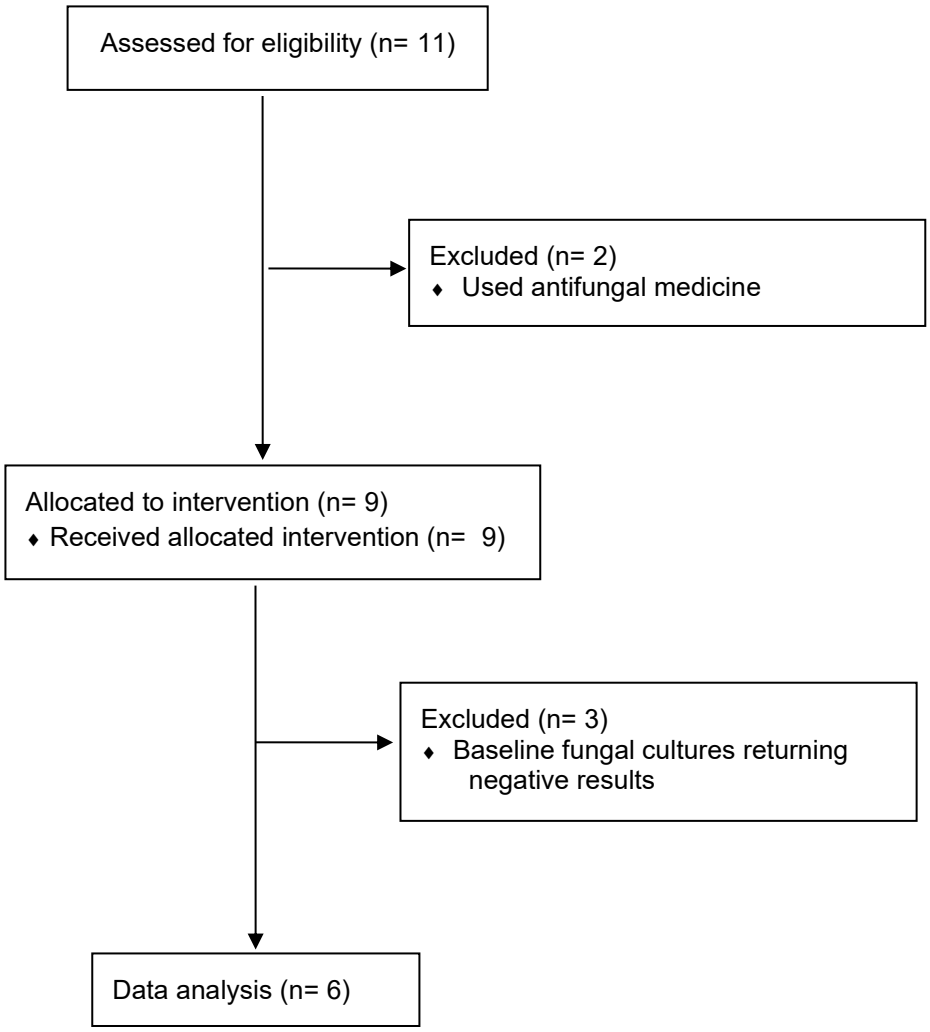
TRIAL INFORMATION

Full title of the trial	**Photodynamic therapy of antifungal resistant dermatophytes**

Summary:
The study aimed to investigate whether photodynamic therapy was efficacious against antifungal resistant fungal skin infections using methylene blue as photosensitizer.
The study initially planned to include 25 patients. Unfortunately, recruitment proved to be challenging, and it was difficult to obtain consent from eligible individuals. A total of 11 participants initially agreed to take part in the study; however, two were excluded immediately because they had recently used antifungal medication (oral or topical). Two individuals were therefore classified as screening failures at the outset. During data analysis, an additional three participants were excluded. This post hoc exclusion was due to their baseline fungal cultures returning negative results. As the fungal cultures required four weeks to process—equivalent to the entire duration of the study—this information was not available until after the study period had concluded. We ultimately decided to terminate the trial early. This decision was based both on the low treatment success rate—only 33% of participants responded to treatment—and on the persistent difficulties with patient recruitment.



Trial Identifiers:

EudraCT no.	2020-002644-23
Sponsor protocol Code	
ISRCTN no.	
Clinicaltrial.gov NCT no.	
WHO Trial no./Universal Reference no. (UTN)	

Sponsor:

Organisation name	Department of Dermatology, Zealand University Hospital
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Contact points – Scientific contact point:

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Result analysis stage:

Analysis stage	Final
Date of interim/final analysis	1st June 2025
Primary completion date reached?	no
Primary completion date	31st December 2021
Global end of trial reached?	Yes
Date of global end of trial	15th August 2024
Was the trial prematurely ended	Yes

General information about the trial:

Main objectives of the trial	We aimed to investigate whether photodynamic therapy is efficacious against antifungal resistant fungal skin infections using methylene blue as photosensitizer.
Actual date of start of recruitment	9th March 2021
Long term follow-up planned?	no

Independent Data-monitoring Committee involvement?	Yes
Protection of subjects	The patients were asked about known allergies that are contradictions to the treatment (light and colour allergy) before study initiation in order to avoid complications. Furthermore, the study staff asked open, non-leading question such as: "How have you been feeling since your last visit?" This helped us to detect and if necessary, treat or react upon adverse events.
Background therapy	An *in vitro* study had demonstrated that photodynamic therapy (PDT) may be useful for the treatment of fungal biofilm. PDT is an approach where a photosensitizer and visible light at specific wavelength generates reactive species of oxygen (ROS) and nitrogen (RNS), which are capable of killing microbes. As the method is a 'physical method' as opposed to medical antifungal treatment, the risk of treatment failure in cases with acquired antifungal resistance should be minimal. The PDT technique is well-established in pre- and skin cancer treatment in dermatology clinics, but its use for the treatment of resistant fungal infections is new. The widespread use of PDT will ensure the simple technique will be broad available for all patients. Furthermore, it will also be useful for the treatment of common fungal infections which is not resistant. Product name: *V03AB17: Methylthioninium chloride Proveblue (methylene blue)* 5mg/ml. Preparation as permitted in Denmark for the acute symptomatic treatment of methaemoglobinemia induced by drugs and chemicals and may be given to children <3 months of age, where it is administered as i.v. injection over 5 minutes. *In the current proposed study methylene blue is used as a photosensitiser and applied superficial on the skin.* Methods: Baseline (week 0) Methylene Blue will be applied at the infection area (target area) and occluded for 3 minutes. PDT illumination with 665 nm, light dose 37 joule / cm2 will be used for 8 minutes and 5 s at a 10 cm distance. Same procedure week 2.
Evidence for comparison(s)	No comparators

Population of trial subjects:

Country	Denmark

Subjects per country:

Trial country	Denmark
Planned number of subjects	25
Actual number of subjects	11

Age of subjects:

	Number of subjects
Between 18-64	11

SUBJECT DISPOSITION

Recruitment details	All patients were recruited from Department of Dermatology, Zealand University Hospital, Roskilde, Denmark.

Screening details	Two individuals were initially classified as screening failures due to use of antifungals less than 14 days before. However, during data analysis, an additional three individuals were excluded. The reason for this late exclusion was that their baseline fungal cultures returned negative results. As the fungal culture results required four weeks to process—equivalent to the duration of the study—the exclusion occurred post hoc.

Pre-assignment period:

		Number of subjects
STARTED		11
Intermediate milestone	Had a laboratory confirmed antifungal resistant dermatophyte infection	11
COMPLETED		6
Reason for non-completion	Other	5 Did not meet the inclusion criteria. Two had used antifungals. Three study subjects were excluded later as their baseline fungal cultures returned negative results. As the fungal culture results required four weeks to process—equivalent to the duration of the study—the exclusion occurred post hoc.

Period table

Title	Overall period
Baseline period	Yes
Allocation method	Not applicable
Blinding used	Not blinded

Arms

Are the arm mutually exclusive? (Only answer no, if the subjects are present in more than one arm in a period. If the arms are not mutually exclusive, the number of subjects in the period, will not be calculated automatically)	Only one arm

Arm table

Arm Title	Treatment Group (Methylene Blue)	
Arm description (provide more information)	Methylene blue treatment of antifungal drug resistant dermatophytosis: Baseline (week 0) Methylene Blue will be applied at the infection area (target area) and occluded for 3 minutes. PDT illumination with 665 nm, light dose 37 joule / cm2 will be used for 8 minutes and 5 s at a 10 cm distance. Same procedure week 2. Follow-up with fungal culture and clinical signs 2 weeks after second treatment (week 4).	
Arm type	Experimental	
	Number of subjects	
STARTED	11 (5 excluded)	
COMPLETED	6	
		Number of subjects
Reason not completed	-	
Reason for joining	Not applicable	

Product used:

IMP Name	Methylthioninium chloride Proveblue
IMP Code	V03AB17
Other names?	methylene blue
Route of administration	Cutaneous use
Pharmaceutical form	Concentrate and solvent for infusion
Dosage and administration details	5mg/ml. Preparation as permitted in Denmark for the acute symptomatic treatment of methaemoglobinemia induced by drugs and chemicals and may be given to children <3 months of age, where it is administered as i.v. injection over 5 minutes. *In current study methylene blue is used as a photosensitiser and applied superficial on the skin.* Week 0: Methylene Blue was applied at the infection area (target area) and occluded for 3 minutes. PDT illumination with 665 nm, light dose 37 joule / cm2 will be used for 8 minutes and 5 s at a 10 cm distance. Same procedure week 2.

BASELINE CHARACTERISTICS

Age:

Report group title	Treatment Group (Methylene Blue)	
Reporting group description	(This is derived from subject disposition)	
Overall number of baseline subjects	(This is derived from subject disposition)	
Age (Categorical)		
	Number of subjects Reporting group	**Total:**
From 18 – 64 years	**6**	**6**

Age (Continuous)	
Units	Years
Central tendency type/Measure type	Mean 49
Dispersion type	Sample min/max 38-60

Gender:

Report group title	Treatment group	
	Number of subjects	**Total:**
Female	2	2
Male	4	4

Study Specific Measure:

Report group title	Physical sign	
Reporting group description		
Overall number of baseline subjects	6 (included in the study)	
Study specific (categorical)		
Characteristic title	Skin scaling	
Units	Clinical grading (see below)	
Category title	None (0), Mild (1), Moderate (2), Severe (3)	
	Number of subjects Reporting group	**Total:**
None	1	1

Mild	3	3
Moderate	2	2

Study specific (Continuous)		
Characteristic title	Fungal culture negative	
Units	Binary outcome (growth/no growth)	
Central tendency type/Measure type	Central tendency	
Dispersion type	Not applicable	
Value of measure type in Reporting group	Value of dispersion type in Reporting group	
growth / no growth	Not applicable	

END POINTS

End point title	Fungal culture negative	
Countable or measurable?	Countable	Measurable
	2 (33%)	
If countable is chosen, enter countable unit	No growth	
If measurable is chosen, enter measurable unit	dichotomous (binary) outcome	
If measurable is chosen, enter measurable type	Number	
If measurable is chosen, enter dispersion type	-	
End point type	Primary	
End point description	No growth of fungal cultures	
End point time frame	4 weeks, 2 weeks after second treatment	

Use categories only if the data for the end point can be categorised (this is only used for countable end points)**	
Proportion with no growth	33% (2/6) of the patients responded to the methylene blue photodynamic treatment.

Statistical Analysis of End Point:

Statistical analysis title	No statistical analysis is applied due to the low numbers of participants and as there was only one treatment arm.

ADVERSE EVENTS

Time frame for adverse event reporting	Study period

Assessment type	Systematic
Frequency threshold for reporting non-serious adverse events	Follow-up consultation (4 weeks)

Adverse Events Reporting group:

Reporting group title	Participants treated
Reporting group description	All participants including those excluded at a later stage
Subjects exposed	6
Number of subjects affected by serious adverse events	0
Number of subjects affected by non-serious adverse events	4
Number of deaths (all causes)	0
Number of deaths resulting from adverse events	0

Serious Adverse Events:

System Organ Class	None

Non-serious Adverse Events:

System Organ Class	Skin Immune system Gastrointestinal disorders
Event term	Grade 1
Additional description	Skin: Pruritus at the treated areas (n= 1) Blisters and sores between the toes (n=1) Skin infection between the toes (n=1) Immune system: Temporary swelling of a regional lymph node (n=1)
Assessment type	Clinical

Limitations and caveats:

Limitations and caveats that apply to the results	The number of patients included in the study was lower than anticipated, which limits the generalizability and statistical analysis of the findings. Further studies involving larger cohorts will be necessary to confirm these results and strengthen the evidence base.

Online references:

PubMED identifier (PMID)	https://pubmed.ncbi.nlm.nih.gov/40587657/ PMID: 40587657