FINAL REPORT

"Efficacy and Safety of Acyclovir 5% Lipstick (Contra) in Treatment of Skin Infections Provoked by Herpes Simplex. A Randomised, Double-blind, Placebo Controlled, Parallel Group Study."

Study Code: 4PH/2011/002

EudraCT number: 2011-002135-26

Version: Final

Date of Report: 12/12/2012

1. TITLE PAGE

Study Title:

Efficacy and safety of Acyclovir 5% Lipstick (Contra) in treatment of skin infections provoked by Herpes Simplex. A randomised, double-blind placebo controlled, parallel group study.

Investigational Medicinal Product (IMPs):

Aciclovir 5% Lipstick: from Aesculapius Farmaceutici Srl (Test formulation).

Indication studied:

Skin infections provoked by Herpes Labialis.

Brief description of study:

A monocentric, randomised, double-blind, placebo-controlled clinical study in parallel groups on both adult male and female (total=70) patients, Caucasian origin to compare the efficacy of a test formulation vs placebo in the treatment of skin infections provoked by Herpes Labialis.

Study population:

70 patients of both sexes have been enrolled and 69 completed the study.

Name of the Sponsor:

Aesculapius Farmaceutici Srl
Via Cozzaglio, 24
25125 Brescia
Tel. 030.89331

Protocol Code:

EudraCT number: 2011-002135-26
Protocol Code: 4PH/2011/002

Development phase of study:

Phase III

Study initiation date:

First patient enrolled: 03/05/2012

Date of early study termination:

N/A

Study completion date:

Last patient completed: 03/12/2012

Principal Investigator :

Dott. Valter Armellani
Via V. Vestina 307, 65015 Montesilvano (PE), Italy
085-4680687

Sponsor signatory:

Name: Dr Enzo Moroni
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This study was performed in compliance with Good Clinical Practices, including the archiving of essential documents.

Date of report:

12/12/2012

2. SYNOPSIS

STUDY TITLE:	Efficacy and safety of Acyclovir 5% Lipstick (Contra) in treatment of skin infections provoked by Herpes Simplex. A randomised, double-blind, placebo controlled, parallel group study.
INVESTIGATIONAL SITE:	Ambulatorio Via V. Vestina 307 65015 Montesilvano (PE), Italy 085-4680687
STUDY PHASE:	III
OBJECTIVE:	Primary study objective: -evaluation of the efficacy of *test formulation* (Acyclovir 5% Lipstick from *Aesculapius Farmaceutici Srl*) in comparison with placebo. Secondary study objective: -evaluation of the general safety of the active treatment (test formulation) in comparison with placebo.
METHODOLOGY:	Randomised double-blind placebo controlled study. Patients have been treated with test formulation or placebo. The test formulation and placebo have been administered five (5) times a day for no more than seven consecutive days.
NUMBER OF SUBJECTS:	Seventy (70).
MAIN CRITERIA FOR INCLUSION:	- Caucasian origin; - Patients with a clinical diagnosis of active infection of recurrent labial herpes in prodromal phase; - Body weight within 20% of ideal body weight; - Normal physical examination, vital signs, ECG; - Negative result of pregnancy test for female patients.
TEST PRODUCT, DOSE, MODE OF ADMINISTRATION, BATCH N°, EXPIRE DATE:	Acyclovir 5% Lipstick, from *Aesculapius Farmaceutici Srl*, topical route, batch n° 011031, expire date 01/2014

CRITERIA FOR EVALUATION:	The efficacy of the formulations has been evaluated by the following endpoints: *1. Primary endpoint:* - Healing time: the number of days from the beginning of the therapy until the complete re-epithelialization of lesions. *2. Secondary endpoints:* - reduction in patients assessed severity of pain (using a Visual Analogue Scale: 0-100 mm); - reduction in lesion size (small, medium, large, very large); - reduction in lesion extension (mm); - reduction of burning and itching (absent, slight, medium and intense). The safety has been evaluated by monitoring: - adverse events during the whole study period; - results of physical examination, vital signs, resting 12-lead ECG obtained and recorded during pre- and post-study visit.
STATISTICAL METHOD:	The statistical analyses have been performed according to the "Intention To Treat" (ITT) and "Per Protocol" (PP) analysis principles. All randomised patients have been included in the Intention-to-Treat population. Only patients showing no/minor protocol deviations have been included in the Per-Protocol population. The results of the two treatment groups have been compared using parametric and non-parametric tests coherently with the type of data. In particular, the difference between the mean of the necessary days for complete re-epithelialization (*healing time*) of the test product and the mean of the necessary days for complete re-epithelialization (*healing time*) of the placebo has been compared using Student's *t*-test. As supportive analysis, a time-to-event analysis has been performed following Kaplan-Meier methodology and Cox proportional hazards method. Non-parametric tests have been used to analyse the secondary efficacy variables. Safety variableshave been fully described. According to CPMP/ICH/363/96 and CPMP/EWP/482/99, both two-sided (at 95% significance level) and one-sided tests (at 97.5% significance level) have been performed. Two-sided

	95% confidence intervals have been used to assess the possible superiority of the test product. A descriptive statistical analysis that involves the determination of mean, median, SD, minimum, maximum, frequency values on primary and secondary efficacy variables have been performed using IBM SPSS 19 for Windows.

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4. LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE(s)	Adverse event(s)
ASL	Agenzia Sanitaria Locale
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CRF	Case Report Form
DBP	Diastolic Blood Pressure
EC	Ethical Committee
ECG	Electrocardiogram
GCP	Good Clinical Practice
GGT	Gamma- Glutamil Transferase
GMP	Good Manufacturing Practice
GOT/AST	Glutamyl Oxalo Acetic Transaminase/Aspartate aminotransferase
GPT/ALT	Glutamyl pyruvic transaminase/Alanine aminotransferase
HCT	Haematocrit
HIV	Human Immunodeficiency Virus
HR	Heart rate
HSV1	Herpes simplex virus 1
ICH	International Conference of Harmonization
IMP	investigational medicinal product
ITT	Intention-to-treat
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MMG	Medici di Medicina Generale
PI	Principal Investigator
PP	Per-protocol
RHL	Recurrent Herpes Labialis
SAE	Serious Adverse Events
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of the Mean
SOPS	Standard Operating Procedures
SPC	Summary of Product Characteristics
QA	Quality Assurance
VAS	Visual Analogue Scale

5. ETHICAL ASPECTS

5.1 Indipendent Ethics Committee

Independent Ethics Committee (IEC) checked the Study Protocol, the Volunteer Information Leaflet, the Informed Consent Form, the Case Report Form (CRF) and the Daily Diary before the beginning of the study. The IEC Approval and a copy of this approval have been provided to the Principal Investigator before the beginning of the study.

5.2 Ethical conduct of the study

The study has been conducted in accordance with the Declaration of Helsinki (revised version of Seoul, October 2008), and is consistent with GCP and applicable regulatory requirements. The study have beewn managed and conducted in compliance with the Protocol. Freely given written consent has been obtained from every subject before his/her effective participation to the study.

The Principal Investigator (PI) ensured that the participants' confidentiality has been maintained throughout the study. The CRFs or other documents only identify participants by their randomization number and their screening number.

5.3 Patient Information and Consent

For patients enrolment, a written consent form in compliance with the current revision of the Declaration of Helsinki and ICH guidelines was obtained from each subject before entering the study. The investigator was responsible for giving to the study participant full and adequate information about the nature, purpose, possible risk and benefit of the study. Study participants were notified that they were free to withdraw from the study at any time. Written information was provided to the participants. A series of questions have been asked to the potential participants in order to confirm that they understood the information provided. The patients had the opportunity to contact the investigator by phone and ask questions before deciding whether to participate or not in the study.

The Principal Investigator or a co-investigator explained in italian the full nature and purpose of the study including possible risks and side effects.

After this explanation and after reading and understanding the patient Information Leaflet, the patient signed the Informed Consent.

The Informed Consent was signed by the patient and by the Co-Investigators.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

INVESTIGATIONAL SITE:	Ambulatorio Via A. D'Andrea 6 65015 Montesilvano (PE), Italy 085-834538
PERSON AUTHORIZED TO SIGN THE PROTOCOL AND AMENDMENTS FOR THE SPONSOR:	Dr. Enzo Moroni Aesculapius Farmaceutici Srl Via Cozzaglio, 24 25125 Brescia Italy Phone + 39 030 89331
PRINCIPAL INVESTIGATOR:	Dr.Valter Armellani Via V. Vestina 307, 65015 Montesilvano (PE), Italy-085-4680687
Co-INVESTIGATORS:	Dr.Aristide Difulvio

For HSV-1 analysis
DOVIM SAS LABORATORIO ANALISI CHIMICO CLINICHE E MICROBIOLOGICHE
Corso Umberto I 219/C, 65015 Montesilvano
Phone: 0854454496
(the laboratory was changed and notified to EC on 11/05/2012):

Ospedale Clinicizzato Santissima Annunziata
Via dei Vestini 31
66100 Chieti Scalo
(thys laboratory was added and notified to EC on 08/11/2012)

The sponsor was responsible for supplying the study center with the investigational products.

7. INTRODUCTION

The test formulation of this study was a lipstick containing aciclovir 5%: Aciclovir 5% Lipstick from Aesculapius Farmaceutici Srl. The aim of this study was to demonstrate the efficacy and safety of the test formulation in comparison with a placebo. Safety was evaluated in terms of AE during the whole study period and by monitoring the results of laboratory tests and vital signs performed at pre- and post-study visit.
The primary endpoint was to evaluate the necessary days from the start of treatment to the complete re-epithelialization (healing time). This study involved 70 adult patients both male and female of Caucasian origin. The study was conducted according to a randomised plan and in according to GCP guidelines.

Recurrent Herpes Labialis (called “RHL”) is a very frequent affection that may strike even several times in life. The cause should be sought in a virus belonging to the herpes viridiae family and which has been recognized as HSV1. HSV1 can be identified by immuno-fluorescence and immuno-enzymatic techniques. The name Herpes Labialis derives from the fact that the lips are frequently concerned, but the infection can also arise around the mouth. However, lesions can also occur on the nose, cheeks, or chin, but they are less common. Most episodes are preceded by a prodromal phase that is characterized by pain, burning, itching, and erythema. These symptoms are followed by lesions on or near the lips: small vesicles develop in clusters along the border of the lips. The vesicles quickly rupture, resulting in erosions that can coalesce to form larger irregular lesions. There is usually just one lesion, in the form of an erythematous-edema mark of small dimensions; in a short time, the mark becomes covered by tight, hemispherical blisters with a diameter of 2-3 millimetres, collected into a bunch. The content is initially clear, and then becomes turbid. The confluence of several blisters can give rise to a bubbly lesion that break up during the course of a week with the evolution of scabs. Over the next 72-96 hours these papules progress to vesicles (blisters) and then ulcers. As the lesions heal they form a soft crust than became hard successively. From the show of the hard crust begin the healing phase. The lesions are generally completely healed by 8 to 10 days. These recurrent infections represent reactivation and not reinfection of RHL which persist in a latent state in the trigeminal (semilunar) ganglion. Here the virus remains dormant for a long period of time, but this latency may be interrupted by numerous circumstances such as exposure to sunlight, stress, fatigue, menstruation and oral trauma. Therefore, the “sleeping” status of the virus may be interrupted and it is capable of re-colonising the site of the primary infection [Siegel 2002].

Acyclovir, 9-(2-hydroxyethoxymethyl)guanine, is an acyclic nucleoside analogue which has a high activity and selectivity for herpes viruses, particularly herpes simplex viruses types 1 and 2 and varicella zoster virus. This selectivity is due to the initial activation of the drug by phosphorylation by a herpes virus-specified thymidine kinase. Normal cellular enzymes do not phosphorylate acyclovir to any significant degree. Acyclovir monophosphate is subsequently converted to a triphosphate which is a more potent inhibitor of herpes virus DNA polymerases than of cellular DNA polymerases. The relationship between the amount of acyclovir triphosphate formed and its inhibition constant (Ki) for the particular viral or cellular DNA polymerase is predictive of the inhibitory activity of acyclovir on DNA replication [Elion 1983].

Acyclovir can be considered a prodrug: it is administered in an inactive (or less active form) and is metabolised into a more active species after administration.
Absorption: it is poorly water soluble and has poor oral bioavailability (10–20%), hence intravenous administration is necessary if high concentrations are required. When orally administered, peak plasma concentration occurs after 1–2 hours. Acyclovir has a high distribution rate, only 30% is protein-bound in plasma.

Metabolism: it is selectively converted into acyclo-guanosine monophosphate (acyclo-GMP) by viral thymidine kinase, which is far more effective (3000 times) in phosphorylation than cellular thymidine kinase. Subsequently, the monophosphate form is further phosphorylated into the active triphosphate form, acyclo-guanosine triphosphate (acyclo-GTP), by cellular kinases. Acyclo-GTP is a very potent inhibitor of viral DNA polymerase; it has approximately 100 times greater affinity for viral than cellular polymerase. As a substrate, acyclo-GMP is incorporated into viral DNA, resulting in chain termination. It has also been shown that viral enzymes cannot remove acyclo-GMP from the chain, which results in inhibition of further activity of DNA polymerase. Acyclo-GTP is fairly rapidly metabolized within the cell, possibly by cellular phosphatases.
Elimination: the elimination half-life of Acyclovir is approximately 3 hours. It is renally excreted, partly by glomerular filtration and partly by tubular secretion [O'Brien J.J 1989].

8. STUDY OBJECTIVES

8.1 Primary objective

The primary study objective was to evaluate the effectiveness of Acyclovir 5% lipstick (Contra) against Herpes Labialis in male and female adult patients in comparison with placebo.

8.2 Secondary objective

The secondary study objective was the evaluation of the safety of Acyclovir 5% lipstick (Contra) against Herpes Labialis in male and female adult patients in comparison with placebo.

9. STUDY PLAN

9.1. Description of the study plan

Type of study:

Randomised double-blind placebo controlled study conducted in compliance with the GCP guidelines.

The active treatment (Acyclovir 5% Lipstick (Contra), from Aesculapius Farmaceutici Srl) and placebo have been topical applied to patients with an active infection of recurrent labial herpes.

Seventy (70) male and female patients have been enrolled and randomized to receive the active treatment or placebo according to a computer-generated randomisation list.

Patients was admitted to Ambulatorio Via Vestina 307, 65015 Montesilvano (the mistake was notified to EC on 11/05/2012), to sign the Informed Consent Form and to perform the following screening activities: physical examination, vital signs and ECG. On Study Day 1, enrolled patients received the first lipstick containing the assigned treatment and the first of five daily scheduled administrations have been performed at the Investigational Site under the supervision of the Principal Investigator/Co-Investigator(s).

Patients enrolled have also been instructed by the Principal Investigator/Co-Investigator(s) to apply the assigned study treatment for five times daily for no more than of seven consecutive days and how to fill the Daily Diary. They came back to the Investigational Site the day 5 to return the first lipstick and to receive the second one. Finally, they came back to the Investigational Site the day after the healing day (see below), or at study day 8 in case of no re-epithelialization, to perform the post-study visit.

Pre-study visit

Potential patients have been screened to ascertain a recurrent Herpes Labialis infection in prodromal phase and the conformance with study inclusion and exclusion criteria.

The initial screening of patients have been conducted by the Principal Investigator/Co-Investigator(s) before the beginning of the study treatments' administrations (Study Day 1) to verify that the enrolled patients fulfilled the inclusion and exclusion criteria.

The following information have been collected for each subject and reported in the CRF by the Principal Investigator/Co-Investigator(s):

- demographic data: sex, age, race;
- medical/Surgical history and underlying disease;
- physical examination: weight [Kg], height [cm], BMI, temperature [°C],

- vital signs: sitting blood pressure (BP): systolic blood pressure (SBP) [mmHg] and diastolic blood pressure (DBP) [mmHg], and hearth rate (HR) [beats/min], 12-leads electrocardiogram (ECG);
- any ongoing organ, tissue, and/or system pathologies;
- prodromal symptoms: itching, pain and/or burning.
- number of hours from the appearance of the first symptom of recurrent Herpes Labialis.
- pregnancy test (only to female participants) by means of stick;

The patients did not undergo any pre and post-laboratory exams since they needed to start treatment administration at prodromal phase of the infection. In fact, laboratory exams typically need a couple of days to be completed, while Acyclovir showed to be more effective if taken at the very first sign of the infection.

Study Development

After screening, patients have been assigned to either active treatment group (Acyclovir 5% lipstick (Contra) from Aesculapius Farmaceutici Srl) or placebo group (biologically inactive lipstick from Aesculapius Farmaceutici Srl without active ingredient) according to a computer-generated randomization list.

Patients enrolled got the first lipstick and have been instructed to initiate the assigned treatment on Study day 1. In particular, the first of the five daily administrations in the Study Day 1 has been performed at the Investigational Site under the supervision of the Principal Investigator/Co-Investigator(s). Patients enrolled have been also instructed by Principal Investigator/Co-Investigator(s) to apply the assigned study treatment for five times daily for five consecutive days (or until complete re-epithelialization), to come back to the Investigational Site on day 5 in order to return the first lipstick and to receive the second one and to apply again the assigned study treatment for the next two days (or until complete re-epithelialization). So, the assigned treatment has been applied for no more than seven consecutive days. They have been instructed on how to fill the Daily Diary. Patients have been informed that the use of any topical agents in the treated area (including cosmetics, lip balms, sunscreens, etc.) during the treatment period is prohibited until healing occurred. Mechanical disruption (i.e., scrubbing, lancing, shaving, etc.) of the prodromal area or lesion is prohibited too.

The total number of visits scheduled by protocol are three: one at the study day 1, one at the study day 5 (if no complete re-epithelialization occurred) when they returned the first

lipstick and received the second one and the last one the day of the complete re-epithelialization.
The following procedure has been conducted during the study as described below:

Study day 0
Randomization day: after the screening period and visit, if the patient meets all the inclusion criteria and none of the exclusion criteria, he/she has been randomized to the next randomization number available (starting from 1). The randomization number has been assigned by the Principal Investigator/Co-Investigator(s) that delivered the correspondent treatment (the treatment with the same randomization number printed on the label) at the Study Day 1. The Principal Investigator filled a register of enrolled patients.

Study day 1

- Assessment of pain measured by Visual Analogue Scale (VAS 0-100 mm), lesion size (small, medium, large, very large), maximum lesion extension (mm) burning (absent, slight, medium, intense) and itching (absent, slight, medium, intense). All data have been recorded and reported in the CRF by Principal Investigator/Co-Investigator(s);
- Application of the first of five scheduled topical daily applications (the next topical administrations have been performed at home by patients);
- Delivery of the Daily Diary and the assigned treatment to the patient in accordance with the randomisation list by the Principal Investigator/ Co-Investigator(s); before the delivery of Daily Diary, the Principal Investigator/Co-Investigator(s) recorded the first application's time. He then explained to the patients that the applications should be performed for five times daily every four hours since the first application (see the instructions on the Daily Diary).
- Topical administrations of the remaining scheduled ones have been done at home by patients every four hours after the first administration;
- Daily diary filling by patient (administration times, occurrence of AEs, formation of hard crust);
- In case of AEs, the patient was instructed to contact the Principal Investigator/Co-Investigator(s). Contact details are reported on the Daily Diary.

Study day 2/3/4 (no complete re-epithelialization)

- Topical administrations of the treatment by patients every four hours; the first application of each treatment day should be performed after eight hours since the last one of the previous day (at about the same time of the previous day);
- Daily diary filling by patient (administration times, occurrence of AEs, formation of hard crust);
- In case of AEs, the patient was instructed to contact the Principal Investigator/Co-Investigator(s). Contact details are reported on the Daily Diary.

Study day 5 (no complete re-epithelialization)

- Patient came back to the Investigational Site in order to return the first lipstick and to receive the second one.
- Topical administrations of the treatment by patients every four hours; the first application of each treatment day should be performed after eight hours since the last one of the previous day (at about the same time of the previous day);
- Daily diary filling by patient (administration times, occurrence of AEs, formation of hard crust);
- In case of AEs, the patient was instructed to contact the Principal Investigator/Co-Investigator(s). Contact details are reported on the Daily Diary.

Study day 6/7 (no complete re-epithelialization)

- Topical administrations of the treatment by patients every four hours; the first application of each treatment day should be performed after eight hours since the last one of the previous day (at about the same time of the previous day);
- Daily diary filling by patient (administration times, occurrence of AEs, formation of hard crust);
- In case of AEs, the patient was instructed to contact the Principal Investigator/Co-Investigator(s). Contact details are reported on the Daily Diary.

Study day 2/3/4/5/6/7/8 (complete re-epithelialization, i.e. the healing day)

The day in which the patient showed a complete re-epithelialization, the patient stopped taking the assigned treatment and he performed the post study visit, in which the complete re-epithelialization has been clinically assessed.

Post-study visit and end of the study

Principal investigator did:

- physical examination:weight [Kg], height [cm], BMI, temperature [°C]
- vital signs: sitting blood pressure (BP): systolic blood pressure (SBP) [mmHg] and diastolic blood pressure (DBP) [mmHg], and hearth rate (HR) [beats/min], 12-leads electrocardiogram (ECG);
- pregnancy test (only to female participants) by means of stick;

The following efficacy evaluations have also been performed:

- Pain measured by Visual Analogue Scale (0-100 mm);
- Lesion size (small, medium, large, very large);
- Maximum lesion extension (mm),
- Burning and Itching (absent, slight, medium, intense).

These evaluations have been assessed by Principal Investigator/Co-Investigator(s) (all data have been reported in the CRF). The patient gave back the Daily Diary and the second lipstick to Principal Investigator/Co-Investigator(s).

Finally, all documents (CRF and Daily Diary) have been handed over to PI at the Investigational Site in order to allow the statistical analysis of clinical data recorded during the treatment study period. The Principal Investigator/Co-Investigator(s) reported the information written by patient in Daily Diary onto CRF.

No follow-up has been defined, following the indication of the ICH guideline "S7A" "SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICAL" in which it is stated (paragraph 2.9) that "Safety pharmacology studies may not be needed for locally applied agents (e.g., dermal or ocular) where the pharmacology of the test substance is well characterized, and where systemic exposure or distribution to other organs or tissues is demonstrated to be low.". Acyclovir is in this category, so if not necessary for the safety of the patients, no follow-up period has been planned.

9.2. Discussion of Study Design

This study has been planned and carried out in order to satisfy the requests of the Swedish Regulatory Agency. This study must be considered an integration study to previous one entitled: "The efficacy and tolerability of Acyclovir 5% Lipstick (Contra) in treating skin infections provoked by Herpes Labialis. A randomised, open, controlled clinical study in parallel groups against an active comparison formulation" (Study Code AC/DIP/2007). In that study the same test formulation (Acyclovir 5% Lipstick (Contra), from Aesculapius

Farmaceutici Srl) has been compared with a marketed reference (Zovirax® 5% cream from GlaxoSmithKline SpA).

The aim of this study was to evaluate of efficacy of the test formulation, Acyclovir 5% lipstick (Contra) from Aesculapius Farmaceutici Srl in comparison with placebo treatment.

A randomised, double-blind, placebo-controlled, parallel study design was selected as the most suitable to evaluate the efficacy of test formulation in the topical treatment of Herpes Labialis. In fact, if we consider only the submitted open-label study without a placebo control (AC/DIP/2007), it cannot be concluded that efficacy has been demonstrated for Contra 5% cutaneous stick. In order to demonstrate efficacy, a double-blind placebo/vehicle-controlled study is necessary.

Both active and placebo formulation have been topically applied as a total cover for the lesion for five times a day, every four hours for no more than seven consecutive days. Even if the treatment period should be 4-5 days (according to SPC of Zovirax® 5% cream from GlaxoSmithKline SpA and of Contra 5% cutaneous stick), we proposed seven days as maximum study length for two reasons: first, the previous study (AC/DIP/2007) showed that the hard crust took 4-5 days to form, so a study length of 5 days could be not sufficient to observe the complete re-epithelialization; second, the seven days study length is a maximum theoretical length, since the patient returned to the Investigational Site the day after the healing day to perform the post-study visit and to discontinue the treatment.

In order to allow for a direct comparison with the primary outcome of the previous study (AC/DIP/2007), in which the healing day was defined as the day of the formation of hard crust, the Principal Investigator/Co-Investigator(s) monitored the formation of hard crust by the daily diary and the visit at Study Day 5. The healing day defined as the day of complete re-epithelialization was used as primary outcome measure to allow for a comparison with other study in literature.

The efficacy of the active treatment was assessed by showing a statistically significant difference between the effectiveness of the test formulation and the placebo. In order to demonstrate a possible superiority of the effectiveness of the test formulation versus the placebo the study design is in accordance with the following EMEA guidelines: Points to consider on switching between superiority and non-inferiority (CPMP/EWP/482/99) and Note for guidance on clinical requirements for locally applied, locally acting products containing known constituents (CPMP/EWP/239/95).

The safety of the test formulation was also evaluated by monitoring the occurrence of AE during the study period.

In accordance to ICH guideline E10 CPMP/ICH/364/96, the placebo-controlled design, by allowing blinding and randomization and including a group that receives an inert treatment, controls for all potential influences on the actual or apparent course of the disease other than those arising from the pharmacologic action of the test drug. Placebo-controlled trials also provide the maximum ability to distinguish AEs caused by a drug from those resulting from underlying disease or intercurrent illness. Moreover, randomization avoids systematic differences between groups with respect to known or unknown baseline variables that could affect outcome and blinding is intended to minimize the potential biases resulting from differences in management, treatment, or assessment of patients, or interpretation of results that could arise as a result of subject or investigator knowledge of the assigned treatment.

9.3. Selection of Study Population

Seventy (70) adult patients of both sexes were enrolled in this study.

9.3.1. **Inclusion criteria**

The patients fulfilled all of the following criteria:

- Males and females aged between 18 (eighteen) and 70 (seventy) able to understand the Volunteer Information Leaflet and to sign the Informed Consent Form with a clinical diagnosis of active infection of recurrent labial herpes in prodromal phase: onset of prodromal symptoms (itching, pain, burning);
- Caucasian origin;
- Body weight within 20% of ideal body weight;
- Normal physical examination , vital signs, ECG;
- History of recurrent herpes labialis with at least 3 recurrences during the past 12 months and typically (>50% of episodes) associated with prodromal symptoms;
- History of at least half of the herpes recurrences producing ulcerative lesions (a recurrence that led to development of a lesion which undergoes vesicle, ulcer/soft crust and/or hard crust formation);
- Females of childbearing potential must have a negative pregnancy test and practice a reliable method of contraception (intrauterine device or combined contraceptives) for all the duration of the study;
- Patients must agree to abstain from the use of anti-inflammatory medications (including aspirin and NSAIDS), systemic steroids and analgesics during the treatment period until healing occurs;

- Patients must agree to abstain from the use of any topical treatments in the lesion area (cosmetics, lip balms, sun screens, etc) during the treatment period until healing occurs
- Patients must agree to abstain from any mechanical disruption of the prodromal area or lesion (i.e., scrubbing, lancing, shaving the area, rubbing with alcohol, etc.).

9.3.2. **Exclusion criteria**

The patients fulfilling any one of the following criteria were not eligible for inclusion in the study:

- Recent infection of Herpes Zoster in the last 4 weeks;
- Treatment with other systemic or topical antiviral agents or systemic corticosteroids within 4 weeks prior to study drug administration;
- Previous herpes vaccine;
- Pregnant, nursing and/or lactating females;
- Patients with herpetic stomatitis;
- Patients with severe symptoms or signs of herpes labialis such as intraoral lesions or lesions in the nostrils;
- Subjects with lesions wider than 2.0cm2;
- Subjects with renal, hepatic or metabolic dysfunction that may pose a risk to the safety;
- Presence of diseases that could interfere with the completion of the study;
- History of drug and alcohol abuse;
- Allergy and/or hypersensibility and/or history of allergic response to active ingredient, related drugs or some of excipient contained in the IMP;
- Related pathologies and use of drugs than can create problems with the therapeutic action of Acyclovir (such as immunodepression);
- Bloodletting (at least 250 ml) or participation in a previous trial within six months before the beginning of the study;
- In the judgement of the PI/co-Investigator(s) patients likely not to be compliant or to be uncooperative during the study.

9.3.3. **Discontinuation criteria**

Study discontinuation by the Patient

A patient admitted to this study was considered as a drop-out if:

- an hypersensitivity or allergic reaction, clearly linked to study medication, has occurred;
- the patient is afflicted with a systemic illness, unrelated to the study medication but occurred during the study period, for which a concomitant medication is required;
- an AE/ADR, that makes it impossible to continue, has occurred;
- poor collaboration of the patient;
- other reasons considered valuable from the PI/Co-Investigator(s) but not specified here.

Patients are free to withdraw from the study at any time if they wish to, in this case a specific reason must be recorded by the PI/Co-Investigator.

Study discontinuation by the Sponsor

The Sponsor may terminate the study at any time, for any of the following reasons:

- violations of inclusion/exclusion criteria to enrol patients;
- major clinical investigation plan violations;
- Study discontinuation by the PI/co-Investigator(s)
- Onset of adverse drug reactions that, endangering the health of patients, make it not ethically acceptable to continue.

9.4.Study treatments

The IMPs with the certificate of analysis have been provided at least three days before study initiation. The study treatments have been sent to Poliambulatorio “San Paolo” located in via San Paolo, 11 – 65015 Montesilvano (PE), Italy. The PI and the Co-investigators, received a quantity of investigational product equal to double the number of planned patients (thirty-five for each treatment group, two lipsticks per patient). Until they are dispensed to the patients, the IMPs have been kept at room temperature (max 25°C) in a securely locked storage facility accessible only to authorized personnel at the Investigational Site and stored in airtight containers and protected from light.

During the study, the PI/Co-investigator(s) or other authorized personnel dispensed the IMPs only to the identified subjects of this study, following the procedures described in this study protocol. In particular, the patients received the first lipstick on study day 1 and the second lipstick on study day 5, they returned the first lipstick on study day 5 and the second lipstick at the end of the study. If the end of the study (ie. complete re-epithelialization) occurred before the study day 5, the patients returned only the first lipstick.

A drug inventory/dispensing record has been maintained by the PI or other authorized personnel and updated in order to verify the delivery, the use and the return of the IMPs.

9.4.1 **Treatments administered**

ACTIVE TREATMENT, DOSE, ROUTE OF ADMINISTRATION, BATCH N°, EXPIRE DATE:

Acyclovir 5% lipstick (Contra), topical route, batch n° 011031, expire date 01/2014.

DESCRIPTION of ACTIVE TREATMENT

Active ingredients: Acyclovir [5% lipstick].

Lipstick: 3 g.

Excipients: ricin oil, carnauba cera, semisynthetic gliceryd solid, white beeswax, octyldodecanol, white vaseline, vanilla flavour, butylated-hydroxytoluene.

PLACEBO TREATMENT, DOSE, ROUTE OF ADMINISTRATION, BATCH N°, EXPIRE DATE:

lipstick without active ingredient, topical route, batch n° 021039, expire date 02/2014.

DESCRIPTION of PLACEBO TREATMENT

Lipstick: 3 g.

Excipients: ricin oil, carnauba cera, semisynthetic gliceryd solid, white beeswax, octyldodecanol, white vaseline, vanilla flavour, butylated-hydroxytoluene.

9.4.2 Identity of Investigational products

Number of units of the pharmaceutical form per primary packaging:

One lipstick.

Number of secondary packaging per volunteer:

One box containing two lipsticks.

Secondary Packaging label

Primary Packaging labels

9.4.3 Method of assigning subjects to treatment groups

The subject treatment was carried out in accordance to a computer generated randomization list.

9.4.4 Selection of dose

In the present study, the applied dose was chosen on the basis of dose used in the previous study titled "The efficacy and tolerability of Acyclovir 5% Lipstick (Contra) in treating skin infections provoked by Herpes Labialis. A randomised, open, controlled clinical study in parallel groups against an active comparison formulation" (Study Code AC/DIP/2007). In this previous study the choice of the dose (120 mg of lipstick per application) was made on the basis of the recommended dosage reported in the Summary of Product Characteristics (SPC) of similar marketed medical products. Anyway, since the total applications are 5 per day, for 7 consecutive days, a dose of 120mg per application results in 120mg*5*7=4200mg. The lipsticks used in this study contain 3000mg, so two lipsticks have been provided to each patients (the first lipstick at study day 1, the second at study day 5, if no complete re-epithelialization occurred). All the patients have been very comfortable with the indicated doses.

9.4.5 Dose regimen

The following selection and timing of dose was valid for all patients: for both active treatment and placebo treatment, five daily administrations have been scheduled every four hours from the first administration (in study day 1) for no more than seven consecutive days. All patients followed the administration plan, due to the fact that a lipstick is easier to use than a cream.

9.4.6 Blinding

Both the Investigator(s) and the patients, who received the assigned treatment in accordance with the randomization list, didn't know the actual treatment used. The Sponsor has handed over 70 closed envelopes to the Principal Investigator, each envelope containing the blinding code for that patient.

There was an adverse event that required the opening of the blinding codes. In this case, the Principal Investigator notified the Sponsor with the randomization number (patient number 2) of the patient, and opened the envelope number 2 in order to get the blinding code for that patient.

The box reported the following wordings (in Italian language): "3g – acyclovir 5% or placebo - lipstick" so, even after opening this blinding code, the Principal Investigator/Co-Investigator(s) cannot distinguish the treatment contained in the following boxes.

9.4.7 Prior and concomitant treatments

Any concomitant therapy was permitted during the whole study period with the exception of immunological drugs.

If a patient needed to submit to a concomitant therapy, in the case of any adverse event or for other reasons, the Principal Investigator evaluated the hypothesis of premature withdrawal. Each concomitant therapy was clearly documented and reported in to CRF. For the List of concomitant medication by subject see appendixes.

9.4.8 Treatment compliance

In this study, the treatment compliance was guarantee by the following methods:

- delivery of Daily Diary by patient to the Principal Investigator/Co-Investigator(s); the Daily Diary allowed the Principal Investigator/Co-Investigator(s) to verify the

treatment compliance because the patient reported the effective time of all applications;

- delivery of boxes and unused or partially or completed used lipstick by patient at the end of the study's treatment period.
- attachment of the primary packaging label of the treatment administered on to CRF at the end of treatment period (for the placebo and test formulation).

Eighteen patients (18) out of 70 (25,7%) failed to return the treatment. Six (6) patients were given both the lipstick at study day 1 because they couldn't return on day 5 for personal motivations and they misunderstood the mode of treatment administration: they consumed both lipsticks alternating them during the day.

9.5. Efficacy and Safety Variables

9.5.1 Efficacy measurements assessed

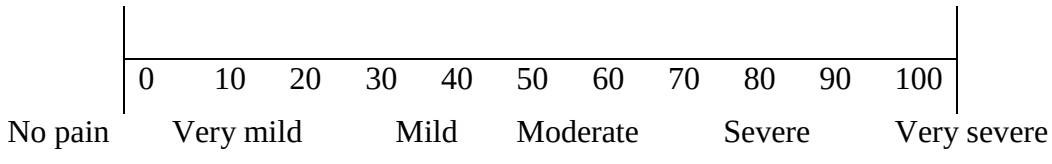
The efficacy variables for the primary objective have been the followings:

Healing time i.e. the number of the days between the beginning of therapy and the complete re-epithelialization of lesions. The time to loss of hard crust or time to normal skin (the healing time) has been monitored and recorded in the following ways:

- By the monitoring and the assessment of the Principal Investigator/Co-Investigator(s) in the post-study visit;
- by CRF: at each study visit, the Principal Investigator/co-Investigator(s) wrote the answer to the following question: "Complete Re-epithelialization of lesions?".

1) Pain Evaluation

The assessment of severity of pain has been performed by using VAS scale reported in the following figure:



2) Lesion Size Evaluation

The assessment of lesion size has been performed by the following scale:

0 = small; 1 = medium; 2 = large; 3= very large.

3) Lesion Extension Assessment

The assessment of maximum lesion extension has been performed in mm.

4) Burning/Itching Evaluation

The assessment of burning/itching has been performed by the following scale:

0 = absent, 1 = slight, 2 = medium, 3= intense.

5) Formation of hard crust

The formation of hard crust has been recorded in the following way:

- By Daily Diary: the patients have been instructed to report the formation of hard crust daily.
- by CRF: at each study visit, the Principal Investigator/co-Investigator(s) wrote the answer to the question: "Formation of hard crust?".

9.5.2 Safety measurements assessed

The safety variables for the secondary objective have been the followings:

- the occurrence of AEs
    - for frequency of AE: (number of subjects with AE/total sample size)*100;
    - for intensity of AE: mild, moderate and severe;
    - for relation with treatments administered of AE: related, possible, unrelated and unclear.
- changes in vital signs (blood pressure and pulse rate) and resting 12-lead ECG between pre- and post-study visit. These data have been reported into CRF by the Co-Investigator(s).

9.5.3 Appropriateness of measurements

The efficacy and safety variables appear suitable for the assessment of the primary and secondary objectives. The choice to assess the possible superiority of the active treatment [test formulation Acyclovir 5% lipstick (Contra), from Aesculapius Farmaceutici Srl] by using a well-established method, such as the evaluation of healing time, seems fitting and it's corroborated by wide spread use in the literature.

9.5.4 Drug concentration measurements

N/A

9.6. Data Quality Assurance

The study was planned to ensure that all data were complete and accurate and in accordance with GCP.

All study data were recorded in the case history and in the Daily Diary and then written out in the CRFs. The Principal Investigator was responsible for the accuracy and completeness of the information, signing the first page of each CRF. The Quality Assurance of CRC examined the following documentation: protocol, blank CRF, blank Daily Diary, blank Informed Consent, and Final Report.

9.7. Statistical methods and determination of Sample Size

9.7.1 Statistical analysis plan

For efficacy evaluation

The effectiveness of the active treatment was assessed using healing time; pain evaluation by VAS scale; lesion size assessment by the following scale: 0 = small, 1 = medium, 2 = large, 3= very large; lesion extension measured in mm and burning and itching evaluated by the following scale: 0 = absent, 1 = slight, 2 = medium, 3= intense.

The statistical analyses were performed according to the "Intention To Treat" (ITT) and "Per Protocol" (PP) analysis principles (Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products - U.S. Department of Health and Human Services Food and Drug Administration, 1998; CPMP/EWP/482/99 and Statistical Principles for Clinical Trials ICH E9). For the primary endpoint, analysis was performed considering patients that terminated the study and all the drop-outs with data related to their specific endpoints.

All randomised patients have been included in the ITT population. Only patients showing no/minor protocol deviations have been included in the PP population. The two treatment groups have been compared for baseline values using a Mann-Whitney U test or a chi-square test.

The results of the two treatment groups have been compared using parametric and non-parametric tests coherently with the type of data. In particular, the difference between the mean of the necessary days from the beginning of therapy until complete re-epithelialization (healing time) of the test product and the mean of the healing time of the placebo have been compared using Student's t test. For supportive analysis, a time-to-event analysis ("survival analysis") has been performed following Kaplan-Meier methodology and a Cox proportional hazards method. For subjects whose duration of episode is unknown, a duration of 15.0 days have been assigned (see the Study Protocol for

the details). Student's t test was used to assess the difference in maximum lesion area and pain score. Other secondary efficacy variables was analyzed using chi-square test16.
According to CPMP/ICH/363/96 and CPMP/EWP/482/99, both two-sided (at 95% significance level) and one-sided tests (at 97.5% significance level) have been performed and the superiority of the test formulation over the placebo was assessed by constructing a two-sided 95% Confidence Interval around the difference between the mean of healing time of the test formulation and the mean of healing time of the placebo.
All statistical computations were performed using IBM SPSS 20 for Windows.

For safety evaluation
The difference between the frequency of AEs occurrence was assessed by descriptive statistics and a chi-square test. Any difference in intensity and relation with treatments administered was assessed by descriptive statistics and a chi-square test.
The presence (or lack) of any statistically significant difference between the values of vital signs in pre and post-study visits was assessed by descriptive statistics and a paired-samples Student's t-test or a non-parametric equivalent test.

9.8. Changes in the conduct of the study or planned analysis

9.8.1 Amendments
No amendments occurred.

9.8.2 Changes in the statistical methods
There were no changes in the statistical methods.

10. STUDY POPULATION

Seventy (70) subjects have been screened, seventy (70) enrolled, seventy (70) have been treated and sixty-nine (69) finished that study according to the protocol (table 10.1.)
Seventy (70) enrolled patients satisfied the inclusion/exclusion criteria according to the study protocol.

Table 10.1

Disposition of subjects	
Screened	70/70
Enrolled	70/70
Dropped-out	1/70
Finished according to protocol	69/70

10.1. Disposition of subjects

Test or placebo formulations were applied for no more than seven consecutive days, according to the Study Flow Chart of the experimental protocol. Drop-outs are summarized in *table 10.2*.

Table 10.2

Randomization Code	**Observations**
2	Adverse Event

Pre-study visit, drug application, and post-study visit dates are reported in the appendices

10.2. Protocol Deviations

10.2.1. Maior deviations from the protocol

Major deviation	**Number of subjects**
Failed treatment's applications for more than 50% of the times;	0
Dysfunctional development of the pathology;	0
Concomitant antiviral therapy.	0

10.2.2. Minor deviations from the protocol

Minor deviation	**Number of subjects**
Failed co-investigator recording on the CRF of the secondary endpoints;	0
Missing of treatment compliance measurement because of failed return of the assigned treatment by patient;	18
Concomitant therapies/medication not related to the pathology.	5

11. EFFICACY EVALUATION

The primary outcome measure was the healing time in each treatment arms. The second outcome measures were the assessment of the severity of the pain, lesion size, maximum lesion extension, burning and itching. For the detailed statistical analysis (see *par. 16.2.10 and 16.2.11*).

Efficacy Variables Listing
Healing time
VAS Pain
Extension
Size
Burning
Itching

11.1. Data set analysed

The evaluable subjects for the main variables were all the subjects randomized (70).

Set	**N**	**Sex (M/F)**
Randomized	70	28/42

11.2. Demographic Characteristics

The population demographic characteristics (all the 70 randomized patients) are reported in the paragraph 16.2.4.

Descriptive demographic statistics for Age, Height and Weight of 70 patients, enrolled and treated are reported in the table 11.1 and 11.2.

TAB.11.1: demographic characteristic of the randomized set (N=70)

Group Statistics

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Age	Test	35	28,49	9,537	1,612
	Placebo	35	26,23	6,436	1,088
Weigh t	Test	35	62,857	9,2264	1,5595
	Placebo	35	66,457	12,7587	2,1566

Heigh t	Test	35	168,171	6,6310	1,1208
	Placebo	35	171,114	8,7473	1,4786
BMI	Test	35	22,169	2,5453	,4302
	Placebo	35	22,617	3,4845	,5890

TAB.11.2: SEX of the randomized set (N=70)

Sex * Treatment Crosstabulation					
			Treatment		Total
			Placebo	Test	
Sex	Female	Count	17	25	42
		% within Sex	40,5%	59,5%	100,0%
		% within Treatment	48,6%	71,4%	60,0%
	Male	Count	18	10	28
		% within Sex	64,3%	35,7%	100,0%
		% within Treatment	51,4%	28,6%	40,0%
Total		Count	35	35	70
		% within Sex	50,0%	50,0%	100,0%
		% within Treatment	100,0%	100,0%	100,0%

The descriptive statistics of vital signs are shown in Table 11.3 and 11.4.

TAB.11.3: Clinical data (vital signs) of the randomized set (N=70) at pre-study.

Group Statistics					
	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Temperature (pre-study)	Test	35	36,631	,1676	,0283
	Placebo	35	36,634	,1939	,0328
Systolic (pre-study)	Test	35	125,23	10,114	1,710
	Placebo	35	124,00	9,570	1,618
Diastolic (pre-study)	Test	35	78,89	7,599	1,285
	Placebo	35	76,06	6,282	1,062
Heart rate (pre-study)	Test	35	68,60	6,222	1,052
	Placebo	35	70,51	5,580	,943

TAB.11.4: Clinical data (vital signs) of the randomized set (N=70) at post-study.

Group Statistics					
	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Temperature (post-study)	Test	35	36,623	,1716	,0290
	Placebo	35	36,646	,1686	,0285
Systolic (post-study)	Test	35	126,00	10,558	1,785
	Placebo	35	124,49	12,763	2,157
Diastolic (post-study)	Test	35	78,43	7,051	1,192
	Placebo	35	76,23	7,538	1,274
Heart rate (post-study)	Test	35	70,51	6,308	1,066
	Placebo	35	70,60	5,542	,937

There weren't statistically significant differences at pre-study and post-study between the two treatments.

11.3 Measurement of treatment compliance

See appendixes.

11.4. Efficacy Results

Efficacy results for the ITT and PP population are given below.

11.4.1. Analysis of efficacy

The randomized groups were compared to assess their homogeneity at baseline. The effect of each treatment was assessed with respect to the baseline values obtained at study entry and the two groups were compared at final visit. The two treatment groups have been compared for baseline values using a Mann-Whitney U test or a chi-square test. The results of the two treatment groups have been compared using parametric and non-parametric tests coherently with the type of data. In particular, the difference between the mean of the necessary days from the beginning of therapy until complete re-epithelialization (healing time) of the test product and the mean of the healing time of the placebo have been compared using Student's t test. For supportive analysis, a time-to-event analysis ("survival analysis") has been performed following Kaplan-Meier methodology and a Cox proportional hazards method. For subjects whose duration of episode is unknown, a duration of 15.0 days has been assigned. This imputation is based on the results from a previous well-known acyclovir study. Student's t test has been used to assess the difference

in maximum lesion area and pain score. Other secondary efficacy variables have been analyzed using chi-square test.

According to CPMP/ICH/363/96 and CPMP/EWP/482/99, both two-sided (at 95% significance level) and one-sided tests (at 97.5% significance level) have been performed and the superiority of the test formulation over the placebo has been assessed by constructing a two-sided 95% Confidence Interval around the difference between the mean of healing time of the test formulation and the mean of healing time of the placebo.

IBM SPSS 20.0 for Windows was used for statistical computations.

Intention to treat Analysis

A total of 70 patients affected by skin infections provoked by Herpes Labialis were enrolled in this study which started in May 2012 and concluded in December 2012. There was one dropped-out patient.

There were no significant differences between the test and placebo groups at study entry:

Group Statistics

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
VAS (basal)	Placebo	35	49,00	16,441	2,779
	Test	35	52,57	14,368	2,429
Extension (basal)	Placebo	35	12,06	4,789	,810
	Test	35	12,20	4,438	,750

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
VAS (basal)	Equal variances	,380	,540	-,968	68	,337	-3,571	3,691	-10,936	3,793

	assumed									
	Equal variances not assumed			-,968	66,801	,337	-3,571	3,691	-10,938	3,795
Extension (basal)	Equal variances assumed	,498	,483	-,129	68	,897	-,143	1,104	-2,345	2,059
	Equal variances not assumed			-,129	67,609	,897	-,143	1,104	-2,345	2,060

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
Size (basal)	70	1,51	,974	0	3	1,00	2,00	2,00
Burning (basal)	70	1,53	,974	0	3	1,00	2,00	2,00
Itching (basal)	70	1,67	,928	0	3	1,00	2,00	2,00
Treatment	70	,50	,504	0	1	,00	,50	1,00

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
Size (basal)	Placebo	35	33,69	1179,00
	Test	35	37,31	1306,00
	Total	70		
Burning (basal)	Placebo	35	35,23	1233,00
	Test	35	35,77	1252,00
	Total	70		
Itching (basal)	Placebo	35	35,74	1251,00

	Test	35	35,26	1234,00
	Total	70		

Test Statistics[a]			
	Size (basal)	Burning (basal)	Itching (basal)
Mann-Whitney U	549,000	603,000	604,000
Wilcoxon W	1179,000	1233,000	1234,000
Z	-,781	-,117	-,105
Asymp. Sig. (2-tailed)	,435	,907	,916
a. Grouping Variable: Treatment			

The descriptive values for VAS, Extension, Size, Burning and Itching scores show pretty similar distributions at baseline:

Descriptives					
	Treatment			Statistic	Std. Error
VAS (basal)	Placebo	Mean		49,00	2,779
		95% Confidence Interval for Mean	Lower Bound	43,35	
			Upper Bound	54,65	
		5% Trimmed Mean		49,72	
		Median		55,00	
		Variance		270,294	
		Std. Deviation		16,441	
		Minimum		15	
		Maximum		70	
		Range		55	
		Interquartile Range		25	
		Skewness		-,628	,398
		Kurtosis		-,616	,778

	Test	Mean		52,57	2,429
		95% Confidence Interval for Mean	Lower Bound	47,64	
			Upper Bound	57,51	
		5% Trimmed Mean		53,17	
		Median		55,00	
		Variance		206,429	
		Std. Deviation		14,368	
		Minimum		20	
		Maximum		70	
		Range		50	
		Interquartile Range		25	
		Skewness		-,489	,398
		Kurtosis		-,884	,778
Extension (basal)	Placebo	Mean		12,06	,810
		95% Confidence Interval for Mean	Lower Bound	10,41	
			Upper Bound	13,70	
		5% Trimmed Mean		12,07	
		Median		12,00	
		Variance		22,938	
		Std. Deviation		4,789	
		Minimum		3	
		Maximum		20	
		Range		17	
		Interquartile Range		7	
		Skewness		-,244	,398
		Kurtosis		-,993	,778
	Test	Mean		12,20	,750
		95% Confidence Interval for Mean	Lower Bound	10,68	

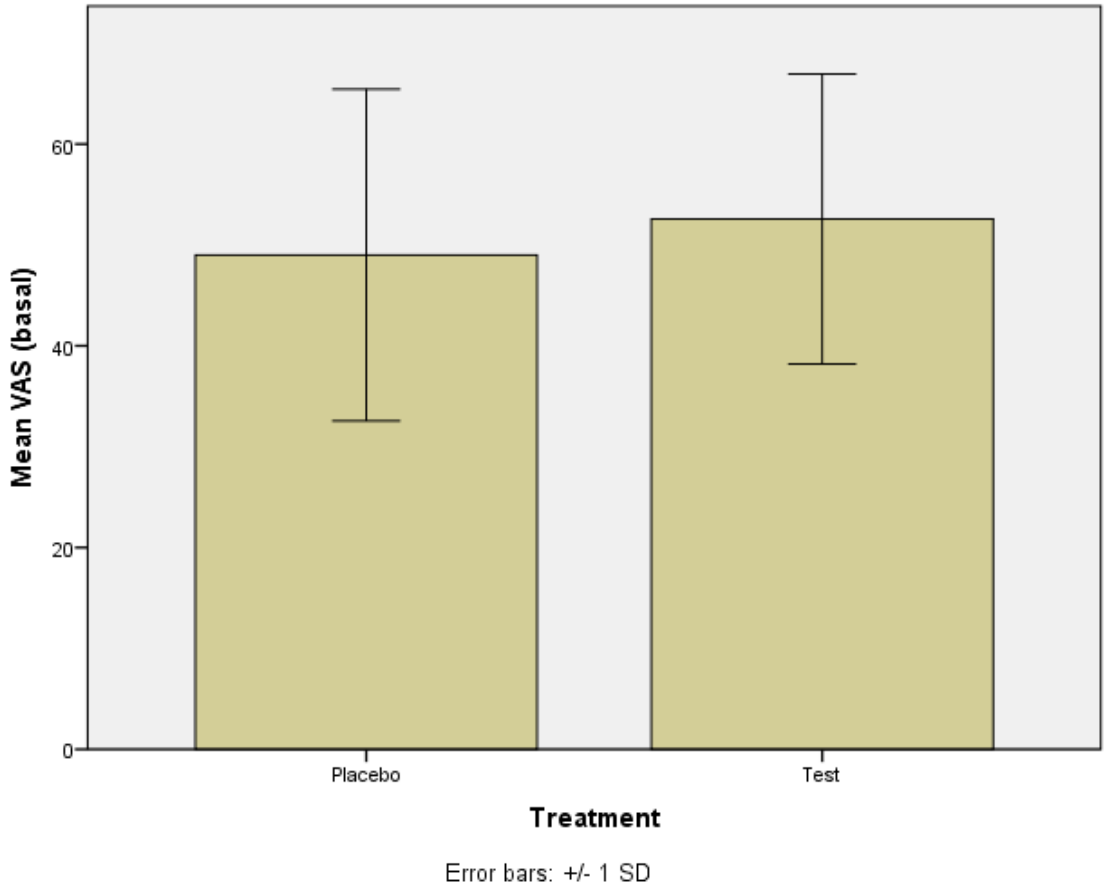
			Upper Bound	13,72	
		5% Trimmed Mean		12,36	
		Median		12,00	
		Variance		19,694	
		Std. Deviation		4,438	
		Minimum		3	
		Maximum		19	
		Range		16	
		Interquartile Range		5	
		Skewness		-,467	,398
		Kurtosis		-,577	,778
Size (basal)	Placebo	Mean		1,43	,165
		95% Confidence Interval for Mean	Lower Bound	1,09	
			Upper Bound	1,76	
		5% Trimmed Mean		1,42	
		Median		1,00	
		Variance		,958	
		Std. Deviation		,979	
		Minimum		0	
		Maximum		3	
		Range		3	
		Interquartile Range		1	
		Skewness		,012	,398
		Kurtosis		-,940	,778
	Test	Mean		1,60	,165
		95% Confidence Interval for Mean	Lower Bound	1,26	
			Upper Bound	1,94	
		5% Trimmed Mean		1,61	

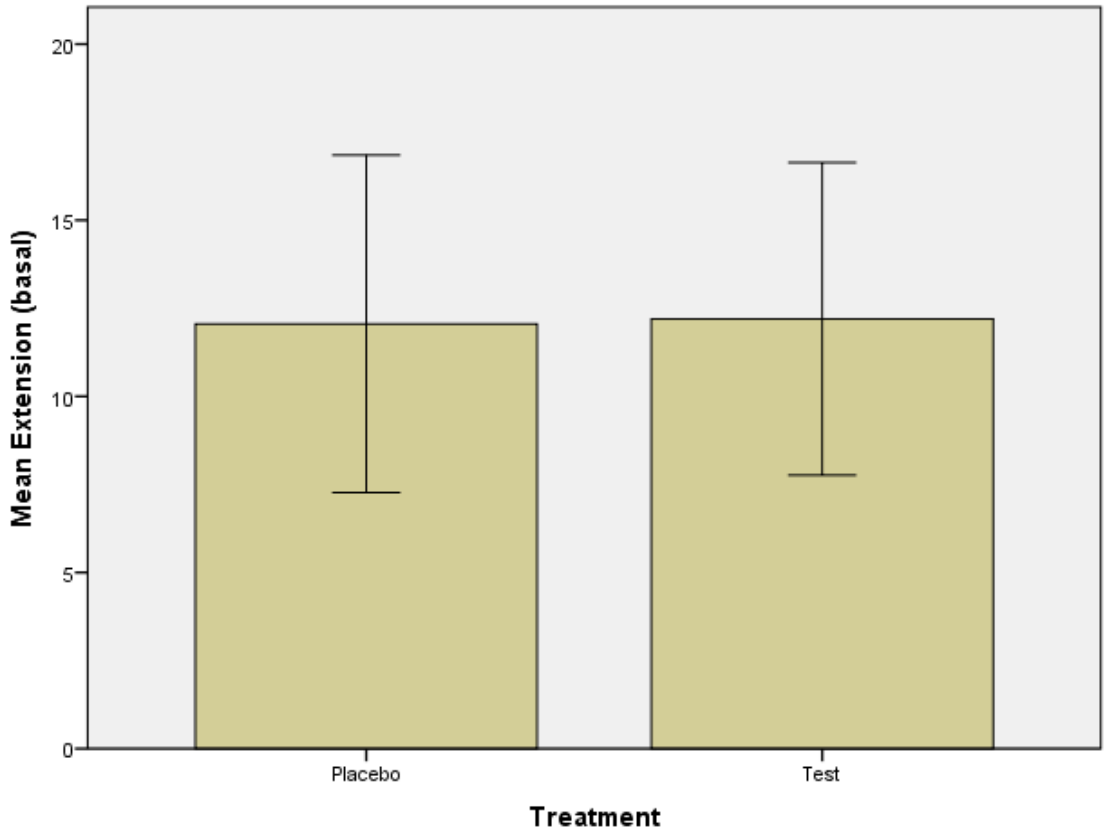
		Median		2,00	
		Variance		,953	
		Std. Deviation		,976	
		Minimum		0	
		Maximum		3	
		Range		3	
		Interquartile Range		1	
		Skewness		-,298	,398
		Kurtosis		-,819	,778
Burning (basal)	Placebo	Mean		1,51	,171
		95% Confidence Interval for Mean	Lower Bound	1,17	
			Upper Bound	1,86	
		5% Trimmed Mean		1,52	
		Median		2,00	
		Variance		1,022	
		Std. Deviation		1,011	
		Minimum		0	
		Maximum		3	
		Range		3	
		Interquartile Range		1	
		Skewness		-,132	,398
		Kurtosis		-1,019	,778
	Test	Mean		1,54	,161
		95% Confidence Interval for Mean	Lower Bound	1,22	
			Upper Bound	1,87	
		5% Trimmed Mean		1,55	
		Median		2,00	
		Variance		,903	

		Std. Deviation		,950	
		Minimum		0	
		Maximum		3	
		Range		3	
		Interquartile Range		1	
		Skewness		-,239	,398
		Kurtosis		-,786	,778
Itching (basal)	Placebo	Mean		1,69	,163
		95% Confidence Interval for Mean	Lower Bound	1,35	
			Upper Bound	2,02	
		5% Trimmed Mean		1,71	
		Median		2,00	
		Variance		,928	
		Std. Deviation		,963	
		Minimum		0	
		Maximum		3	
		Range		3	
		Interquartile Range		1	
		Skewness		-,146	,398
		Kurtosis		-,890	,778
	Test	Mean		1,66	,153
		95% Confidence Interval for Mean	Lower Bound	1,35	
			Upper Bound	1,97	
		5% Trimmed Mean		1,67	
		Median		2,00	
		Variance		,820	
		Std. Deviation		,906	
		Minimum		0	

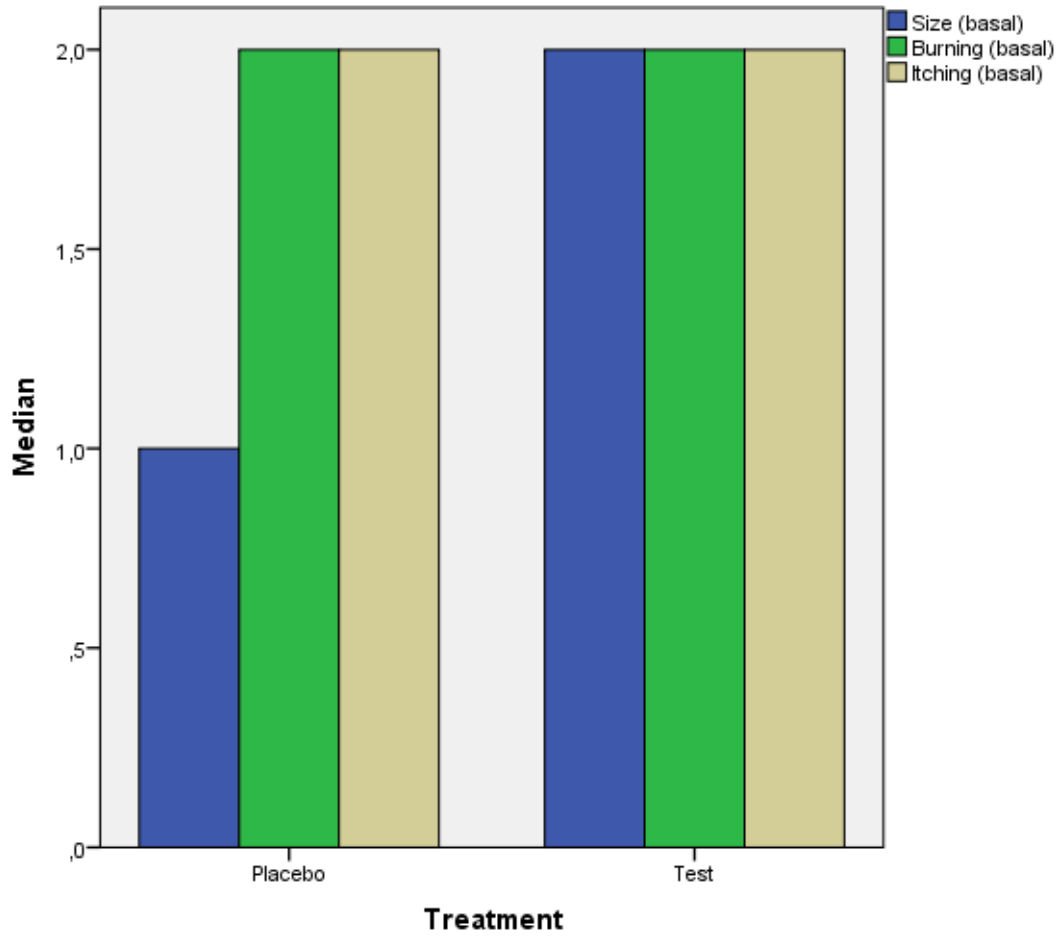
		Maximum	3	
		Range	3	
		Interquartile Range	1	
		Skewness	-,248	,398
		Kurtosis	-,584	,778

The following bar graphs show the values at baseline of all variables for each treatment. Again, the two groups weren't different:





Error bars: +/- 1 SD



As showed by the following tables, there were significant differences between the two groups at the final visit (VAS p<0,05 Student's *t* test; Extension p<0,05 Student's *t* test; Size p<0,05 Mann-Whitney U test; Burning p<0,05 Mann-Whitney U test; Itching p<0,01 Mann-Whitney U test):

Group Statistics

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
VAS (final)	Placebo	35	5,00	11,440	1,934
	Test	35	,43	1,867	,316
Extension (final)	Placebo	35	1,23	2,840	,480
	Test	35	,00	,000	,000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
VAS (final)	Equal variances assumed	23,045	,000	2,333	68	,023	4,571	1,959	,662	8,481
	Equal variances not assumed			2,333	35,811	,025	4,571	1,959	,597	8,546
Extension (final)	Equal variances assumed	33,097	,000	2,560	68	,013	1,229	,480	,271	2,186

	Equal variances not assumed			2,560	34,000	,015	1,229	,480	,253	2,204

Descriptive Statistics								
	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
Size (final)	70	,11	,401	0	2	,00	,00	,00
Burning (final)	70	,10	,347	0	2	,00	,00	,00
Itching (final)	70	,11	,320	0	1	,00	,00	,00
Treatment	70	,50	,504	0	1	,00	,50	1,00

Ranks				
	Treatment	N	Mean Rank	Sum of Ranks
Size (final)	Placebo	35	38,50	1347,50
	Test	35	32,50	1137,50
	Total	70		
Burning (final)	Placebo	35	38,50	1347,50
	Test	35	32,50	1137,50
	Total	70		
Itching (final)	Placebo	35	39,50	1382,50
	Test	35	31,50	1102,50
	Total	70		

Test Statistics[a]			
	Size (final)	Burning (final)	Itching (final)
Mann-Whitney U	507,500	507,500	472,500

Wilcoxon W	1137,500	1137,500	1102,500
Z	-2,541	-2,542	-2,984
Asymp. Sig. (2-tailed)	,011	,011	,003
a. Grouping Variable: Treatment			

The descriptive values for VAS, Extension, Size, Burning and Itching scores show the different distributions at final visit:

Descriptives[a,b,c,d]					
	Treatment			Statistic	Std. Error
VAS (final)	Placebo	Mean		5,00	1,934
		95% Confidence Interval for Mean	Lower Bound	1,07	
			Upper Bound	8,93	
		5% Trimmed Mean		3,25	
		Median		,00	
		Variance		130,882	
		Std. Deviation		11,440	
		Minimum		0	
		Maximum		50	
		Range		50	
		Interquartile Range		0	
		Skewness		2,625	,398
		Kurtosis		6,962	,778
	Test	Mean		,43	,316
		95% Confidence Interval for Mean	Lower Bound	-,21	
			Upper Bound	1,07	
		5% Trimmed Mean		,04	
		Median		,00	

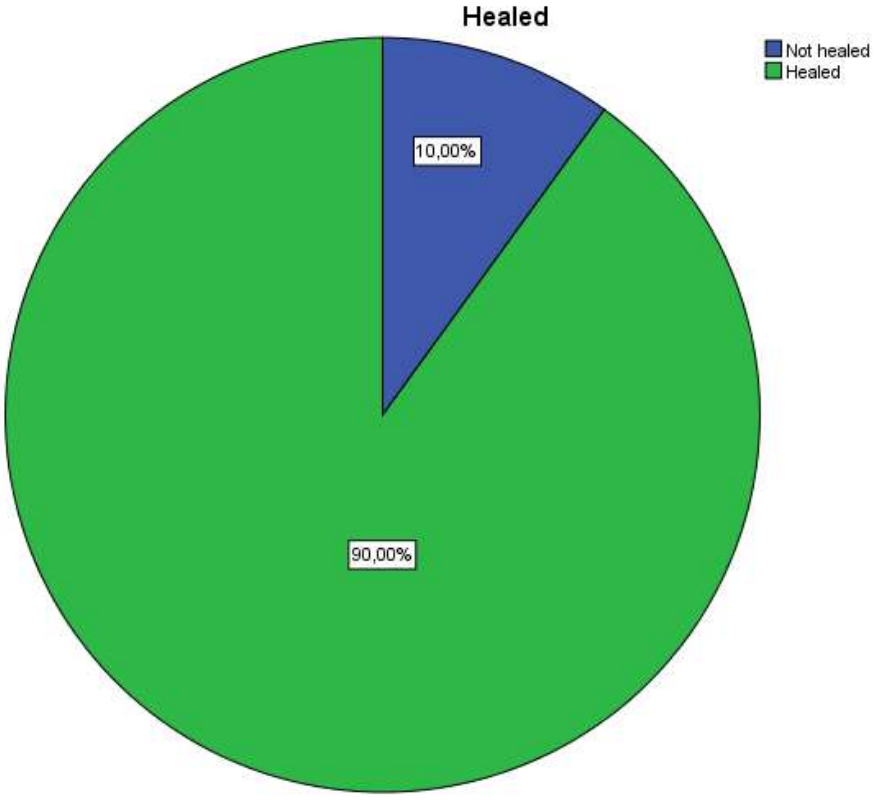
		Variance		3,487	
		Std. Deviation		1,867	
		Minimum		0	
		Maximum		10	
		Range		10	
		Interquartile Range		0	
		Skewness		4,645	,398
		Kurtosis		22,197	,778
Extension (final)	Placebo	Mean		1,23	,480
		95% Confidence Interval for Mean	Lower Bound	,25	
			Upper Bound	2,20	
		5% Trimmed Mean		,81	
		Median		,00	
		Variance		8,064	
		Std. Deviation		2,840	
		Minimum		0	
		Maximum		10	
		Range		10	
		Interquartile Range		0	
		Skewness		2,345	,398
		Kurtosis		4,498	,778
Size (final)	Placebo	Mean		,23	,092
		95% Confidence Interval for Mean	Lower Bound	,04	
			Upper Bound	,42	
		5% Trimmed Mean		,14	
		Median		,00	
		Variance		,299	
		Std. Deviation		,547	

		Minimum		0	
		Maximum		2	
		Range		2	
		Interquartile Range		0	
		Skewness		2,404	,398
		Kurtosis		5,025	,778
Burning (final)	Placebo	Mean		,20	,080
		95% Confidence Interval for Mean	Lower Bound	,04	
			Upper Bound	,36	
		5% Trimmed Mean		,13	
		Median		,00	
		Variance		,224	
		Std. Deviation		,473	
		Minimum		0	
		Maximum		2	
		Range		2	
		Interquartile Range		0	
		Skewness		2,409	,398
		Kurtosis		5,560	,778
Itching (final)	Placebo	Mean		,23	,072
		95% Confidence Interval for Mean	Lower Bound	,08	
			Upper Bound	,37	
		5% Trimmed Mean		,20	
		Median		,00	
		Variance		,182	
		Std. Deviation		,426	
		Minimum		0	
		Maximum		1	

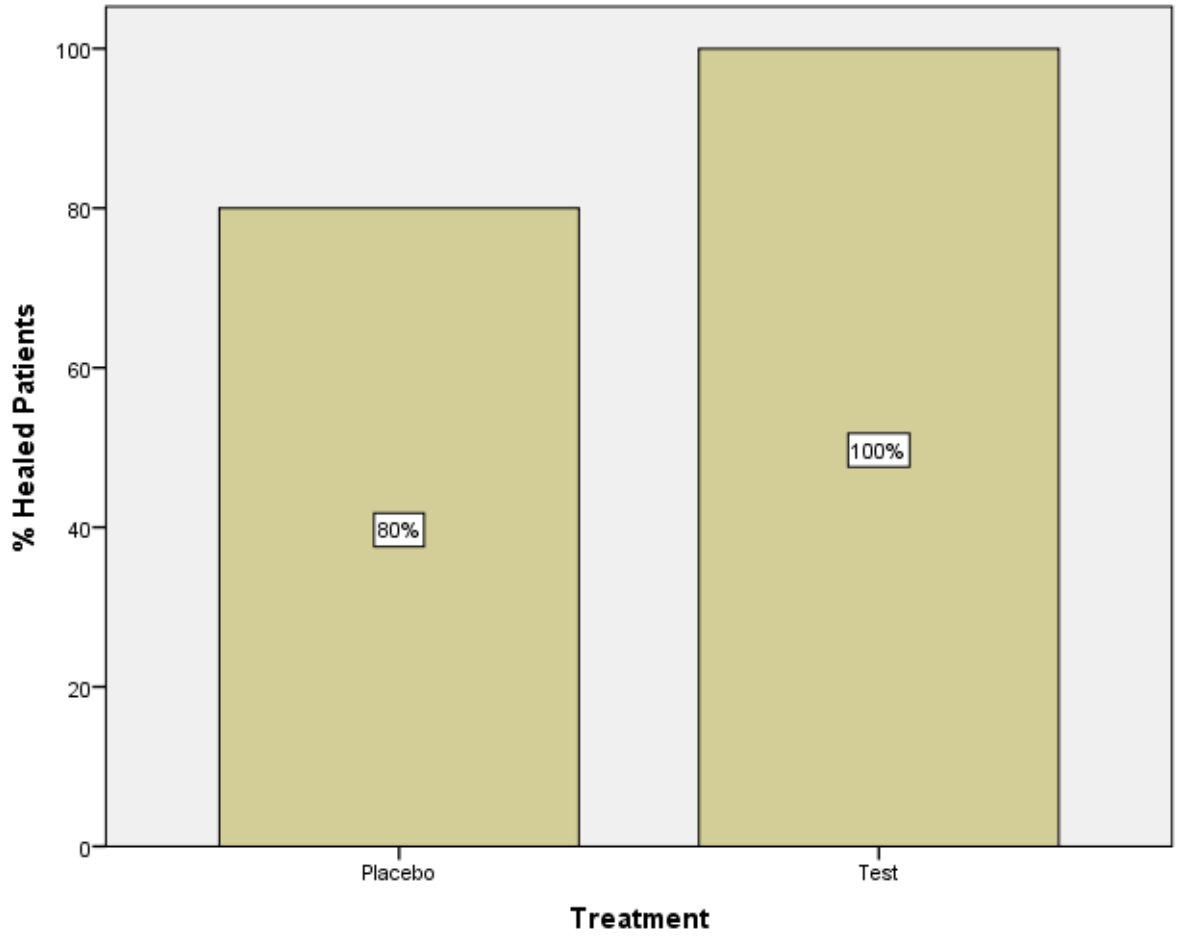
		Range	1	
		Interquartile Range	0	
		Skewness	1,351	,398
		Kurtosis	-,188	,778
a. Extension (final) is constant when Treatment = Test. It has been omitted.				
b. Size (final) is constant when Treatment = Test. It has been omitted.				
c. Burning (final) is constant when Treatment = Test. It has been omitted.				
d. Itching (final) is constant when Treatment = Test. It has been omitted.				

Healed patients were 63 out of 70 (90%):

Healed					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Not healed	7	10,0	10,0	10,0
	Healed	63	90,0	90,0	100,0
	Total	70	100,0	100,0	



The test treatment showed a 100% of healed patients (35 out of 35) while the placebo treatment showed a 80% of healed patients (28 patients out of 35):



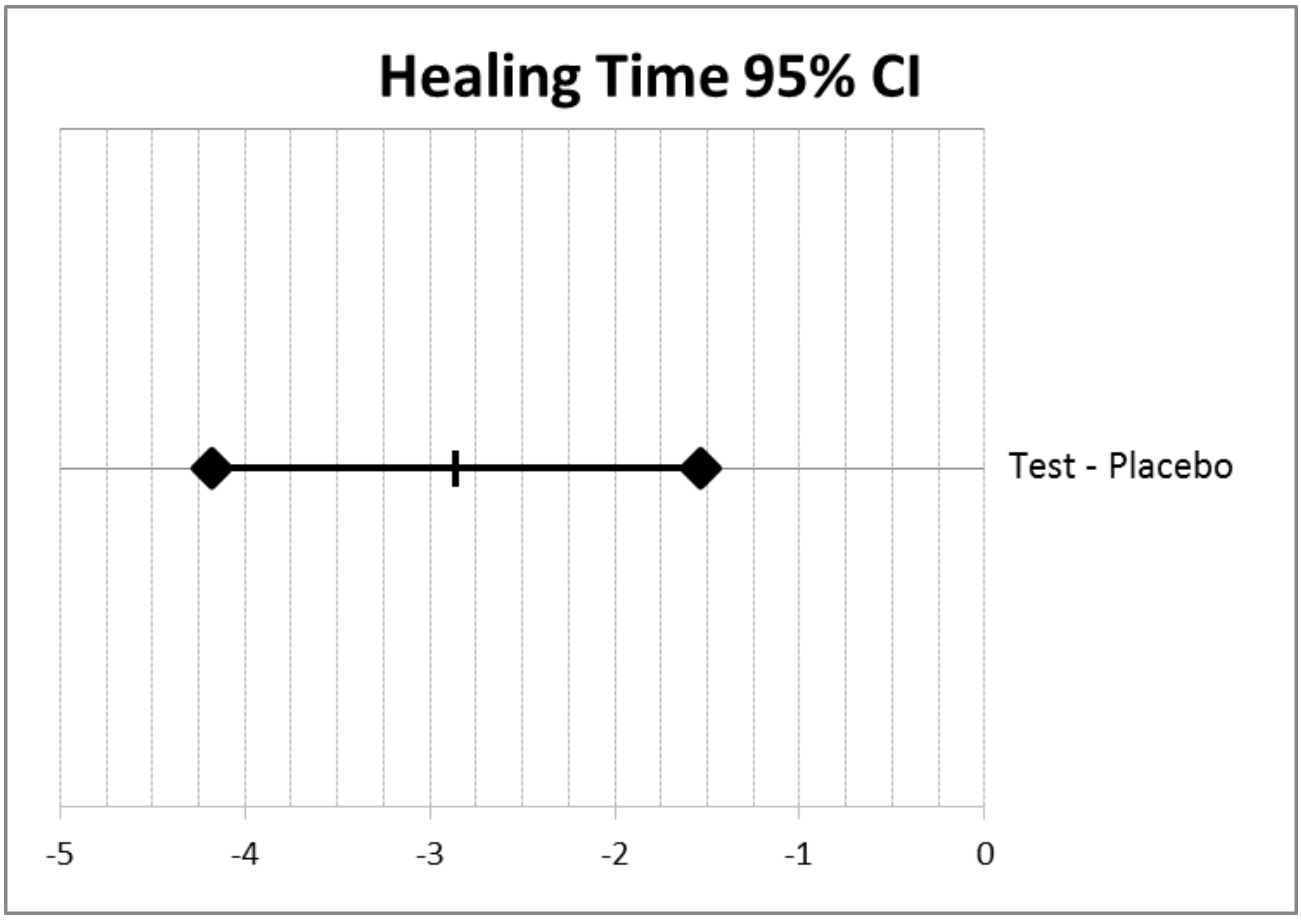
With regards for healing time, the primary efficacy endpoint, the following graphs and tables show that there were significant differences between the two treatments (p<0,001 Student's *t* test):

Group Statistics					
	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Healing Time (days)	Test	35	4,94	,968	,164
	Placebo	35	7,80	3,740	,632

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Healing Time (days)	Equal variances assumed	27,139	,000	-4,375	68	,000	-2,857	,653	-4,160	-1,554
	Equal variances not assumed			-4,375	38,539	,000	-2,857	,653	-4,179	-1,536

The mean difference was more than 1.1 day used as margin of clinical meaningfulness.

In particular, the 95% Confidence Interval of the difference of healing times between the two treatments (Test minus Placebo), lied entirely to the left of the zero value (in this case, i.e. healing time, lesser values are better, so the confidence interval is to be found at the left of the zero), demonstrating that the test treatment is superior to the placebo in treating the skin infection under study:



The Kaplan-Meier survival test shows different survival curves for the two treatments (p<0,001 Log-rank test):

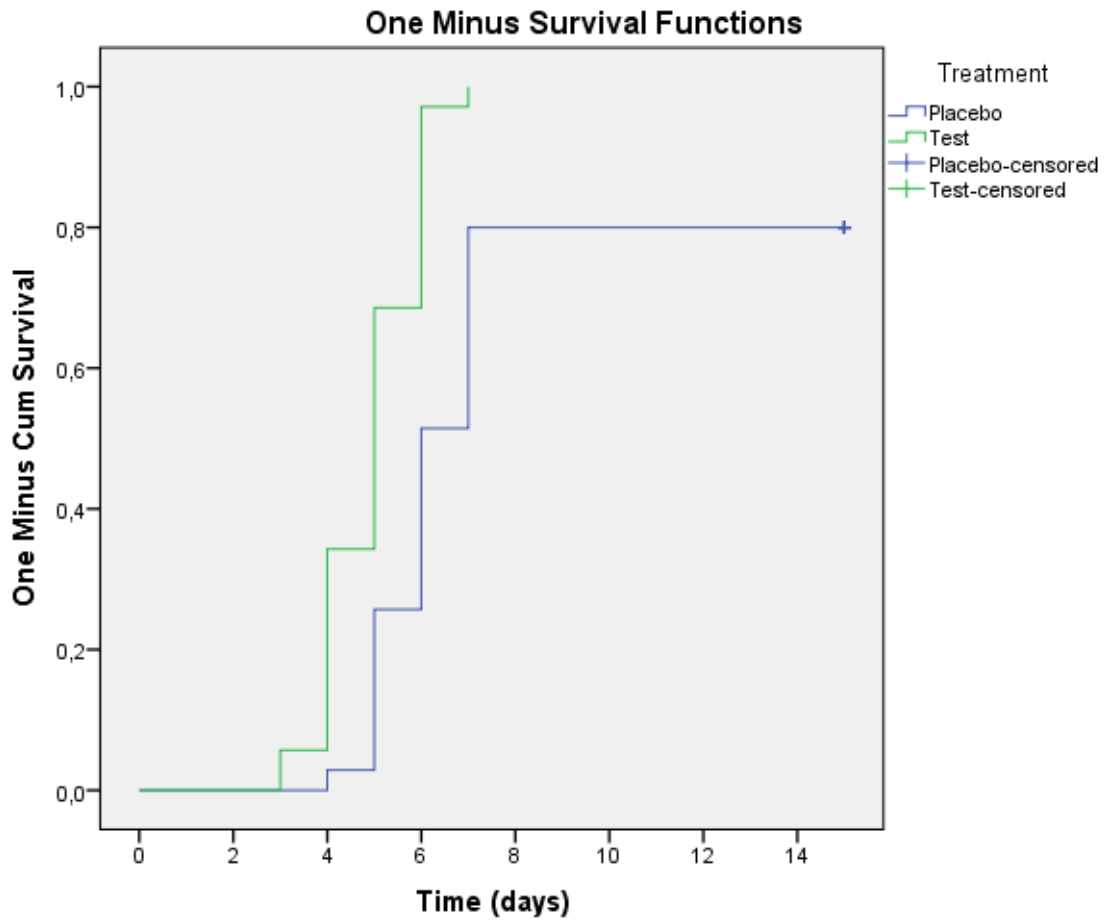
Case Processing Summary				
Treatment	Total N	N of Events	Censored	
			N	Percent
Placebo	35	28	7	20,0%
Test	35	35	0	0,0%
Overall	70	63	7	10,0%

Means and Medians for Survival Time								
Treatment	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Placebo	7,800	,623	6,579	9,021	6,000	,311	5,390	6,610
Test	4,943	,164	4,622	5,264	5,000	,229	4,551	5,449
Overall	6,371	,364	5,657	7,085	6,000	,188	5,632	6,368
a. Estimation is limited to the largest survival time if it is censored.								

Percentiles						
Treatment	25,0%		50,0%		75,0%	
	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error
Placebo	7,000	,237	6,000	,311	5,000	,304
Test	6,000	,099	5,000	,229	4,000	,281
Overall	7,000	,167	6,000	,188	5,000	,209

Overall Comparisons			
	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	24,948	1	,000

The vector of trend weights is -1, 1. This is the default.



The survival curve for Test treatment is above the survival curve for placebo, showing a faster healing time with respect to the placebo.

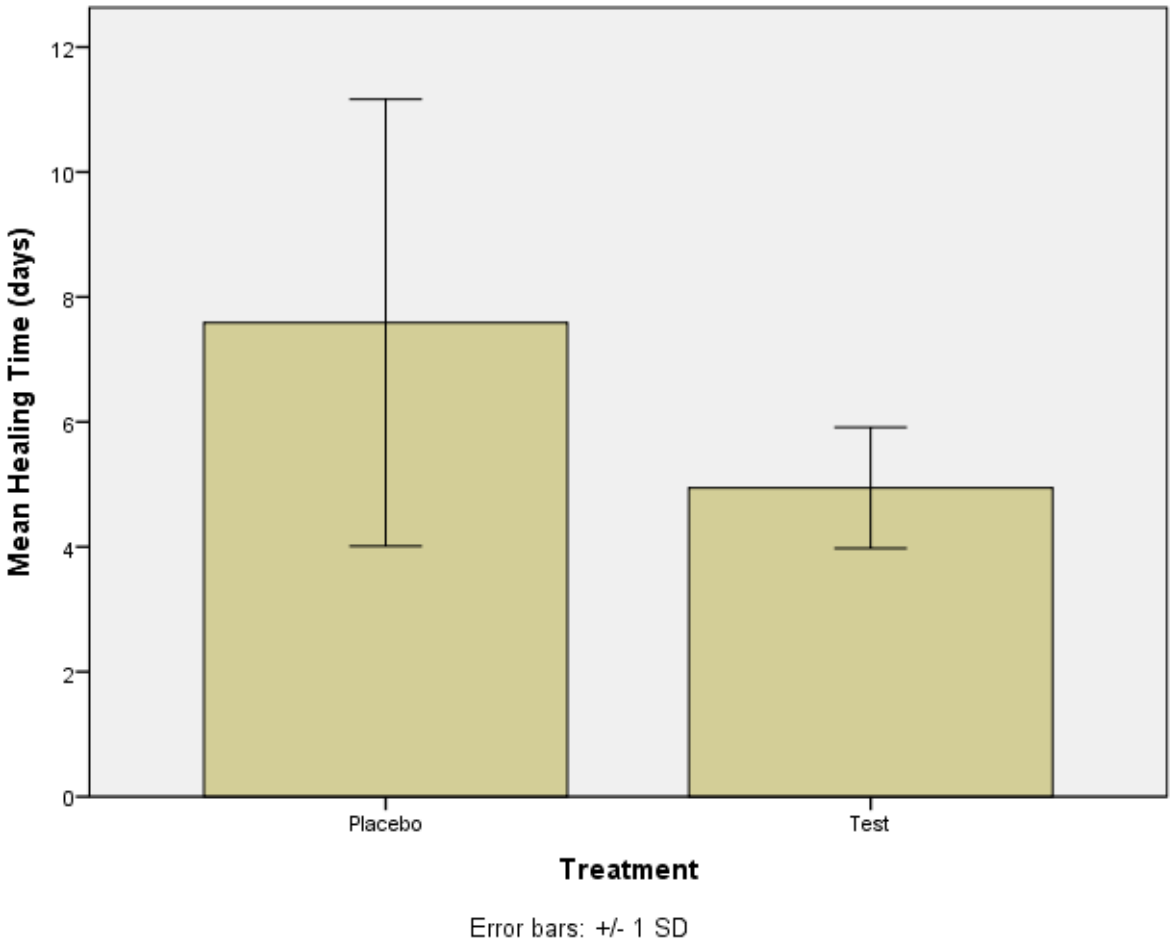
PER-PROTOCOL ANALYSIS

Only patients showing no or minor protocol deviations have been included in the PP population. Since there weren't any major deviations and there were only 1 drop-out, only the primary endpoint has been re-analysed. In fact, one dropped-out patient doesn't influence the ITT results.

With regards for healing time, the primary efficacy endpoint, the following graphs and tables show that there were significant differences between the two treatments (p<0,001 Student's *t* test):

Group Statistics					
	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Healing Time (days)	Test	35	4,94	,968	,164
	Placebo	34	7,59	3,577	,613

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Healing Time (days)	Equal variances assumed	19,764	,000	-4,220	67	,000	-2,645	,627	-3,897	-1,394
	Equal variances not assumed			-4,167	37,681	,000	-2,645	,635	-3,931	-1,360


Mean Healing Time (days)
12
10
8
6
4
2
0
Placebo
Test
Treatment
Error bars: +/- 1 SD

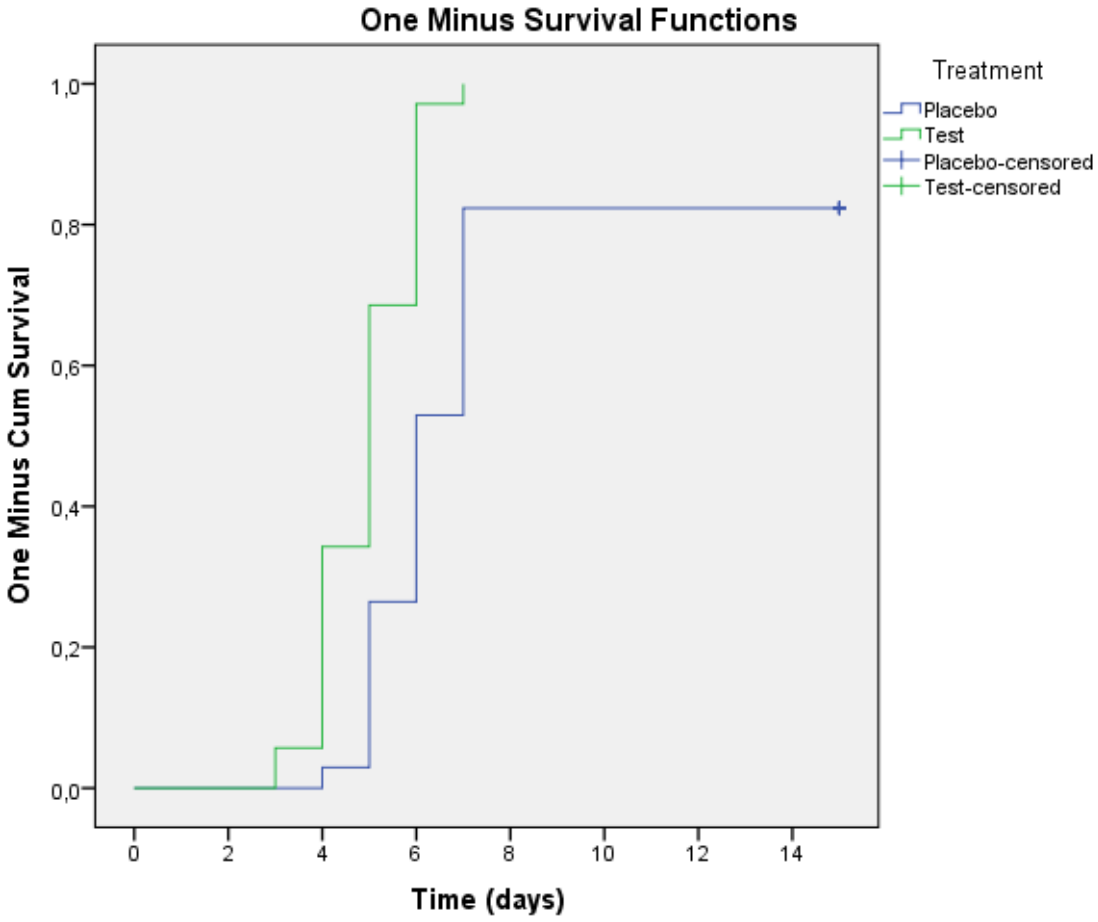
The Kaplan-Meier survival test shows different survival curves for the two treatments (p<0,001 Log-rank test):

Case Processing Summary				
Treatment	Total N	N of Events	Censored	
			N	Percent
Placebo	34	28	6	17,6%
Test	35	35	0	0,0%
Overall	69	63	6	8,7%

Means and Medians for Survival Time								
Treatment	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Placebo	7,588	,604	6,404	8,773	6,000	,306	5,400	6,600
Test	4,943	,164	4,622	5,264	5,000	,229	4,551	5,449
Overall	6,246	,347	5,565	6,927	6,000	,184	5,640	6,360
a. Estimation is limited to the largest survival time if it is censored.								

Percentiles						
Treatment	25,0%		50,0%		75,0%	
	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error
Placebo	7,000	,222	6,000	,306	5,000	,303
Test	6,000	,099	5,000	,229	4,000	,281
Overall	6,000	,239	6,000	,184	5,000	,207

Overall Comparisons			
	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	23,621	1	,000
The vector of trend weights is -1, 1. This is the default.			



The survival curve for Test treatment is above the survival curve for placebo, showing a faster healing time with respect to the placebo.

11.4.2 Statistical/analytical issues

N/A

11.4.3 Tabulation of individual response data

See appendixes

11.4.4 Drug dose, drug concentration and relationships to response

N/A

11.4.5 Drug-drug and Drug-disease interactions

N/A

11.4.6 By-patient displays

N/A

11.4.7 Efficacy conclusions

The two groups were homogeneous at baseline. The test group showed significant improvement at final visit with respect to the placebo group in all the variables under study, in particular in the continuous data (VAS and lesion extension). The three categorical variables, size, burning and itching, were too sparse at the final visit to be analysed correctly. Anyway, the test treatment have a mean healing time of about 5 days (in accordance with the literature), while the placebo group have a mean healing time of about 8 days. The placebo healing time are greater than those found in literature because a "default" duration of 15 days was assigned to the patients who not healed in 7 days. This imputation doesn't change the clinical results since the Kaplan-Meyer curves showed that the test's curve is always above the placebo one, demonstrating faster healing times for the test treatment. Moreover, only the placebo group had not healed patients, while all the patients in the test group healed within 7 days. The ITT results coincides with the PP results, thus incrementing the strength of the results. The significant difference between the test and placebo groups validate the study and the chosen sample size.

12. SAFETY EVALUATION

The safety evaluation was based on the following variables:

Safety Variables Listing
Adverse events
Serious adverse events

12.1 Extent of exposure

The extent of exposure to the test formulation (Aciclovir 5% Lipstick from Aesculapius Farmaceutici Srl) and to the placebo formulation was no more than seven consecutive days with a dose of 600 mg/die of acyclovir per patient.

70 patients were exposed to the test or placebo formulation; in particular 35 were exposed to the test formulation and 35 were exposed to the placebo formulation.

12.2 Adverse events (AEs)

12.2.1 Brief summary of adverse events

Tolerability was good because the incidence and severity of the adverse events that occurred in the two active treatment arms did not differ significantly.

Treatment	Total n. of AE	AE probably correlated to drug
Aciclovir 5% Lipstick, from Aesculapius Farmaceutici Srl	0/35	0/35
Placebo	4/35	0/35

The total number of AE was 4 (5,7%).

12.2.2 Analysis of adverse events

The adverse events occurred were not related to the treatments in study, they were single occurrences, of slight intensity and all resolved at the end of the study.

12.2.3 Listing of adverse events by patient

See appendixes.

12.3 Death, Other serious adverse events, and other significant adverse events

No serious adverse events occurred.

12.4 Clinical laboratory evaluation

No laboratory measurements have been done.

12.5 Vital signs, physical finding and other observation related to safety

Vital signs, *i.e.* blood pressure, heart rate and body temperature have been measured before the beginning of the study and at the end of the study.

Pregnancy tests were not done for women in menopause (5) and for women taking contraceptives (2). For all other women, the pregnancy test have been. All these tests were negative.

HSV1 test have been done for 55 out of 70 patients. For ten (15) patients, the prodromal phase was too advanced, so the principal investigator(s) decided to not perform the test.

12.6 Safety conclusions

Tolerability was good. The four adverse events that occurred during the study were not related to the test treatment. The dose was comfortable.

13. DISCUSSION AND OVERALL CONCLUSIONS

The two groups were homogeneous at baseline. The test group showed significant improvement at final visit with respect to the placebo group in all the variables under study, in particular in the continuous data (VAS and lesion extension). The three categorical variables, size, burning and itching, were too sparse at the final visit to be analysed correctly. Anyway, the test treatment have a mean healing time of about 5 days (in accordance with the literature), while the placebo group have a mean healing time of about 8 days. The placebo healing time are greater than those found in literature because a "default" duration of 15 days was assigned to the patients who not healed in 7 days. This imputation doesn't change the clinical results since the Kaplan-Meyer curves showed that the test's curve is always above the placebo one, demonstrating faster healing times for the test treatment. Moreover, only the placebo group had not healed patients, while all the patients in the test group healed within 7 days. The ITT results coincides with the PP results, thus incrementing the strength of the results.

These data validate the present study as it demonstrated that the selected clinical outcome measures and the study design were sufficiently sensitive and therefore suitable to detect the observed difference between the two treatments as statistically significant (indicating that the test treatments were indeed more effective than placebo in treating skin infections provoked by Herpes Labialis). In addition, the results indicate that the sample size selected for the present study gave enough power to the selected statistical tests.

It can be concluded that:

- The present study is valid as it allowed the detection of a statistically significant difference in efficacy outcome measure between the two treatments.
- Based on both ITT and PP population, the efficacy of the test medication observed in the present study did differ significantly from the efficacy of the placebo medication indicating that the test medication is superior to the placebo in treating skin infections provoked by Herpes Labialis based on the selected primary outcome measure.
- No significant difference in the occurrence and severity of adverse events was observed between the two treatement arms indicating that the safety and tolerability of the test treatment were good.