# 1 Title page

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| **Title:** | Explorative analysis of topical miltefosine application in adult patients with atopic dermatitis |
| **Sponsor:** | Prof. Dr. med. Margitta Worm |
| **Sponsor's contact person:** | Prof. Dr. med. Margitta Worm |
| **Investigational products:** | Miltefosine (Miltex®) |
| **Indication studied:** | Atopic Dermatitis |
| **Development phase of the study:** | Therapeutic exploratory Phase II |
| **Principal investigator according to § 4 AMG:** | Prof. Dr. med. Margitta Worm |
| **Study centres:** | Mono-centre |
| **Study number:** | Miltefosin bei AD; Eudra-CT Number: 2007-003471-39 |
| **Study dates:** | Start: September 2007 End: January 2008 |
| **Design:** | Randomised, active-controlled, double-blind, parallel group design |
| **Report date:** | 29.01.2009 |
| **GCP-compliance:** | The investigation was carried out and essential documents are archived in accordance with Good Clinical Practice (GCP) Guideline. |
| **Confidentiality:** | This document is a confidential communication of X-pert Med GmbH. No unpublished information contained herein will be published or disclosed without prior approval by the sponsor. However, this document can be disclosed to authorized representatives of national or international regulatory authorities under the condition that they respect its confidential nature. |

# 2 Synopsis

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| **NAME OF SPONSOR:**<br>**Prof. Dr. med. Margitta Worm** | **NAME OF FINISHED PRODUCT:**<br>**Miltex®**<br>**NAME OF ACTIVE INGREDIENT(S):**<br>**Miltefosine** | **(FOR NATIONAL AUTHORITY USE ONLY)** |
| **Title of clinical trial:**<br>Explorative analysis of topical miltefosine application in adult patients with atopic dermatitis | | |
| **Principal Investigator:** | Prof. Dr. med. Margitta Worm | |
| **Study center:** | Mono-centre, please refer to section 6 for details about the investigators and other important participants. | |
| **Publication (reference):** | - Congress-Abstract: Dölle S, Hoser D, Rasche C, Lee H.-H, and Worm M.: "Clinical efficacy of miltefosine in atopic dermatitis" XXVII. EAACI Congress, Abstract Book<br><br>- Manuscript in preparation | |
| **Study period (years):**<br>**Date of approval:**<br><br>**Date of first patient enrolled:**<br>**Date of last patient completed:** | September 2007 – January 2008<br>22nd August 2007 (Version 1.1, 21st June 2007)<br>8th October 2007 (Amendment, Version 1.3, 31st August 2007)<br>14th September 2007<br>23rd January 2008 | |
| **Development phase of the study:** | Therapeutic exploratory (phase II) Study | |
| **Objectives**<br>Primary: | - To determine the clinical responsiveness of the skin of patients with atopic dermatitis to miltefosine using the three item severity (TIS) score. | |
| Exploratory: | - To determine the clinical responsiveness to miltefosine compared to an active control (hydrocortisone).<br>- To support the TIS score the objective SCORAD (Severity Scoring of Atopic Dermatitis) was assessed.<br>- Analysis of immunohistological parameters like $CD4^+/CD8^+$ T-cells infiltration, mast cells and also measurement of the epidermal thickness.<br>- Analysis of the skin physiological and thermographic parameters. | |

**Methodology:**

**Methods for evaluating the primary objective**

- Evaluation of the TIS score, which is based on 3 items; erythema, oedema/papulation and excoriations. The 3 items are scored on a 4-point-scale from 0 to 3 (0=none, 1=mild, 2=moderate and 3=severe). At screening 2 comparable target lesions were defined and randomised. The TIS score were evaluated at screening, visit 1, 2, 3 and 4 as well as follow up 1 and 2.

**Methods for evaluating the exploratory objective**

- The objective SCORAD were evaluated for global disease severity at screening, visit 1, 2, 3 and 4 as well as follow up 1 and 2.
- The skin biopsies were taken before and after the treatment. The skin biopsies were frozen, cut in 4 µm thick sections and stained for $CD4^+$/$CD8^+$ T-cells and mast cells infiltration and for measuring the epidermal thickness.
- The skin barrier function (skin physiology) was assessed with following measurements: transepidermal water loss (TEWL), the sebum level (SEBUM), the skin hydration (CORNEUM) and pH-value. The measurements were performed at visit 1, 2, 3 and 4.
- Thermographic imaging was used to detect variations in temperature of the target lesion. Thermographic measurements were performed at visit 1, 2, 3 and 4).

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| **Number of patients (planned and analyzed):** | Planned: 16<br>Included: 16<br>Drop-outs: 0<br>Analyzed: 16 |
| **Diagnosis and main criteria for inclusion:** | - Diagnosis of atopic dermatitis according to Hanifin&Rajika<br>- Chronic course of disease<br>- Age ≥18 years<br>- 2 comparable skin lesions of 10 $cm^2$ with a TIS score between 5 and 7 points appropriated to take biopsies<br>- Negative pregnancy test<br>- High reliable method of contraception for women of childbearing potential as well as for the female partner of sexual active men who participate in this trial<br>- Inform content according to AMG §40 (1) 3b |

**Test product, dose and mode of administration, batch no.:**

Miltefosine as 6% solution (Miltex®)

- 2 drops on 10 cm² once daily in the first treatment week and twice daily in the second and third treatment weeks

**Reference product, dose and mode of administration, batch no.:**

Hydrocortisone as 1% solution (Hydrogalen®), active control

- 2 drops on 10 cm² once daily in the first treatment week and twice daily in the second and third treatment weeks

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| **Duration of treatment:** 3 weeks | |
| **Criteria for evaluation:** | |
| Primary effect parameters: | - Clinical evaluation of treatment response to miltefosine over a period of 3 weeks. Evaluations of the TIS score before, during, and after the treatment as well as in the follow up. The TIS score assess the disease intensity of the target lesion evaluating erythema, edema/papule and excoriation each on a 4-point scale (0=no, 1=mild, 2=moderate, 3=severe). |
| Exploratory effect parameters: | - Clinical evaluation of treatment response to miltefosine compared to the active control, hydrocortisone.<br>- Additionally the objective SCORAD was assessed.<br>- Number of immune effector cells ($CD4^+$/$CD8^+$ T-cells, mast cells) and epidermal thickness before compared to after the treatment.<br>- Change from baseline of skin physiological and thermographic parameters. |
| Safety parameters: | - Evaluation of adverse events (AE) that are summarized as general AEs and local skin-related AEs with definite drug relation. |
| **Statistical methods:**<br>- No formal sample size calculation was performed because of the exploratory character of this clinical trial. However, an exemplary sample size calculation was done. With a median effect size of 1.5% standard deviation detected with a two-sided significance level of 5% and a power of 80% a study population of 16 patients was calculated.<br>- The clinical parameter (TIS) from baseline was compared with the TIS from after the 3-week treatment using the non-parametric paired Wilcoxon test.<br>- A change from baseline to the end of treatment of the clinical parameter (TIS score) was compared between miltefosine and hydrocortisone using the non-parametric unpaired Mann-Whitney-U test.<br>- All exploratory analysis, e.g. the number of immune effector cells ($CD4^+$/$CD8^+$ T-cells, mast cells) and the epidermal thickness as well as the skin physiological and thermographic parameters were evaluated using non-parametric test either Wilcoxon test or Mann-Whitney-U test. | |

**SUMMARY - CONCLUSIONS:**

**Effect results:**

1. Significant decrease of TIS score over the time of miltefosine treatment;
2. Less effectiveness on the clinical outcome in the exploratory comparison to hydrocortisone;
3. The objective SCORAD improved in almost all patients over the 3-week intervention period.
4. The immunhistological parameters were hardly changed, except of the epidermal thickness that tended to reduce to normal thickness in the miltefosine-treated lesions and were significantly reduced in the hydrocortisone treatment indicating an atrophic characteristic;
5. No major significant changes were observed in the skin physiological parameters. Only the TEWL significantly reduced over the time in both treatments.
6. The thermography represented by the maximum temperature was significantly reduced in the miltefosine-treated lesions.

**Safety results:**

The overall treatment was well tolerable. No systemic general AE occurred during the treatment. No general or local skin-related AE caused a pre-termination of the intervention. However, the local skin-related AEs were mainly due to the miltefosine treatment. Especially, symptoms of dry skin were exclusively caused by the miltefosine treatment indicating that improved basic formulations are necessary for further clinical trials.

**Conclusions:**

The primary effect criterion was reached with this exploratory design.
The exploratory effect parameter underline the primary effect in particular the thermographic parameter which in turn underpin the immunhistological trends (reduced epidermal thickness, reduced CD4+ T-cells and mast cell counts) in the miltefosine-treated lesions.
In general, both treatments were well tolerated, however, local skin-related AEs were observed in the miltefosine treatment more often.

Date of the report: 29th January 2009

# 3 Table of contents

# 4 List of abbreviations and definition of terms

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| AD | Atopic Dermatitis |
| AE | Adverse Event |
| AMG | Arzneimittelgesetz (German Drug Law) |
| ALP | Alkaline Phosphatase |
| ALT | Alanine Aminotransferase |
| Approx. | Approximately |
| CD | Cluster of Differentiation |
| CORNEUM | Skin Hydration or Water-binding Property |
| CRF | Case Report Form |
| GCP | Good Clinical Practice |
| GGT | Gamma Glutamyltransferase |
| ICH | International Conference of Harmonisation |
| IEC | Independent Ethics Committee |
| IMP | Investigational Medicinal Product |
| ISF | Investigator Site File |
| ITT | Intention To Treat |
| MCH | Mean Corpuscular Haemoglobin |
| MCHC | Mean Cellular Haemoglobin Concentration |
| MCV | Mean Cellular Volume |
| MPV | Mean Platelet Volume |
| PP | Per Protocol |
| RDW | Red Cell Distribution Width |
| SAE | Serious Adverse Event |
| SCORAD | Severity Scoring of Atopic Dermatitis |
| SEBUM | Sebum Level or Skin Surface Lipid Content |
| SOP | Standard Operating Procedure |
| TIS | Three Item Severity |
| TEWL | Transepidermal Water Loss |

# 5 Ethics

## 5.1 Independent Ethics Committee (IEC)

The protocol of the clinical trial, the patient information and informed consent, and any other written information provided to the patients was approved by the local Independent Ethics Committee (IEC).

The principal investigator (here also sponsor: Prof. Dr. med. M. Worm) was responsible for submitting the documents to the IEC.

During the trial no documents were sent to the IEC for reviewing.

At the end of the clinical trial, the investigator notified the IEC about the trial completion. The synopsis of the final report will be provided to the IEC within approximately 30 days after signing of the final report if requested by the IEC.

The address and chairmen of the IECs are given in section 6 and in Appendix 16.1.3.

## 5.2 Ethical conduct of the clinical trial

The clinical trial was conducted in accordance with applicable regulations governing the protection of human patients, such as national drug laws (German Drug Law: AMG [2]), ICH-GCP guidelines [1; 5] and the Declaration of Helsinki [3].

## 5.3 Patient information and consent

IEC approval of the written patient information and informed consent was obtained prior to their use. This consent form contains a phrase by which consent was given for the access to the non-personalized data by the sponsor, national and regulatory authorities. In addition, it states that the patient was free to withdraw from the clinical trial at any time without any negative consequences. The patient information gives a complete and comprehensive explanation of the significance, nature, extent and possible risks of the clinical trial. For details of the informed consent procedure please refer to chapter 8.2 of the protocol (provided in Appendix 16.1.1). It complied with all applicable regulations governing the protection of human patients, such as national drug laws (German Drug Law: AMG [2], ICH-GCP guidelines[1; 5] and the Declaration of Helsinki [3]. A sample of patient information and informed consent are provided in Appendix 16.1.1.

# 6 Investigators and trial administrative structure

## Sponsor/Principal Investigator

Prof. Dr. med. Margitta Worm
Charité – Universitätsmedizin Berlin
Department of Dermatology and Allergology
Allergy-Center-Charité (ACC)
Charitéplatz 1, 10117 Berlin, Germany
Phone.: +49 (0)30-450 518 105
Fax: +49 (0)30-450 518 919
margitta.worm@charite.de

## Sub-Investigators

Dr. med. Claudia Rasche
Charité – Universitätsmedizin Berlin
Department of Dermatology and Allergology
Allergy-Center-Charité (ACC)
Charitéplatz 1, 10117 Berlin, Germany
Phone: +49 (0)30-450 618 305
Fax: +49 (0)30-450 518 968
claudia.rasche@charite.de

Dr. med. Hae-Hyuk Lee
Charité – Universitätsmedizin Berlin
Department of Dermatology and Allergology
Allergy-Center-Charité (ACC)
Charitéplatz 1, 10117 Berlin, Germany
Phone: +49 (0)30-450 618 098
Fax: +49 (0)30-450 518 968
h.lee@charite.de

## Project Coordination

Sabine Dölle
Charité – Universitätsmedizin Berlin
Department of Dermatology and Allergology
Allergy-Center-Charité (ACC)
Charitéplatz 1, 10117 Berlin, Germany
Phone.: +49 (0)30-450 618 367
Fax: +49 (0)30-450 518 968
sabine.doelle@charite.de

## Project Partner

JADO Technologies GmbH
BioInnovationszentrum
Tatzberg 47-51, 01307 Dresden, Germany
Phone: +49 (0)351 796 3801
Fax: +49 (0)351 796 3801
tg@biosciencemanagers.com

## Blinding and Randomisation

OUTPUT Pharma Services GmbH
Pauwelsstrasse 19, 52074 Aachen, Germany
Stephan Heckler
Phone: +49 (0)241-96 32 681
Fax: +49 (0)241-96 32 689
s.heckler@output.eu

## Monitoring/Sample Size Calculation

KKS Charité (Coordinating Centre for Clinical Trials)
Campus Virchow-Klinikum
Augustenburger Platz 1, 13353 Berlin, Germany
Phone: +49 (0)30 450 553 016
Fax: +49 (0)30 450 553 937
kks@charite.de

## Leading Ethics committee

Landesamt für Gesundheit und Soziales Berlin (LaGeSo)
Agency of the ethic committee of Berlin
Sächsische Str. 28, 10707 Berlin, Germany

For the chairmen and member of the responsible committee of the local IEC participating in the clinical trial, please refer to Appendix 16.1.3.

The curricula vitae of the investigators and other important participants of the clinical trial are provided in Appendix 16.1.4.

# 7 Introduction

## 7.1 Basics

The clinical trial protocol contains all information to perform the clinical trial according to the legal requirements, and to the current revisions of the recommendations of the Declaration of Helsinki [3], Good clinical Practice (GCP)- and International Conference on Harmonization (ICH)-guidelines [1; 5].

## 7.2 Risk-benefit evaluation

Miltefosine is given orally for the treatment of visceral leishmaniasis (marketed under the trademark Impavido® in Germany since 2004). Most common adverse events at dosages of up to 150 mg/day are gastro-intestinal side effects like nausea, diarrhoea and loss of appetite (SmPC Impavido). In addition, increase of liver enzymes and serum creatinine were reported. In general, the side effects are mild to moderate and transient and do not cause discontinuation of therapy. Impavido® at a standard dosage of 100 mg/day for visceral leishmaniasis causes plasma concentration of 70 µg/ml. Reported bioavailability was 82 % (dogs) or 94 % (rats; SmPC Impavido).

In the present trial miltefosine was used topically as 6% solution (Miltex®). Miltex® was applied according to the allowed doses of 2 drops per 10 cm² in a frequency of once daily in the first treatment week and twice daily in the second and third treatment week. A target lesion of 10 cm² was assumed as adequate for this explorative analysis. This treated 10-cm² skin area is much smaller then the treated skin area in the allowed indication. For the treatment of malignant changed skin by breast cancer, Miltex® is extensively used over a period of at least 8 weeks.

The so far reported circulating drug levels are under 1 nmol/ml in men after dermal application, mainly due to a very low relative bioavailability of topical used miltefosine. Even a dermal application of up to 7.5 ml Miltex® (450 mg miltefosine/day) did not elevated he serum drug level over 1 nmol/ml (SmSC Miltex®).

Over the whole period of treatment (3 weeks) every patient applied a total of 70 drops (equals 1.75 ml or 105 mg miltefosine). This dose is far below the allowed maximum dose of 5 ml Miltex®/d. Therefore the risk of systemic toxicity is acceptably low and the safety margin for dermal application is sufficiently wide from the toxicological point of view.

Miltex® application frequently causes skin irritation causing pruritus, erythema, and tightness of the skin and drying. Those side effects typically do not cause discontinuation of treatment. Dry skin can be treated with emollients 4 hours after the application. Gastro-intestinal side effects like nausea, diarrhoea and loss of appetite rarely possible. The clinical trial was performed by qualified dermatologists. By severe allergic or irritative skin reactions the treatment had to be discontinued and the investigators were responsible to re-establish the health status of the patient of baseline.

Systemic side effects could occur after overdosage but treatment compliance was monitored throughout the clinical trial. Liver enzymes, serum creatinine and haematology were measured before and after the treatment.

Hydrogalen® is available in various dosage forms (e.g. as crème, ointment or solution). To have an appropriate control, Hydrogalen® solution was used. Investigations to the lo-

cal tolerance of Hydrogalen® provided no evidence for irritative or allergic skin reactions (SmSC Hydrogalen®). A higher sensitivity to UV radiation exists as it is for all corticosteroids. Hydrocortisone showed no toxicity or systemic activity in animal experiments.

The study population consisted of 16 patients with AD. Since all subjects received active treatment there might be a direct benefit to the subjects participating in the clinical trial. Due to the topical application of an active control, the investigator was able to compare the treatment results and could terminate the treatment for this individual any time when the risk benefit ratio seemed to be unfavourable.

The skin biopsies and the blood withdrawals displayed no additional study specific risk factor. However, the risk of local infections, marginal bleeding, transient pain or scarring could not be excluded. Usual side effects of biopsies are intolerance reactions to local anesthetics, local pruritus and erythema.

### 7.3 Overall clinical trial description

The clinical trial was conducted to evaluate the anti-inflammatory and immunmodulatory effect of topical-used miltefosine in patients with AD. The design was double-blind, active-controlled with intra-individual comparison of two target-lesions. Miltefosine and the active-control (hydrocortisone) were topically applied on a defined small target lesion (10 $cm^2$) over a period of 3 weeks.

Since, this clinical trial was the first time that miltefosine was used in patients with AD, nothing was known about the kind of application. Therefore, dosage and treatment period were derived from previous published experiences in dermato-oncology. One small target lesion was chosen for this exploratory analysis.

The aim of this clinical trial was to investigate the anti-inflammatory potential of miltefosine in inflamed skin of patients with AD over a period of 3 weeks. The primary endpoint was the clinical assessment of the disease severity of a defined target lesion before and after the treatment by using the TIS score. The topical application was exploratory compared to hydrocortisone used as active control. To underpin the clinical TIS score used in the clinical trial, the objective SCORAD was assessed. Additionally, skin physiology and thermography during the course of treatment and immunhistological biomarkers in the skin biopsies before and after the treatment were exploratory analysed.

## 8 Study objectives

The objective of this clinical trial was to evaluate the anti-inflammatory effect of miltefosine on inflammatory skin of patients with AD.

- Primary Objective

  Evaluation of the clinical response of inflamed skin of patients with AD to miltefosine using the TIS score (before, during, and after the treatment as well as in the follow up).

- Exploratory Objectives

  Evaluation of the clinical response to miltefosine compared to an active control (hydrocortisone).

  Evaluation of the objective SCORAD to support the TIS score result.

Evaluations of immunhistological parameters like $CD4^+/CD8^+$ T-cells infiltration, mast cells and measurement of the epidermal thickness.

Evaluations of changes in skin physiology and thermography.

- safety

  Evaluation of adverse events (AE) that are summarized as general AEs and local skin-related AEs with definite drug relation.

# 9 Investigational plan

## 9.1 Overall study design and plan-description

Patients with diagnosed AD were recruited for this randomised, double-blind, active-controlled trial. The clinical trial hat an exploratory character with an intra-individual comparison of two target lesions.

Patients underwent complete medical history, physical examination, and laboratory evaluation before treatment started. Eligibility was checked by the investigator on the basis of the in- and exclusion criteria.

Two target lesions (inflammatory lesions) with a size of 10 $cm^2$ each were determined per patient. Only lesions with a TIS score between 5 to 7 points were examined in the clinical trial. The patients had to have two comparable skin lesions also appropriate for skin biopsies.

Study medication was applied as 2 drops once daily in the first and twice daily in the second and third treatment week (for description of application procedure please refer to chapter 7.1.2 of the protocol provided in Appendix 16.1.1). The first application was performed under supervision of the investigator at baseline. Clinical evaluations (TIS score, SCORAD) were done at baseline, during and at the end as well as at the follow up. Skin physiology and thermography were also done to every visit. Two 4 mm punch biopsies were taken before and at the end of treatment.

## 9.2 Discussion of study design, including the choice of control groups

The clinical trial was designed to show the supposed immunmodulatory and anti-inflammatory effect of miltefosine in patients with AD. For the detailed study design please refer to chapter 5 of the protocol (provided in Appendix 16.1.1). Briefly, 16 patients with diagnosed AD were included in this randomised, double-blind, active-controlled, exploratory clinical trial. A 3-week treatment was planned with 2 follow up visits, 2 and 4 weeks after the end of treatment, respectively (**Figure 14.1.1**). Patients underwent complete medical history, physical examination, and laboratory evaluation at screening. Eligible subjects were enrolled at visit 1 within a maximum of 4 weeks after screening. First application was performed under supervision of an investigator at visit 1.

An active-controlled design was chosen. Hydrocortisone is a low-potential steroid used topically in the treatment of AD. The guidelines for treatment of AD suggest topical corticosteroids as a first line treatment for AD [6; 8]. In this exploratory clinical trial we have chosen hydrocortisone as an active-control to get information about the dimension of the anti-inflammatory and immunmodulatory effect of miltefosine. We wanted to compare exploratory the effect of miltefosine with hydrocortisone. Furthermore, our primary endpoint assessed the clinical immunmodulatory effect of miltefosine over a defined time pe-

riod. For this purpose the TIS score was compared at the beginning and end of the treatment.

We conducted a clinical trial with intra-individual comparison of test areas. That means one patient was treated with both trial medication. The different treated lesion had appropriate distance in between, which was satisfactorily for our exploratory conclusions. Besides, only small lesions were treated and a systemic effect was not expected. Therefore, an interaction of trial medication was minimised. Further clinical trials treating larger skin areas should be conducted without intra-individual comparisons to avoid the dependence of the investigated parameters like miltefosine and active-control or placebo.

The skin biopsies were taken directly before and after the treatment and were taken from the same lesion treated with trial medication. The reason was to have biopsies exactly from the area treated with the trial medication. The first biopsies were taken with spitting distance of the target lesion but not directly in the target lesion. Therefore, the treatment with the trial medication could start at the same time. The biopsies after the treatment were taken directly from the treated lesion advantageous for the immunhistological results. However, the clinical assessments of the TIS score could not be done in a part of patients at follow up 1 (2 weeks after the end of treatment) because of inflammatory processes caused by the biopsies.

## 9.3 Selection of study population

### 9.3.1 Inclusion criteria

- Diagnosis of atopic dermatitis according to Hanifin&Rajika
- Chronic course of disease
- Age ≥18 years
- 2 comparable skin lesions of 10 $cm^2$ with a TIS score between 5 and 7 points appropriated to take biopsies
- Negative pregnancy test
- High reliable method of contraception for women of childbearing potential as well as for the female partner of sexual active men who participate in this trial
- Inform content according to AMG §40 (1) 3b

### 9.3.2 Exclusion criteria

Patients meeting any of the following exclusion criteria were not to be included into the trial:

<u>General exclusion criteria:</u>

- Pregnancy or lactation
- Participation in another clinical trial within the last 30 days
- Subjects who are inmates of psychiatric wards, prisons, or other state institutions (according AMG §40 (1) 4)
- Clinically significant laboratory abnormalities (biochemistry and haematology)
- Other reasons like mental disorders, Drug or alcohol dependency
- Clotting diseases

<u>Medical exclusion criteria:</u>

- Erythrodermia
- Other chronic skin diseases
- malignant skin diseases

- specific skin processes (skin tuberculosis), mycosis, rosacea, periorale dermatitis, …
- bacterial skin infections
- viral skin infections like herpes simplex, zoster, varicella
- History or concomitant renal pathology
- Known hypersensitivity to study drugs or their components
- Any other chronic or acute illness requiring systemic treatment which might have any influence on the outcome of the clinical trial (investigator's decision)
- Immunodeficiency including HIV
- Target lesions covering breast implants

Prohibited concomitant medication:

- Antihistamines during the past **3 days** before start of treatment
- Topical emollients (urea < 3%) on the target lesions during the past **3 days** before start of treatment and during the clinical trial
- Topical corticosteroids on the target lesions during the past **14 days** before start of treatment and during the clinical trial
- Systemic treatment with corticosteroids or immunosuppressive agents including immunomodulators during the past **4 weeks** before start of treatment and during the clinical trial
- UV radiation during the past **4 weeks** before start of treatment and during the clinical trial
- Medication against blood clotting during the past **7 days** before the biopsies

### 9.3.3 Removal of patients from treatment or assessment

The criteria for withdrawal of a subject from the clinical trial were the following:

- Personal desire of the patient
- Pregnancy
- Non-compliance
- Strengthened irritation or pruritus at target lesions during the trial procedures
- Local reactions which lead to a worsening of TIS score ≥9 points
- Any other situation which might make the further participation of the patient difficult or unethical (investigator's decision)
- Use of a moderate or high potential corticosteroids at the target lesions

None of this situations occurred during the clinical trial.

### 9.3.4 Premature termination of the clinical trial

The sponsor reserved the right to cancel the clinical trial at any time if:

- unjustified risk factors occur during the trial which lead to a negative risk/benefit analysis
- new findings comprise the safety of the patients and if the continuation of the clinical investigation is judged to be inappropriate.

In addition, at the discretion of ethics committee the entire clinical trial may be cancelled for important reasons at any time.

None of this situations occurred and the clinical trial was not terminated premature by the sponsor or the ethics committee.

## 9.4 Treatments

### 9.4.1 Treatments administered

The Investigational Medicinal Product (IMP), Miltex® is a product marketed by Baxter Oncology GmbH and contains the active ingredient miltefosine (approval number: 24202.00.00, 24204.00.00).

The IMP, Hydrogalen® solution is a product market by GALENpharma and contains the active control substance hydrocortisone (approval number: 34546.00.03).

Both IMPs were supplied by JADO Technologies GmbH, Germany and randomised by OUTPUT Pharma Services GmbH.

### 9.4.2 Identity of investigational products

Both medications were administered topical in a dosage of 2 drops per target lesion once daily in the first and twice daily in the second and third treatment week. All patients received appropriate dispensers (on-way pipettes see Chapter 7.1.2 of the protocol provided in Appendix 16.1.1) for both solutions.

Miltefosine is concentrated at 60 mg per ml. All patients received 70 drops over the whole treatment period of 3 weeks (corresponds approx. 1.75 ml or 105 mg of miltefosine).

All patients also received 70 drops Hydrogalen® over the whole treatment period. Hydrogalen® in solution containing 10 mg per g.

All IMPs had the same batch number and expiry date:

Batch number: 230807
Expiry date: 05/2009

For detailed information about the labelling of trial medication see section 9.4.6 and Appendix 16.1.6.

### 9.4.3 Method of assigning patients to patient numbers

Since, it was a mono-centre trial all 16 patients had to be recruited in the Allergy-Centre-Charité. Patients were recruited from the consulting hours specialised for AD. Recruited patients obtained a screening and patient number in ascending sequence (see section 9.4.5).

Prior to randomisation, 2 target lesions per patient were determined by the investigator and assigned sequential letters (A and B). A randomization list assigned the different treatments (active control or miltefosine). The randomisation list is provided in Appendix 16.1.7 including the patient identifier, and treatment assigned. Trial medication was randomised using the software "nQuery Advisor 6.01". After patients enrolment the randomisation code was assigned in ascending sequence to the patients. The randomisation code consisted of the patient number and the letters A and B.

### 9.4.4 Selection of doses in the clinical trial

Since, there is limited data on the immunmodulatory effect, the tolerability and efficacy of miltefosine in AD; we used the approved dosage of Miltex®. Thus, we applied 2 drops (related to 50 µl Miltex® or 3 mg miltefosine) per 10 cm². Our target lesion were 10 cm²,

therefore, the daily dose were 2 drops in the first and 4 drops in the second and third treatment week. The same application schedule was used for hydrocortisone.

### 9.4.5 Selection and timing of dose for each patient

Patients recruited to this clinical trial received a screening number in ascending order, beginning with S01. After obtained informed consent and proven inclusion/exclusion criteria, 2 target lesions were defined and were assigned to A and B. The patient were enrolled to the clinical trial and obtained a patient number. For treatment the patient received the trial medication with the corresponding patient number.

All patients had to use 2 drops of the corresponding trail medication per target lesion once daily in the first and twice daily in the second and third treatment week.

### 9.4.6 Blinding

Trial medication was packaged, coded and labelled by OUTPUT Pharma Services GmbH, Germany. Primary packaging of both IMPs was covered by an identical fixed secondary package. The secondary package was labelled according to local law and identified the treatment with A or B. An example for labelling is provided in Appendix 16.1.6.

Trial medication was randomised using the software "nQuery Advisor 6.01" (see section 9.4.3).

One set of sealed envelopes were prepared by OUTPUT Pharma Services GmbH, Germany and stored at the study centre in the Investigator's Side File (ISF). The randomisation list was kept by OUTPUT Pharma Services GmbH, Germany until the database was closed. The database was locked after entering all relevant items and after a check through a trial independent person.

Premature unblinding:

The randomisation code had only to be unblinded in case of an emergency due to a serious adverse event (SAE) which led to premature termination of the clinical trial for this patient. The randomisation code had to be opened in case of a suspected unexpected serious adverse reaction (SUSAR).

Opening of the code envelope must be documented in the CRF and on the code envelope by dating and signing it, and by giving a reason for opening the envelope.

The sponsor (here also Principal investigator) had to be immediately informed about any unblinding and were responsible to forward the information to the IEC/IRB.

For this clinical trial no premature unblinding was necessary.

Regular Unblinding:

After terminating the clinical trial, entering all relevant items in the data base, locking the data base, and checking the data through an trial independent person, a routine unblinding was performed by the sponsor (here also Principal investigator).

### 9.4.7 Prior and concomitant therapy

For prohibited concomitant medication please refer to section 9.3.2.

Any other concomitant medication taken, as well as any changes in concomitant medication were documented in the CRF indicating the

- trade name of medication
- indication for use
- route of administration
- daily dose
- start date
- end date
- was the reason an AE
- on-going

A list of concomitant medication per patient can be found in Appendix Table 16.3.6 and the CRF page of concomitant medication in Appendix 16.1.2.

### 9.4.8 Treatment compliance

The treatment compliance was controlled by diary cards, which had to be filled in every day by the patient and were inspected at every visit. In the diary cards, the patients had to document any changes in their health, concomitant medications as well as had to assess the tolerability of trial medication. An example of diary card can be found in Appendix 16.1.2. The compliance was additionally monitored by checking the consumed one-way pipettes at every visit.

### 9.4.9 Measurements to assess the immunmodulatory effect and the safety of miltefosine

#### 9.4.9.1 Immunmodulatory effect

**Methods for evaluating the primary objective**

The primary objective was the assessment of the target lesion by use of the **Three Item Severity (TIS) score.**

The TIS score is a simplified system derived from the SCORAD [9]. The scoring system is based on 3 items; erythema, oedema/papulation and excoriations.

The choice of the items used in the TIS score was based on the following criteria [9]:
- The items should be relevant for all age groups.
- If two items are highly correlated only one is scored.
- The items should reflect disease severity and should be independent of other interfering factors.
- The items should be subject to change and improve when AD improves.
- No combination of objective signs with subjective symptoms.

Dryness, as a characteristic feature of AD, was not included in the TIS score, because it largely depends on when the emollient was last applied. Furthermore, lichenification is not suitable for evaluating a short-term therapy, because this feature responds rather slowly to therapy. Moreover, oozing is closely linked to the feature erythema and is therefore already represented by erythema in the TIS score. Finally, subjective symptoms like pruritus or sleep-loss are strongly influenced by psychological factors and can cause large variations.

The 3 items are scored on a 4-point-scale from 0 to 3 (0=non, 1=mild, 2=moderate and 3=severe).

At screening 2 comparable target lesions were defined, randomised to A or B, and had to be treated with the corresponding trial medication over a period of 3 weeks. The target lesions were assessed before, during and after as well as 2 to 4 weeks after the treatment.

To include patients with comparable target lesions, a TIS score between 5 and 7 was determined as inclusion criteria (see section 9.3.1).

### Methods for evaluating the exploratory objective

The standardized assessment method of AD is the **SCORAD**, one of the best validated systems. The SCORAD combines extent, severity and subjective symptoms and is suited for clinical trials assessing the global severity of AD. In this cause we decided to use the TIS score because of the very small target lesion to be evaluated. However the objective SCORAD were used to have a overall impression of disease severity during the treatment.

Pre- and post-treatment 4-mm skin punches were embedded in O.C.T. medium and placed in a disposable vinyl mold (Tissue-Tek, Sakura). The samples were slowly frozen in liquid nitrogen and stored at –80°C. The skin biopsies were cut into 4 µm thick sections and stained for **immunhistology** analysis. The staining for $CD4^+$ and $CD8^+$ T-cells were carried out by the streptavidin/biotin complex method using monoclonal antibodies to CD4 (clone MT310; Dako) and CD8 (C8/144B; Dako). The signals were detected by an alkaline phosphatase/red detection kit (Dako, Hamburg, Germany) and by hematoxylin counterstaining. The cells were counted in 200x300-µm-fields at 100x magnification and the averages were calculated.

To detect infiltrating mast cells, the 4 µm skin sections were stained for 1 hour with o-toluidine blue. Positively stained cells were counted in 3 300x500-µm-fields at 100x magnification and the averages were calculated.

For measurements of the epidermal thickness the skin sections were stained with hematoxylin (Papanicolaou´s solution; Merck) and eosin (Eosin-Phloxin-Lösung; Dr. K. Hollborn & Söhne). Measurements were taken using the Axiovision measuring-tools (Zeiss, Berlin, Germany) at 100x magnification.

The impaired skin barrier is a known characteristic in patients with AD. The skin barrier can be characterised by non-invasive measurement of physical skin parameters like transepidermal water loss (TEWL), the sebum level, the skin hydration and pH-value. All measurements of **skin physiology** were made in the same room with a constant air temperature and the relative humidity after about 15 minutes rest.

**Thermography**, or thermographic imaging, detects radiation in the infrared range of the electromagnetic spectrum with a special thermographic camera (Flier). Infrared radiation is emitted by all objects based on their temperature. The amount of radiation emitted by an object increases with temperature, therefore, thermography allows to detect variations in temperature. Thermographic imaging was performed at each visit (Visit 1 to 4) if possible. Both target lesions were photographed from the same distance: 30 cm.

## 9.4.9.2 Safety

All safety laboratory evaluations (except of urine pregnancy test) were performed by the certified laboratory of the Charité – Universitätsmedizin Berlin at screening and visit 4.

Following safety laboratory parameters were determined:

| | |
|---|---|
| Haematology profile | Differential blood count (white blood cell count), platelet count, erythrocyte count |
| Biochemical profile | alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), serum creatinine, calcium, sodium, potassium |

For female patients of child bearing potential a commercial urine pregnancy test was performed at screening and visit 4.

All abnormal laboratory findings that were determined to be clinically significant by the investigator were considered as AE. In cause of pregnancy, the clinical trial had to be stopped immediately for those patients.

At each visit (screen, visit 1 to 4) physical examination were performed and vital signs were measured. Any changes from baseline which were determined clinically significant by the investigator had to be documented as AE.

No clinically relevant abnormal laboratories were detected, no clinically relevant changes in physical examination and vital signs were documented and non female patient became pregnant during this clinical trial.

### 9.4.9.3 Study flow chart

See **Table 14.2.1**

## 9.4.10 Appropriateness of measurements

Most methods used in this clinical trial were widely used and generally recognized as reliable. Two methods, the TIS score for measuring the primary endpoint and the thermography used as an exploratory measurement were not valid enough and are explained in this section.

### 9.4.10.1 Method for evaluating the primary objective

The primary effect parameter was a clinical parameter. Changes of the TIS score over the 3-week treatment were evaluated. This score was firstly described by Wolkerstorfer et al. [9]. The TIS score is a simplified system derived from the SCORAD (see section 9.4.9.1) and is suitable for routine clinical use. But, the TIS score is not often used in clinical trials and therefore not a valid parameter. It is recommended to use the objective SCORAD which offers a more detailed and comprehensive assessment. However, in the case of this clinical trial the TIS score provided a better method to assess the clinical changes of a small target lesion.

### 9.4.10.2 Method for evaluating the exploratory objective

The **SCORAD** is a well standardized assessment method for the severity of AD and often used in clinical trials.

The **immunhistological analysis** focused on immune effector cells ($CD4^+$/$CD8^+$ T-cells, mast cells) and on epidermal thickness. To measure immunhistological parameters, skin biopsies were taken before and at the end of treatment. One local side effect of corticosteroids is the skin atrophy which we planned to assess by measurement of epidermal thickness. Immunmodulating properties can impact the function and behaviour of immune effector cells. Thus, the effect of miltefosine may be seen by changes in

$CD4^+/CD8^+$ T-cells and mast cell infiltrations that are exploratory analysed in this clinical trial.

AD is associated with disturbances of skin barrier function as evidenced by an increase in transepidermal water loss (TEWL), a decrease in water-binding properties (corneometry), a reduction in skin surface lipids, particularly ceramides (sebumetry) and elevated pH-values (pH-metry). The development or establishment of new treatment strategies have to consider parameters of **skin physiology**. Therefore, we planned to measure non-invasively these skin physiological parameters. To date, these measurements are not validated and only used in clinical trials. Merely, the TEWL is assessed in the occupational dermatology standardised as alkaline resistance test. Thus, in this clinical trial the skin physiological parameters were exploratory evaluated.

In recent times **thermography** has been used in medical research. The development and regression of inflammatory processes in the skin of patients with AD are accompanied by temperature changes due to increased vasodilatation and extravasation at the skin surface. Since thermographic imaging is able to display temperature changes, it might be a highly accurate method to visualize and quantify the target skin lesions. Until now, thermography was not used as an objective tool to show treatment success. Therefore, it is not a valid method and only used exploratory.

## 9.5 Data quality assurance

Quality control

Data quality assurance was performed according to international guidelines (GCP, ICH), standard operating procedures (SOP) or working instructions. The data were documented first in the source data and afterwards in the CRF by the investigator or designed personnel. Monitoring was performed to inspect the correct transfer and plausibility of the data. The monitoring visits were performed according to monitoring visit plan and a monitoring report was prepared.

The data were entered in a database and checked by a trial independent person. Once all the data quality control steps were performed, the database was locked and released for reporting and statistical evaluation.

Quality assurance

External audits were not performed on this clinical trial.

## 9.6 Statistical methods planned in the protocol and determination of sample size

### 9.6.1 Statistical and analytical plans

All data obtained in this clinical trial and documented in the CRFs and patient diaries were analyzed with descriptive group statistics.

All randomised subjects with at least one application of the trial medication were included into the primary and exploratory effect analysis population (‘intention-to-treat’ - ITT). It was intended to exclude the drop-outs during the investigated treatment form the per-protocol (PP) population. Since, that was not the case the PP population was defined to exclude subjects which refused at least one biopsy. With the PP population all immunohistological parameters were analysed.

The safety population included all subjects with at least one application of the trial population. This population were used for all safety analyses and baseline characteristics.

The excluded patients, visits and measurements from the ITT and PP population are listed in Appendix Table 16.2.1 to 16.2.4.

### 9.6.2 Determination of sample size

A formal sample size calculation was not performed for this exploratory clinical trial. There was no data accessible about the mean or standard deviation of the TIS score. Further, no previous data about the effect of miltefosine in AD was known. The planned sample size of 16 patients was justified by means of an exemplary sample size discussion. We calculated the TIS score from previous SCORAD-data [7] and assumed a medium effect size of 1.5 standard deviation (two-sided significance level of 5%, power of 80%).

## 9.7 Changes in the conduct of the clinical trail or planned analyses

The clinical trial was conducted according to the clinical trial protocol; version 1.3 dated 31st August 2007. No further formal protocol amendments were made.

The protocol with the revision points which were made to get the protocol; version 1.3, dated 31st August 2007 approved are provided in Appendix 16.1.1.

# 10 Study patients

## 10.1 Disposition of patients

Forty-six patients with a diagnosis of AD were screened for study participation. Twenty-four patients were excluded during the interview with the investigator and 2 were not randomised (screening failures, Appendix Table 16.2.1).

Overall, 16 patients were enrolled and treated with miltefosine and hydrocortisone. All 16 patients completed the clinical trial, and nobody was withdrawn prematurely.

An overview over the patients is provided in the flow chart (**Figure 14.1.2**).

The first signed informed consent was on 11th September 2007 and the corresponding first randomisation was the 14th September 2007, last patient last visit was on the 23rd January 2008.

## 10.2 Protocol deviations

All patients who entered the clinical trial fulfilled the inclusion and exclusion criteria.

With randomisation 3 patients completely refused the biopsies and one patient refused the second both biopsies after the treatment.

All patients had the protocol deviation that the termination examination was done at follow up and not at visit 4 which was originally defined in the trial protocol.

None of the randomised patient used an excluded concomitant treatment.

The protocol deviations are listed and summarized by patient in Appendix Table 16.2.5.

# 11 Evaluation of the immunmodulatory effect

## 11.1 Data sets analyzed

Altogether 16 patients were treated in this clinical trial. They constituted the full analysis set for safety evaluation and the ITT population for the primary and exploratory effect analysis. Two screened patients could not be randomised (screening failures) and were excluded from the ITT (Appendix Table 16.2.1). One patient at follow-up 1 and 4 patients at follow-up 2 had to be excluded from the primary effect analysis (Appendix Table 16.2.2). The exclusion of patients and observations from the exploratory analysis of skin physiology and thermography were only because of organisational reasons (listed in Appendix Table 16.2.3).

From 3 of 16 patients no biopsies were taken before and after the 3-week treatment. One patient refused the second both biopsies after the treatment. Thus, these 4 patients were excluded from the PP population (Appendix Table 16.2.4, see also section 9.6.1).

## 11.2 Demographic and other baseline characteristics

The age of the 5 male and 11 female patients of the ITT population ranged from 18 to 58 years (**Table 14.2.2**). All patients were Caucasian except for one with Asian parentage. Only patients with moderate or severe AD were included indicated by the SCORAD points ranging from 34 to 69 points. The TIS score for both target lesions was comparable in each patient at baseline (median TIS score = 6 points).

The safety laboratory parameters of all patients were normal before starting the clinical intervention. Since, it is recommended to monitor kidney and liver function parameters [4], the main safety laboratory parameters creatinine and liver enzymes (ALT, AP, GGT) were tabulated in the baseline characteristics (**Table 14.2.2**).

Individual listings of demographic information and adherence to inclusion and exclusion criteria are provided in Appendix Table 16.3.7.

## 11.3 Measurement of treatment compliance

Compliance was checked by record of applications in the patient diary. The overall compliance was very good. In the first treatment week all patients used the trial medication correct, two drops once daily. In week 2 and 3 the trial medication had to be used twice daily. Patient 013 forgot the morning application an day 8, 15 and 25. Patient 012 forgot the evening application on day 16 and the morning application on day 17.

## 11.4 Results of the primary and exploratory effect and tabulations of individual subject data

### 11.4.1 Analysis of immunmodulatory effect

#### 11.4.1.1 Primary effect criterion

**Course of TIS score of the miltefosine treatment**

The primary effect parameter was the clinical response to the miltefosine treatment over a period of 3 weeks. This was evaluated by means of the TIS score measured before, dur-

ing, and after the treatment as well as in the follow up. The TIS score assess the disease intensity of a target lesion evaluating erythema, edema/papule and excoriation each on a 4-point scale (0=no, 1=mild, 2=moderate, 3=severe).

An effect size of a standard deviation of 1.5 was supposed to show a clinical relevant effect of miltefosine treatment over a time period. This could be seen already after 1 week of treatment with miltefosine (**Figure 14.1.3**). The TIS score decreased in all patients during the 3-week treatment with miltefosine. The median decline the TIS score was 2 points ranging from 1 to 6 points. The TIS score before treatment was defined as the TIS score from visit 1. For those patients who had only one visit for screening and visit 1, the TIS score from the screening (= visit 1) was used.

Individual listings of primary effect criteria by patient are provided in Appendix Table 16.2.6.

### 11.4.1.2 Exploratory effect criteria

#### TIS score comparison between miltefosine and active control

The treatment response to miltefosine was exploratory compared to the active control, hydrocortisone. The TIS scores were reduced in both treatment interventions. Significant differences were detected after 2 weeks of treatment and were persistent after 3 weeks **Figure 14.1.4**. After the 2$^{nd}$ treatment week a continuous reduction of the TIS score was seen in both treatments with an ongoing course beyond the end of treatment only in the miltefosine treatment (**Figure 14.1.4**, indicated by the red dotted lines).

#### Objective SCORAD

Additionally to the TIS score, the objective SCORAD was assessed. The TIS score is a simplified system derived from the SCORAD and more appropriate to assess clinical changes of a small lesion. To get a global impression of disease severity during the treatment, the objective SCORAD was considered. The course of the median objective SCORAD is depicted in **Figure 14.1.5**. A significant improvement over the intervention period was observed in almost all patients. Regarding the difference between before to after the treatment one patient had no major change (P.-Nr. 010: objective $SCORAD_{before}$ = 25.3; objective $SCORAD_{after}$ = 25.4) and only one patient showed a decline of 12 points (P.-Nr. 012: objective $SCORAD_{before}$ = 37; objective $SCORAD_{after}$ = 49). The objective SCORAD before treatment was defined as the objective SCORAD from visit 1. For those patients who had only one visit for screening and visit 1, the objective SCORAD from the screening (= visit 1) was used.

Individual listings of the objective SCORAD are provided in Appendix Table 16.2.7.

#### Immunhistological parameters

For 12 of 16 patients the number of immune effector cells ($CD4^+$/$CD8^+$ T-cells, mast cells) and the epidermal thickness were compared from the beginning until the end of treatment with miltefosine or hydrocortisone, respectively.

The epidermal thickness of normal skin is ranging between 30 and 200 µm. The epidermal thicknesses were comparable between both treatment groups in the beginning. The median with 205.65 µm (hydrocortisone) and 205.74 µm (miltefosine) were above the upper limit (**Table 14.2.8**). A positive effect was seen in the miltefosine-treated lesions causing a median reduction to normal status after the 3-week treatment. In contrast, the

hydrocortisone treatment showed atrophic characteristics by means of significant thinner epidermal layer after treatment ($p = 0.012$, **Figure 14.1.6**).

Both treatments tended to reduce $CD4^+$ T-cells infiltrations (**Figure 14.1.7**). No changes in $CD8^+$ T-cells were detected in during the course of treatment neither for hydrocortisone or miltefosine (data not depicted).

The mast cells numbers were low and an oppositional trend was detected in the two treatments (**Figure 14.1.8**). However, the differences between before and after the treatment were marginal and no statistical significances were calculated.

Individual listings of immunhistological parameters are provided in Appendix Table 16.2.8 to 16.2.11.

**Skin physiology**

As described before in section 9.4.10.2 the skin of patients with AD is characterised by an abnormal skin barrier function. The development and establishment of new treatment drugs should also consider the physiological skin parameters and should have a positive impact.

Because of technical problems with our skin physiology equipment, we could not perform all measurement with all patients and at all visits. The missing measurements are listed in Appendix Table 16.2.3.

For the TEWL, a significant reduction was monitored for both treatments (**Figure 14.1.9**). The TEWL of both target lesions were comparable before treatment. The hydrocortisone treatment reduced the TEWL stronger compared to miltefosine. To mention is the high variations indicating the sensitivity of the measurements. The latter fact applied to all physiological skin parameters.

The content of skin surface lipids (SEBUM, **Figure 14.1.10**) differed between the hydrocortisone and miltefosine target lesion. An elevation of lipid content in the skin was observed in the hydrocortisone-treated lesion at visit 2 and 3. However, the lipid content at visit 4 was the same like before the treatment. Non crucial changes were observed in the miltefosine-treated lesion. It has to be mentioned, that the values for the sebumetry were surprisingly very high and were not comparably to data from the literature. This may be due to general technical problems.

The water-binding properties (CORNEUM, **Figure 14.1.11**) are comparable between both target lesions before starting different treatments. The course of CORNEUM differed between both treatments. A tendency of increased water-binding property was observed only in the hydrocortisone-treated lesion. In comparison, the miltefosine treatment leads to a decrease. After the 3-week intervention significant differences were observed between both treatments.

The pH-values of normal skin are about 5.6. The median pH of both target lesions was not patho-physiologically changed before the treatment (**Figure 14.1.12**). Additionally, almost no changes were observed during the course of treatment.

The descriptive statistic can be found in **Table 14.2.9**. The individual listings of the physiological skin parameters are provided in Appendix Table 16.2.12.

## Thermography

Temperature changes due to inflammatory processes can be detected with thermographic imaging. The thermographic measurements were performed on visit 1, 2, 3 and 4.

Because of organisational reasons some measurements are missing and are listed in Appendix Table 16.2.3.

Until now, no validated analysis method exists. However, we evaluated the maximum temperature in the 10 cm² field. Significant changes were only detected for the miltefosine treatment (**Figure 14.1.13**).

The descriptive statistics can be found in **Table 14.2.10**. The individual listings of the thermographic parameters are provided in Appendix 16.2.13.

### 11.4.2 Statistical/analytical issues

Statistical analysis of primary and exploratory effect data were performed as following:

Because of the exploratory character the effect parameters were analysed by using non-parametric tests. Calculations were performed with SPSS Statistics Base 17.0 (SPSS Inc, Chicago, IL, USA). Results are given as median (minimum to maximum). *P*-values ≤ 0.05 were considered statistically significant (2-sided significance). *P*-values are given either as value or as asterisk with * *p*-value ≤ 0.05, ** *p*-value < 0.01 and *** *p*-value < 0.001.

Statistical differences between baseline and end of treatment as well as differences between the individual visits were evaluated with the paired non-parametric Wilcoxon test.

The statistical differences between hydrocortisone and miltefosine were only exploratory evaluated. The analyses were done by using the unpaired non-parametric Mann-Whitney-U test.

The statistical analyses of all parameters were done not until all parameters were collected and entered in the data bases. The clinical trial was regularly unblinded after locking the data base (see section 9.4.6).

### 11.4.3 Immunmodulatory effect conclusions

The primary outcome defined as a significant improvement of the clinical picture assessed by the TIS score was reached. The TIS score significantly decreased in all patients during the 3-week treatment with miltefosine. The effect was already observed after 1 week and retained at least 4 weeks after the treatment stopped.

The exploratory comparison to hydrocortisone showed that the miltefosine treatment was less effectively on the clinical outcome. Significant differences were observed after the 2$^{nd}$ treatment week until the end of treatment. In comparison to miltefosine, the effect of hydrocortisone was not retained after the end of treatment.

The objective SCORAD improved in all patients, except of 2 patients, over the 3-week intervention period indicating a global improvement of disease severity. This effect is very likely due to the intensive observation by clinician every week which in turn encouraged the patient to a better basic care.

By the investigation of the immunhistological parameters a reduction of the epidermal thickness were detected in both treatments. The miltefosine treatment caused a median reduction to a normal epidermal thickness. In comparison, the hydrocortisone treatment caused a significant reduction with atrophic characteristics.
The $CD4^+$ T-cell infiltrations were reduced in both treatments, but no significant changes were assessed. Also, changes in the $CD8^+$ T-cell counts were detected in neither group.

A trend of reduced mast cells were observed in the miltefosine-treated target lesions. By contrast, a mild increase was observed in the hydrocortisone treatment. Both findings were not statistical significant.

For the skin physiological parameters significant changes were observed for the TEWL in both treatments. An increased TEWL is a typical sign of disturbed skin barrier function. Therefore, the reduced TEWL is a positive marker for the miltefosine treatment. In the exploratory comparison to hydrocortisone no major differences in the course of TEWL were detected.
For the two parameters sebum level and pH-value no major significant changes were observed over the intervention period and in the group comparison.
There were no significant changes of water-binding properties over the treatment period. However, a significant different course was observed in the exploratory comparison of hydrocortisone and miltefosine. The CORNEUM tended to reduce in the miltefosine intervention and to increase in the hydrocortisone intervention.

The thermography were analysed by measuring the maximum temperature of the treated target lesion. Here, a significant median reduction was observed in the miltefosine-treated lesions. This parameter indicates that less inflammatory processes happened. This result underpins the positive changes by trend of the immunhistological parameters (reduced epidermal thickness, reduced $CD4^+$ T-cell infiltrations and reduced mast cell numbers) although these effects were not statistically significant.

# 12 Safety evaluation

## 12.1 Adverse events (AEs)

### 12.1.1 Brief summary of general adverse events

General AEs were observed in 11 (69%) of the 16 patients treated.

Most frequently observed symptoms were headache (8 of 32 general AEs). The general AEs were mainly moderate (50%) or mild (47%) in intensity (**Table 12.1.2**). Only one general AE was defined as severe (jammed nerve in the back) which was not related to one of the treatments. No general AE were definitely related to miltefosine. Most events with a possible relationship to miltefosine were headache (7 of 32 AEs), pruritus (5 of 32 AEs) and exacerbation of AD (3 of 32 AEs). Those general AEs were recovered at the end of the clinical trial, except of 4 which were stabilised (Appendix Table 16.3.1).

Expected local side effects of miltefosine and the application in form of solutions were defined as local skin-related AEs. These were recorded by the patients as side effects in the diary cards. The local skin-related AEs which are certainly related to miltefosine are displayed in section 12.1.3.

No AE leads to change in dose or discontinuation. No SAE was observed and no death entered.

### 12.1.2 Display of general adverse events

**Table 12.1.1 General adverse events:** Summary of concerned system organ classes

| **All treatment-emergent adverse events** | **Total 16 patients** | |
|---|---|---|
| **System organ class** | **F** | **N** |
| Musculoskeletal and connective tissue disorders | 2 | 2 |
| Skin and subcutaneous tissue disorders | 15 | 7 |
| Nervous system disorders | 8 | 4 |
| Respiratory, thoracic and mediastinal disorders | 6 | 5 |
| Gastrointestinal disorders | 1 | 1 |
| **TOTAL** | **32** | **19** |

Source: Appendix Table 16.3.1
F = number of adverse events, N = number of patients with adverse events

**Table 12.1.2 General adverse events:** Summary of characteristics

| | | **Total 16 patients** | |
|---|---|---|---|
| **Category** | | **F** | **N** |
| Related | No | 17 | 9 |
| | Possible | 15 | 9 |
| | Yes | 0 | 0 |
| **TOTAL** | | **32** | **18** |
| Intensity | mild | 15 | 8 |
| | moderate | 16 | 8 |
| | severe | 1 | 1 |
| **TOTAL** | | **32** | **17** |
| Outcome | recovered | 24 | 8 |
| | ongoing | 3 | 2 |
| | stabilised | 5 | 5 |
| | sequelae | 0 | 0 |
| | patient died | 0 | 0 |
| | unknown | 0 | 0 |
| **TOTAL** | | **32** | **15** |
| Action taken – concerning study treatment | treatment unchanged | 32 | 11 |
| | drug reduced | 0 | 0 |
| | permanently discontinued | 0 | 0 |
| | temporarily discontinued | 0 | 0 |
| **TOTAL** | | **32** | **11** |
| Action taken – other | medication | 26 | 9 |
| | none | 6 | 5 |
| | other | 0 | 0 |
| **TOTAL** | | **32** | **14** |

Source: Appendix Table 16.3.1
F = number of AEs, N = number of patients with AEs

### 12.1.3 Display of local skin-related AE

**Table 12.1.3 Local skin-related AE:** Significantly related to miltefosine

| **Local skin-related AEs significantly related to miltefosine** | **Total 16 patients** | | |
|---|---|---|---|
| **characteristics** | **F** | **S** | **N** |
| Pruritus | 10 | 13 | 3 |
| Dry skin | 3 | 26 | 3 |
| Burning | 8 | 15 | 3 |
| Tingling | 7 | 19 | 4 |
| **TOTAL** | **28** | **73** | **13** |

Source: Appendix Table 16.3.2
F = number of local skin-related AEs, S = number of days with local skin-related AEs,
N = number of patients with local skin-related AEs

### 12.1.4 Listing of general and local skin-related adverse events by patient

General and local skin-related AEs are listed by patient in Appendix Table 16.3.1 and 16.3.2.

### 12.1.5 Other treatment related local skin-related AEs

Most local skin-related AEs were also caused by application of the active control. The hydrocortisone application led to pruritus in 2, burning in 3 and tingling in 3 patients. Only the dry skin was exclusively due to miltefosine application. The numbers of local skin-related AEs caused by hydrocortisone were less compared to the miltefosine treatment.

**Table 12.1.4 Local skin-related AE:** Significantly related to hydrocortisone

| **Local skin-related AEs significantly related to hydrocortisone** | **Total 16 patients** | | |
|---|---|---|---|
| **characteristics** | **F** | **S** | **N** |
| Pruritus | 8 | 10 | 2 |
| Dry skin | 0 | 0 | 0 |
| Burning | 6 | 6 | 3 |
| Prickle | 5 | 9 | 3 |
| **TOTAL** | **18** | **15** | **8** |

Source: Appendix Table 16.3.2
F = number of local skin-related AEs, S = number of days with local skin-related AEs,
N = number of patients with local skin-related AEs

### 12.1.6 Analysis and discussion of significant adverse events during the clinical trial

Both treatments were well tolerated. No systemic general AEs occurred during the treatment and no general AE was linked to the IMP or the active control. The most frequently observed symptom was headache with the most possible connection to the treatment, too. No general or local skin-related AE caused a pre-termination of the intervention.

Both treatments caused local pruritus, burning and tingling. However, the miltefosine treatment caused more local skin-related AEs compared to the hydrocortisone. Especially, dry skin was exclusively caused by the miltefosine treatment indicating that improved basic formulations are necessary for further clinical trials.

## 12.2 Clinical laboratory evaluation

Safety laboratory parameters (blood count, hepatic and renal function parameters; see section 9.4.9.2) were determined at screening for eligibility and after the clinical trial for control.

Some laboratory values were out of reference range, but were not defined as clinical relevant by the investigator.

AEs associated with abnormal laboratory renal or liver function parameters with a causal relationship to the IMP were not reported.

Results of clinical laboratory evaluation are summarized overall and by subgroups in the following tables:

**Table 14.2.3**

**Table 14.2.4**

**Table 14.2.5**

**Table 14.2.6**

### 12.2.1 Listing of individual laboratory measurements by patients and each abnormal laboratory value

Values of individual laboratory measurements are provided for each parameter and patient in Appendix Table 16.3.3.

### 12.2.2 Evaluation of each laboratory parameter

#### 12.2.2.1 Laboratory values over time

No marked changes in safety laboratory values were observed. For male patients 3 laboratory parameters significantly changed from screening to visit 4. However, those changes were not medically relevant for the individual patient (**Table 14.2.5**). Descriptive statistics of laboratory values are provided in **Table 14.2.6**.

#### 12.2.2.2 Individual patient changes

Individual patient changes can be found in Appendix Table 16.3.3. Laboratory values out of range are listed by patient and visit in **Table 14.2.3** and **Table 14.2.4**. Regarding the liver function enzymes, no parameter increased in more then two times the ULN during the clinical trial. An increased ALT at visit 4 was observed in one female patient (P-Nr.:

009, more then 1.5 from screening). For AP no values above the ULN were detected. One patient (P-Nr.: 009) had an increased GGT values but also at screening, which was stated as clinical not relevant by the investigator.

Actually, all detected serum creatinine values were under the ULN. Only one female patient had a value out of range at screening, which was not clinically relevant (P.-Nr.: 009).

Regarding the differential blood count at visit 4, the leucocytes were increased in one male patient (P.-Nr.: 008) and decreased in one male patient (P.-Nr.: 011). The lymphocytes were decreased in 4 male patients (P.-Nr.: 004, 007, 011, and 012). In 2 of these patients reduced lymphocyte numbers were already observed at screening without clinical relevant changes to visit 4 (P.-Nr.: 007 and 012). Eosinophiles above the ULN were detected in 2 male patients (P.-Nr.: 007 and 012) which were already observed at screening. Increased neutrophiles and reduced erythrocytes were observed only in one male patient (P.-Nr.: 008). All observed values out of range were not clinical relevant.

## 12.3 Vital signs, physical findings and other observations related to safety

### 12.3.1 Listing of individual measurements of vital signs and physical examination, by patient

Individual results of vital sign measurements and physical examination are provided in Appendix Table 16.3.4 and 16.3.5.

At Screening 3 patients (P.-Nr.: 005 and 014, female; 017, male), at visit 2 3 patients (P.-Nr.: 005, female; 017 and 016, male), at visit 3 4 patients (P.-Nr.: 005 and 014, female; 013 and 017, male), at visit 4 and at follow-up 1 only patient (P.-Nr.: 016, male and 017, male, respectively) and at follow-up 2 4 patients P.-Nr.: 005, female; 007, 015 and 017, male) had increased pulse rates without clinical relevance.

Regarding the blood pressure, one patient had continuously increased systolic and diastolic blood pressure (P.-Nr.: 007, male). Six other patients (P.-Nr.: 006 and 009, female; 004, 015, 016 and 017, male) had increased systolic and/or diastolic blood pressure minimum at one visit and maximum at 4 visits. One male patient (P.-Nr.: 010) had only once an increased systolic blood pressure at follow-up 1, and 2 male patients (P.-Nr.: 008 and 012) had only once an increased diastolic blood pressure at visit 1 and visit 2, respectively.

All observed vital signs out of range were not estimated as clinical relevant in this clinical trial.

### 12.3.2 Evaluation of parameters of vital signs and physical examination

#### 12.3.2.1 Vital signs

Median values of systolic and diastolic blood pressure did not change markedly during the clinical trial as compared to screening. The heart beat was significantly reduced at visit 4, but did remain in the normal range. Descriptive statistics of vital signs are provided in **Table 14.2.7**. The vital signs before treatment were defined as the values from visit 1. For those patients who had only one visit for screening and visit 1, the vital signs values from the screening (= visit 1) were used.

Adverse events associated to vital sign values changes with causal relationship to the IMP were not reported.

### 12.3.2.2 Physical examination

A complete physical examination was performed at all visits. Abnormalities were observed by all patients in examination of the skin, of course due to the underlying and investigated disease, AD. One male patient had additionally a vitiligo recorded once at screening (P.-Nr.: 007). Other abnormalities were a high blood pressure disease of one patient (P.-Nr.: 009). Although it was a known underlying disease, it was misleadingly recorded at all visits. Finally, one male patient (P.-Nr.: 016) had an existing prolapse at lumbar vertebra 5 and sacrum 1.

## 12.4 Safety conclusions

In general, both treatments were well tolerated by all patients. No systemic general AE occurred during the treatment and no general AE was certainly linked to the IMP or the active control. No general or local skin-related AE caused a pre-termination of the intervention. However, the local skin-related AEs (pruritus, burning and tingling) were mainly due to the miltefosine treatment. Additionally, symptoms of dry skin were exclusively caused by the miltefosine treatment.

All safety laboratory values were more or less normal before and after the intervention. Some laboratory values that were a little out of range, were not defined as clinical relevant. No marked changes in safety laboratory were observed.

All vital signs were in a normal range before, during and after the intervention. Values out of range were not defined as clinical relevant. Regarding the physical examinations, no abnormal changes were identified during the treatment period.

# 13 Discussion and overall conclusions

## 13.1 Effect

The primary outcome was reached represented by the significantly reduced TIS score >1.5 points over the time of miltefosine treatment. A rapid treatment response already after 1 week and a lasting effect after the end of treatment were observed. In the exploratory comparison to hydrocortisone, miltefosine was less effective. However, the lasting effect was not seen for the hydrocortisone treatment. The clinical improvement was underlined by the coexisting improvement of the objective SCORAD.

The immunhistological investigations revealed changes in the epidermal thickness. The miltefosine treatment led to a normalization of epidermal thickness, whereas the hydrocortisone treatment caused a significant reduction of epidermal thickness indicating atrophic characteristics. Reduced $CD4^+$ T-cell infiltrations were observed in both treatments and no major changes were found in the $CD8^+$ T-cell distribution. The mast cell counts showed oppositional trend without significant differences.

Changes in the skin physiological parameters were observed in both treatments. The TEWL were significantly reduced over time in both treatments indicating an advantage of both formulations. The water-binding capacity (CORNEUM) showed oppositional trend. No major significant changes in the sebum levels and pH-values were observed.

The thermography represented by the maximum temperature was significantly reduced only in the miltefosine-treated lesions. This result indicates less inflammatory processes in the skin and underpins the positively changed clinical outcome. Furthermore, the weak non-significant changes of the immunhistological parameters are strengthen.

## 13.2 Safety

In general, bothe treatments were well tolerated by all patients. No systemic general AE occurred during the treatment and no general AE was certainly linked to the IMP or the active control. No general or local skin-related AE caused a pre-termination of the intervention. However, the local skin-related AEs (pruritus, burning and tingling) were mainly due to the miltefosine treatment. Additionally, symptoms of dry skin were exclusively caused by the miltefosine treatment.

All safety laboratory values were more ore less normal before and after the intervention. Some laboratory values that were a little out of range, were not defined as clinical relevant. No marked changes in safety laboratory were observed.

All vital signs were in a normal range before, during and after the intervention. Values out of range were not defined as clinical relevant. Regarding the physical examinations, no abnormal changes were identified during the treatment period.

# 14 Figures and tables referred to but not included in the text

## 14.1 Figures

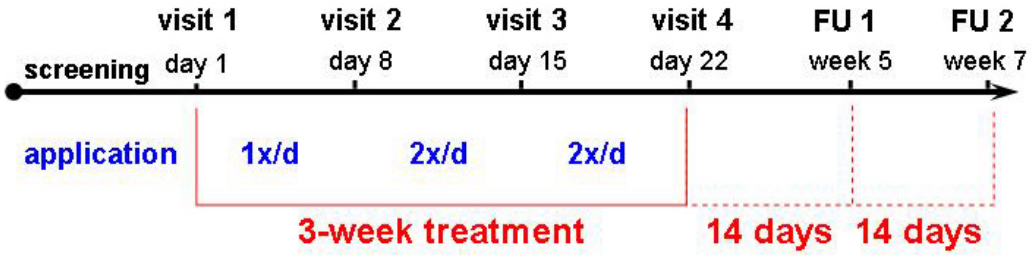


**Figure 14.1.1: Study design of the clinical trial 'Miltefosine bei AD'**

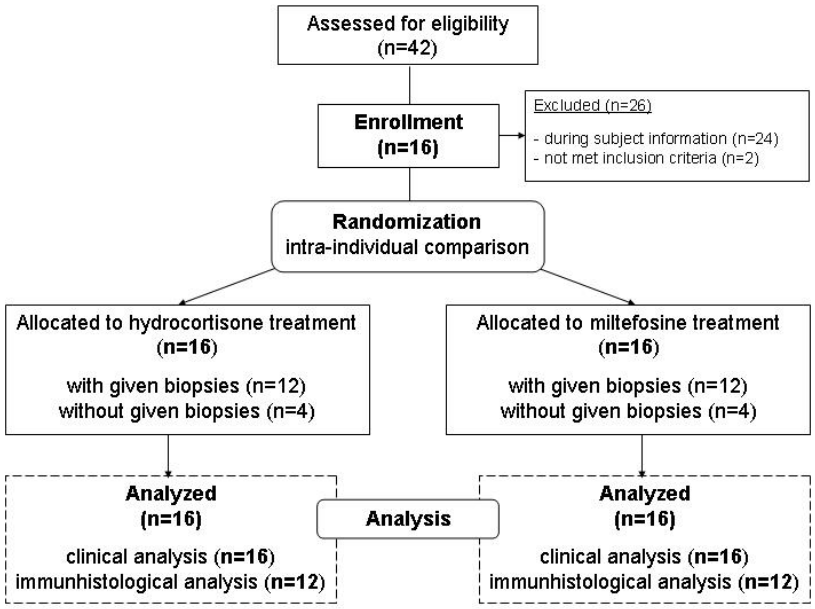


**Figure 14.1.2: Flow chart of the clinical trial 'Miltefosine bei AD'**

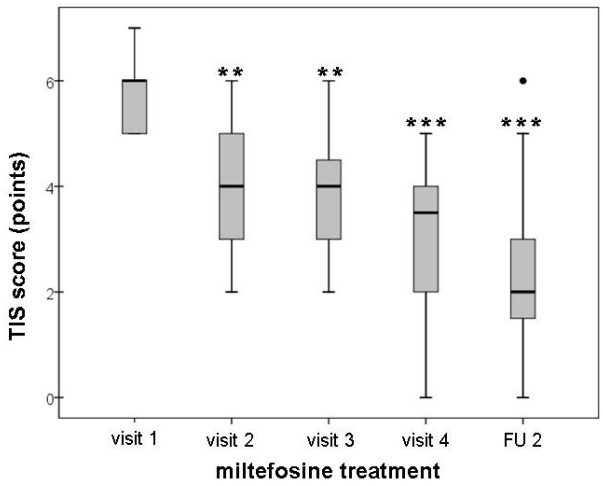


**Figure 14.1.3:** **TIS score over the course of miltefosine treatment**;
Follow up 2 (FU 2) was performed 4 weeks after the end of treatment. 4 patients had no follow up 2 and the values of the follow up 1 were used. Asterisks indicate significant difference between the visits with ** *p*-value < 0.01, *** *p*-value < 0.001

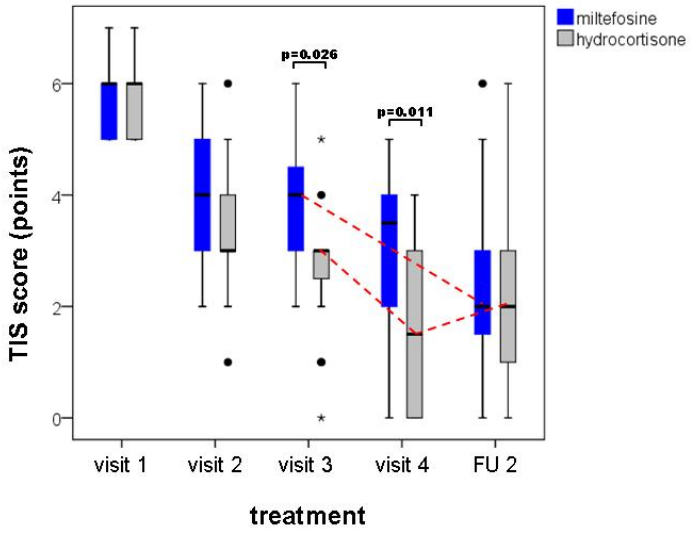


**Figure 14.1.4:** **TIS score over the course of intervention compared between miltefosine and hydrocortisone treatment**;
Median is shown as black line; Outliers are shown as black dots; Asterisks are extreme outliers; The red dotted lines indicate the course of TIS score beyond the end of treatments; FU 2 = follow up 2

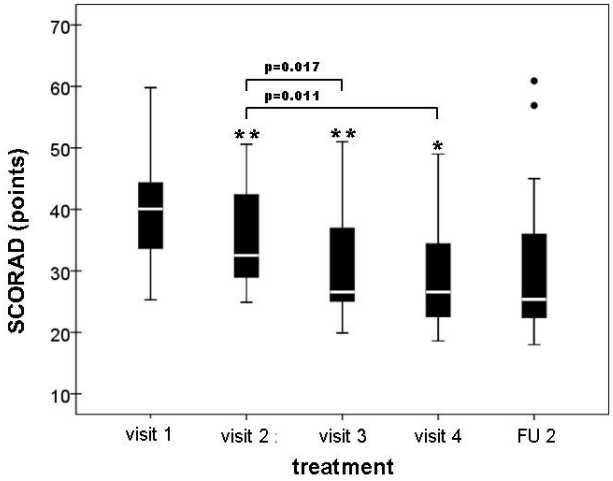


**Figure 14.1.5: SCORAD before, during and after treatment**;
Median is shown as white line; Outliers are shown as black dots; Asterisks give the significant difference to the SCORAD before treatment with ** *p*-value < 0.01, *** *p*-value < 0.001; FU 2 = follow up 2

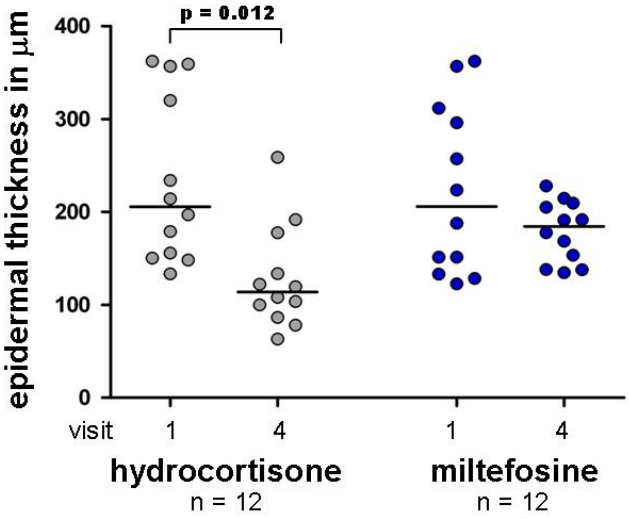


**Figure 14.1.6: Epidermal thickness before and after treatment;**
Median is shown as black line; Dots indicate the individual values

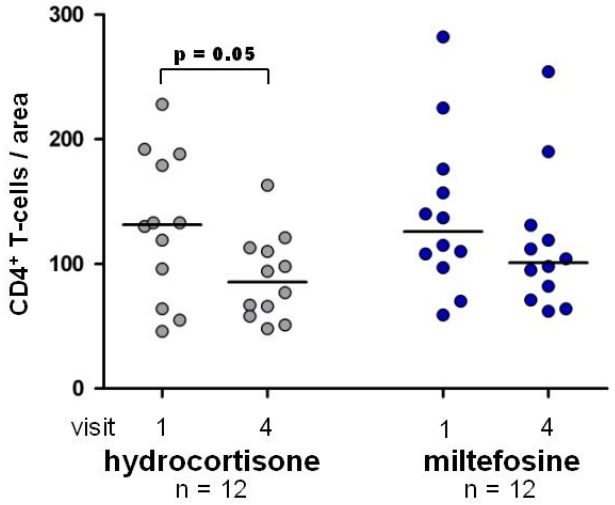


**Figure 14.1.7:** **$CD4^+$ T-cells in the skin sections before and after treatment;**
Median is shown as black line; Dots indicate the individual values; Area = 0.06 mm²

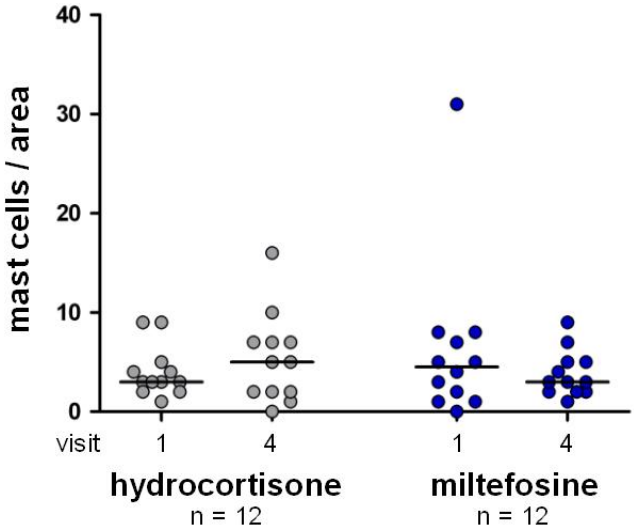


**Figure 14.1.8:** **Mast cells counts in the skin sections before and after treatment;**
Median is shown as black line; Dots indicate the individual values; area = 0.24 mm²

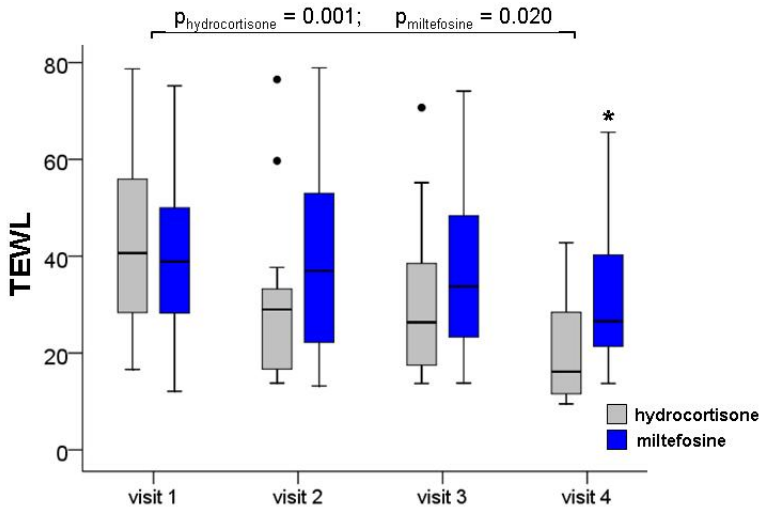


**Figure 14.1.9** **Course of TEWL during the treatment**;
Median is shown as a black line; Asterisk indicates significant differences between hydrocortisone and miltefosine with * *p*-value < 0.05; Outliers are shown as black dots

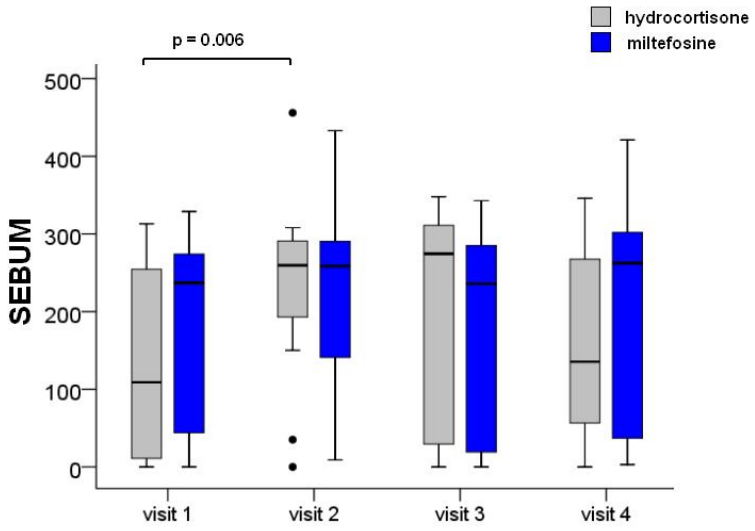


**Figure 14.1.10** **Course of sebum level during the treatment**;
Median is shown as a black line; Outliers are shown as black dots

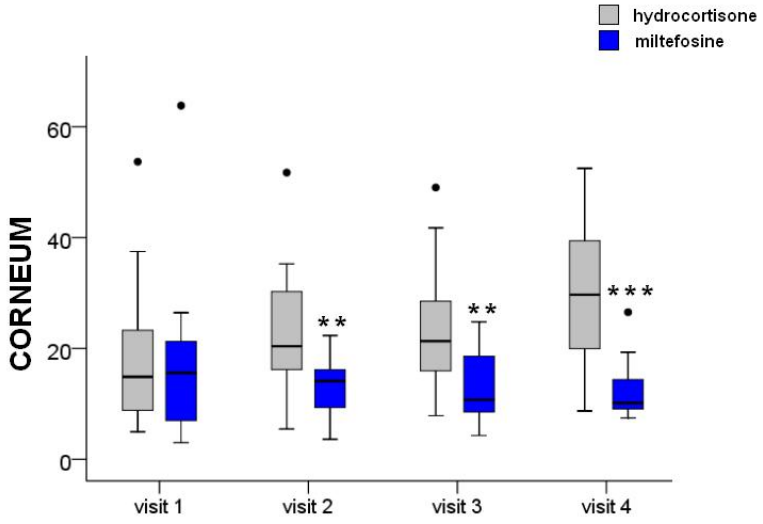


**Figure 14.1.11 Course of water content of the stratum corneum (CORNEUM) during the treatment**;
Median is shown as a black line; Asterisks indicate significant differences between hydrocortisone and miltefosine with ** *p*-value < 0.01 and *** *p*-value < 0.001; Outliers are shown as black dots

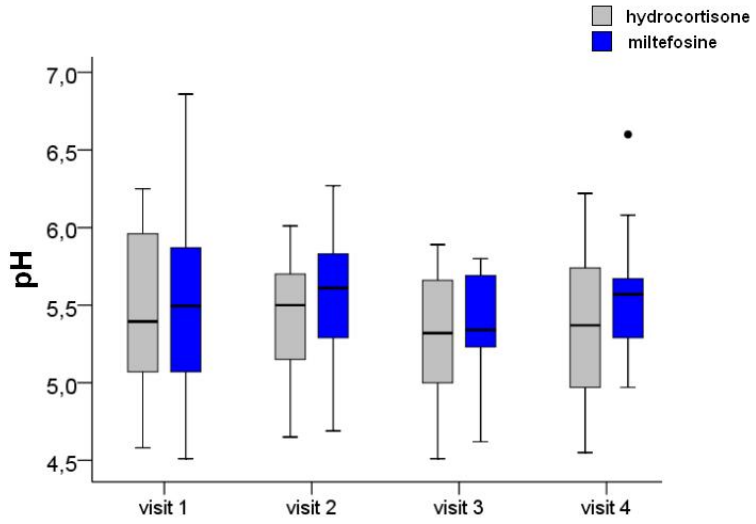


**Figure 14.1.12 Course of skin pH during the treatment;**
Median is shown as a black line; Outlier is shown as black dot

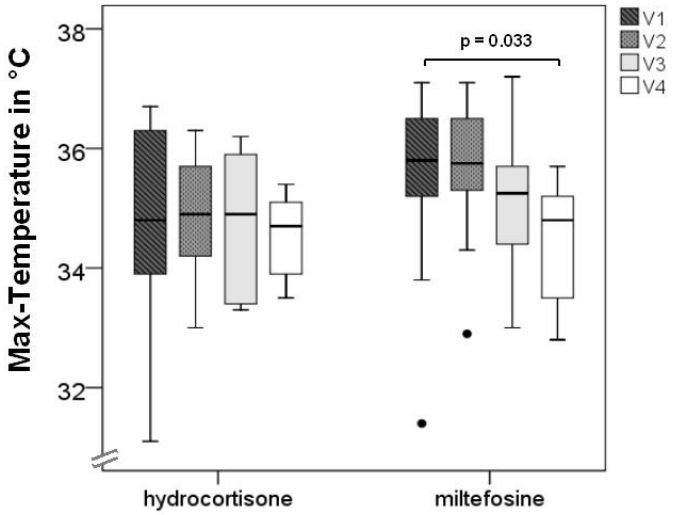


**Figure 14.1.13 Thermographic measurements during the course of treatment;**
Maximum temperature of the 10 cm² target lesion are depicted; Median is shown as a black line; Outlier is shown as black dot

## 14.2 Tables

### Table 14.2.1: Time schedule of study procedures

| **Treatment period** | **Screen** | **visit 1** | **visit 2** | **visit 3** | **visit 4** | **follow-up 1** | **follow-up 2** |
|---|---|---|---|---|---|---|---|
| day (s) or week (s) | d-28 to d1 | d1 | d8 ±1d | d15 ±1d | d22 ±1d | w5 ±3d | w7 ±3d |
| **Investiations/Procedures** | | | | | | | |
| Subject information | ✓ | | | | | | |
| Informed consent | ✓ | | | | | | |
| Anamnesis | ✓ | | | | | | |
| Inclusion/exclusion criteria | ✓ | | | | | | |
| Vital signs[#] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ethical origin | ✓ | | | | | | |
| Physical examination | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pregnancy test* | ✓ | | | | | | |
| IgE | ✓ | | | | | | |
| Safety laboratory | ✓ | | | | ✓ | if required | |
| SCORAD | ✓ | ✓ | | | ✓ | | |
| Definition of target lesion | ✓ | ✓[i.n.] | | | | | |
| Assessment of target lesion | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Skin physiology | | | ✓ | ✓ | ✓ | | |
| Thermography | | | ✓ | ✓ | ✓ | | |
| Photography | | ✓ | ✓ | ✓ | ✓ | | |
| Biopsies | | ✓ | | | ✓ | | |
| Dispense of patient's diary | | ✓ | | | | | |
| Control of patient's diary | | | ✓ | ✓ | ✓ | | |
| Return of patient's diary | | | | | ✓ | | |
| Dispense of trial medication | | ✓ | | | | | |
| Return of trial medication | | | | | ✓ | | |
| Dispense of pipettes | | ✓ | ✓ | ✓ | | | |
| Return of pipettes | | | ✓ | ✓ | ✓ | | |
| Start of treatment | | ✓ | | | | | |
| End of treatment | | | | | ✓ | | |
| Record of tolerability | | | ✓ | ✓ | ✓ | | |
| Documentation of AE | | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Record of Co-medication | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Termination visit** | | | | | ✓ | | |

[#] Vital signs comprise body height and weight, blood pressure and hard beat.
[##] Safety laboratory comprise haematology and clinical chemistry.
* for women with child bearing potential
**In terms of a premature trial termination this termination visit should be performed.
[i.n.] if necessary

## Table 14.2.2: Demographic Data at baseline

| Variable | patients (n=16) |
|---|---|
| Sex (female/male): | 11 / 5 |
| Age (years): | 29 [18 – 58] |
| Height (cm): | 175 [155 – 193] |
| Weight (kg): | 71 [57 – 110] |
| Ethical origin | 15 Caucasian / 1 Asian |
| SCORAD (points):<br><25 (mild)<br>≥25 and <50 (moderate)<br>≥50 (severe) | 51 [34 – 69]<br>n=0 (0 %)<br>n=6 (37 %)<br>n=10 (63 %)6 [5 – 7] |
| Median TIS score (points):<br>- 5 points<br>- 6 points<br>- 7 points | <br>n=7 (44 %)<br>n=7 (44 %)<br>n=2 (12 %) |
| Safety laboratory:<br>- creatinine (mg/dl)<br>- ALT (U/l)<br>- AP (U/l)<br>- GGT (U/l) | <br>0.87 [0.68 – 1.08]<br>19 [9 – 56]<br>62.5 [47 – 101]<br>15.5 [11 – 55] |

* TIS – Three item severity; ALT – alanine aminotransferase, AP – alkaline phosphatase, GGT – gamma glutamyltranspeptidase; values in median [minimum – maximum]

**Table 14.2.3 Laboratory values at screening:** Values out of reference range but not clinical significant (each patient).

| Screening | | | Value out of reference range | | |
|---|---|---|---|---|---|
| **P.-No.** | **Age (years)** | **Sex** | **1** | **2** | **3** |
| 003 | 41 | M | Leucocytes (per nl)<br>range: 4.50 to 11.00<br>3.84 | | |
| 005 | 28 | F | RDW (%)<br>range: 11.9 to 14.5<br>15.4 | | |
| 006 | 45 | F | Eosinophiles (per nl)<br>normal: < 0.45<br>0.89 | | |
| 007 | 39 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.08 | Eosinophiles (per nl)<br>normal: < 0.45<br>0.87 | |
| 008 | 28 | M | RDW (%)<br>range: 11.9 to 14.5<br>14.6 | | |
| 009 | 58 | F | Creatinine (mg/dl)<br>normal: < 1.0<br>1.05 | GGT (U/l)<br>normal: < 38<br>55 | MCH (pg)<br>range: 26.0 to 34.0<br>35.0 |
| 010 | 25 | M | Potassium (mmol/l)<br>range: 3.40 to 5.20<br>3.20 | | |
| 012 | 38 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.37 | Eosinophiles (per nl)<br>normal: < 0.45<br>0.81 | |
| 013 | 23 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.16 | | |
| 016 | 28 | M | ALT (U/l)<br>normal: < 45<br>56 | Eosinophiles (per nl)<br>normal: < 0.45<br>0.72 | |

Source Appendix Table 16.4.3
P.-No. = Patient number, RDW - Red Cell Distribution Width, GGT – Gamma Glutamyltransferase, MCH - Mean Corpuscular Haemoglobin, ALT – Alanine Aminotransferase

**Table 14.2.4 Laboratory values at visit 4:** Values out of reference range but not clinical significant (each patient).

| Visit 4 | | | Value out of reference range | | | |
|---|---|---|---|---|---|---|
| **P-No.** | **Age (years)** | **Sex** | **1** | **2** | **3** | **4** |
| 004 | 42 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.21 | | | |
| 006 | 45 | F | Calcium (mmol/l)<br>range: 2.15 to 2.45<br>2.14 | | | |
| 007 | 39 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.04 | Eosinophiles (per nl)<br>normal: < 0.45<br>0.90 | | |
| 008 | 28 | M | Leucocytes (per nl)<br>range: 4.50 to 11.00<br>12.25 | Erythrocytes (per pl)<br>range: 4.60 to 6.20<br>4.5 | Haemoglobin (g/dl)<br>range: 14.0 to 17.5<br>13.7 | Neutrophiles (per nl)<br>range: 1.80 to 7.70<br>9.60 |
| 009 | 58 | F | ALT (U/l)<br>normal: < 34<br>59 | GGT (U/l)<br>normal: < 38<br>42 | MCH (pg)<br>range: 26.0 to 34.0<br>35.0 | MCV (fl)<br>range : 81 to 100<br>101 |
| 011 | 28 | M | Leucocytes (per nl)<br>range: 4.50 to 11.00<br>4.32 | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.12 | | |
| 012 | 38 | M | Lymphocytes (per nl)<br>range: 1.40 to 3.70<br>1.18 | Eosinophiles (per nl)<br>normal: < 0.45<br>0.78 | | |

Source Appendix Table 16.4.3
P.-No. = Patient number, MPV – Mean Platelet Volume, ALT – Alanine Aminotransferase, GGT – Gamma Glutamyltransferase, MCH - Mean Corpuscular Haemoglobin, MCV – Mean Cellular Volume

**Table 14.2.5 Laboratory values:** Changes from screening to visit 4 of values with statistical significant differences; Could only be detected in the group of male patients.

| | **Differences from screening to visit 4** | | |
|---|---|---|---|
| **P.-No.** | **GGT (U/l)** | **MCV (fl)** | **RDW (%)** |
| 003 | 4 | -1 | 0 |
| 004 | 7 | 0 | 0.4 |
| 007 | 3 | -1 | 0.2 |
| 008 | 2 | -1 | 0.2 |
| 010 | N.P. | 0 | -0.1 |
| 011 | 3 | 0 | 0.1 |
| 012 | 2 | -1 | 0.1 |
| 013 | -3 | -2 | 0 |
| 015 | 0 | 0 | 0 |
| 016 | 5 | 0 | 0.1 |
| 017 | -1 | -1 | 0.4 |
| **Median** | **2.5** | **-1.0** | **0.1** |
| **Range (min to max)** | **-3.0 to 7.0** | **-2.0 to 0.0** | **-0.1 to 0.4** |
| ***p*-value** | **0.050** | **0.020** | **0.028** |

Source Appendix Table 16.4.3
P.-No. = Patient number, GGT – Gamma Glutamyltransferase, MCV - Mean Cellular Volume, RDW - Red Cell Distribution Width, N.P. – Not Possible

**Table 14.2.6 Laboratory values:** Descriptive statistics, considered separately for female and male, median [minimum to maximum]

| Laboratory value | sex | Screening | Visit 4 | *p*-value |
|---|---|---|---|---|
| Sodium (mmol/l) | F | 138 [136 to 141] | 141 [136 to 141] | N.S. |
| | M | 141 [136 to 143] | 138 [136 to 142] | N.S. |
| Potassium (mmol/l) | F | 4.1 [3.7 to 4.2] | 3.9 [3.6 to 4.2] | N.S. |
| | M | 3.9 [3.2 to 4.8] | 4.1 [3.5 to 4.3] | N.S. |
| Calcium (mmol/l) | F | 2.3 [2.2 to 2.4] | 2.3 [2.1 to 2.5] | N.S. |
| | M | 2.3 [2.2 to 2.5] | 2.3 [2.2 to 2.4] | N.S. |
| Creatinine (mg/dl) | F | 0.74 [0.68 to 1.05] | 0.77 [0.65 to 0.88] | N.S. |
| | M | 0.93 [0.75 to 1.08] | 0.87 [0.75 to 0.98] | N.S. |
| ALT (U/l) | F | 17 [10 to 33] | 18 [12 to 59] | N.S. |
| | M | 23 [9 to 56] | 25 [11 to 38] | N.S. |
| AP (U/l) | F | 53 [47 to 64] | 51 [48 to 58] | N.S. |
| | M | 72 [50 to 101] | 63 [48 to 111] | N.S. |
| GGT (U/l) | F | 14 [11 to 55] | 13 [12 to 42] | N.S. |
| | M | 16 [11 to 42] | 18 [12 to 37] | **0.050** |
| Leucocytes (/nl | F | 6.7 [5.7 to 8.8] | 6.7 [5.3 to 9.5] | N.S. |
| | M | 6.5 [3.8 to 8.3] | 6.8 [4.3 to 12.3] | N.S. |
| Erythrocytes (/nl | F | 4.5 [4.1 to 4.6] | 4.5 [3.9 to 4.5] | N.S. |
| | M | 4.9 [4.6 to 5.5] | 4.9 [4.5 to 5.7] | N.S. |
| Haemoglobin (g/dl) | F | 13.2 [13.0 to 14.3] | 13.1 [12.6 to 13.7] | N.S. |
| | M | 14.8 [14.2 to 16.2] | 14.8 [13.7 to 16.5] | N.S. |
| Haematocrit (l/l) | F | 0.39 [0.38 to 0.41 | 0.39 [0.38 to 0.39 | N.S. |
| | M | 0.44 [0.42 to 0.48] | 0.44 [0.41 to 0.49] | N.S. |
| MCH (pg) | F | 29.3 [28.4 to 35.0] | 29.0 [29.0 to 35.0] | N.S. |
| | M | 29.9 [28.5 to 30.9] | 30.0 [28.3 to 30.9] | N.S. |
| MCHC (g/dl) | F | 34.0 [33.8 to 35.1] | 34.0 [33.2 to 34.8] | N.S. |
| | M | 34.0 [33.3 to 34.6] | 33.8 [33.3 to 34.7] | N.S. |
| MCV (fl) | F | 86 [84 to 100] | 87 [85 to 101] | N.S. |
| | M | 88 [84 to 91] | 89 [84 to 92] | **0.020** |
| RDW (%) | F | 12.8 [12.2 to 15.4] | 12.6 [12.2 to 15.0] | N.S. |
| | M | 13.2 [12.5 to 14.6] | 13.0 [12.4 to 14.4] | **0.028** |
| Thrombocytes (/nl) | F | 254 [194 to 376] | 275 [169 to 389] | N.S. |
| | M | 270 [215 to 355] | 272 [207 to 347] | N.S. |
| MPV (fl) | F | 10 [9 to 12] | 11 [10 to 12] | N.S. |
| | M | 11 [9 to 11] | 11 [9 to 11] | N.S. |
| Neutrophiles (/nl) | F | 3.6 [3.2 to 5.6] | 4.0 [2.3 to 5.3] | N.S. |
| | M | 3.8 [1.9 to 5.2] | 4.3 [2.6 to 9.6] | N.S. |
| Lymphocytes (/nl) | F | 1.9 [1.6 to 2.9] | 2.0 [1.5 to 3.4] | N.S. |
| | M | 1.5 [1.1 to 2.5] | 1.6 [1.0 to 2.6] | N.S. |
| Monocytes (/nl) | F | 0.50 [0.43 to 0.76] | 0.51 [0.44 to 0.72] | N.S. |
| | M | 0.49 [0.26 to 0.77] | 0.54 [0.30 to 0.90] | N.S. |
| Eosinophiles (/nl) | F | 0.33 [0.21 to 0.89] | 0.31 [0.23 to 0.63] | N.S. |
| | M | 0.63 [0.14 to 0.87] | 0.33 [0.13 to 0.90] | N.S. |
| Basophiles (/nl) | F | 0.02 [0.02 to 0.04] | 0.03 [0.02 to 0.04] | N.S. |
| | M | 0.03 [0.01 to 0.08] | 0.02 [0.01 to 0.07] | N.S. |

Source Appendix Table 16.4.3
ALT – Alanine Aminotransferase, AP – Alkaline Phosphatase, GGT – Gamma Glutamyltransferase, MCH – Mean corpuscular haemoglobin, MCHC – Mean Cellular Haemoglobin Concentration, MCV – Mean Cellular Volume, RDW – Red Cell Distribution Width, MPV – Mean Platelet Volume, N.S. – Not Significant

**Table 14.2.7 Vital signs:** Descriptive statistics, median [minimum to maximum]

| Vital signs | Heart beat *p*-value | Blood pressure Systolic *p*-value | Blood pressure Diastolic *p*-value |
|---|---|---|---|
| Visit 1 | 76 [52 to 96] | 120 [110 to 145] | 80 [70 to 100] |
| Visit 2 | 80 [60 to 92] N.S. | 120 [105 to 140] N.S. | 80 [70 to 100] N.S. |
| Visit 3 | 76 [60 to 92] N.S. | 120 [100 to 160] N.S. | 180 [70 to 110] N.S. |
| Visit 4 | 72 [48 to 84] **0.032** | 120 [110 to 160] N.S. | 80 [70 to 100] N.S. |
| Follow-up 1 | 78 [52 to 96] N.S. | 125 [100 to 160] N.S. | 80 [60 to 110] N.S. |
| Follow-up 2 | 80 [68 to 84] N.S. | 130 [110 to 160] N.S. | 80 [70 to 110] N.S. |

Source Appendix Table 16.4.4
N.S. – Not Significant

**Table 14.2.8 Immunhistological parameters:** Descriptive statistics, median [min to max]

| Immunhistological parameter | Visit | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | Hydrocortisone | | | Miltefosine | | |
| | | Median | Min | Max | Median | Min | Max |
| Epidermal thickness | 1 | 205.65 | 133.37 | 362.29 | 205.74 | 122.69 | 362.29 |
| | 4 | 113.86 | 63.36 | 258.83 | 184.36 | 134.71 | 228.14 |
| $CD4^+$ T-cells | 1 | 132 | 46 | 228 | 126 | 59 | 282 |
| | 4 | 86 | 48 | 163 | 101 | 62 | 254 |
| $CD8^+$ T-cells | 1 | 7 | 1 | 50 | 7 | 2 | 411 |
| | 4 | 8 | 1 | 23 | 7 | 2 | 23 |
| Mast cells | 1 | 3 | 1 | 9 | 5 | 0 | 31 |
| | 4 | 5 | 0 | 16 | 3 | 1 | 9 |

Min = minimum, Max = maximum

**Table 14.2.9 Skin physiology:** Descriptive statistics, median [min to max]

| Skin physiological parameters | Visit | Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hydrocortisone | | | | | Miltefosine | | | | |
| | | N | Median | Min | Max | *p*-value | N | Median | Min | Max | *p*-value |
| TEWL | 1 | 16 | 40.65 | 16.60 | 78.70 | | 16 | 38.90 | 12.10 | 75.20 | |
| | 2 | 16 | 29.00 | 13.80 | 76.50 | 0.008 | 16 | 36.95 | 13.20 | 78.90 | |
| | 3 | 16 | 26.35 | 13.70 | 70.70 | 0.026 | 16 | 33.75 | 13.80 | 74.10 | |
| | 4 | 16 | 16.15 | 9.50 | 42.80 | 0.030 | 16 | 26.60 | 13.70 | 65.60 | 0.034 |
| SEBUM | 1 | 15 | 109.00 | 0 | 313.00 | | 15 | 237.00 | 0 | 329.00 | |
| | 2 | 16 | 259.50 | 0 | 456.00 | 0.006 | 16 | 258.50 | 0 | 434.00 | |
| | 3 | 16 | 274.50 | 0 | 348.00 | | 16 | 364.50 | 0 | 343.00 | |
| | 4 | 16 | 135.50 | 0 | 346.00 | | 16 | 262.50 | 3.00 | 421.00 | |
| CORNEUM | 1 | 13 | 14.88 | 4.98 | 53.70 | | 13 | 15.60 | 3.00 | 63.82 | |
| | 2 | 15 | 20.13 | 5.48 | 51.74 | | 14 | 14.13 | 3.60 | 22.34 | |
| | 3 | 15 | 21.32 | 7.88 | 49.04 | | 15 | 10.76 | 4.30 | 24.82 | |
| | 4 | 13 | 29.70 | 8.74 | 52.50 | | 13 | 10.18 | 7.48 | 26.55 | |
| pH | 1 | 10 | 5.40 | 4.58 | 6.25 | | 10 | 5.5 | 4.51 | 6.86 | |
| | 2 | 10 | 5.50 | 4.65 | 6.01 | | 10 | 5.61 | 4.69 | 6.27 | |
| | 3 | 13 | 5.35 | 4.51 | 5.89 | | 9 | 5.34 | 4.62 | 5.80 | |
| | 4 | 13 | 5.37 | 4.55 | 6.22 | | 13 | 5.57 | 4.97 | 6.60 | |

N = number of patients, Min = minimum, Max = maximum;
p-values only describe the differences between the single visits, e.g. visit 1 to vivit2, visit 2 to visit3 and visit3 to 4 because these *p*-values are not depicted in **Figure 14.1.9** to **Figure 14.1.12**.

**Table 14.2.10 Thermographic imaging:** Descriptive statistics, median [min to max]

| Visit | Maximum Temperature of the lesion | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Hydrocortisone | | | | Miltefosine | | | |
| | N | Median | Min | Max | N | Median | Min | Max |
| **1** | 15 | 35.0 | 31.1 | 41.9 | 15 | 35.5 | 31.4 | 35.5 |
| **2** | 11 | 34.9 | 31.3 | 36.3 | 12 | 35.5 | 32.7 | 35.5 |
| **3** | 13 | 34.9 | 31.9 | 36.2 | 12 | 35.2 | 33.0 | 35.2 |
| **4** | 13 | 34.2 | 31.9 | 35.4 | 13 | 34.8 | 32.1 | 34.8 |

N = number of patients, Min = minimum, Max = maximum

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