1. Clinical Study Report Title Page

A randomized, placebo-controlled, evaluator-blinded, study to assess the anti-inflammatory effects of topical erythromycin and clindamycin in patients with inflammatory facial acne.

Investigational Drug:	
CHDR Number	CHDR1732
EudraCT number:	2017-003105-18
Toetsing Online number:	NL62760.056.17
Date of Report:	24-Dec-2019
Study Dates:	First subject, first dose: 03Apr2019
	Last subject, last visit: 08May2019
Indication:	Inflammatory facial acne
Principal Investigator:	R. (Robert) Rissmann, PhD

CHDR template v2019.9

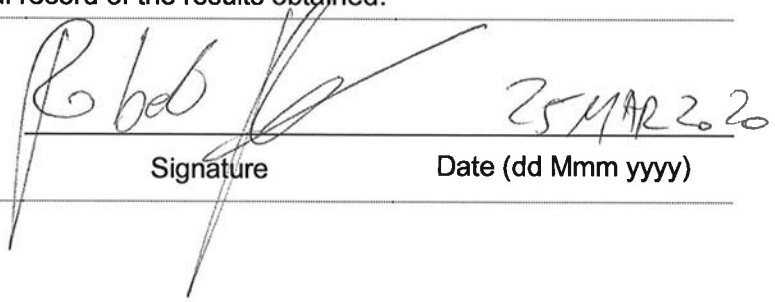
This study was performed in compliance with Good Clinical Practice.

SIGNATURE PAGE - PRINCIPAL INVESTIGATOR

Study Title

I hereby declare that the work was performed according to the procedures herein described and that the report provides a correct and faithful record of the results obtained.

R. (Robert) Rissmann, PhD
Principal investigator



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2. Summary

Title
A randomized, placebo-controlled, evaluator-blinded, study to assess the anti-inflammatory effects of topical erythromycin and clindamycin in patients with inflammatory facial acne.

Short Title
Anti-inflammatory effects of topical erythromycin and clindamycin in acne patients.

Principal investigator & Trial Site
R. Rissmann, PhD (Principal investigator), J. Burggraaf MD, PhD (Medical responsibility), Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands

Background & Rationale
Acne vulgaris (AV) is a cutaneous disease of the pilosebaceous follicles. In adolescents it is very common, the prevalence ranges from 35 to over 90% (1, 2). Acne vulgaris typically affects the face, neck, chest, upper back and upper arms. Clinical features include non-inflammatory lesions (closed and/or open comedos) and inflammatory lesions (papules, pustules and nodules). When becoming extensive, inflammatory lesions can lead to scarring and post-inflammatory hyperpigmentation and it is known that acne can have a significant impact on patients self-esteem and social life (3).
Four factors are involved in the pathophysiology of AV, i) follicular hyperkeratinisation, ii) increased sebum production, iii) Propionibacterium acnes (P. acnes) colonization within the follicle and iv) inflammation. The exact role of P. acnes in acne is an ongoing debate, however P. acnes is able to stimulate the immune system in several ways: stimulation of Toll-like receptors 2 and 4, direct stimulation of T lymphocytes, and the activation of the NRLP3-inflammasome via various NLRs (4). Furthermore recent investigations suggest that the pro-inflammatory cytokine IL-1beta may play an important role in the development of inflammation in AV (5, 6).
Antibiotics including erythromycin (a macrolide antibiotic) and clindamycin (a lincosamide antibiotic) via topical and systemic administration route play a major role in the treatment of AV. Both erythromycin and clindamycin are bacteriostatic by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. Furthermore, anti-inflammatory and immuno-modulating properties of these antibiotics have been described *in vitro* and *in vivo*, mostly in the field of respiratory medicine. A recent in-vitro study showed that erythromycin reduces IL-1beta in LPS stimulated PMBCs (7).
However, currently there is no mechanistic evidence of those anti-inflammatory properties *in vivo* in skin diseases such as acne. Therefore, the objective of this study was to assess the anti-inflammatory and immunomodulatory properties of topical erythromycin and clindamycin in patients with inflammatory acne.
In patients with inflammatory facial acne, the combined bacteriostatic and immunomodulatory effects of erythromycin and clindamycin were explored. Treatment effects were extensively characterized by conventional methods including lesion counts, global assessment scales and visual grading as well as state-of-the-art methodology, including perfusion by laser speckle contrast imaging, analysis of local skin surface, biopsy biomarkers and skin microbiota. This extensive response profiling, combined with the mechanistic insights from concurrent in vitro and in vivo studies in healthy volunteer challenges, increased the understanding of erythromycin's and clindamycin's effects in acne vulgaris.

Objectives

- To evaluate the effects of topically applied erythromycin and clindamycin in patients with facial AV
- To explore skin and faecal microbiota in patients with AV;
- To evaluate the effects of topically applied erythromycin and clindamycin on skin and faecal microbiota.

Design

This is a randomized, open-label, placebo-controlled, evaluator-blinded study.

Investigational drug

Erythromycin 4% topical gel formulation

Erythromycin is a bacteriostatic antibiotic that belongs to the macrolide group of antibiotics. Macrolides act as antibacterial by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. A topical gel formulation with hyprolose and ethanol.

Clindamycin 1% topical lotion formulation:

Clindamcin is a bacteriostatic antibiotic that belongs to the lincosamide group of antibiotics. Lincosamides act as bacteriostatic by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. An aqueous topical lotion formulation with ethanol.

Comparative drug

Seventy (70) % topical ethanol solution served as placebo.

Participation and demographics

100 subjects were screened of which 30 subjects were enrolled into the trial. 30 subjects were randomized to 1 of the 3 treatment arms. Demographics were comparable across the three treatment groups. Baseline characteristics were slightly different across the treatment groups: the placebo group had the highest mean total inflammatory lesion count, followed by clindamycin and then erythromycin. This was also reflected in the investigator global assessment (IGA): the placebo group had the most patients with category ‘moderate’ followed by clindamycin and erythromycin.

The analysis populations consisted of 30 subjects for both the Intent-to-treat (ITT) and the Clinical Evaluable population.

Results and Discussion

Pharmacokinetic results:

No pharmacokinetic investigations were performed.

Efficacy/pharmacodynamics

In the clinical evaluation of the acne vulgaris as assessed by inflammatory lesion count (IFLC) and IGA, all treatments including placebo led to either i) a reduction of the IFCL or ii) an improvement of the IGA. However, erythromycin treatment and clindamycin treatment did not result in statistically significant reduced counts compared to placebo. For the patient reported outcome, measured by the patient global assessment (PGA), erythromycin performed statistically significant better than placebo and clindamycin ($p = 0.0136$ and $P = 0.0469$, respectively), no statistically significant difference was found between clindamycin and placebo ($p = 0.1703$). No substantial differences were observed in sebum measurements by sebumeter, Laser Speckle Contrast Imaging (LSCI), Transdermal Analysis Patch (TAP), and biopsy biomarkers, when comparing the two active treatments to placebo. In microbiological endpoints, clindamycin showed the strongest effect against P. acnes quantified by

culture, which was significant when compared to placebo (p = 0.0295). The data of the microbiome demonstrated a high degree of variability between subjects. In general the presence of the genus cutibacteria or staphylococci dominated lesional skin. No clear treatment effect could be observed on the reduction of either cutibacteria (formerly proprionibacteria) or staphylococci. The composition of the gut microbiome at day 28 was comparable to predose, hence no treatment effect was observed.

Safety and Tolerability
This study showed that the two active treatments and placebo were well tolerated by the subjects. No treatment related study discontinuation or treatment related SAE occurred. The AE profile was comparable for all subjects across treatment groups. The most frequent occurring treatment-emergent AEs were headache/migraine, nasopharyngitis and influenza like illness. All TEAEs were of mild or moderate severity and self-limiting.

Adherence / exposure
In total, 30 subjects were included in the randomized ITT population. Twenty (20) subjects were randomized to one of the two active treatment groups, i.e. erythromycin or clindamycin and 10 subjects were randomized to placebo. Administrations were performed on consecutive days and only sporadically subjects did not comply to the twice daily treatment regimen. All dose administrations at home were recorded via a mobile app. The average daily dose applied per treatment was 386.8mg for clindamycin, 498.0mg for clindamycin, and 2919.5mg for placebo.

Safety results:
The results from the current study show that clindamycin and erythromycin are safe and well tolerated for BID administration up to 28 days to subjects with mild to moderate acne vulgaris. The overall incidence of TEAEs was similar among subjects receiving active treatment and vehicle. No clinically significant changes were attributable to treatment with clindamycin or erythromycin.

Overall conclusion:
The aim of this study was to extensively characterize the effect of topically applied erythromycin and clindamycin in acne vulgaris patients. Although erythromycin and clindamycin reduced the total inflammatory lesion count and IGA, both active treatments did not perform statistically significantly better than placebo. No other pharmacological effect of erythromycin or clindamycin was found other than the reduction of P. acnes in culture by clindamycin. This study did not provide clear evidence for other mechanisms of action other than the antimicrobial effect, which was most notable for clindamycin.

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4. List Of Abbreviations And Definition Of Terms

AE	Adverse Event
ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee; in Dutch, ABR = Algemene Beoordeling en Registratie
ALT	alanine aminotransferase/serum glutamic pyruvic transaminase (SGPT)
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase/serum glutamic oxaloacetic transaminase (SGOT)
b.i.d.	bis in diem / twice a day
BMI	Body Mass Index
BP	Blood Pressure
bpm	beats per minute
CA	Competent authority (also CCMO)
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CHDR	Centre for Human Drug Research
CK	creatine kinase
CRF	Case Report Form
EC	Ethics Committee (also Medical Research Ethics Committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC).
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbant assay
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IB	investigator's Brochure
IMPD	Investigational Medicinal Product Dossier
IFLC	Inflammatory Lesion Count
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
AE	Adverse Event
ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee; in Dutch, ABR = Algemene Beoordeling en Registratie
ALT	alanine aminotransferase/serum glutamic pyruvic transaminase (SGPT)
ANCOVA	Analysis of Covariance

ANOVA	Analysis of Variance
AST	aspartate aminotransferase/serum glutamic oxaloacetic transaminase (SGOT)
ATC	Anatomic Therapeutic Chemical
b.i.d.	bis in diem / twice a day
BMI	Body Mass Index
BP	Blood Pressure
bpm	beats per minute
TAP	Transdermal Analysis Patch
LSCI	Laser Specke Contrast Imaging
MIC	Minimum Inhibitory Concentration

5. Ethics

5.1. Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The protocol of this study was submitted to and reviewed by the Ethics Committee (EC) Stichting Beoordeling Ethiek Biomedisch Onderzoek (PO box 1004, 9400 BA Assen, The Netherlands) and the Competent Authority (CA) Central Committee on Research involving Human Subjects (PO box 16302, 2500 BH Den Haag, The Netherlands).
The CA provided a declaration of no objection on 06Dec2017 and the EC approved the protocol on 13Dec2017. An amendment was submitted to the EC and CA and the CA provided a declaration of no objection on 23Feb2018 and the EC approved the amended protocol on 08Mar2018. The study did not commence before formal approval was granted.

A list of the members of the EC is provided in the letters of approval.

5.2. Ethical Conduct of the Study

The study was conducted under ethical principles that have ethical origins in the Declaration of Helsinki according to the Dutch Medical Research in Human Subjects Act (WMO). The study was performed in compliance with good clinical practice (GCP).

5.3. Patient Information and Consent

Subjects were given oral and written information about the study prior to screening. The subjects were permitted to ask questions to qualified staff and were given ample opportunity to carefully consider participation in the trial. After they gave written acknowledgement of informed consent to participate, a medical screening took place. After approval by the subjects, their general practitioners were notified.

7. Introduction

Acne vulgaris (AV) is a cutaneous disease of the pilosebaceous follicles. In adolescents it is very common, the prevalence ranges from 35 to over 90% (1, 2). Acne vulgaris typically affects the face, neck, chest, upper back and upper arms. Clinical features include non-inflammatory lesions (closed and/or open comedos) and inflammatory lesions (papules, pustules and nodules). When becoming extensive, inflammatory lesions can lead to scarring and post-inflammatory hyperpigmentation and it is known that acne can have a significant impact on patients self-esteem and social life (3).

Four factors are involved in the pathophysiology of AV, i) follicular hyperkeratinisation, ii) increased sebum production, iii) Propionibacterium acnes (P. acnes) colonization within the follicle and iv) inflammation. The exact role of P. acnes in acne is an ongoing debate, however P. acnes is able to stimulate the immune system in several ways: stimulation of Toll-like receptors 2 and 4, direct stimulation of T lymphocytes, and the activation of the NRLP3-inflammasome via various NLRs (4). Furthermore recent investigations suggest that the pro-inflammatory cytokine IL-1beta may play an important role in the development of inflammation in AV (5, 6).

Antibiotics including erythromycin (a macrolide antibiotic) and clindamycin (a lincosamide antibiotic) via topical and systemic administration route play a major role in the treatment of AV. Both erythromycin and clindamycin are bacteriostatic by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. Furthermore, anti-inflammatory and immuno-modulating properties of these antibiotics have been described *in vitro* and *in vivo*, mostly in the field of respiratory medicine. A recent in-vitro study showed that erythromycin reduces IL-1beta in LPS stimulated PMBCs (7).

However, currently there is no mechanistic evidence of those anti-inflammatory properties *in vivo* in skin diseases such as acne. Therefore, the objective of this study was to assess the anti-inflammatory and immunomodulatory properties of topical erythromycin and clindamycin in patients with inflammatory acne.

8. Study Objectives

- To evaluate the effects of topically applied erythromycin and clindamycin in patients with facial AV;
- To explore skin and faecal microbiota in patients with AV;
- To evaluate the effects of topically applied erythromycin and clindamycin on skin and faecal microbiota.

9. Investigational Plan

9.1. Overall Study Design and Plan - Description

This is was randomized, placebo-controlled, evaluator-blinded, study to evaluate the anti-inflammatory effects of topical erythromycin and clindamycin in acne in patients with inflammatory facial acne.

Erythromycin, clindamycin or placebo was administered by the subjects at home BID for 4 weeks.

Erythromycin 4% topical gel formulation
Erythromycin is a bacteriostatic antibiotic that belongs to the macrolide group of antibiotics. Macrolides act as antibacterial by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. Each gram of the 4% gel contains 40mg of erythromycin in a formulation of 96% ethanol and hyprolose. The product specification in French is provided in D2. of the submission dossier.

Clindamycin 1% topical lotion formulation:
Clindamycin is a bacteriostatic antibiotic that belongs to the lincosamide group of antibiotics. Lincosamides act as bacteriostatic by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. Each liter of the 1% lotion contains 10mg of clindamycin in a formulation of 96% ethanol, 5% methanol, propyleneglycol (E1520) and purified water. The product specification in Dutch is provided in D2. of the submission dossier.

Placebo
70% topical alcohol formulation served as placebo.

For this trial with the administration of topical erythromycin and clindamycin BID for 4 weeks, 30 acne patients between the age of 18 and 45 were included.

The total duration of the study for each subject will be up to 8 weeks divided as follows:

- Screening: Up to 21 days before dosing;
- Treatment and study assessments: Days 0 to 28 (weekly visits)
- Follow-up: day 42

9.1.1. Medical screening

Within 3 weeks prior to study baseline visit (Day 0), patients underwent a medical screening. Screening was performed in a fasting state (≥4 hours), and consisted of medical history, physical examination, 12-lead ECG, vital signs, weight, height, heart rate, blood sampling (haematology, biochemistry, virology) and urinalysis. In addition, skin types were assessed according to the Fitzpatrick classification and acne was assessed (inflammatory lesion count and investigator global assessment). Only subjects who were found to be eligible were enrolled in the study.

9.1.2. Treatment and observation period

The time schedule of study periods in general is provided in Visit and Assessment Schedule (section 9.5.1). Subjects visited the clinical unit on days 0, 7, 14, 21, and 28 during the treatment period from day 0 through day 28. One follow up visits was scheduled on day 42.

9.1.3. Follow-up

There was one follow-up visit at day 42, which included efficacy and PD assessments.

9.2. Discussion of Study Design, Including the Choice of Control Groups

Because the formulations of the treatments were not identical (clindamycin was a lotion and erythromycin a gel) and the packaging was different as well, a double blind design was not feasible. All subjective clinical scores (lesion counts and IGA scorings) were done by blinded CHDR research physicians or nurse practitioners. These investigators were not involved in other parts of the study (single-blind design).

A 70% topical alcohol formulation served as placebo as the formulations of both clindamycin and erythromycin contained ethanol which is a known antibacterial agent.

9.3. Selection of Study Population

9.3.1. Inclusion Criteria

Eligible subjects had to meet *all* of the following inclusion criteria:

1. Healthy male and female subjects, 18 to 45 years of age. The health status is verified by absence of evidence of any clinical significant active or uncontrolled chronic disease other than AV following a detailed medical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, virology and urinalysis;
2. Mild to moderate inflammatory acne vulgaris on the face, ≥5 inflammatory lesions (papules and/or pustules), present at screening and baseline visit
3. A maximum of 5 nodules present at screening and baseline visit
4. Inflammatory acne present for at least 6 months
5. Fitzpatrick skin type I-II (Caucasian)
6. Able and willing to give written informed consent and to comply with the study restrictions.
7. Willing to comply with 2x2mm facial skin punch biopsies

9.3.2. Exclusion Criteria

Eligible subjects had to meet *none* of the following exclusion criteria:

1. Severe acne where systemic treatment is needed
2. Use of any topical (anti-acne) medication (prescription or OTC) within 2 weeks prior to baseline
3. Use of any oral/systemic treatment for acne, including oral antibiotics, excluding OAC, within 4 weeks prior to baseline
4. Use of systemic isotretinoin within 6 months prior to baseline
5. History of pathological scar formation (keloid, hypertrophic scar)
6. Known hypersensitivity to erythromycin or clindamycin, drugs of the same class, or any of their excipients.
7. Known contact dermatitis reaction to any product
8. Tanning due to sunbathing, excessive sun exposure or a tanning booth within 3 weeks of enrollment.
9. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year.

10. Loss or donation of blood over 500 mL within three months (males) or four months (females) prior to screening
11. Pregnant, a positive pregnancy test, intending to become pregnant, or breastfeeding

9.3.3. Removal of Patients from Therapy or Assessment

Subjects could leave the study at any time for any reason if they wished to do so without any consequences. If a subject decided to withdraw from the study, all efforts would be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible.

The investigator could also withdraw a subject if continuing participation was, in his opinion, deleterious to the subject's well-being or could temporally interrupt or permanently discontinue the study drug if continued administration of the study drug was believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study drug could be triggered by an Adverse Event (AE), a diagnostic or therapeutic procedure, an abnormal assessment (e.g. ECG or laboratory abnormalities), or for administrative reasons in particular withdrawal of the subject's consent. The reason for study drug interruption or premature discontinuation would be clearly documented. Subjects could also be withdrawn in case of a protocol violation and/or non-compliance.

9.4. Treatments

9.4.1. Treatments Administered

Erythromycin, clindamycin or placebo was applied BID for 28 days on the face.
All formulations were dispensed by the Pharmacy of Leiden University Medical Centre, The Netherlands.

9.4.2. Identity of Investigational Product(s)

Erythromycin 4% topical gel formulation

Erythromycin is a bacteriostatic antibiotic that belongs to the macrolide group of antibiotics. Macrolides act as antibacterial by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. A topical gel formulation with hyprolose and ethanol.

Clindamycin 1% topical lotion formulation:

Clindamcin is a bacteriostatic antibiotic that belongs to the lincosamide group of antibiotics. Lincosamides act as bacteriostatic by reversibly binding to the P site on the 50S subunit of bacterial ribosomes. An aqueous topical lotion formulation with ethanol.

Placebo:

Seventy (70) % topical ethanol solution served as placebo.

9.4.3. Method of Assigning Patients to Treatment Groups

Subjects in this study were numbered sequentially from 1 to 30. Treatments were randomized.

The randomization code were generated using SAS version 9.1.3 by a study-independent, CHDR statistician. The randomization code was kept strictly confidential. Sealed individual randomization codes, per subject and per treatment, were placed in a sealed envelope containing the and labelled 'emergency decoding envelopes' were kept in a safe cabinet at CHDR.

9.4.4. Selection of Doses in the Study

For both erythromycin and clindamycin the highest dose available for topical solution (4% and 1% respectively) was used in order to maximize the possible anti-inflammatory effects.

9.4.5. Selection and Timing of Dose for Each Patient

All subjects were to apply the treatment BID for 28 consecutive days with approximately 12 hours. between each treatment.

9.4.6. Blinding

Study treatments were randomized. Treatments were not identical but were distinguishable, therefore a double blind design was not feasible. All subjective clinical scores (lesion counts and IGA scorings) were be done by blinded CHDR research physicians or nurse practitioners. These investigators were not involved in other parts of the study. The study was conducted in a single-blind (evaluator blinded) fashion.

9.4.7. Prior and concomitant therapy

Concomitant medication registration was done in the appropriate section of the CRF until the subject's last visit.

Acne treatments and antibiotics were prohibited during the course of the study. Any other medication that did not interfere with the study objectives, as judged by the investigator, was allowed.

9.4.8. Treatment Compliance

Treatment compliance was monitored via a mobile app, the app also sent reminders in order to monitor and increase treatment compliance.

9.5. Safety and efficacy variables and flowchart

Table 1 Visit and Assessment Schedule

Time point / Assessment	Up to -21 d	Day 0	Day 7	Day 14	Day 21	Day 28 EOT	Day 42 EOS
Informed consent	X						
Demography	X						
Inclusion and exclusion criteria	X	X					
Medical history	X						
Physical examination	X						
Fitzpatrick skin type assessment	X						
ECG	X						
Height and weight	X						
Concomitant medication	X						
Vital Signs (HR, BP, temperature)	X						
BsHaem, BsChem, BsGluc, Virology	X						
Urinalysis, UrPregnancy	X						
Drug (-placebo) administration at clinic		X					
Drug (-placebo) administration at home twice a day			X	X	X	X	
Weight drug tubes		X	X	X	X	X	
Clinical assessments (lesion count, IGA)	X	X	X	X	X	X	X
Standardized facial photography (VISIA)		X	X	X	X	X	X
Sebum measurements (Sebumeter)		X	X	X	X	X	X
Laser Speckle Contrast Imaging (LSCI)		X	X	X	X	X	X
Optical Coherence Tomography (OCT)		X	X	X	X	X	X
Transdermal Analysis Patch (TAP)		X	X	X	X	X	X
Skin punch biopsy (2mm)		X				X	
Microbiota skin swab (lesional and non-lesional skin)		X	X	X	X	X	X
Comedo extraction		X	X	X	X	X	X
Faecal sampling for microbiota at home		X[1]				X[1]	
Faeces questionnaire at home		X				X	
Patient reported outcome (sPGA)		X				X	
E-diary (twice daily for treatment compliance and selfies)				X			
E-diary satisfaction questionnaire						X	
(S)AE/Con-meds	X	X	X	X	X	X	X

BP = Blood Pressure, HR = Heart Rate, SCR = Screening, = AE = Adverse Event, BsHaem = Blood Sample Haematology, BsChem = Blood Sample Chemistry, BsGluc = Blood Sample Glucose

1: faecal samples were collected at home, within 7 days before the first application and after the last application.

9.5.1. Safety assessments

9.5.1.1. Vital Signs

Evaluations of systolic and diastolic blood pressure, pulse rate, and temperature were performed at screening. Pulse and blood pressure were taken after 5 minutes in the supine position. Automated oscillometric blood pressures were measured using a Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400.

9.5.1.2. Weight and Height

Weight (kg) and height (cm) were recorded and body mass index (BMI) was calculated at screening.

9.5.1.3. Physical Examination

Physical examination (i.e., inspection, percussion, palpation and auscultation) was performed during screening. Clinically relevant findings that were present prior to study drug initiation were be recorded with the subject's Medical History. Clinically relevant findings found after study drug initiation and meeting the definition of an AE (new AE or worsening of previously existing condition) were recorded.

9.5.1.4. Electrocardiography

12-lead electrocardiographs (ECGs) were obtained at screening using Marquette 800/2000/5500 or Dash3000 and stored using the MUSE Cardiology Information System. The investigator assessed the ECG recording as 'normal', 'abnormal - not clinically significant', or 'abnormal - clinically significant' and include a description of the abnormality as required. The ECG parameters assessed included heart rate, PR, QRS, QT, and QTc (calculated using Bazett's and Friedericia's method).

9.5.1.5. Laboratory Assessments

Blood and other biological samples were collected at screening for the following clinical laboratory tests.

Lab	Tests	Collection & Analysis
Haematology	Haemoglobin [including Mean Corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC)], haematocrit, red cell count (RBC), total white cell count (WBC) and Platelet count. Differential blood count, including: basophils, eosinophils, neutrophils, lymphocytes, and monocytes.	2 mL of venous blood in a BD Vacutainer® K2EDTA tube. Samples will be analysed by the Clinical Chemistry Laboratory (AKCL) of Leiden University Medical Center (LUMC).
Chemistry and electrolytes	Sodium, potassium, calcium, inorganic phosphate, total protein, albumin,	3.5 mL of venous blood in a BD Vacutainer® SST

	triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin[1], alkaline phosphatase, AST, ALT gamma-GT and LDH.	Gel and Clot Activator tube. Samples will be analysed by the AKCL of LUMC.
Glucose	Glucose[2]	2 mL of venous blood in a BD Vacutainer® Sodium Fluoride tube. Samples will be analysed by the AKCL of LUMC.
Virology (serology)	HIV1 and HIV2 antibodies, Hepatitis B antigen, and Hepatitis C antibodies	5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the Microbiology Laboratory (CKML) of the Leiden University Medical Center.
Urinalysis	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose. If there is a clinically significant positive result, urine will be sent to the AKCL for microscopy and/or culture.	A midstream, clean-catch urine specimen will be analysed by dipstick (Multistix® 10 SG, Siemens Healthcare Diagnostics, Frimley, UK).
Pregnancy[3]	hCG. If there is a clinically significant, positive result, urine will be sent to the AKCL for confirmation.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
[1]Conjugated bilirubin was reported only when total bilirubin was outside the reference range. [2]After 4-hours fasting. [3]Pregnancy test for women of childbearing potential was performed at screening and if pregnancy was suspected during the study.		

9.5.2. Pharmacodynamic assessments

9.5.1. Standardized facial photography

A standardized set of 3 facial photos (front, left, right) were taken every study visit by Canfield Visia CR according to SOP CGEVISIA. Furthermore, patients were instructed to take daily selfies with a validated mobile app.

9.5.2. E-diary

All subjects were asked to fill in an e-diary twice daily from the first dose administration to EOT (Day 28). For this purpose subjects made use of an app. The app was intended to be used as e-diary in order to monitor and promote treatment compliance in the clinical trial. The e-diary app captures data of the treatment application (twice daily) by means of a photo, records the time and the date of the photo. Furthermore, daily selfies (once daily) were made. When the photos were taken and data was entered into the app, the electronic data was sent to the CHDR SQL server via a secured connection (SSL encryption). At EOT (day 28) subjects will fill in an evaluation form. The evaluation form is provided in English in appendix A in the protocol and in Dutch as separate document in the submission dossier (F1).

9.5.3. Sebum measurements

Sebum excretion were measured every study visit by Sebumeter® according to SOP CGESEBUM. The measurement were repeated for 3 times and the average was used for the analysis.

9.5.4. Perfusion by LSCI

Cutaneous microcirculation was assessed using the laser speckle imager (LSCI; PeriCam PSI System, Perimed Jäfälla, Sweden), and was done according to the SOP CGELSCI every study visit. Measurements were performed in a temperature-controlled room with a temperature around 22°C. The subject had to get accommodated to the room temperature for a minimum of 15 minutes prior to testing. If no suitable area could be identified, the measurement was not performed and data was entered as missing. Analysis of the data was done according to the pertaining SOP.

9.5.5. Morphology by OCT

Skin morphology was assessed by optical coherence tomography at every study visit according to SOP CGEOCT. Optical coherence tomography uses reflected light returning from skin tissue to create an image of the skin and 2 mm below the skin. The visualization can be done because different skin structures reflect light in a different way and can therefore be distinguished. Optical coherence tomography is similar to ultrasound however instead of sound it uses light refraction to visualize tissue.

9.5.6. Skin punch biopsies

9.5.6.1. Sample collection

Two-millimetre punch biopsies were taken at day 0 and day 28 from a papule or pustule. With this size, the risk of scarring is minimal. The most favourable facial biopsy location was chosen and discussed with the patient, e.g. in the hairline or jawline. Moreover, at day 0 a biopsy was taken from non-lesional non-facial skin (upper back) as healthy control. The biopsy procedure was performed according to Standard Operating Procedure for skin punch biopsies with a local anaesthetics (CGESPBIO). The biopsies were placed in RNAlater medium directly after harvest of the biopsy and stored at 4°C. The biopsy sample were analysed at the Immunology Laboratory at Erasmus MC, Rotterdam, The Netherlands for local biomarkers.

9.5.6.2. Biomarker sequencing

RNA extraction and quantitative PCR were performed for a subset of immunomodulatory biomarkers (IL-1b, IL-1a, TNF-a, IL-6, IL-8, IL-10, IL-17a, IFN-g, ICAM1).

9.5.7. Transdermal Analysis Patch (TAP)

Skin biomarkers were measured pre-dose and after 7, 14, 21, 28, and 42 days by TAP (FibroTx, Estonia). TAP consists of a multiplex capture-antibody micro-array that is supported by a dermal adhesive bandage for fixture to skin. When TAP was applied to skin and left on for 20 minutes, the antibodies printed on the micro-array captured biomarkers from skin through immune recognition. Biomarkers (IL-1a, IL-1b, TNF-a, IL-8, IL-6, IL-17) captured from skin by TAP were qualitatively and quantitatively analyzed by spot-ELISA by a specific TAP analyzer. Each TAP kit was labelled and stored at 4 ºC overnight and after that frozen at -20ºC until shipment.

9.5.8. Skin microbiome

9.5.8.1. Sample collection

Collection of skin culture samples is a non-invasive procedure where a sterile polyester flock tip (Puritan Sterile Polyester Tipped Applicators REF 25-3206-H 20MM) per site is passed along the

surface of predefined lesional (papule of pustule) and non-lesional skin according to SOP CGESWAB. This was performed every study visit. The skin swab was placed in a 2 ml lysis tube (REF ZY-R1103, Zymo Research) containing DNA/RNA shield to stabilize and preserve the DNA. The tubes were stored in the freezer at -80°C, and shipped to BaseClear at the end of the study. The microbiology samples were analyzed at BaseClear Laboratories, The Netherlands.

9.5.8.2. DNA extraction

The DNA extraction was performed using adapted DNA extraction method based on the Zymo Research fecal DNA extraction methodology. In short, the swabs in the 2 ml lysis tubes underwent a mechanical shearing procedure that lyses cells from micro-organisms captured by the swab. DNA was eluted in a volume of 50µl.

9.5.8.3. Microbiome analysis

After DNA extraction, the variable regions 3 and 4 of the 16S rRNA gene were amplified giving an amplicon of around 450 base pairs. This amplicon was analyzed by capillary systems using standard protocols, to confirm successful amplification of a PCR fragment of the expected size. PCR products were cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small a-specific PCR products and the purified PCR products are quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies). Subsequently, the PCR products were diluted and an equal mass for each sample was used as template in a 2nd PCR where sample specific barcodes (Index primers (Nextera). XT Index kit) were appended to the PCR products using a 2nd PCR with a limited number of cycles. Following an PCR purification step as above, the PCR products were equimolarly pooled and sequenced on the Illumina MiSeq platform using the MiSeq v3 sequencing kit generating paired end 300 nt sequence reads. De-multiplexed FASTQ files were generated as output. Paired end reads were assembly into 'pseudoreads' followed by removal of chimeric sequences and taxonomic classification. In addition, sequence reads were clustered together based on sequence similarity.

9.5.9. Microbiological culture for p. acnes quantification

Swabs of predefined lesional (papule of pustule) and non-lesional skin were taken with a sterile cotton swab according to SOP CGESWAB. The skin swab was shipped to the Microbiology department of the Alrijne Hospital, Leiden, The Netherlands for cultures and susceptibility tests. Colony numbers (colony forming units – CFU) and minimal inhibitory concentrations (MIC) were reported. Moreover, in order to study P. acnes in the pilosebaceous unit a comedo extraction was performed and the sebum was cultured for P. acnes. Comedo extraction was performed if applicable (i.e. if the patient has comedones) and according to SOP CGECOMED.

9.5.10. Faecal microbiome

9.5.10.1. Sample collection

Faecal samples were collected at home. Subjects used a 'faeces catcher' in their toilet and afterwards used a cotton swab to transfer a scoop of faeces to a 2 ml lysis tube (REF ZY-R1103, Zymo Research) containing DNA/RNA shield to stabilize and preserve the DNA. The tube was then placed in a plastic protection bag and placed in an envelope. The samples were sent to BaseClear Laboratories, The Netherlands for analysis.

Faeces questionnaire
Pre-dose and at EOT patients were asked to fill in a faeces questionnaire in order to get insight in the patients' dietary behaviour and bowel habits at the time of faeces sample collection. The Dutch questionnaire is provided in the submission dossier under F1.

9.5.10.2.DNA extraction
The DNA extraction was performed using adapted DNA extraction method based on the Zymo Research fecal DNA extraction methodology. In short, the swabs in the 2 ml lysis tubes underwent a mechanical shearing procedure that lyses cells from micro-organisms captured by the swab. DNA was eluted in in a volume of 50µl.

9.5.10.3.Microbiome analysis
After DNA extraction, the variable regions 3 and 4 of the 16S rRNA gene were amplified giving an amplicon of around 450 base pairs. This amplicon was analyzed by capillary systems using standard protocols, to confirm successful amplification of a PCR fragment of the expected size. PCR products were cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small a-specific PCR products and the purified PCR products were quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies). Subsequently, the PCR products were diluted and an equal mass for each sample waa used as template in a 2nd PCR where sample specific barcodes (Index primers (Nextera). XT Index kit) were appended to the PCR products using a 2nd PCR with a limited number of cycles. Following a PCR purification step as above, the PCR products were equimolarly pooled and sequenced on the Illumina MiSeq platform using the MiSeq v3 sequencing kit generating paired end 300 nt sequence reads. De-multiplexed FASTQ files were generated as output. Paired end reads were assembled into 'pseudoreads' followed by removal of chimeric sequences and taxonomic classification. In addition, sequence reads were clustered together based on sequence similarity.

9.5.11. Pharmacokinetic assessments
Not applicable.

9.5.12. Appropriateness of Measurements
In this study, standard assessments were used to assess tolerability and safety of the study drug. A battery of validated or exploratory objective and subjective PD tests was included to assess the pharmacodynamic effect of the study drugs.

9.6. Quality Assurance

The study was conducted in compliance with the pertaining CHDR Standard Operating Procedures and CHDR's QA procedures.

Before enrollment of any subject, the investigators reviewed the submission dossier including the protocol.

All appropriate CHDR personnel was trained for study specific procedures according the applicable SOPs

Prior to the commencement of the study, items to be included in the clinical database were determined and suitable documents as electronic data collection forms were created to ensure the appropriate collection of all required data. All electronic CRFs (eCRFs) were reviewed by the clinic staff and a Data Manager. The study site reviewed source data according to GCP and internal procedures to ensure their accuracy, completeness and verifiability.

The accredited laboratories were used to ensure that laboratory and bio-analytical samples were handled in accordance with GLP and to ensure consistency of analysis and reporting of results.

9.7. Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1. Statistical and Analytical Plans

Continuous demographic variables (e.g., age, height, weight, BMI) were summarized by descriptive statistics (n, mean, SD, median, Min, Max). Qualitative demographic characteristics (sex, race/ethnicity) were summarized by counts and percentages.

The AE coding dictionary for this study was the Medical Dictionary for Regulatory Activities (MedDRA). It was used to summarize AEs by primary system organ class (SOC) and preferred term (PT). All adverse events were displayed in listings.

A treatment-emergent adverse event (TEAE) was defined as an adverse event observed after starting administration of the specific treatment, or up to 5 days (96 hours) after study drug administration. If a subject experiences an event both prior to and after starting administration of a treatment, the event was considered a TEAE (of the treatment) only if it had worsened in severity (i.e., it is reported with a new start date) after starting administration of the specific treatment, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period were summarized.The number of subjects with treatment emergent AEs were summarized by cohort, MedDRA, SOC, PT and drug relatedness.

The final analysis was preceded by an administrative blind data review which consisted of individual graphs per visit by time of all pharmacodynamic measurements by time. The graphs were used to detect outliers and measurements unsuitable for analysis.

The PD parameters were listed by treatment, subject, visit and time. Individual graphs by time were be generated. All PD endpoints were summarised (n, mean, SD, SEM, median, Min and Max values) by treatment and time, and were presented graphically as mean over time, with standard deviation as error bars. Both nominal results, and log-transformed results and change from baseline were utilized in all data summaries. All categorical PD endpoints were summarised by frequencies.
Parameters were initially analyzed without transformation, but if the data suggested otherwise, log-transformation was applied. Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change.
To establish whether significant treatment effects were detected on the repeatedly measured PD and efficacy parameters, each parameter was analyzed with a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors and subject as random factor and the (average) baseline measurement as covariate.
The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters will be estimated using the restricted maximum likelihood method.
Biopsy parameters were analyzed with a mixed model analysis of covariance (ANCOVA) with treatment as fixed factor and the baseline measurement as covariate. The general treatment effect and specific contrasts were reported with the estimated difference and the 95% confidence interval, the least square mean estimates and the p-value. Graphs of the Least Squares Means (LSM) estimates over time by treatment were presented with 95% confidence intervals as error bars, as well as change from baseline LSM estimates.
The following contrasts were calculated within the model overall and for EOT and EOS:

- Erythromycin - Placebo
- Clindamycin – Placebo
- Erythromycin – Clindamycin

The following additional metrics were calculated for each treatment:

- Proportion of patients who achieved lesion count 0 at day 28 and day 42 (EOT/EOS)
- Proportion of patients who achieved IGA 0 to 1 at day 28 and 42 (EOT/EOS)

9.7.2. Determination of Sample Size

This was an exploratory study; therefore, the sample size was not based on statistical considerations.

9.8. Changes in the Conduct of the Study or Planned Analyses

9.8.1. Changes in Conduct of the Study – protocol amendments

The original protocol was dated 07Dec2017. A total of 1 amendment was written in addition to the study protocol. A summary of the main items of the amendment are described below.

Change	Rationale	Justification & Classification	Changed Document(s), Section
Screening period extended from 14 days to 21 days	Screening period was too short to obtain all screening data to include patients.	Substantial; increased study duration	- ABR - Protocol, Section 3.1 - SIS & ICF - Advertentiedocument
Comedo extraction for P. acnes culture is added	P. acnes occurs not only on the skin surface but also in the pilosebaceous unit.	Substantial; addition pharmacodynamic endpoint	- ABR - Protocol, Section 7.3.8 - SIS & ICF
Faeces questionnaire added	Provides insight in bowel habits and diet.	Substantial; additional patient questionnaire	- ABR - Protocol, Section 7.4.2 - SIS & ICF

Table 2. Item listing as per amendment of the clinical trial application. Source: Appenix16.1.1

9.8.2. Changes in Data

Not applicable.

9.8.3. Changes in the Planned Analysis

No changes in the planned analysis.

10. Subjects

10.1. Disposition of Subjects

Hunderd (100) subjects signed the informed consent form and underwent a medical screening. Seventy (70) subjects were excluded based on the inclusion and exclusion criteria. A total of thirty (30) subjects were enrolled in the study.

Thirty (30) subjects completed the treatment and follow-up period per protocol as presented in Table 3.

Table 3. Subject disposition per treatment (Safety population).

	Erythromycin	Clindamycin	Placebo
Subjects dosed	10 (100%)	10 (100%)	10 (100%)
Subjects completed	10 (100%)	10 (100%)	10 (100%)

10.2. Protocol Deviations

Protocol deviations were identified based on conditions related to the categories below:

- Protocol entry criteria
- Forbidden concomitant medications
- Missing evaluations for relevant endpoints
- Other protocol deviations occurring during study conduct.

Major protocol deviations were identified before the study closure, and listed where appropriate. The following overview summarizes the protocol deviations considered most important and/or (clinically) most relevant by the investigators. Overall, no deviation impacted subject safety or was considered to have a significant impact on the study results.

Subject number	Protocol deviation	Classification
7	Faeces sample of day 28 EOT is missing	Minor
11	Predose faeces sample is missing	Minor
	EOT visit performed outside 28+/- 3 time window (performed at 28 + 5 days)	Major
20	EOT visit performed outside 28+/- 3 time window (performed at 28 – 5 days)	Major
21	Predose faeces sample is missing	Minor

Table 4. Protocol deviations.

Occasionally procedures were missing or not performed in the allowed time window. Explanatory notes were added to the CRF in these cases. These events are not described in the table and were not considered to have had any consequences on study outcome.

11. Pharmacokinetic/Pharmacodynamic Evaluation

11.1. Data Sets Analysed

Data of all subjects participating in the study were included in the analyses if the data could meaningfully contribute to the objectives of the study.

- Safety population - all subjects who were validated (randomized) and received at least one topical administration of study medication.
- Intent-to-treat Population – All subjects who received at least two topical administrations of study medication.
- Clinical Evaluable Population – All subjects who completed three weeks of treatment (at least 42 administration) and the EOT visit and have no major protocol deviations

11.2. Demographics

The demographic are summarized in the following table:

Summary of subject demographics

	All subjects	Clindamycin	Erythromycin	Placebo
Age (years)				
N	30	10	10	10
Mean (SD)	20.4 (2.2)	20.9 (2.6)	20.6 (2.4)	19.6 (1.6)
Median	20	21	20	20
Min, Max	18, 25	18, 25	18, 25	18, 22
Height (cm)				
N	30	10	10	10
Mean (SD)	177.84 (11.28)	173.97 (12.89)	178.95 (10.89)	180.60 (9.95)
Median	177.6	175.1	180.1	178.1
Min, Max	153.5, 197.2	153.5, 195.3	160.4, 197.2	168.5, 194.1
Weight (kg)				
N	30	10	10	10
Mean (SD)	68.597 (8.990)	65.725 (11.363)	71.360 (9.965)	68.705 (3.788)
Median	69.48	63.75	70.55	69.20
Min, Max	49.65, 88.00	49.65, 80.60	51.30, 88.00	63.15, 76.00
BMI (kg/m2)				
N	30	10	10	10
Mean (SD)	21.77 (3.03)	21.79 (3.59)	22.31 (3.25)	21.22 (2.34)
Median	20.6	20.8	20.6	20.9
Min, Max	16.9, 29.5	16.9, 29.5	19.6, 28.4	18.7, 26.6
Sex				
Female	14 (46.7%)	6 (60.0%)	5 (50.0%)	3 (30.0%)
Male	16 (53.3%)	4 (40.0%)	5 (50.0%)	7 (70.0%)
Race				
Mixed	1 (3.3%)	0 (0%)	0 (0%)	1 (10.0%)
White	29 (96.7%)	10 (100%)	10 (100%)	9 (90.0%)

Table 4. Demographics
Source data: Safety Report page 111 of 115.

No important differences were noted between treatment groups.

11.3. Baseline characteristics

Although subjects were randomized over the three treatment groups, not all three treatment groups were identical in terms of disease severity at baseline (table 5.): the placebo group had the highest mean total inflammatory lesion count, followed by clindamycin and then erythromycin. This was also reflected in the IGA: the placebo group had the most patients with category ‘moderate’

followed by clindamycin and erythromycin.

Treatment group	Placebo N=10	Clindamycin N=10	Erythromycin N=10
Total inflammatory lesion count (mean ± SD)	20.6 ± 7.3	17.1 ± 7.5	12.3 ± 6.0
IGA (N subjects)	Mild: 6 (60%) Moderate: 4 (40%)	Mild: 7 (70%) Moderate: 3 (30%)	Mild: 9 (90%) Moderate: 1 (10%)
PGA (N subjects)	Mild: 0 (0%) Moderate: 4 (40%) Severe: 5 (50%) Very severe: 1 (10%)	Mild: 0 (0%) Moderate: 5 (50%) Severe: 5 (50%)	Mild: 1 (10%) Moderate: 8 (80%) Severe: 1 (10%)

Table 5: Baseline characteristics
Source data: PD report page 321 and 338 - 339 of 404.

11.4. Measurements of Treatment Compliance

Treatment compliance was monitored using a validated app. Treatment compliance was high (>90%) in all three treatment groups (Safety Report page 112 of 115).

11.5. Analysis of Efficacy/Pharmacodynamics

The Efficacy/Pharmacodynamics analyses were conducted after completion of the study. All pharmacodynamics results are provided in the Statistics Report. Relevant results are included in this report.

11.5.1. Inflammatory lesion count

The inflammatory lesion count (IFLC) was performed at every visit. In figure 1 the change from baseline the IFCL over time per treatment is presented. All treatments led to a reduction in inflammatory lesion count (placebo CFB -2.6 at EOT, clindamycin CFB -5.7 at EOT, and erythromycin CFB -5.2 at EOT). Clindamycin and erythromycin did not yield a statistical significant lesion count reduction compared to placebo at EOT (p = 0.3421 and p = 0.4329 respectively).

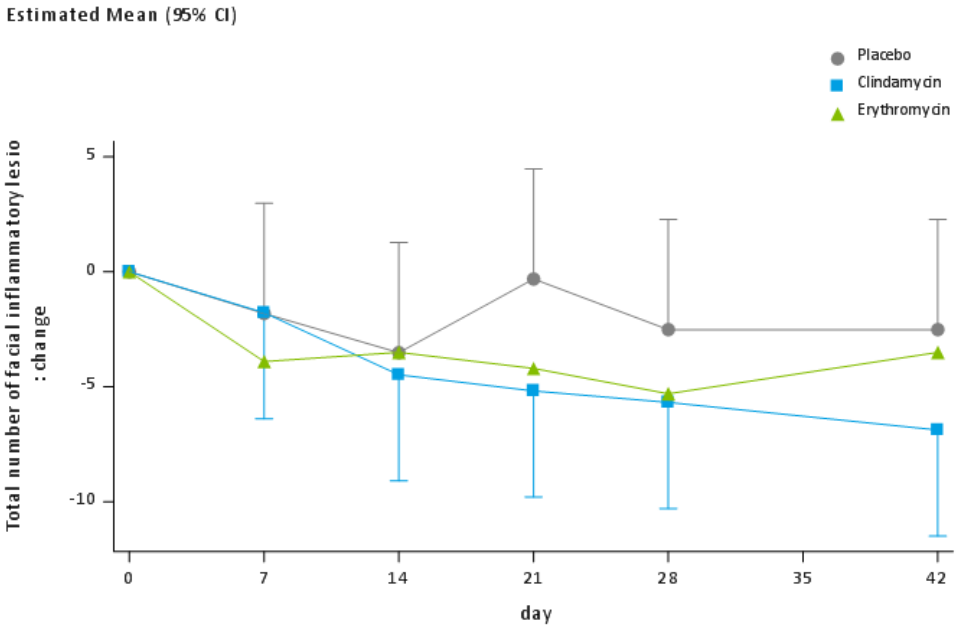

Figure 1: Inflammatory Lesion Count LSM change from baseline, source: PD report page 356 of 404.

11.5.2. Investigator global assessment

The investigator global assessment (IGA) was performed at every visit. In the placebo group 4/10 patients reached a score of clear or almost clear. In the clindamycin group 2/10 patients reached clear or almost clear, and in the erythromycin group 5/10 patients reached clear or almost clear. No statistical significance was found between any of the groups (using Fisher's Exact Test). Source: PD report page 393 – 395 of 404.

11.5.3. Patient global assessment

The patient global assessment (PGA) was performed at day 0 and at day 28 (EOT). All three groups showed improvement on the PGA, but only patients treated with erythromycin reached almost clear (4/10). Erythromycin performed significantly better than placebo and clindamycin (p = 0.0136 and P = 0.0469, Fisher's Exact Test). There was no statistically significant difference between clindamycin and placebo (p = 0.1703).

	Day	Clear	Almost clear	Mild	Moderate	Severe	Very severe
Placebo	0	-	-	-	4	5	1
	28	-	-	2	5	3	-
Clindamycin	0	-	-	-	5	5	-
	28	-	-	5	5	-	-
Erythromycin	0	-	-	1	8	1	-
	28	-	4	5	1	-	-

Table 6: PGA, source data: PD report page 339 of 404.

11.5.4. Local biomarkers by TAP

No notable changes were observed in the skin surface biomarkers as assessed by TAP. Likewise no significant differences among treatments were found.

11.5.5. Biopsy biomarkers

Predose and at end of treatment (day 28) a 2mm skin punch biopsy was taken and analyzed for inflammatory biomarkers (IL-1b, IL-1a, TNF-a, IL-6, IL-8, IL-10, IL-17a, IFN-g, ICAM1). No significant differences were observed pre and post treatment. Source: PD report page 159 – 183 of 188.

11.5.6. LSCI

LSCI measurements were performed at every visit. Although all treatments show a decrease in skin perfusion of lesional skin (corrected for non lesional perfusion levels), no significant differences were observed between placebo and active treatment.

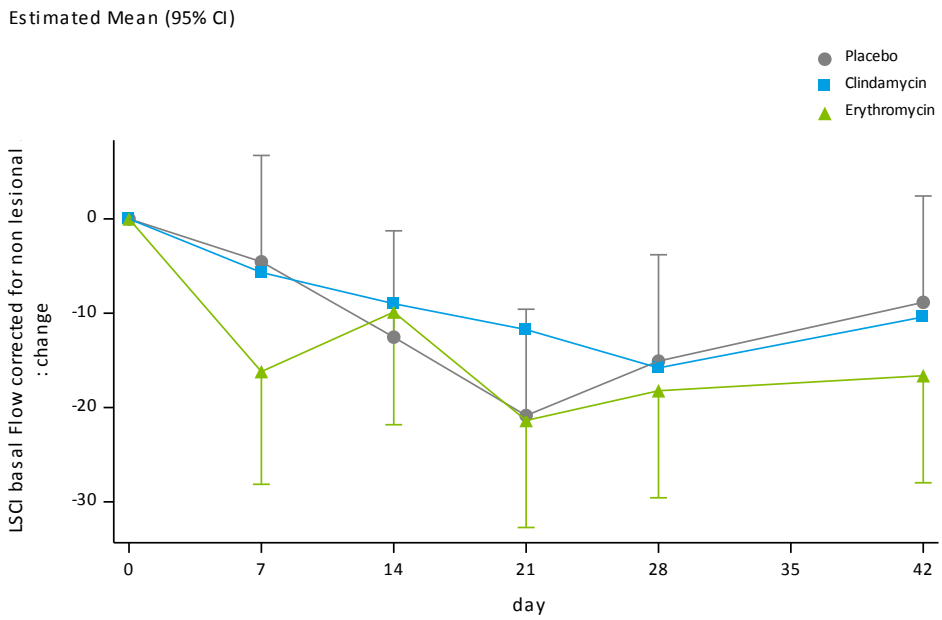

Figure 2: LSCI of lesional skin over time corrected for a control region, source PD report page 158 of 188

11.5.7. P. acnes in culture

P. acnes in culture was shown to be highly variable among subjects and over time (source: PD report page 342 of 404). All treatments including placebo led to a reduction of P. acnes in culture at day 28 EOT. However, the reduction in P. acnes over time was most noticeable and consistent in the clindamycin group and was found significant when compared to placebo (p = 0.0295).

11.5.8. Minimum inhibitory concentration for erythromycin and clindamycin

From P. acnes that was cultured from skin swabs, the minimum inhibitory concentration (MIC) for erythromycin and clindamycin was determined. The MIC for erythromycin and clindamycin in all three treatment groups was variable over time, and no clear treatment effect could be observed (source: PD report page 344 – 353 of 404).

11.5.9. Microbiome

In general lesional and non-lesional skin were characterized by a high percentage of cutibacteria and staphylococci. Clindamycin and erythromycin showed a trend in decreasing the relative abundance of staphylococci after 28 days. No treatment led to a clear decrease in the relative abundance of cutibacteria. Clindamycin led to a slight increase of cutibacteria relative abundance, compared to erythromycin and placebo. Source: Microbiome report.

11.5.10. Statistical/Analytical issues

None.

11.5.10.1. Adjustments For Covariates

For the repeated measures analysis on efficacy endpoints, the effect of treatment was tested, adjusting for day and Baseline value, where treatment and day were categorical fixed factors and Baseline value was a continuous covariate.

11.5.10.2. Handling Of Dropouts Or Missing Data

Missing data were not imputed before summary or analysis. Data below the limit of quantification

(LOQ) was set to 50% of the LOQ for summary purposes only, and was entered as missing in the analysis.

11.5.10.3. Interim Analyses And Data Monitoring

No interim analysis was performed. Data monitoring was performed to CHDR standard operating procedures and according to the study specific Data Monitoring Plan.

11.5.10.4. Multicentre Studies

Not applicable.

11.5.10.5. Multiple Comparison/Multiplicity

There was no adjustment for multiplicity due to the exploratory nature of the study.

11.5.10.6. Use Of An "Efficacy Subset" Of Patients

Not applicable.

11.5.10.7. Active-Control Studies Intended To Show Equivalence

Not applicable.

11.5.10.8. Examination Of Subgroups

Not applicable.

11.5.11. Tabulation of individual response data

Listings of individual response data are provided in the Statistics Report

11.5.12. Drug dose, Drug concentration, and relationships to response

Not applicable.

11.5.13. Drug-drug and drug-disease interactions

Not applicable.

11.5.14. By-patient displays

Not applicable.

11.5.15. Pharmacodynamic/Pharmacokinetic conclusions

12. Safety Evaluation

12.1. Extent of Exposure

In total 20 subjects received active topical treatment with either erythromycin 4% or clindamycin 1% twice a day. Per treatment group the following extent of exposure was obtained by photo-documentation with the mobile e-diary app: at least 49 (average 52.9) and 43 (average 51.7) applications out of 56 for clindamycin and erythromycin respectively. The placebo was at least 51 (average 52.6) times applied by 10 subjects. Administrations were performed twice a day for 28 consecutive days and only sporadically subjects did not comply with the application as per protocol. The low minimum of applications in the erythromycin group can be explained by a single subject (S20) who scheduled his end of study visit several days earlier due to personal reasons. The average daily dose applied per application was 386.8mg for clindamycin, 498.0mg for clindamycin, and 2919.5mg for placebo.

12.2. Adverse Events (AEs)

12.2.1. Brief Summary of Adverse Events

One serious adverse event (SAE), unrelated to the study drug, occurred during this study: subject 20 suffered from a concussion after a skiing accident. Treatment-emergent AEs by system organ class and Medical Dictionary for Regulatory Activities (MedDRA) preferred term are displayed on page 114 of 114 of the Safety Report. All but two of the TEAEs were considered as unrelated (n = 28) to treatment. One TEAE was probably related and was an application site AE. And one TEAE was unlikely related to the treatment (eyelid dermatitis). All TEAEs were mild (n = 26) or moderate (n = 4).

12.2.2. Display of Treatment Emergent Adverse Events (AE's)

A display of the Treatment Emergent Adverse Events is provided in the Safety Report, page 114 and 115 of 115.

12.2.3. Listing of Adverse Events by Subject

Listings of adverse events by subject are provided in the Safety Report.

12.3. DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

12.3.1. Listing of Deaths, other Serious Adverse Events and Other Significant Adverse Events

12.3.2. Deaths

Not applicable

12.3.3. Other Serious Adverse Events

As stated in 12.2.1 one serious adverse event occurred which was not related to study treatment.

12.3.4. Other Significant Adverse Events

Not applicable.

12.4. Vital Signs and Physical Findings and Other Observations Related to Safety

Not applicable.

12.4.1.1. ECG recordings

Not applicable.

12.5. Safety Conclusions

No clinically significant changes or TEAEs related to the study drug occurred during the study.

13. Discussion And Overall Conclusions

Efficacy/pharmacodynamics
In the clinical evaluation of the acne vulgaris as assessed by IFLC and IGA, all treatments including placebo led to a reduction of the IFCL or an improvement of the IGA, however, erythromycin and clindamycin did not perform statistically significant better than placebo. For the patient reported outcome, measured by the patient global assessment (PGA), erythromycin performed significantly better than placebo and clindamycin ($p = 0.0136$ and $P = 0.0469$, respectively), no statistically significant difference was found between clindamycin and placebo ($p = 0.1703$). No substantial differences were observed in sebum measurements by sebumeter, LSCI, TAP, and biopsy biomarkers, when comparing active treatment to placebo. In microbiological endpoints, clindamycin showed the strongest effect against P. acnes quantified by culture, which was significant when compared to placebo ($p = 0.0295$). The data of the microbiome demonstrated a high degree of variability between subjects. In general the presence of the genus cutibacteria or staphylococci dominated lesional skin. No clear treatment effect could be observed on the reduction of either cutibacteria or staphylococci. The composition of the gut microbiome at day 28 was comparable to predose, hence no treatment effect was observed.

Safety and Tolerability
This study showed that the two active treatments and placebo were well tolerated by the subjects. No treatment related study discontinuation or treatment related SAE occurred. The AE profile was comparable for all subjects across treatment groups. The most frequent occurring treatment-emergent AEs were headache/migraine, nasopharyngitis and influenza like illness. All TEAEs were of mild or moderate severity and self-limiting.

Adherence / exposure
In total, 30 subjects were included in the randomized ITT population. 20 subjects were randomized to one of the two active treatment groups, i.e. erythromycin or clindamycin and 10 subjects were randomized to placebo. Administrations were performed on consecutive days and only sporadically subjects did not comply to the twice daily treatment regimen. All dose administrations at home were recorded via a mobile app. The average daily dose applied per treatment was 386.8mg for clindamycin, 498.0mg for clindamycin, and 2919.5mg for placebo. A possible explanation for the high amount of placebo used is that the tubes containing the placebo, which consisted of an ethanol 70% solution, was not designed for facial application and subjects had to use cotton pads to apply the solution, which can lead to spilling or saturation of the cotton pads.

Safety results:
The results from the current study show that clindamycin and erythromycin are safe and well tolerated for BID administration up to 28 days to subjects with mild to moderate acne vulgaris. The overall incidence of TEAEs was similar among subjects receiving active treatment and vehicle. No clinically significant changes were attributable to treatment with clindamycin or erythromycin.

Overall conclusion:
The aim of this study was to extensively characterize the effect of topically applied erythromycin and clindamycin in acne vulgaris patients. Although erythromycin and clindamycin reduced the total inflammatory lesion count and IGA, both active treatments did not perform significantly better than placebo. No other pharmacological effect of erythromycin or clindamycin was found other than the reduction of P. acnes in culture by clindamycin. This study did not provide clear evidence for other mechanisms of action other than the antimicrobial effect, which was most notable for clindamycin.

14. References

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15. Appendices

15.1. Safety report

15.2. Microbiome report

15.3. Statistics report

The following documents are part of the clinical study file and available on request.:

- Protocol and protocol amendments
- Sample case report form (unique pages only)
- List of IECs or IRBs (plus the name of the committee Chair if required by the regulatory authority)
- Representative written information for patient and sample consent forms
- List and description of investigators and other important participants in the study, including CVs or equivalent summaries of training and experience relevant to the performance of the clinical study
- Listing of patients receiving test drug(s)/investigational product(s) from specific batches, where more than one batch was used.
- Randomization scheme and codes (patient identification and treatment assigned)
- Audit certificates (if available)
- Documentation of statistical methods
- Excluded Subjects / Data Form
- Documentation of laboratories
- Publications based on the study