Two dose levels of once-weekly fosravuconazole versus daily itraconazole in combination with surgery in patients with eumycetoma in Sudan: a randomised, double-blind, phase 2, proof-of-concept superiority trial

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Summary

Background Eumycetoma is an implantation mycosis characterised by a large subcutaneous mass in the extremities commonly caused by the fungus *Madurella mycetomatis*. Despite the long duration of treatment, commonly a minimum of 12 months, treatment failure is frequent and can lead to amputation. We aimed to compare the efficacy of two doses of fosravuconazole, a synthetic antifungal designed for use in onychomycosis and repurposed for mycetoma, with standard-of-care itraconazole, both in combination with surgery.

Methods This phase 2, randomised, double-blind, active-controlled, superiority trial was conducted in a single centre in Sudan. Patients with eumycetoma caused by *M mycetomatis*, who were aged 15 years or older, with a set lesion diameter (>2 cm and ≤16 cm) requiring surgery were included. There was a limit of 20 female patients in the initial enrolment, owing to preclinical toxicity concerns. Exclusion criteria included previous surgical or medical treatment for eumycetoma; presence of loco-regional lymphatic extension; osteomyelitis, or other bone involvement; pregnancy or lactation; severe concomitant diseases; a BMI under 16 kg/m²; contraindication to use of the study drugs; pre-existing liver disease; lymphatic extension; osteomyelitis; transaminase levels more than two times the laboratory's upper limit of normal, or elevated levels of alkaline phosphatase or bilirubin; or any history of hypersensitivity to any azole antifungal drug. Patients were randomly allocated in a 1:1:1 ratio to 300 mg fosravuconazole weekly for 12 months (group 1); 200 mg fosravuconazole weekly for 12 months (group 2); or 400 mg itraconazole daily for 12 months (group 3) using a random number list with non-disclosed fixed blocks of size 12, with equal allocation to each of the three arms within a block. To ensure masking between groups, placebo pills were used to disguise the difference in dosing schedules. All groups took pills twice daily with meals. In all groups, surgery was performed at 6 months. The primary outcome was complete cure at end of treatment at the month 12 visit, as evidenced by absence of mycetoma mass, sinuses, and discharge; normal ultrasonography or MRI examination of the eumycetoma site; and, if a mass was present, negative fungal culture from the former mycetoma site. The primary outcome was assessed in the modified intention-to-treat (mITT) population (all patients who received one or more treatment dose with one or more primary efficacy assessment). Safety was assessed in all patients who received one or more doses of the study drug. This study is registered with ClinicalTrials.gov (NCT03086226) and is complete.

Findings Between May 9, 2017, and June 10, 2021, 104 patients were randomly allocated (34 in group 1 and 2, respectively, and 36 in group 3). 86 (83%) of 104 patients were male and 18 (17%) patients were female. After an unplanned second interim analysis, the study was terminated early for futility. Complete cure at 12 months in the mITT population was 17 (50%) of 34 (95% CI 32–68) for group 1, 22 (65%) of 34 (47–80) for group 2, and 27 (75%) of 36 (58–88) in group 3. Neither dose of fosravuconazole was superior to itraconazole (p=0·35 for 200 mg fosravuconazole *vs* p=0·030 for 300 mg fosravuconazole). 83 patients had a total of 205 treatment-emergent adverse events, and two patients had serious adverse events that led to discontinuation, neither related to treatment.

Interpretation Treatment with either dose of fosravuconazole was not superior to itraconazole, and the two doses had a numerically lower efficacy. However, fosravuconazole presented no new safety signals, and its lower pill burden and reduced risk of drug–drug interactions compared with the relatively expensive and inaccessible itraconazole suggests further research into effective treatments with a shorter duration and higher cure rate, without the need for surgery are warranted.

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Introduction

Mycetoma is an implantation mycosis of bacterial or fungal origin (actinomycetoma and eumycetoma, respectively).[1] It is distributed worldwide, with locally acquired cases reported from 102 countries, including emerging foci outside the tropics. By 2019, around 20 000 cases had been reported in the literature, mostly from Mexico, India, and Sudan.[2] In the Americas, actinomycetoma dominates, whereas in Asia and Africa the presence of actinomycetoma or eumycetoma is more varied. Under-reporting, misdiagnosis, and non-confirmed cases mean that epidemiological data are incomplete, making it difficult to estimate the global burden.[2] Lesions often occur on the foot after a thorn prick, but can occur elsewhere on the body. The infection spreads among subcutaneous tissues, leading to a painless mass, deformity, and loss of function. Filament-containing fungal grains are discharged through multiple openings in the skin, referred to in this Article as sinuses.[1,3,4] Secondary bacterial infection is common.[5] Distant spread can occur along the lymphatic or circulatory systems. The disease typically affects male adults aged 15 to 30 years living in poor rural communities.[6] The social and economic consequences of disability are severe, and include dropping out of school or inability to work; stigma is common, contributing to psychosocial consequences including depression.[7] No case of spontaneous infection clearing has been reported in the literature.

Mycetoma was added to WHO's Neglected Tropical Disease list in 2016 and eumycetoma was included in the WHO list of fungal priority pathogens in 2022.[8,9] *Madurella mycetomatis* is the most common causative agent of eumycetoma; its treatment, mainly restricted to azoles, is unsatisfactory and often lasts for 12 months followed by surgery. The fungus can often still be cultured

Research in context

Evidence before this study

Eumycetoma has been so neglected that there are serious gaps in our understanding about how it is transmitted, its incidence, and its prevalence. There is also no effective medical treatment, and patients often present after having the disease for long periods of time. As a result, surgical removal is often necessary even after 12 months of drug treatment. Postoperative antifungal therapy can also be given to prevent recurrence. The current first-line antifungal treatment in low-income and middle-income countries, itraconazole, is thought to be only about 40% effective, although there were no clinical trial data to support this figure. The drug requires twice daily ingestion for at least 12 months with food, making adherence difficult and dropping out of care common. The availability of itraconazole is limited, so ketoconazole, a drug withdrawn in many jurisdictions due to adrenal and hepatic toxicity, is often used. A new and effective treatment for eumycetoma that is affordable and appropriate for all endemic areas is urgently needed. To fulfil this unmet need, and based on supportive in vitro efficacy for *Madurella mycetomatis*, the Drugs for Neglected Diseases initiative has repurposed the broad-spectrum antifungal agent fosravuconazole that was developed by Eisai for onychomycosis. We searched PubMed from Jan 1, 2019, to Dec 31, 2024 using the search terms ("mycetoma" OR "eumycetoma" OR "itraconazole" OR "fosravuconazole" OR "ravuconazole") AND ("drug treatment") for articles published in English. We found 22 articles, none of which referred to eumycetoma or mycetoma.

Added value of this study

To our knowledge, this single-centre, comparative, randomised, double-blind, parallel-group, active-controlled, clinical superiority trial conducted in Sudanese patients with eumycetoma is the first of its kind on the efficacy of two doses of fosravuconazole alongside surgery compared with standard care. Although the trial was terminated early, owing in part to the COVID-19 pandemic and civil unrest in Sudan making recruitment unfeasible, and in part because we could not demonstrate superiority between either fosravuconazole dose, all drug regimens were well tolerated with no new safety signals. Despite this, our per-protocol analysis showed an efficacy for 200 mg fosravuconazole over the 70% targeted complete cure rate. Although we were unable to demonstrate superiority, fosravuconazole also has other advantages over standard care; it requires weekly rather than twice daily doses reducing cost and increasing chances of adherence, can be taken without food, has no new safety signals, and has a low risk of drug–drug interactions.

Implications of all the available evidence

We demonstrate that both itraconazole and fosravuconazole, combined with surgery, have satisfactory efficacy in patients with eumycetoma. Efforts need to be made to promote early treatment to avoid extensive lesions, and to increase the chance of a successful outcome. Furthermore, more data are needed for patients with larger lesions, including sites other than extremities, and potentially with bone involvement in other endemic areas, and with eumycetoma caused by other fungal strains. We also need studies on more diverse patient populations, such as children, women of childbearing potential, and patients with disease recurrence. Clarification is needed on the respective contributions of drug treatment and surgery (as stand-alone treatments and in combination), in order to guide research into effective treatments with a shorter duration and higher cure rate, without the need for surgery.

from the well-encapsulated grains of surgical specimens.[10] Recurrences are common and often lead to amputation.[11,12] Evidence is limited to case reports or case series.

In a large retrospective review of 1242 patients in Sudan, with varied doses and durations of ketoconazole or itraconazole and timing of surgery (at month 6 or 12), 321 (25·9%) were cured, 35 (2·8%) had amputations, and more than half the patients (671 [54%]) dropped out, probably because of the low levels of response and high cost of the drug.[12] In practice, despite being withdrawn in many jurisdictions due to concerns about adrenal and hepatic toxicity, ketoconazole is often the only treatment available in endemic countries in Africa.[13,14] Itraconazole is the first-line drug in low-income countries, but the currently available originator drug can be expensive and thus seldom accessible. Despite the long—often 12-month—treatment duration, no evidence for resistance has been reported, and all measured in vitro susceptibilities have been below the therapeutic serum levels for itraconazole, but this remains a risk.[15,16] A new and effective treatment for eumycetoma that is appropriate for affected populations in all endemic areas is urgently needed.

Fosravuconazole, a prodrug of the active compound ravuconazole, was discovered by Eisai (Tsukuba, Japan) and approved in Japan as a 3-month oral treatment for onychomycosis. It has been investigated for use in Chagas disease and repurposed for mycetoma.[13,14] This triazole has broad spectrum antifungal activity, a long half-life, large volume of distribution, good safety profile, can be taken without food, and has low risk for drug–drug interactions.[14] In a phase 3 trial of 12 weeks of fosravuconazole for onychomycosis, all adverse drug reactions were mild to moderate and reversed to normal after end of treatment.[15] Compared with ketoconazole and itraconazole, which both have minimal inhibitory concentrations (MICs) to inhibit 50% of *M mycetomatis* strains (MIC_{50}) of 0·063 µg/mL, ravuconazole has a much lower MIC_{50} of 0·004 µg/mL.[16] After long-term preclinical toxicology studies, two doses were selected for assessment in a clinical trial: 200 mg, with expected exposure above MIC_{50} for all patients, and 300 mg, with expected exposure above MIC_{90} but not in all patients (a higher dose would unacceptably increase the risk of toxicity; unpublished data). Given the prolonged half-life of ravuconazole, a loading dose strategy allows rapid attainment of steady state conditions, after which, weekly administration maintains plasma concentrations above the MIC. Phase 1 studies demonstrated this was achievable in 7 days with a two-stage dosing regimen (unpublished data).

In this trial, patients received fosravuconazole for 6 months, then had surgery to excise the mycetoma mass, followed by 6 months of fosravuconazole to treat any remaining fungus. The trial was designed to select an effective fosravuconazole dosing regimen (200 mg or 300 mg, once per week for 12 months) with a favourable risk to benefit profile for patients with *M mycetomatis* who require surgery, and to demonstrate superiority over the standard-of-care, 400 mg itraconazole daily for 12 months (assuming itraconazole efficacy to be 40%, based on literature reports and an extensive review of unpublished data at the Mycetoma Research Centre, Khartoum). It was conducted in patients with small-to-medium sized lesions to ensure a relatively homogeneous population: larger lesions might require a different approach. The trial was also designed to determine whether the three regimens were compatible with the targeted efficacy rate of over 70% patients being cured at the end of treatment, based on MIC in vitro. We used a superiority, rather than a non-inferiority, design because of the excellent fosravuconazole profile and the target product profile, and also given the unacceptable low efficacy of itraconazole from existing literature and review of the Mycetoma Research Centre database, and an in vitro study showing *M mycetomatis* was highly susceptible to ravuconazole.

Methods

Study design and participants

This single-centre, comparative, randomised, double-blind, parallel-group, active-controlled, clinical superiority phase 2 trial in patients with eumycetoma requiring surgery was conducted at the Mycetoma Research Centre, Soba University Hospital, Sudan. The study protocol was approved by the National Medicines and Poisons Board and the Soba University Hospital Ethics Committee.[17] The study was designed and performed in accordance with the Helsinki Declaration and the International Council for Harmonisation E6 Good Clinical Practice Guidelines. All patients provided written informed consent; for patients younger than 18 years this was provided by a parent or legal representative; a literate witness signed the form for illiterate patients. Patients under 18 years old also provided written assent, alongside the informed consent provided by their guardian. This study is complete and registered with ClinicalTrials.gov (NCT03086226).

Investigators used active community screening to identify potential patients. Patients were pre-screened per standard of care to confirm eligibility and mycetoma diagnosis, using ultrasonography and biopsy for histopathology of deep tissue. Initially, patients aged 18 years or older with eumycetoma caused by *M mycetomatis* only and a single lesion with a diameter of at least 2 cm but not reaching 10 cm, requiring surgery were eligible for inclusion to ensure a uniform patient population. After an amendment on April 27, 2018, eligibility criteria were adjusted to patients aged 15 years and older with a greater lesion diameter (≥2 to <16 cm) to increase the enrolment rate. The number of female patients was initially restricted to 20, due to preclinical data suggesting increased hepatic and adrenal toxicity in female animals, but this restriction was lifted following

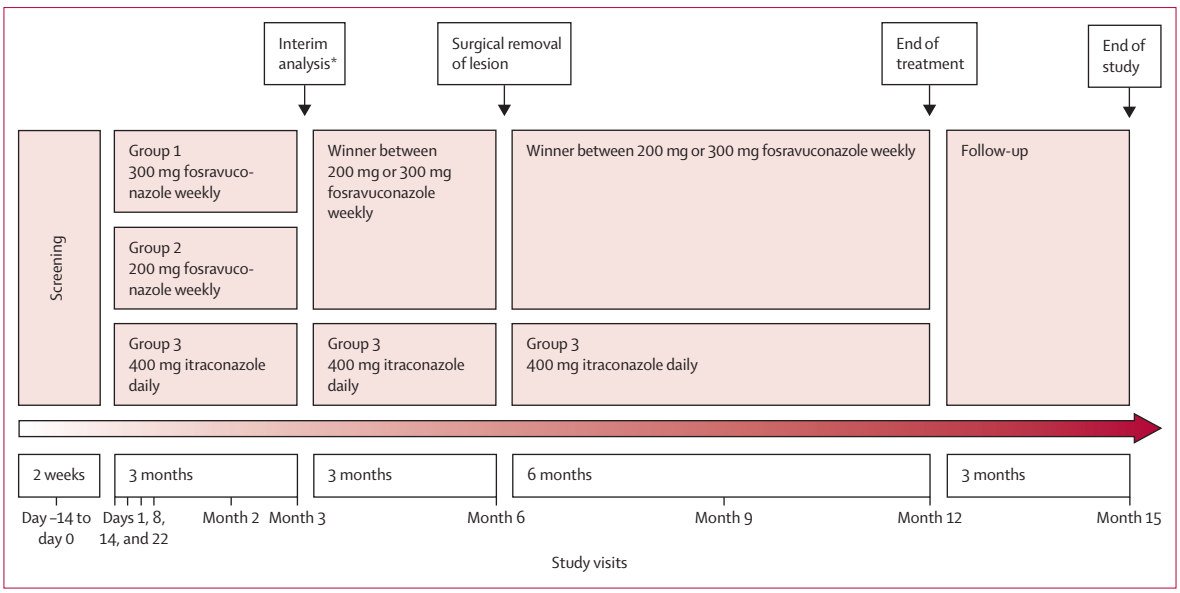

Figure 1: **Study design**
*The second round of random allocation was not performed, following a Data Safety Monitoring Board recommendation.

an interim safety review. Female patients required a negative pregnancy test and, if of childbearing potential, were required to use adequate contraception during the trial and for 2 months afterwards. Exclusion criteria included previous surgical or medical treatment for eumycetoma; presence of loco-regional lymphatic extension, osteomyelitis, or other bone involvement; pregnancy or lactation; severe concomitant diseases; a BMI under 16 kg/m²; contraindication to use of the study drugs; pre-existing liver disease, transaminase levels more than two times the laboratory's upper limit of normal, or elevated levels of alkaline phosphatase or bilirubin; receiving or likely to require drugs that were either a substrate for cytochrome P450 family 3 subfamily A member 4 (CYP3A4), metabolised by CYP3A4, or both; Fridericia's corrected QT interval longer than 450 msec; familial short QT syndrome or corrected QT interval prolongation; or any history of hypersensitivity to any azole antifungal drug.[17]

Randomisation and masking

Patients were randomly allocated in a 1:1:1 ratio to a loading dose of 300 mg fosravuconazole (Eisai, Tsukaba, Japan) orally once a day on days 1, 2, and 3, followed by a single 300 mg oral dose taken on day 8, then weekly for 12 months (group 1); a loading dose of 200 mg fosravuconazole orally once a day on days 1, 2, and 3, followed by a single 200 mg oral dose taken on day 8, then weekly for 12 months (group 2); or the active control 400 mg itraconazole (Janssen Pharmaceutica, Beerse, Belgium) administered as 200 mg orally twice a day daily for 12 months (group 3). Following a planned interim analysis, patients were to be randomly allocated in a 1:1 ratio once one of the fosravuconazole groups was dropped (figure 1). Unique allocation numbers were prepared by an independent statistician (Creapharm, Reims, France) using a random number list with non-disclosed fixed blocks of size 12, with equal allocation to each of the three groups within a block. Patients, caregivers, investigators, and outcome assessors were masked to treatment assignment. Placebo capsules, identical in appearance to the study drugs, were used to mask the frequency of administration in groups 1 and 2, and itraconazole was over-encapsulated to match fosravuconazole. To conform with the standard administration of itraconazole, patients took fosravuconazole plus a placebo dose, or two placebo doses when the weekly dose was not being taken, twice daily after a full meal, despite it not requiring to be taken with food. Doses were not repeated even after vomiting or losing the capsule.

Procedures

To identify the causative agent, at pre-screening, during surgery, and in case of recurrence, a surgical or needle (Estacore automatic biopsy needle, Geotek, Ankara, Türkiye) biopsy from deep tissue was performed to obtain black grains, which were used for histopathology, culture, PCR, and molecular typing. For PCR and typing, DNA was isolated from the grain using the Quick-DNA Fungal/Bacterial Kit (Zymo Research, Irvine, CA, USA).[18] Isolated DNA was amplified using *M mycetomatis*-specific primers 26.1A (5′-AATGAGTTGGGCTTTAACGG-3′) and 28.3A (5′-TCCCGGTAGTGTAGTGTCCCT-3′), and confirmed by sequencing the internally transcribed spacer (ITS) region.[18] To determine the genetic diversity of *M mycetomatis* between patients, DNA was typed using the *M mycetomatis* short tandem repeat (*Mmy*STR) genotyping assay.[19] To determine whether prolonged

exposure to itraconazole or fosravuconazole would lead to decreased susceptibility of the pathogen, the *M mycetomatis CYP51A* gene was sequenced at screening and after surgery, and in vitro susceptibilities were determined for available isolates.[20]

Adherence to trial medication was assessed via questionnaire and pill count at each visit. All patients were admitted to hospital on the month 6 visit for surgery to remove any mycetoma mass.

Assessments were conducted during the screening period (days –14 to 0); during treatment on day 1; weeks 2, 3, and 4; and months 2, 3, 6, 9 and 12; and for follow-up at 15 months (for the assessments performed at all scheduled visits see appendix pp 4–5).

At every scheduled visit, (1,3)-β-D-glucan levels in serum were measured[21] and the lesion was physically examined to assess whether it was improving, static, worsening, or cured based on predefined criteria (absence of mass, sinuses, and discharge), or had recurred after apparent cure. When a mass was present at months 6, 12, or 15, a culture from a surgical biopsy was performed. Ultrasonography, MRI examination, or both were performed at screening, and at months 6 (before surgery), 12, and 15. X-rays were taken at screening to exclude bone involvement. Clinical photographs were taken at screening and at all scheduled trial visits. If relapse occurred, patients received voriconazole or itraconazole, surgery, or both as rescue treatment.

Safety was assessed by monitoring adverse events (graded using NCI CTCAE Version 4.03), laboratory parameters, vital signs, physical examinations, electrocardiograms (ECGs), and serum or urine pregnancy tests.

At each pharmacokinetics timepoint (appendix p 4–5), 3 mL whole blood was collected into a heparin-lithium tube and centrifuged for 5 min at 1900 g at room temperature to obtain plasma, which was stored in polypropylene cryotubes at –40°C until shipment for analysis of ravuconazole or itraconazole content to the Radboud University Medical Centre (Nijmegen, Netherlands). Analysis was done following a protein precipitation method using ultraperformance liquid chromatography with fluorescence detection (appendix p 2).[22]

Outcomes

The primary efficacy endpoint was complete cure at end of treatment at the month 12 visit, as evidenced by absence of mycetoma mass, sinuses, and discharge; normal ultrasonography or MRI examination of the eumycetoma site; and, if a mass was present, negative fungal culture from the former mycetoma site. Secondary efficacy endpoints included assessing whether efficacy was compatible with the targeted efficacy rate of over 70% of patients for each of the three treatment groups, based on the primary endpoint; time to complete cure, time to effective treatment (combination of mycological eradication and clinical improvement in the eumycetoma lesion of at least 50% reduction in size), and time to failure; eumycetoma lesion size, clinical symptoms, signs, or both (change in initial mycetoma lesion size over time and change from baseline in the number of clinical symptoms, signs, or both); durability, determined as the difference between time to cure and time to relapse up to month 15; and decrease or disappearance of detectable (1,3)β-D-glucan from serum. Safety endpoints were occurrence of treatment-emergent adverse events (TEAEs) and abnormal laboratory values, and the severity grade of adverse events per trial treatment. The pharmacokinetics endpoint was analysis of fosravuconazole trough concentrations on weeks 2 and 3, and months 6 and 12. Other secondary endpoints not reported here were comparative efficacy and toxicity of 200 mg and 300 mg fosravuconazole at months 3 and 15 (no evaluable data); comparative efficacy of each group by aetiologic pathogen and correlated with in vitro susceptibility (pre-treatment, pre-surgery, and post-treatment for failures; not performed because there were >90 fungus subtypes); comparative pathogen eradication rates for each regimen at 6 months and end of treatment and end of study (this, and the remaining endpoints are to be analysed and reported separately); relationships between ravuconazole and itraconazole pharmacokinetics and host genetics; and immune response before, and at months 3, 6, 9 and 12 after treatment initiation; and potential relationships between treatment exposure and pharmacodynamic parameters by using population pharmacokinetic–pharmacodynamic modelling.

See Online for appendix

Statistical analysis

A drop-the-loser design was used with an interim analysis when 28 patients per treatment group had completed the 3-month evaluation period, to select the best fosravuconazole dose for progression in the trial (with discontinuation of the losing dose group, and the winning group and active control group continuing recruiting) and to evaluate lifting the restriction on enrolling a maximum of 20 female patients.

Given an estimated itraconazole efficacy rate of 40%, based on existing literature and review of the Mycetoma Research Centre database, and a targeted cure rate of 70% for the winning dose of fosravuconazole, a sample size of 49 patients in the winner and control groups was estimated to provide a power of 85% at a 5% significance level. The planned total sample size was 138, comprising 28 patients for the fosravuconazole losing dose group and 55 patients each for the fosravuconazole winning dose group and the standard care groups, including a 12% provision for loss to follow-up to preserve adequate power.

The primary efficacy endpoint was assessed in the modified intention-to-treat (mITT) population of patients (all randomly allocated patients receiving at least one dose of the study medication in the 300 mg and 200 mg groups, and with at least one assessment after random

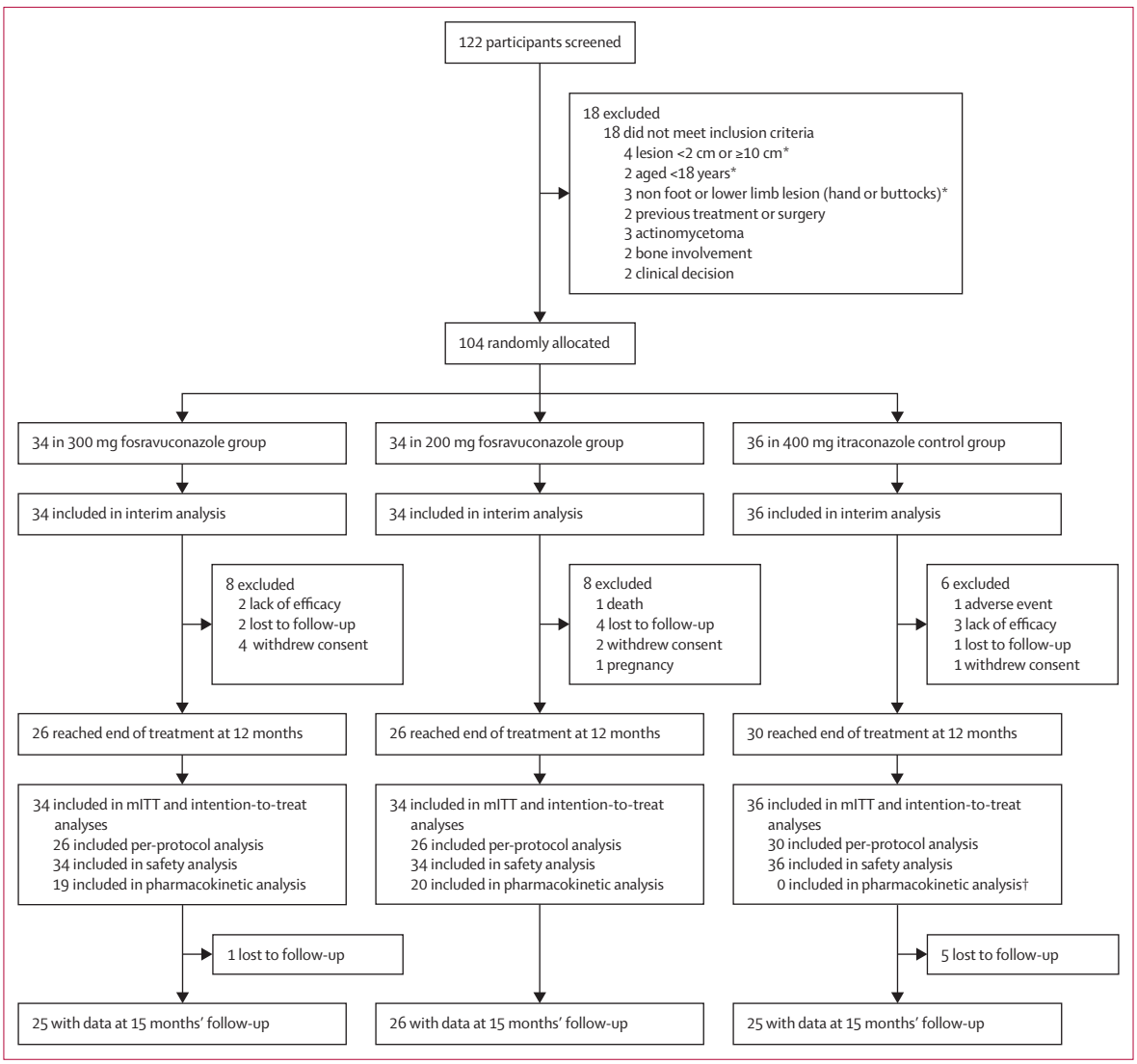

Figure 2: **Trial profile**
mITT=modifed intention-to-treat. *Before protocol amendment. †No pharmacokinetic analysis was planned for the control group; this will be analysed and reported separately.

allocation for the primary efficacy variable) using a likelihood ratio test, with target α set to 0·022 (two-sided). The set α was obtained based on Pocock's adjustment for the three analyses (two interim and one final), and comparison of the two doses of fosravuconazole with itraconazole was made using the Hochberg step-up procedure. Missing data for primary analysis were considered as failure. Sensitivity analyses of the primary efficacy endpoint were performed on the per-protocol population (patients who met all inclusion criteria and no exclusion criteria, with no protocol deviations that were considered to probably influence the study outcome) and patients in whom clinical assessment at month 12 showed absence of any mycetoma lesion. Safety was assessed in all patients who received at least one dose of study drug and had at least one post-dose safety assessment. The remaining analysis sets can be found in the appendix (p 2).

The 95% CI was used to determine whether any of the treatment groups were compatible with the targeted efficacy rate of over 70% of cured patients at month 12. Time in months to complete cure and time in months to treatment failure were evaluated by using Kaplan–Meier curves with corresponding log-rank test.

The mean change from baseline in lesion size, calculated as the surface area (cm²) using the formula surface area=$\pi \times 0 \cdot 25 \times$length$\times$width (under the assumption that the shape would be elliptical) was presented with 95% CI at each post-baseline visit. The percentage change from baseline in the number of pre-therapy clinical symptoms, signs, or both over time for each treatment group was presented with 95% CI at each post-baseline visit.

The safety analysis consisted of descriptive analysis of each TEAE. TEAEs, serious adverse events (SAEs), and adverse drug reactions (ADRs) were summarised by the Medical Dictionary for Regulatory Activities system organ classes and preferred terms, preferred terms only, and severity. Each adverse event reported was characterised for seriousness, relationship to study drug, and outcome. Liver and adrenal toxicity were considered adverse events of special interest (AESIs).

Ravuconazole plasma concentration-time data were subjected to pharmacokinetics analysis. Trough concentrations were summarised for the various pharmacokinetics sampling days. Dose proportionality between 300 mg and 200 mg of ravuconazole was assessed using the ratio of median dose-corrected trough concentrations. Trough concentrations were further used to evaluate exposure–response relationships using logistic regression. Pharmacokinetics and exposure–response analyses were performed using R version 4.2.3. All other statistical analyses and data processing were performed using SAS version 9.4.

Role of the funding source

The study sponsor, DNDi, was responsible for the study design, collection of data, interpretation of results, and reviewing the report.

Results

Between May 9, 2017, and June 10, 2021, 122 patients were screened and 104 were randomly allocated (34 in group 1 and 2, respectively, and 36 in group 3). 76 (73%) of 104 patients completed the trial at 15 months' follow-up (figure 2).

The first interim analysis, in the 84 patients who had completed at least 3 months of treatment, showed no evidence of increased drug-related toxicity or non-compliance in one treatment group over another. Since it was unlikely that superiority of one fosravuconazole dosage regimen would be shown (due to higher than expected efficacy in the itraconazole comparator group), and that the safety parameters were similar for the two fosravuconazole groups, all three groups were continued, following Data Safety Monitoring Board (DSMB) recommendation. The DSMB saw no signal of concern of increased risk for toxicity in women, the restriction on recruitment was lifted. As the trial went ahead with all three groups, the sample size was increased from 138 to 165 (55 patients per group), irrespective of lifting the restriction on recruitment of female patients. The second interim analysis was unplanned and led to stopping the study; it was followed by the final analysis.

A second, unplanned, interim analysis was conducted by the DSMB, due to slow recruitment because of the COVID-19 pandemic and political unrest in Sudan. Following DSMB advice, the trial was terminated early for futility to demonstrate superiority of either fosravuconazole dose over itraconazole. The statistical plan was revised to conduct the superiority analysis on the patients recruited by the second interim analysis (n=104) instead of on the sample size planned after the first interim analysis (n=165).

	300 mg fosravuconazole (N=34)	200 mg fosravuconazole (N=34)	400 mg itraconazole (N=36)
Age, years			
Mean (SD)	30·5 (12·9)	25·0 (7·3)	28·5 (12·5)
Median (IQR)	29·0 (22·0–33·0)	23·0 (20·0–29·0)	24·5 (19·5–33·0)
Range	15·0–77·0	16·0–44·0	15·0–58·0
Sex*			
Male	31 (91%)	28 (82%)	27 (75%)
Female	3 (9%)	6 (18%)	9 (25%)
Ethnicity			
African	34 (100%)	34 (100%)	36 (100%)
Weight, kg			
Mean (SD)	60·1 (12·0)	60·2 (13·2)	60·5 (11·4)
Median (IQR)	60·5 (52·0–67·0)	57·3 (52·0–70·0)	60·0 (53·5–68·8)
Range	36·0–95·5	38·9–88·5	36·0–85·5
Height, m			
Mean (SD)	1·7 (0·1)	1·7 (0·1)	1·7 (0·1)
Median (IQR)	1·7 (1·7–1·8)	1·7 (1·6–1·8)	1·7 (1·6–1·8)
Range	1·2–1·9	1·5–1·9	1·5–1·9
BMI (kg/m²)			
Mean (SD)	20·9 (3·5)	20·9 (3·6)	20·9 (3·8)
Median (IQR)	20·8 (18·2–23·0)	20·7 (18·5–22·5)	19·9 (18·4–22·2)
Range	16·0–31·2	16·0–28·7	16·1–31·0
History and symptoms			
Duration of illness, months			
Mean (SD)	44·7 (38·0)	47·1 (49·6)	41·7 (44·1)
Range	2·0–168	2·0–240·0	0·0–180·0
History of trauma	14 (41%)	13 (38%)	12 (33%)
Family history of eumycetoma	6 (18%)	9 (26%)	13 (36%)
Local swelling	34 (100%)	34 (100%)	36 (100%)
Sinuses†	25 (74%)	27 (79%)	23 (64%)
Discharge	20 (59%)	14 (41%)	19 (53%)
Pain	7 (21%)	5 (15%)	5 (14%)
Other symptoms	0	0	1 (3%)

Data are n (%) unless otherwise specified. *Biological sex assigned at birth was assessed by principal investigators, with the options male and female. †Openings on the skin.

Table 1: **Demographic and baseline characteristics**

Patients' ages ranged from 15·0 to 77·0 years and BMI ranged from 16·0 to 31·2 kg/m². The predominance of male patients (86 [83%] of 104 *vs* 18 [17%] female patients) was primarily due to initial restriction on female recruitment (table 1). Mean duration of eumycetoma at baseline was 44·5 months, ranging from 2·0 months to 20 years. At baseline, the most frequent eumycetoma symptom was local swelling present in all 104 patients, followed by sinuses, discharge, and pain. All patients were infected with *M mycetomatis*, based on PCR and ITS sequencing.

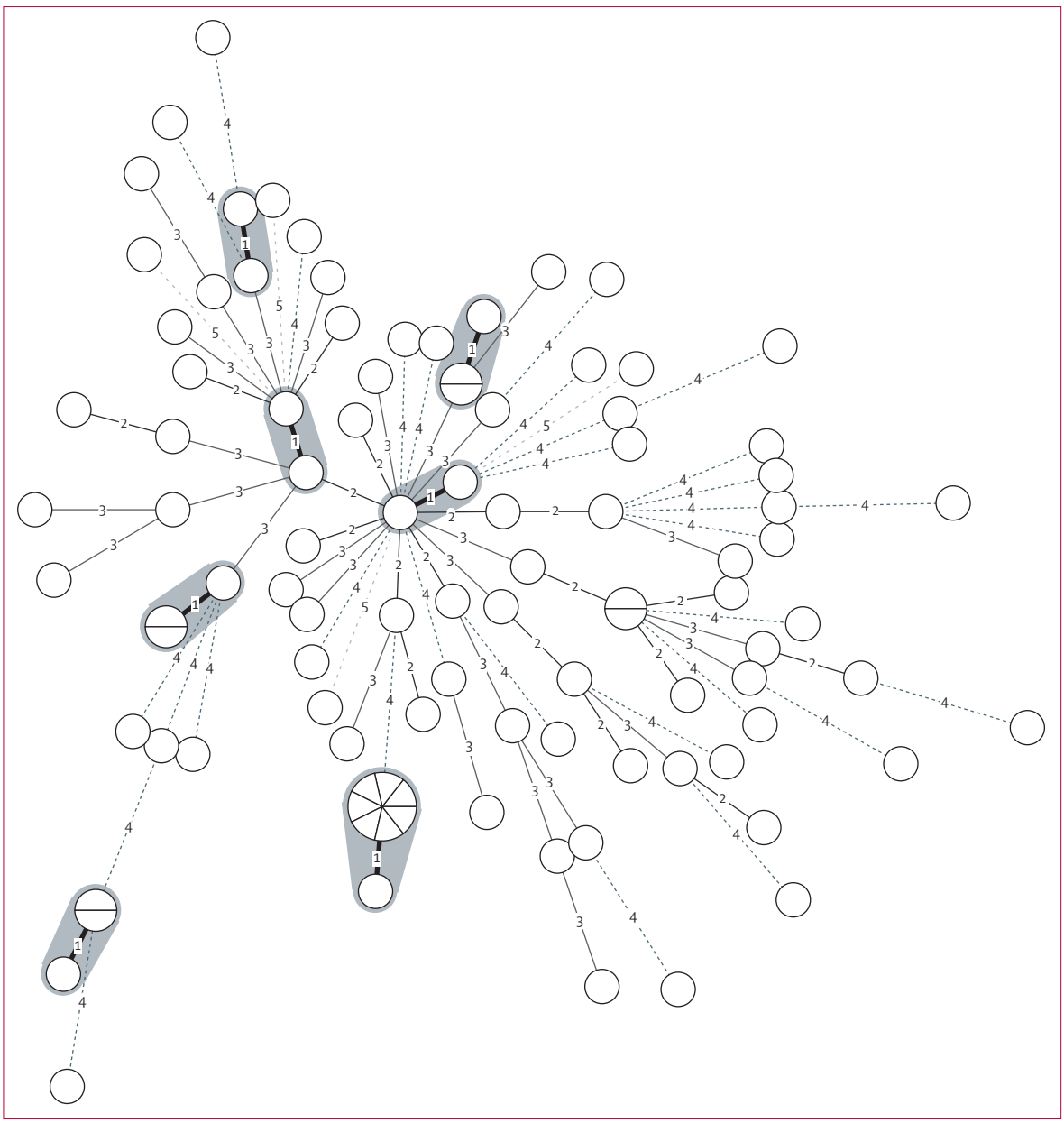

Figure 3: **Genetic diversity of the *Madurella mycetomatis* isolates from the different patients**
Minimum spanning tree based on the 11 short tandem repeats of the *M mycetomatis*-specific typing technique *Mmy*STR. In this tree, the genetic diversity of *M mycetomatis* grains is shown. Each circle represents a genotype. The size of each circle corresponds to the number of patients with a grain of that genotype. The number on connecting lines indicates the number of different markers between the genotypes. Genotypes with a grey background differ in only a single marker and are considered to be related. *Mmy*STR=*M mycetomatis* short tandem repeat assay.

Genetic sequencing was generated from viable grains taken from surgical or needle biopsy at month 0 (104 [100%] of 104 patients), month 6 (93 [100%] of 93 patients), at month 12 (11 [12%] of 90 patients), and at month 15 (three [4%] of 76 patients). There was high genetic diversity among the *M mycetomatis* isolates (figure 3). 11 [92%] of 12 patients for whom *M mycetomatis* DNA was isolated from a recurrent lesion had a *Mmy*STR genotype identical to the original isolate, and the one other patient with a recurrent lesion had a *Mmy*STR genotype which differed, indicating potential re-infection with another strain. The *M mycetomatis* genotype within lesions remained stable over time; there was no genetic alteration in the *MmySTR* genotype or in the *M mycetomatis CYP51* target gene between the different visits in any patient. No significant rise in MIC was observed in the isolates from the 13 patients tested.

Treatment adherence was high, with only three patients in the 400 mg itraconazole group (8%) not reaching full adherence and full adherence in the other groups. In the mITT population, complete cure at end of treatment, comprising surgery at 6 months, absence of mycetoma mass, sinuses, and discharge, normal ultrasonography or MRI, and if a mass was present, negative fungal culture from the former mycetoma site was 17 (50%) of 34 (95% CI 32–68) for group 1, 22 (65%) of 34 (47–80) for group 2, and 27 (75%) of 36 (58–88) in group 3. Neither dose of fosravuconazole was superior to itraconazole (p=0·35 for 200 mg fosravuconazole *vs* p=0·030 for 300 mg fosravuconazole). All groups had a higher than expected efficacy, but only itraconazole demonstrated an efficacy rate of over 70% in the mITT population. Sensitivity analysis in the mITT population for clinical assessment only (with absence of any mycetoma lesion at month 12) showed a significantly lower efficacy (p=0·010) of 300 mg fosravuconazole compared to itraconazole (table 2). Patients who were lost to follow-up were considered as missing information, and thus their data were counted as failures in the analysis. While no patient was excluded from the mITT population (n=104), the per-protocol analysis excluded 22 patients with a major protocol deviation (n=82). Major protocol deviations occurred in one patient who, at the end of treatment, developed a new mycetoma lesion in a pre-existing swelling in another anatomical site; other major protocol deviations were medication dispensing or dosing error (four patients), missed procedures at visit 9 (seven patients), or missed visit 9 (ten patients). By the per-protocol population, complete cure at the end of treatment was 17 (65%) of 26 patients (95% CI 44–83) in group 1, 22 (85%) of 26 patients (65–96) in group 2, and 24 (80%) of 30 patients (61–92) in group 3. The number of recurrences at end of treatment in the mITT population was five (15%) of 34 for group 1, eight (24%) of 34 in group 2, and five (14%) of 36 in group 3.

Time to cure was not significantly different between the treatments (p=0·23; appendix p 8). Mean change in mycetoma lesion size or (1,3)-β-D-glucan concentration in serum from baseline to month 6 was similar for all treatment groups; the mean difference in lesion size in cm² was 3 (95% CI –7 to 13) in group 1, –0·3 (–4 to 4) in group 2, and –0·2 (–7 to 6) in group 3 (appendix pp 6, 8). There were no significant differences in symptoms (local swelling, sinuses, discharge from the sinuses, pain, and other) in each of the groups from baseline at month 6; similarly, there were no significant differences in signs, except for a significant reduction in discharge from sinuses in the 300 mg fosravuconazole group (59% to 27% [p=0·0361]) and a reduction in number of sinuses in the 200 mg fosravuconazole group 2 (appendix p 6).

Median ravuconazole trough concentrations throughout the treatment period ranged at a steady state between the month 6 and 12 visits. For group 1 (300 mg fosravuconazole), median trough concentrations ranged from 2·77 to 3·65 mg/L over this period; for group 2 (200 mg fosravuconazole) the range was 1·50–1·70 mg/L. These trough concentrations indicated a higher than dose-proportional exposure in the observed fosravuconazole dose range, with dose proportionality ratios for 300 mg versus 200 mg ranging from 1·03–1·42 (appendix p 7). Despite this large range in individual exposures, no exposure–response relationship between ravuconazole trough concentrations and complete cure at end of treatment at the month 12 visit was identified, suggesting exposure with 300 mg was no more beneficial than with 200 mg.

83 patients had a total of 205 TEAEs (table 3); malaria was most common (20 [19%] of 104 patients), followed by bacterial infection, influenza, headache, pyrexia, and back pain (>10% of patients). Grade 1 ADRs were reported in three patients in group 3 (one electrocardiogram QT prolonged; two of decreased cortisol [one discovered at end of treatment, one during treatment]) and two grade 2 ADRs (nausea and vomiting) were reported in one patient in group 2. Permanent treatment discontinuation due to TEAEs was reported for two patients (one patient had four SAEs: a fatal event

	Group 1 300 mg fosravuconazole (N=34)	Group 2 200 mg fosravuconazole (N=34)	Group 3 400 mg itraconazole (N=36)
mITT population, n	34	34	36
Complete cure*, n (% [95% CI])	17 (50% [32 to 68])	22 (65% [47 to 80])	27 (75% [58 to 88])
Difference in efficacy (95% CI)	–25 (–47 to –3)	–10 (–32 to 11)	Ref
p-value	0·030	0·35	Ref
Per-protocol population, n	26	26	30
Complete cure*, n (% [95% CI])	17 (65% [44 to 83])	22 (85% [65 to 96])	24 (80% [61 to 92])
Difference in efficacy (95% CI)	–15 (–38 to 9)	55 (–15 to 25)	Ref
p-value	0·22	0·65	Ref
mITT population: clinical assessment only, n	34	34	36
Complete cure†, n (% [95% CI])	23 (68% [50 to 83])	27 (79% [62 to 91])	33 (92% [78 to 98])
Difference in efficacy (95% CI)	–24 (–42 to –6)	–12 (–29 to 4)	Ref
p-value	0·010‡	0·14	Ref
Complete case population§, n	25	27	32
Complete cure*, n (% [95% CI])	18 (72% [51 to 88])	21 (78% [58 to 91])	28 (88% [71 to 97])
Difference in efficacy (95% CI)	–16 (–37 to 6)	–10 (–29 to 10)	Ref
p-value	0·14	0·32	Ref

mITT=modified intention-to-treat. *Complete cure was defined by absence of mycetoma mass, sinuses, and discharge; normal ultrasound examination of the mycetoma site or normal MRI; and a negative fungal culture from a surgical biopsy if a mass was present. Target significance α level was set at 0·022 after adjusting for three analyses (two interim and final). No p value was less than 0·022, so no adjustment of α for comparison of the two doses of fosravuconazole to itraconazole by using the Hochberg step-up procedure was performed. †Clinical assessment was absence of mycetoma mass, sinuses, and discharge. 14 patients (five patients in group 1, six patients in group 2, and three patients in group 3) with missing outcome had their data considered as failures. ‡p-value evaluating the difference in efficacy between each of the fosravuconazole treatment groups and itraconazole. α was set at 0·022 after adjusting for three analyses (two interim and final). Adjusting for the two comparisons, the difference between 300 mg fosravuconazole and 400 mg itraconazole is statistically significant. §All patients with information at the primary outcome (month 12), excluding those with missing information or lost to follow-up.

Table 2: **Primary efficacy outcome at end of treatment**

	300 mg fosravuconazole (N=34)	200 mg fosravuconazole (N=34)	400 mg itraconazole (N=36)
At least one TEAE	25 (74% [62])	29 (85% [78])	29 (81% [65])
Infections and infestations	14 (41% [23])	18 (53% [32])	17 (47% [21])
Malaria	7 (21% [8])	8 (24% [8])	5 (14% [6])
Bacterial infection	7 (21% [7])	6 (18% [7])	3 (8% [4])
Hepatitis B	1 (3% [1])	2 (6% [2])	2 (6% [2])
Urinary tract infection	2 (6% [2])	3 (9% [3])	0
Upper respiratory tract infection	1 (3% [1])	1 (3% [1])	2 (6% [2])
Schistosomiasis	2 (6% [2])	0	0
Tonsillitis	0	2 (6% [2])	0
Respiratory, thoracic, and mediastinal disorders	4 (12% [4])	5 (15% [5])	11 (31% [12])
Influenza	3 (9% [3])	3 (9% [3])	8 (22% [8])
Lower respiratory tract infection	0	2 (6% [2])	2 (6% [2])
Cough	0	0	2 (6% [2])
Gastrointestinal disorders	6 (18% [8])	5 (15% [8])	5 (14% [7])
Nausea	0	3 (9% [3])	3 (8% [3])
Abdominal pain	3 (9% [3])	1 (3% [1])	1 (3% [1])
Vomiting	1 (3% [1])	2 (6% [2])	1 (3% [1])
Abdominal pain upper	2 (6% [2])	0	1 (3% [1])
Injury, poisoning, and procedural complications	2 (6% [2])	6 (18% [6])	1 (3% [1])
Injury	0	2 (6% [2])	0
Nervous system disorders	5 (15% [7])	5 (15% [8])	4 (11% [5])
Headache	4 (12% [6])	5 (15% [6])	3 (8% [4])
General disorders and administration site conditions	5 (15% [6])	4 (12% [5])	3 (8% [3])
Pyrexia	5 (15% [5])	2 (6% [2])	3 (8% [3])
Blood and lymphatic system disorders	4 (12% [4])	3 (9% [3])	2 (6% [2])
Lymphadenopathy	2 (6% [2])	2 (6% [2])	1 (3% [1])
Musculoskeletal and connective tissue disorders	2 (6% [2])	2 (6% [4])	4 (11% [4])
Back pain	1 (3% [1])	1 (3% [1])	4 (11% [4])
Pain in extremity	0	2 (6% [3])	0
Investigations	2 (6% [2])	0	3 (8% [3])
Cortisol decreased	0	0	2 (6% [2])
Skin and subcutaneous tissue disorders	0	3 (9% [3])	2 (6% [2])
Vascular disorders	1 (3% [1])	0	3 (8% [3])
Hypertension	0	0	2 (6% [2])
Renal and urinary disorders	1 (3% [1])	2 (6% [2])	0

Data are number of patients (% [number of events]). TEAEs are Medical Dictionary for Regulatory Activities system organ class preferred terms. TEAE=treatment-emergent adverse event.

Table 3: **Summary of TEAEs reported in more than one patient by treatment group**

unrelated to treatment caused by cardiac arrest, tetanus, and sepsis; and HIV infection; and one patient had an ADR of decreased cortisol). No safety signals emerged from haematology, chemistry, or ECG assessments (appendix pp 3–4).

Discussion

To our knowledge, this is the first ever randomised, controlled, double-blind trial conducted in patients with eumycetoma. It provides a benchmark for the efficacy of antifungal agents in addition to surgical excision of lesions, and is the first in human administration of fosravuconazole over a prolonged period. The primary objective was to demonstrate superior efficacy of weekly dosing with either 200 mg or 300 mg fosravuconazole over the standard-of-care 400 mg daily itraconazole (Sporanox; Janssen Pharmaceutica, Beerse, Belgium), for 12 months in patients with small-to-medium sized eumycetoma lesions requiring surgery. The efficacies of 50% for 300 mg fosravuconazole and of 65% for 200 mg fosravuconazole in the mITT analysis are encouraging, but the higher than expected 75% efficacy of itraconazole meant it was not possible to demonstrate superiority of either fosravuconazole dose over itraconazole. Efficacies in the per-protocol population, which more closely resembles real-life use, are greater than 70% for both 200 mg fosravuconazole (85%) and itraconazole (80%). The secondary efficacy endpoints (time to complete cure, time to treatment failure, and time from surgery to recurrence) also showed little difference between treatments. The much higher than expected efficacy of itraconazole may be due to the less homogeneous population and higher drop-out rates in clinical practice than in a controlled, blinded phase 2 trial with more robust follow-up and free administration of this otherwise expensive treatment.

All regimens were well tolerated, with no new safety signals, although not statistically significant, there appeared to be fewer patients with TEAEs and fewer individual TEAEs with 300 mg fosravucanazole. The incidence of ADRs was low, and none were serious. There were no obvious differences between treatment groups regarding incidence of SAEs, AESIs, severe TEAEs, or TEAEs leading to treatment interruption or discontinuation. The three TEAEs leading to death in one patient were not treatment-related.

Exposure following both doses of fosravuconazole appeared higher than dose-proportional, but no obvious exposure–response relationship was observed, suggesting exposure levels had achieved maximum effect. Given this, and the similar efficacy and safety data for the two fosravuconazole arms, the 200 mg dose might be selected for future studies, which has additional advantages in terms of pill burden and cost. The lack of clinical response (symptoms and signs and lesion surface area) in the first 6 months was remarkable, although this was only assessed for the mass above the level of the skin. Similarly, we did not observe a significant decrease in (1,3)-β-D-glucan levels in serum during the first 6 months of treatment. Future studies should address volumetric assessment of the whole lesion during treatment, possibly using biomarkers and exploring the relationship to drug exposure. Drug exposure seems to induce a biofilm-like encapsulation of the eumycetoma mass with extensive fibrosis, making surgical removal easier; however, as shown in this study, this does not completely prevent relapse even after prolonged drug treatment. It is unclear whether encapsulation prevents drug penetration, and whether nanoparticle delivery could be more effective.

The patients were infected with a genetically diverse range of *M mycetomatis* isolates, similar to those reported in other populations with eumycetoma in Sudan.[19] Only one patient had the genotype differing from the original at end of treatment, perhaps due to re-infection with a new *M mycetomatis* strain or a mixed infection, although mixed infections have not previously been documented.[23] Prolonged exposure to azoles did not alter the *M mycetomatis CYP51A* nucleic acid sequence over time and no rise of MIC above the epidemiological cutoff values set for *M mycetomatis* exposed to itraconazole or ravuconazole was noted.[20] For most filamentous fungi, resistance to azoles is linked with alterations of the target gene; however, other routes of potential resistance, such as enhanced activity of drug efflux pumps, could only be ruled out if MICs were determined for all patients.[20]

Our study has some limitations. This was a single-centre trial; the results cannot be extrapolated to other endemic areas without further studies. The study was restricted to patients aged 15 years and older with lesion diameters of 2 cm or larger but smaller than 16 cm caused by *M mycetomatis*; further study is required on children, women of childbearing potential, lesions that are more extensive, or caused by other fungal pathogens, and recurrences. Despite these limitations and the lack of superiority, the combination of 200 mg fosravuconazole with surgery showed positive aspects such as the good safety profile and low risk of drug–drug interaction, plus it can be taken without food, and administration is only once a week compared with twice daily for the first-line treatment. Dosing could be planned for a preferred day, or as a weekly recurring event, reducing the risk of forgetting one of the twice daily itraconazole doses or taking it without food, leading to lower exposure. Data in support of weekly administration from high-income countries are scarce, and there are no studies from low-resource settings.[24]

Effective treatments with combinations of drugs with a shorter duration and higher cure rate, without the need for surgery for early forms, could be explored in the future. New and promising compounds have been reported from MycetOS, an open-source drug discovery initiative,[25] and new classes of antifungal drugs already in phase 2 or 3 trials for other indications may be explored for their potential in eumycetoma.[26] Preclinical models for eumycetoma will be key to guiding selection of the most promising compounds.[27] Community education and awareness is essential, as chances of success might be higher if early diagnosis and treatment are implemented. While fosravuconazole might provide an effective and safe monotherapy in patients with small-to-medium size eumycetoma, further research efforts should focus on combination therapies of shorter duration with improved efficacy across the clinical spectrum, taking into account other causative fungi, other endemic regions, and all patient groups.

Contributors

The study was conceived and designed by AHF, NS-W, BS, and EEZ. The principal investigator, AHF, provided oversight on all aspects of the study. The co-principal investigators, SMB and ESWM, managed the investigators' team and oversaw the study at the clinical site. BS and TWE provided statistical advice in the study design and interim analyses. PO provided data management. KH provided oversight on the use of fosravuconazole as a study drug. The study physicians, ESA, OEB, LAF, and AAM, performed all clinical and safety assessments. The laboratory analyses were performed by WWJvdS, NAM, and EES. MENB provided radiological assessments. Pharmacy services of the investigational products were provided by HYA. The immunological assessments were performed by AMM. The pharmacokinetic analyses were performed by RJB, W-YC, and TPCD. KOO and BAN provided oversight on practical aspects and quality control. AHF, SMB, and EEZ reviewed all study data and analysis and had final responsibility for the decision to submit for publication. All authors had full access to all the data in the study and accepted responsibility to submit for publication. EEZ and AHF prepared the original draft. All authors commented on the draft and approved the final version of the manuscript.

Declaration of interests

BS declares consulting fees and other payments from Drugs for Neglected Diseases initiative (DNDi). KH is employed by the company that invented the study drug. All other authors declare no competing interests.

Data sharing

Interested researchers may request access to de-identified patient data from Vivli, the data-sharing partner of the DNDi, commissioner of this study, at https://vivli.org/ourmember/dndi/.

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