalities should be assessed as “clinically significant” (and therefore have to be reported as an AE) only if they meet AT LEAST ONE of the following conditions:

- The abnormality suggests a disease and/or organ toxicity AND this abnormality was not present at the screening visit or is assessed as having evolved since the screening visit
- The abnormality results in discontinuation of the study drug
- The abnormality requires medical intervention or concomitant therapy
- Abnormal ECG results judged clinically significant by the investigator (therefore an AE) and which are classified with regards of severity as CTCAE v4.03 Grade 3 or Grade 4 must be carefully assessed, as they are likely to require medical intervention and/or be life-threatening.
- ECGs that attain exclusion criteria levels ie. QTcF >450 msec after the patient has been enrolled must be reported as abnormal and clinically significant. They must be reported as adverse events.

Note: a confirmatory retest of clinically significant grade 3 and 4 laboratory abnormalities should be performed within 48 hours to verify the value. Beyond 48 hrs the clinically significant grade 3 and 4 laboratory values recorded will be reported even if it has not been verified. The exact timing of the repeat test should be based on appropriate medical judgment.

Grade 3 or Grade 4 AEs must be carefully assessed, as they are likely to require medical intervention and/or be life-threatening.

When reporting a clinically significant abnormal laboratory results or ECG abnormalities as AEs, a clinical diagnosis should be recorded rather than the abnormal value itself, if available (for example, “hypokalemia” rather than “decreased potassium levels”). Furthermore, lab abnormalities which present with clinical signs and symptoms will also be considered clinically significant. However, in these cases, the adverse event should be recorded as the syndromic clinical diagnosis (*ex.* acute pancreatitis instead of each finding separately: high levels of amylase, high levels of lipase, abdominal pain and vomiting).

8.10.10. Other Ultrasound, Magnetic Resonance Imaging (MRI) and X-ray assessment, the investigator will evaluate whether the findings are normal or abnormal. If abnormal, the investigator will assess if this finding is “clinically significant” or not. If the finding is abnormal and clinically significant, it should be reported as an adverse event (AE).

Primary abnormalities related to the mycetoma lesion should not be reported as AEs except if there are secondary complications e.g. secondary infection or abscess formation.

8.10.11. Exposure in utero

Female subjects can be included in the study. There are stringent criteria for female subjects of child bearing age. These include

- Inclusion criteria: If female of child bearing potential, using adequate contraception for the period of the trial plus 12 ± 2weeks.

- Exclusion criteria: Pregnancy at inclusion, intent to become pregnant, or use of a new method of contraception within 3 months prior to study entry

Exposure in utero during the treatment period can occur if the subject becomes pregnant. The study is being conducted on outpatient basis and based on studies with similar eligibility contraception criteria, pregnancy is not uncommon.

If pregnancy is identified during study participation while receiving an investigational drug (from day start of last menstruation period = LMP) or within 12 weeks of discontinuing the investigational drug, the investigator must submit the event on the **Pregnancy Report form,** together with the first day of last menstruation period and the expected date of birth. This must be done irrespective of whether an adverse event has occurred. The information submitted should include the anticipated date of delivery.

Pregnant subjects will be withdrawn from study treatment and continue safety follow up and appropriate rescue treatment as judged by the investigator.

The investigator will follow the subject until completion of the pregnancy or until pregnancy termination (i.e., induced / spontaneous abortion). The investigator will provide pregnancy outcome information in the **Pregnancy Outcome report form** as a follow-up to the initial pregnancy reporting form.

Note: A pregnancy is not an SAE. Any unfavorable outcome meeting a seriousness criterion, i.e., in the case of unfavorable pregnancy outcome (miscarriage, stillbirth), birth defect occurs or congenital abnormality shall be reported using the SAE form (in addition to the Pregnancy outcome form).

In the case of a live birth, a paediatrician should assess the infant at the time of birth and submit a Child Surveillance form. New-borns exposed during pregnancy will be followed up until the age of 2 years.

In the case of a live birth, a paediatrician should assess the infant from the time of birth until the child reaches 24 months of age. Because of the risk of development of lenticular opacity (cataract) found in animal studies with E1224, an ophthalmologist will also assess the infant from the time of birth until the child reaches 24 months of age. The principal investigator must gather information on the child's health status from birth to 24 months of age by the child's treating physicians (paediatrician, ophthalmologist). She/he must inform and advise the mother in order to ensure the monitoring of the child's health status, as well as complete the safety information of the child's health status in the corresponding form (Child Surveillance Form).

If an event presented by child during the 2 years of child monitoring is identified as a relevant medically important event, it shall be reported using the SAE form (in addition to the Child Surveillance form).

8.10.12. Adverse event follow-up

All adverse events should be followed until they are resolved or the investigator assesses them as chronic or stable or the subject participation in the trial ends (i.e., until a final report is completed for that subject).

In addition, all serious adverse events and those non-serious events assessed by the investigator as related (with reasonable possibility) to the investigational drug must continue to be followed even after the subject participation in the trial is over. Such events should be followed until they resolve or until the investigator assesses them as “chronic” or “stable.” Resolution of such events is to be documented on the CRF.

9. Withdrawal criteria

A subject should be withdrawn from the study treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the subject ie. subject choice. Any other circumstances that result in early withdrawal will be reported.

Early withdrawal visit study procedures shall be conducted for early withdrawals. If the reason for early withdrawal is subject choice, agreement of the study participant will be required for any early withdrawal procedures to be conducted.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible.

When it is a subject choice to withdraw, the subject will be asked whether they are withdrawing the entire study participation or they would like to continue some components of study follow up procedures. Follow up can continue if the subject wishes to continue components of follow up and their data will be included in the database. If the subject withdraws consent for the entire study participation, no further study evaluations should be performed and no attempts should be made to collect additional data beyond the early withdrawal visit, with the exception of safety data, which should be collected if possible and in accordance with patient consent.

If a subject is withdrawn from the study, the reason must be recorded on the CRF e.g. died, lost to follow up, patient withdrew etc. and date of event recorded.

If a subject is withdrawn from the treatment because of a treatment limiting adverse event, thorough efforts should be made to clearly document the outcome of AE.

Use early withdrawal (VZ) procedures in the schedule of events.

9.1. Rules for permanently interrupting study treatment

If a subject is withdrawn from the study before the full course of the treatment is completed, the physician must make all necessary arrangements to ensure that the subject receives the appropriate treatment for the relevant medical condition (e.g. with drug/s currently recommended by the national policy).

The Investigator will permanently interrupt the treatment based on the following:

- ALT or AST >8x ULN (to be reported as Adverse Event of Special Interest see section 8.10.3)
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TBL >2x ULN or INR >1.5): to be reported as serious adverse event; see section 8.10.2)

- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- QTc post dosing <310 msec or <350 msec accompanied of cardiac symptoms
- QTc> 500 msec at any time during treatment
- QTc post dosing delta >60msec
- Adverse event or any other condition which, in the investigator's opinion, would put the patient at undue risk by continuing the trial treatment
- Major protocol deviation incompatible with the continuation/participation on the trial
- Any condition that the investigators considers medically necessary to interrupt the treatment, such as:
    - Significant leukopenia (<2,500 cells/mm3)
    - Severe gastrointestinal symptoms
    - Severe allergic dermopathy
    - Peripheral sensitive neuropathy
- Pregnancy.

9.2. Subject withdrawal from the study and subject replacement

Subjects withdrawn from the study will not be replaced.

If the serum ALT exceeds 8 X ULN at any time or elevation of serum bilirubin >2 X ULN, the patient should be immediately withdrawn from the study.

10. Data Analysis and Statistical Methods

10.1. Sample size determination

Sample size

Sample size calculation is based on "Pick the winner or drop the loser design". Two doses will be tested at the time of interim analysis that will be performed when half of subjects will have reached the 3-month evaluation. The loser will be dropped at that time and the type one error has to be adjusted for that reason. The critical value of the final analysis will be $z_{0.030}$ = 2.168 instead of $z_{0.05=}$ 1.96.[37] Limited information is available on the efficacy of itraconazole for the treatment of eumycetoma: in one study, 38% of subjects were cured at 12 months (n = 13 subjects) and 25.9% in another study. Given an optimistic efficacy rate of 40% for the reference drug (positive control) and a targeted cure rate of 70% for the winner, a sample size of 49 subjects in the winner arm and the control arm will provide a power of 85% at the final analysis. The total sample size without provision for non-evaluable subjects should therefore be 123 (49 + 49 + 25). The interim analysis (drop-the-loser) will be done when 25 subjects per arm (n= 75) have reached 3 months follow-up.

A 12% provision will be made for the decrease of power due to early lost to follow-up or non-evaluable but randomized subjects receiving at least one dose. This provision will be applied to the control and the winner arm after the interim analysis.

The total sample size of **138 subjects** is therefore composed of **28** subjects at **interim** analysis per arm and **55** subjects for the winner and the control arm at the **final** analysis (winner= 55, control= 55 and loser= 28).

The final analysis is conservative because the adjustment of alpha is based on half of information collected at the interim analysis. Because the assessment at interim is done at 3 months instead of 12 months and the provision for early lost to follow-up or

non-evaluable subjects is, at the end of recruitment, the available proportion of information at the interim analysis is clearly smaller than 50%. The true proportion of information is not easy to assess without any knowledge of the predictive value of the 3-month assessment.

10.2. Definition of study populations included in the analysis

Population of interest

- Intent To Treat (ITT): All randomized subjects receiving at least one dose of the study drug. It will be the primary population.
- Full Analysis (mITT) Population: All randomized subjects receiving at least one dose of study medication and had at least 1 post-randomization assessment for the primary efficacy variable.
- Clinically Evaluable (CE) (Per Protocol) Population: A subset of subjects from the mITT population who met all inclusion and who had no exclusion criteria, had no protocol deviations likely to influence the study outcome. Details of the evaluability criteria will be determined prior to study unblinding and specified in the Statistical Analysis Plan (SAP).
- Safety Population: The group of subjects that received at least one dose of study drug and had at least 1 post-dose safety assessment.
- Pharmacokinetic Population: The PK Analysis Set is the group of randomized subjects who receives at least one dose of either fosravuconazole or itraconazole and has at least one quantifiable ravuconazole or itraconazole concentration with a documented dosing history.
- Pharmacokinetic/Pharmacodynamic Population: The PK/PD Analysis Set is the group of randomized subjects who receive at least one dose of either fosravuconazole or itraconazole, have at least one quantifiable ravuconazole or itraconazole concentration, and at least one PD measurement, with a documented dosing history

10.3. Subject Disposition

All subjects entering the recruitment sites will be screened for eligibility and their consent sought as appropriate. The site will report subject flow to trial monitors on a weekly basis.

10.4. Baseline

Routine baseline characteristics including age, gender, height, weight, BMI for age, and symptoms (Local swelling, Skin changes, Pain Discharge Ulceration) will be documented and summarised as appropriate according to data variable type (e.g. mean for normally distributed continuous data).

10.5. Treatment Compliance

Drug adherence is critical to the success of any treatment regimen. Poor or even sub-optimal adherence can lead to treatment failure, recurrence and resistance. To optimize adherence in this trial, counselling should be planned by the investigator/study nurse/social worker with the subject, and caregivers at the screening visit to discuss the individual issues that may affect each subjects' chances of successfully adhering to treatment.

A number of key issues should be considered and/or addressed: A form named Medication Adherence Counselling that covers these key issues will be used as a

reference for the site staff. This completed form should be kept in the source notes of the subject from baseline visit, adherence counselling session should take place and clear instructions need to be given regarding the intake of the treatment regimen. If applicable, a follow-up telephone call could be given by the investigator/ study nurse/ social worker to the person responsible for giving the medications, three days after the start of the initiation of treatment.

Adherence counselling should take place at each visit. Also, subjects, must be reminded to bring all unused trial medication to every visit. Adherence should also be assessed via pill count and an adherence questionnaire completed. Drug accountability (medication dispensed and returned, and the number of days of treatment) will be recorded in the CRF. If adherence issues are identified or suspected, a new session with investigator/study nurse/social worker must take place trying to identify issues and working actively to find a solution, while using the Medication Adherence Counselling Form.

Pill-counts will take place at each visit, and will be recorded in the drug accountability form. It is possible that subjects need to come to the site before their next scheduled visit in case of problem with prescribed medicine. Trial medication dispensation should be planned carefully.

All initial trial drugs will be supervised on administration by study medical staff. The dose given will be recorded in the source document /CRF and CRF. Full compliance will be considered as 90-110% of the prescribed dose used. Self-administered overdose considered medically important should be reported as an SAE.

10.6. Efficacy Analysis

Primary Efficacy Analysis

The **primary question of interest** is to determine whether, in addition to surgery the retained dose (the winner at the interim analysis) of fosravuconazole short-course monotherapy is more effective than the standard-of-care 12-month regimen of itraconazole monotherapy.

Before answering the primary question, it will be determined in an interim analysis which is the fosravuconazole dose with the best observed efficacy (the winner) and what is the safety of such a dose. The interim analysis will be performed on the set of evaluable subjects assessed during the blind review.

The **Primary analysis** will be performed on the ITT set of subjects and on the primary endpoint using a likelihood ratio test. The adjusted alpha is set to 0.030 (two-sided).

The **Primary efficacy variable:** is Complete cure at the End-of-Treatment (EOT; 52-week time point) in the mITT population.

a. negative fungal culture from a surgical biopsy from the former mycetoma site, and
b. clinical cure (no clinical evidence of mycetoma mass, sinus tract, or discharge) and
c. normal lesion site or only fibrosis on MRI

The **Primary endpoint** is a binary variable (cured or not cured). Cure is a complete cure at the 12-month End-of-Therapy.

Other efficacy analyses
Sensitivity analysis

The main sensitivity analysis will be identical to the primary analysis but performed on the per protocol set of subjects.

Secondary objectives and analyses

- Determine which each of the three regimens is compatible with the targeted efficacy rate of >70% of "cured" subject at EOT. This will be done by the estimation of the cure rate at end of treatment.
- Assess the comparative efficacy and toxicity of fosravuconazole 200 mg and 300 mg weekly doses at 3 months. The response rate will be estimated at 3 months for each of the three regimens and the 95%confidence interval will be provided (Clopper Pearson confidence interval). Distribution of toxicity grades will be displayed and compared through a Krukal Wallis test.

Secondary efficacy variables:

Secondary efficacy endpoints
Clinical

- Effective treatment (combination of mycological eradication and clinical improvement in the mycetoma lesion) at EOT and EOS visits in the mITT and CE Populations
- Overall improvement (mycological eradication and ≤10% of the mycetoma mass remaining) at EOT and EOS visits in the mITT and CE Populations
- Complete cure, effective treatment, and overall improvement categorized by etiologic fungal genus and species at EOT and EOS visits in the mITT and CE populations
- Time to complete cure, time to effective treatment, and time to failure.
- Lack of AEs leading to treatment discontinuation.
- Lack of SAEs.

Microbiological

- To assess clearance of the fungus from the lesion by PCR, culture and histology at various time-points during and after treatment.
- To assess homogeneity of *Madurella mycetomatis* strains collected
- To assess decrease or disappearance of detectable beta-glucan from serum
- To assess development of drug resistance against study drugs in strains isolated before, during and after treatment

Immune responses

- To assess the change in immune response during and after end of treatment by measuring cell types, cytokine profiles level and immunoglobulins in the peripheral blood

Pharmacokinetics /pharmacodynamics

- To measure drug levels and their metabolites at various time-points
- To use population PK/PD modeling to explore potential relationships between clinical, microbiological and pharmacokinetic parameters with clinical outcomes at time point 3 months and at the end of treatment

Statistical Methods

- Assess the comparative pathogen eradication rates for each of the three regimens at EOT and for the winner and control group at 6 Months. The rate of eradication will be estimated and compared through a likelihood ratio test. The adjustment for multiplicity will be done at 6 months using alpha = 0.030. The Hochberg procedure will be used for the adjustment at EOT.
- Assess the comparative efficacy of each arm by etiologic pathogen (subtype of fungus) and correlate outcome with *in vitro* susceptibility (pre- and post-treatment for failures). The cure rate will be compared after post-stratification on the type of fungus using a CMH test. A Breslow day test will be performed to verify whether the treatment effect is dependent upon the fungus.
- Evaluate the comparative rates of change in initial mycetoma lesion size over time and the rate of resolution of any pre-therapy clinical signs and/or symptoms over time for each regimen. The time course of the lesion size will be compared through a mixed model for repeated measures. The same approach for signs and symptoms will be applied.
- Assess the comparative safety and tolerability profile for each regimen based on the incidence of adverse events and laboratory test abnormalities. The safety will consist in the description of each serious adverse event at Month 3, the incidence rate of each adverse event (preferred term) per treatment group and the incidence rate of the adverse event classified by system organ. Laboratory abnormalities will be considered as AEs. Shift tables and Stuart Maxwell test will be performed.
- Evaluate the comparative durability of response for each treatment regimen at the End-of-Study (EOS) visit. The time to onset of cure and the time to relapse, if any, will be calculate for each subject. The difference of time to cure to time to relapse will be calculated and the durability will be censored if the subject is still cured at Month 12. Subjects who were not cured during the trial will be excluded from the analysis. Durability will be compared through a Gehan test for censored data.

10.7. Safety Analysis

Adverse events will be collected as defined in section 8.10.4.

All subjects enrolled and who have been started on study medication will be included in the safety analysis (treatment-emergent AEs). The AE data will be coded using MedDRA dictionary.

AE’s will be tabulated by System Organ Class (SOC), by relationship to study drug (ADR) and severity.

The proportion of subjects experiencing at least one AE will also be presented. The frequency and severity of the AEs will be described as well as the frequency of SAEs or AEs that lead to treatment discontinuation.

Safety will be assessed as follows:

- Continuous monitoring and recording of all serious adverse events (SAEs) and adverse events (AEs)/AESIs

- Regular monitoring of haematology, urinalysis, and blood chemistry values (with clinical significance if abnormal)
- Regular measurement of vital signs
- Performance of limited physical examinations
- Performance of ECGs at selected study visits (with clinical significance if abnormal)
- Serum or urine pregnancy tests performed monthly

10.8. Analysis of other endpoints

Ravuconazole and itraconazole plasma concentration-time data will be subjected to population PK analysis using nonlinear mixed effects modeling. The effect of covariates, such as baseline characteristics/demographics, on PK will be explored. Derived exposure parameters of ravuconazole and itraconazole, including, but not limited to, C_{max} and AUC at steady-state (AUCs), will be calculated from the final PK model using individual posterior estimates of the PK parameters and from dosing history.

Pharmacokinetic Analysis:

Plasma concentrations of ravuconazole and itraconazole will be measured on Day 1 of Week 1, and on Weeks 2, 3, 4, and Months 2, 3, 6, and 12 (at end of treatment) for analysis of population PK, as described below:

- On Day 1, Week 1, subjects will take the dose of either fosravuconazole or itraconazole in the morning, and three blood samples for determination of plasma concentrations of ravuconazole and itraconazole will be taken, one at each of the following collection time windows: 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-dose.

- At Weeks 2 and 3, and on Month 12, at end of treatment (EOT), a single sample will be taken prior to the morning dose (pre-dose sample).

- On Week 4, subjects will be instructed to take the morning dose and come to the investigational site for collection of a single blood sample within 2 to 6 hours post-dose.

- On Month 3, subjects will be instructed to take the morning dose and come to the investigational site for collection of 3 blood samples, one at each of the following collection time windows: 2 to 4 hours, 6 to 12 hours, and 24 to 96 hours post-dose.

- On Month 6, following surgery, four samples will be taken, each one at the following collection time windows: prior to the morning dose (pre-dose sample), 2 to 4 hours, 6 to 12 hours, and 24 to 120 hours post-dose.

The exact time of dosing on the days of sample collection, and the exact time of sample collection within the collection time window, should be recorded.

Ravuconazole and itraconazole plasma concentration-time data will be subjected to population PK analysis using nonlinear mixed effects modeling. The effect of covariates, such as baseline characteristics/demographics, on PK will be explored. Derived exposure parameters of ravuconazole and itraconazole, including, but not limited to, Cmax and AUC at steady-state (AUCs), will be calculated from the final PK model using individual posterior estimates of the PK parameters and from dosing history.

Plasma concentrations of ravuconazole and itraconazole will be quantified using Ultraperformance Liquid Chromatography with fluorescence detection (UPLC-UV)

Pharmacokinetic/Pharmacodynamic Analyses

Population PK/PD modelling will be used to explore the relationship between exposure to ravuconazole and itraconazole and response, including but not limited to reduction in lesion size, reduction in viable pathogens, and the probability of achieving clinical endpoints (>50% reduction in lesion size with closure of >50% of sinuses at 3 months, and cure at 12 months). Additionally, exposure-safety relationships for selected safety parameters may be explored graphically. Any emergent relationship may be modelled as appropriate.

10.9. Interim analysis

See study design section 3.1 for planned interim analysis, the timing of the analysis, the statistical methods and the decision rules regarding study continuation, or premature termination and the reasons for performing the interim analysis.

10.10. Missing, unused, and spurious data

Primary imputation rule: For all analyses, a lost to follow-up or withdrawal will be considered a failure regardless of the reason. Lost to follow up will only be determined at D358 (V9) i.e. the primary endpoint.

An investigation will be made concerning the sensitivity of the results of analysis to the method of handling missing values, especially if the number of missing values is substantial. Sensitivity analysis for the primary and secondary outcomes will consider patients who do not have a measurement taken at some follow-up visit to have their last observation carried forward (LOCF).

Clear identification of a particular value as an outlier will be most convincing when justified medically as well as statistically. However, graphical displays will used visualize and identify. Cases that visually “stand out” will be assessed for possible influence on results and conclusions by comparing results from analyses with and without the outlier(s).

10.11. Deviations from the original statistical plan

Any deviation(s) from the original statistical plan will be described and justified in the final report.

11. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB), independent of the investigator and sponsors, will be set up.

The DSMB monitors the study in order to ensure that harm is minimised and benefits maximised for the study subjects. They will review the study data at pre-determined intervals and issue recommendations about the study. The data and intervals will be agreed prior to or soon after the study initiation and documented in the DSMB Charter.

12. Quality Assurance and Quality Control Procedures

12.1. Investigator’s file

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigator’s Site File, subject clinical source documents

and screening / enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRF and query forms, IEC and regulatory approval with correspondence, sample informed consent, drug accountability records, staff curriculum vitae and authorization forms and other appropriate documents/ correspondence etc.

12.2. Case report forms (CRFs).

Data will be collected by laboratory technicians, medical doctors, clinical officers and nurses authorized by the investigator. It will be supervised by the Investigator and signed by the investigator or by an authorised staff member. Study-specific information will be entered into the Case Report Form (CRF).

An Openclinica database will be developed and used in this study to enter information collected from paper CRF; this integrated workflow management system, is secure, flexible and will improve the data quality. Furthermore, it will optimize time effectiveness as the project progresses.

The investigator and the study staff will be trained to resolve queries according to the Data management plan. At the end of the study, the CRFs and queries copies will be kept in the site for archiving.

Data that are derived should be consistent with the source documents or the discrepancies should be explained. All CRF data should be anonymised, i.e. identified by study patient number only.

The investigator at the trial site should ensure the accuracy, completeness, legibility, and timelines of all data reported to the sponsor in the CRFs (including consistency with Forms/documents sent to DNDi PV, E.g. SAE forms) and any other additional information that is required. The investigator is responsible for keeping all consent forms, screening forms, source documents / CRF and the completed subject identification code list in a secure location.

In-case of any changes to the above. Any change must be updated in the data management plan and/or documented by a note to file that clearly states the change, reason and where the record all of the protocol-required information to be reported to DNDi on each trial subject's information will be entered in accordance with ICH GCP guidelines.

12.3. Source documents

The verification of the CRF data must be by direct inspection of source documents. Source documents include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrolment logs.

The investigator must maintain source documents such as laboratory and consultation reports, history and physical examination reports, etc., for possible review and/or audit by DNDi and/or Regulatory Authorities. The Investigator / designee will record the date of each subject's visit together with a summary of their status and progress in the study.

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not

obscure the original entry, and should be explained if necessary (e.g., *via* an audit trail).

12.4. Record Retention

The investigator/Institution should maintain a record of the location(s) of their respective essential documents including source documents, for at least 25 years after completion or discontinuation of the study. The storage system used during the trial and for archiving (irrespective of the type of media used) should provide for document identification, version history, search, and retrieval.

After that period the documents may be destroyed with prior permission from DNDi, subject to local regulations. Should the investigator wish to assign the study records to another party or move them to another location, DNDi must be notified in advance.

12.5. Monitoring

Monitoring visits to the trial site will be made periodically by DNDi representatives or designated clinical monitors to ensure that GCPs and all aspects of the protocol are followed. Source documents will be reviewed for verification of consistency with data on CRFs. The investigator will ensure direct access to source documents by DNDi or designated representatives. It is important that the investigators and their relevant personnel are available during the monitoring visits.

The investigators will permit representatives of DNDi and/or designated clinical monitors to review all source documents / CRFs/ Forms and documents sent to DNDi PV, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each subject will be made available provided that subject confidentiality is maintained in accord with local regulations. The reviews are for the purpose of verifying the adherence to the protocol and to ensure the study is conducted according to GCP. It is important that the investigators and other trial site staff are available at these visits.

The monitoring visits provide DNDi with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of CRFs (including consistency with Form and documents sent to DNDi PV), resolve any inconsistencies in the study records, as well as to ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four visit types are planned: pre-study, study start, during the study, and study end.

It will be the clinical monitor’s responsibility to review the source documents / CRF at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

12.6. Audits and inspections

The trial site may also be subject to quality assurance audits by DNDi or designated representatives and/or to inspection by regulatory authorities or Independent Ethics Committees (IEC). Auditors and inspectors will also require direct access to documents in order to review source & trial documents. They may also visit the trial facility and will inspect the overall management of the trial in order to verify compliance with the protocol, GCP and local regulations.

It is important that the investigators and their relevant personnel are available for possible audits or inspections.

12.7. Data Management

The investigator/institution should have control of all essential documents and records generated by the investigator/institution before, during, and after the trial. The sponsor should ensure that the investigator has control of and continuous access to the CRF data reported to the sponsor. The sponsor should not have exclusive control of those data.

Data will be transcribed from the source documents to the CRF. The MRC study coordinator or designee will enter the data into the CRF. The clinical trial monitor will conduct Source Document Verification and validate all entries to the CRF and any query resolutions. The trial data will be stored in a computer database maintaining confidentiality in accordance with national data legislation.

To ensure data quality, a uniform hard copy source document / CRF will be designed for use in developing the CRF. Data centre will use only the validated entries in the database for data cleaning and analysis accordingly.

12.8. Confidentiality of trial documents and subjects' records

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor, subjects should not be identified by their names, but exclusively by an identification code. The investigator should keep a subject enrolment list showing codes, names, and addresses. The investigator should ensure that the identity of trial subject is maintained in strict confidence and is not revealed to the sponsor (except for the monitor and auditor). If a document containing subject identity needs to be provided to the sponsor, this information must be blacked-out before submission to the sponsor.

13. Protocol Amendments

The Principal investigator will ensure that the study protocol is strictly adhered to throughout, and that all data are collected and recorded correctly on the source documents / CRF. The Principal investigator may contact the medical coordinator for a protocol waiver for minor deviations from the protocol, e.g. patient unable to attend during visit window.

All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the sponsor and the Principal investigator and is to be submitted to the appropriate IEC for information and approval in accordance with local requirements, and to regulatory agencies if required. Approval by IEC (and Regulatory Authority, if applicable) must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial [e.g. change in clinical monitor[s], change of telephone number[s].

The protocol amendment can be initiated by either sponsor or by any Principal investigator. The sponsor or investigator will provide in writing the reasons for the proposed amendment and will discuss and document any new changes in the protocol section covering changes made to the protocol.

14. Early Termination of the Study

Both the sponsor and the investigator reserve the right to terminate the study at any time prior to inclusion of the intended number of subjects, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the sponsor and the investigator will assure that adequate consideration is given to the protection of the subject's interest.

Reasons for early termination by the sponsor(s) may include but not be limited to:

- Too low enrolment rate.
- Protocol violations.
- Inaccurate or incomplete data.
- Unsafe or unethical practices.
- Questionable safety of the test article.
- Suspected lack of efficacy of the test article.
- Following the recommendation of the DSMB or IEC
- Administrative decision.

Reasons for early termination by the investigator may be:

- Insufficient time or resource to conduct the study
- Lack of eligible patients

If a study is early terminated either by the sponsor or by the investigator, the investigator must:

- Complete all source documents / CRFs to the greater extent possible
- Return all test articles, and related study materials to the sponsor who provided them
- Answer all questions of the sponsors or their representatives related to data of subjects enrolled at the site prior to study termination
- Ensure that subjects enrolled in the study who had not yet reached end of treatment time point are followed up with the necessary medical care.
- Provide in writing the reasons for his decision to the national health authority and the sponsor.

15. Ethics

The experimental protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and ICH guidelines for Good Clinical Practice (International Committee for Harmonization). DNDi assures that it will comply with all applicable state, local and foreign laws for protecting the rights and welfare of human subjects. This protocol and any protocol amendments will be reviewed / approved by an IEC before its implementation.

It is the responsibility of the Global or National Coordinating Investigator/Investigator to apply for review to the IEC of the country where the study takes place regarding local rules and regulations. Written approval from all involved IECs must be obtained before implementation of any protocol-specified intervention /investigation provided to the subject [such as subject information sheets or descriptions of the study].

Any modifications made to the protocol after receipt of the IEC approval must also be submitted by the investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

15.1. Informed consent process

Inclusion in the study will occur only if the subject (for adults) or the parent/guardian (for children) gives/signs written informed consent before the start of any study specific procedures. If the subject is illiterate or unable to write, a literate witness must sign (this person should have no connection to the research team and the sponsor, and, if possible, should be selected by the subject). The investigator should also obtain the for children, an assent of children but their assent must be completed by the signed permission (ICF document) of a parent or guardian.

will also be required. Minors less than 18 years who are considered emancipated minors (e.g. less than 18 years and married) can give and sign informed consent form to participate in the trial.

It is the responsibility of the investigator / designee to obtain written informed consent and assent (if applicable), from each subject participating in this study, after adequate presentation of aims, methods, anticipated benefits, and potential hazards of the study.

The written informed consent and assent form (if applicable) document will be translated into the local language or a language understood by the subject(s). The subject will be given all the necessary time to discuss the information received with members of the community or family before deciding to consent or not. The subject or their impartial witness (if applicable) will be asked to provide written and signed consent. The same rights will be applied to the minors.

A new Informed Consent will be obtained in subjects who were recruited as minors, if they reach their 18th birthday while still in the study. If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated as needed. All subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

15.2. Ethical aspects of subject inclusion and study procedures

Potential study subjects will be selected from individuals who are 15 years or older and diagnosed with mycetoma at the MRC clinic. The majority of the patients affected by mycetoma are 15-30 years old. Patients travel for treatment from Sennar and other endemic areas to the MRC which is well known for specializing in treatment of mycetoma. These patients will be screened to join the study.

Patients in this study may also be recruited from on-going epidemiological surveys in the endemic areas thus also ensuring that sufficient patients with moderate lesions are identified and recruited. Previous experience shows that often patients with smaller lesions postpone seeking medical help until the lesions are extensive.

When patients arrive at the centre, routine procedures are followed to diagnose and treat mycetoma. The routine procedures conducted for diagnosis at MRC include history taking, physical examination, routine labs, ultrasound, plain x-ray and biopsy. In order to ensure study efficiency a pre-screening checklist will be developed and used. The pre-screening checklist is designed to check rapidly assessable protocol criteria, filter and reduce number of patients who will undergo consent process. After confirmation of mycetoma diagnosis, subjects who meet pre-screening criteria will be invited to participate in the study. The ICF process will be conducted and documented in a language understood by the participant. No research components of the study can be done without documented informed consent from the patient. After consenting,

inclusion/exclusion criteria will be systematically assessed during screening phase and only those meeting the study criteria will be eligible for enrolment. Roles of investigators are provided in Appendix I.

The diagnosis and treatment of mycetoma require invasive tests and surgery which require anaesthesia, local, loco-regional or general anaesthesia depending on the procedure to be done. Procedure and anaesthesia risks will be explained in the informed consent. The risks will be minimized by using qualified and experienced staff for these procedures during the study.

The following patients will be specifically excluded:

- Females of childbearing age who are not considered to have adequate contraception to participate in the study due to known embryotoxic drug effects and children less than 15 years old due to the insufficiency of safety data to support their inclusion. For the same reason pregnant females and lactating mothers will be excluded.
- HIV co-infected patients are excluded from the study, as they are likely to present different profile in terms efficacy, toxicity and potentially require ARV therapy.

Study procedures are designed to ensure accurate assessment efficacy and safety. Patients will be treated as outpatients up to the end of month 12.

Biological samples will be drawn only for the purpose of clinical assessments, diagnosis, pharmacokinetic (PK) and immune responses. The volume of blood collected will be minimised as much as possible. One puncture into the vein should be performed as much as possible e.g. by placement of an indwelling catheter.

The total volume of blood to be collected will be approximately 187mL over the 15 months participation. The maximum amount of blood draw occurs at month 6 visit (Day 176-V7) when 32ml of blood is drawn. One study visit will have no blood draw.

There may be slight variations in the blood volume due to tubes available in the market but the maximum volume of blood to be collected in a study visit will not be more than 20mL.

Biological samples may be kept after the completion of the study to evaluate how other mycetoma biomarkers or immunological markers respond following treatment with trial medication. Shipment will be minimized and reserved for quality control or for standardized testing of key efficacy end points.

Subjects participating in this study may experience discomfort during examination and blood sampling. Most blood draws/laboratory will follow standard phlebotomy practices. To minimize discomfort, phlebotomy personnel should be trained or experienced in drawing blood.

The risks are summarized as a) risks related to blood sampling (temporary discomfort, pain, bleeding, and rarely infection; b) risks related to biopsy, surgery and the anaesthesia; c) risks related to study drugs (the more common side effects of fosravuconazole and itraconazole are nausea, diarrhoea and vomiting with the rare but serious side effect is liver failure which can lead to death) d) risks from pregnancy (due to potential harmful effect to the unborn child).

15.3. Ethical aspects of study treatments

Experimental data suggests that fosravuconazole has potential as a new oral treatment

for eumycetomata caused by *Madurella mycetomatis*. Phase I studies in healthy volunteers receiving fosravuconazole suggest that the benefit/risk ratio of the selected dose is acceptable. Itraconazole with surgery, perceived as the best treatment for eumycetoma in Sudan is the comparator arm. Any patients that fail to respond or have recurrence following treatment with fosravuconazole will be treated with rescue medication (see section 7.9 for details).

Placebo will be required to ensure the same numbers of tablets are administered to each subject to mask drug regime allocated. All screened, but ineligible patients will be treated with the current standard treatment according to Sudanese Mycetoma National treatment guidelines. All patients will be treated.

If trial results show statistical superiority of fosravuconazole over itraconazole, subjects who complete treatment in the fosravuconazole arm and were cured but ultimately have recurrence or possibly subjects who fail itraconazole (at PI discretion) will be offered extension with fosravuconazole in a study setting post trial.

15.4. HIV status and VCT

All patients will be offered counselling and screening for HIV through provider initiated counselling and testing program (PICT) which is routine practice in hospital. This will be done at the same time as consent is obtained for inclusion in the trial and at the end of the trial. The Informed Consent Form will be the same form to document consent for HIV test. Patients who decline HIV testing cannot participate in the trial.

Patients who are found to be HIV positive will not be eligible to participate in the trial but will receive appropriate referral for HIV care and treatment, according to national treatment guidelines.

15.5. Patient costs

Patients with mycetoma often travel long distances to reach the mycetoma centre. Mycetoma is generally treated on an outpatient basis except when surgery is required. Surgery will be done using general anaesthesia or spinal block (locoregional). This necessitates an overnight hospitalization. Hospitalization may be done for convenience of the subject. Subjects will be reimbursed for travel to and from the study site but will not receive any payment for trial participation. All the hospitalization and medication needs during surgery will be provided for free of charge. In addition, any medication that is required during the trial period will be provided free of charge to the patient. Food during the in-patient treatment phase will also be provided free of charge to the subject. This is an essential part of the patient care plan bearing in mind the high prevalence of malnutrition. Education and nutritional guidance will be offered. An appointment card used by the clinic for routine bookings will be used to remind subjects of the dates they are required to return to the centre. To minimize loss to follow up, additional measures may be included e.g. home visit to map the residence, telephone calls if the subject agrees to these.

16. Insurance and Liability

DNDi is insured to indemnify the investigator against any claim for damages brought by a research subject who suffers from a research related injury during the performance of the trial according to the protocol. The insurance will also cover for the costs of medical care required as a result of the subject participation in the trial (ex. AE related to study drug or study procedure).

17. Reporting and publication

The clinical trial will be registered with a recognised clinical trial registry such as www.clinicaltrials.gov and www.pactr.org/ and the results will be provided in a Clinical Trial Report.

The results of this trial may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to DNDi prior to submission. Feedback of the study results and implication of the results to the community will be through the Ministry of Health and community outreach meetings with the involvement of local authorities.

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19. APPENDICES

19.1. APPENDIX 1: ROLES OF INVESTIGATORS

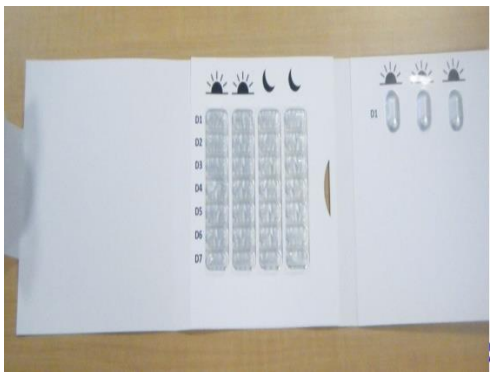
[redacted]

19.2. APPENDIX 2: SAMPLE DRUG CHART AND CALENDAR

These tools will be used to facilitate better understanding for the patient during the informed consent process.

Figure 4: Sample Drug Chart showing weekly study doses (same for the 3 study arms).

Drug wallet and box design example (one box is designed to have four wallets)



One week 2-52 wallet with 31capsules

Box with one wallet for demo

Figure 5: Sample theoretical calendar showing anticipated study visits.

This calendar is for demonstration purposes only. It is based on a hypothetical enrolment Monday 01 Jan in a non-leap year. The circles represent study visits 1 to 10 and correspond to study visit day 1,8,15,22,85,57,176,267, 358, 455 or week 1,2,3,4 month 2,3,6,9,12 and 15. It will be noted that participant visits recur on the same weekday to sync the PK and active study drug. A visit planner must be used to determine the exact visit dates for enrolled study participants.

YEAR 1

Month 1

M	T	W	T	F	S	S
(1)	2	3	4	5	6	7
(8)	9	10	11	12	13	14
(15)	16	17	18	19	20	21
(22)	23	24	25	26	27	28
29	30	31				

Month 2

M	T	W	T	F	S	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
(26)	27	28				

Month 3

M	T	W	T	F	S	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
(26)	27	28	29	30	31	

Month 4

M	T	W	T	F	S	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30						

Month 5

M	T	W	T	F	S	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

Month 6

M	T	W	T	F	S	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
(25)	26	27	28	29	30	

Month 7

M	T	W	T	F	S	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

Month 8

M	T	W	T	F	S	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

Month 9

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
(24)	25	26	27	28	29	30

Month 10

M	T	W	T	F	S	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Month 11

M	T	W	T	F	S	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

Month 12

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
(24)	25	26	27	28	29	30
31						

YEAR 2

Month 13

M	T	W	T	F	S	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

Month 14

M	T	W	T	F	S	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29		

Month 15

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
(31)						