STATISTICAL ANALYSIS PLAN

A RANDOMISED, EVALUATOR BLINDED, WITHIN SUBJECT, SINGLE CENTRE EVALUATION OF THE VASOCONSTRICTION PROPERTIES OF MC2-01 CREAM, COMPARED TO 5 OTHER CORTICOSTEROIDS IN HEALTHY SUBJECTS

SPONSOR N°	MC2-01-C4
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VERSION HISTORY

Version	Date of Issue	Reason for change
Version #1.0	30-Oct-2018	Anne-Claire Cathelineau issued the first version.
Version #2.0	05-Dec-2018	Anne-Claire Cathelineau implemented the changes required by Irene Sandholdt and Morten Præstegaard and issued the second version.

SAP APPROVALS

The following persons have approved this Statistical Analysis Plan by manually signing in the Tables below:

On behalf of DDS	
Name & Title	Date & Signature
Johan SELMER, MD VP Medical Affairs	

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Table of contents

List of Tables

List of Figures

1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADR	Adverse Drug Reaction
AE	Adverse Event
BDP	Betamethasone dipropionate
CPCAD	Centre de Pharmacologie Clinique Appliquée à la Dermatologie = name of the trial site
FAS	Full Analysis Set
IMP	Investigational Medicinal Product
IP	Investigational Product
IRB	Institutional Review Board
LOCF	Last Observation Carried Forward
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
N	Number of subjects
PP	Per Protocol
PT	Preferred Term
SAE	Serious Adverse Event
SD	Standard Deviation
SOC	System Organ Class

2 STUDY OBJECTIVES

2.1 Primary study objective

The objective of this trial is to compare the vasoconstriction potential (skin blanching effect) of MC2-01 cream with Clobex® 0.05% (clobetasol propionate) lotion, Betamethasone Dipropionate (Augmented) cream, Triamcinolone Acetonide 0.1% (triamcinolone acetonide) cream, Locoid® 0.1% (hydrocortisone butyrate) cream, Desowen® 0.05% (desonide) cream and MC2-01 vehicle using the human skin blanching test (McKenzie-Stoughton's test).

2.2 Secondary study objectives

The objective of this trial is to assess the local tolerability of MC2-01 cream and the matching vehicle.

3 STUDY DESIGN

3.1 Study type

This is a single center, investigator blinded, active - and vehicle-controlled, single application, intra-individual comparisons on mini-test sites (n=7), involving 36 healthy subjects meeting specific inclusion/exclusion criteria.

Eligible subjects will receive a single application of each of the following:

- **MC2-01 cream** containing calcipotriol and betamethasone dipropionate, w/w 0.005% / 0.064%.
- **MC2-01 cream vehicle**
- **Clobex® lotion** containing clobetasol propionate 0.05%
- **Betamethasone Dipropionate (Augmented) cream**
- **Triamcinolone Acetonide** containing triamcinolone acetonide 0.1%
- **Locoid® cream** containing hydrocortisone-17-butyrate 0.1%
- **Desowen® cream** containing desonide 0.05%

The trial will employ an unoccluded McKenzie-Stoughton trial design based on topical application of a corticosteroid-containing formulation for a period of 16 hours in healthy human volunteers followed by visual estimation of the degree of blanching on a multiple unit scale (0 to 4) by two independent trained observers 2 hours after the end of the application period. The trial will be conducted with three successive individual phases:

- **Screening phase** with a screening blanching test.
- **Test phase**
- **Follow-up phase**. For subjects with a local tolerability scores >0 at Day 2 or if a non-serious adverse event classified as reasonably or possibly related to the investigational products or a Serious Adverse Event (SAE) is ongoing once a subject has completed the test phase (Day 2), a follow-up visit/contact will take place up to 14 (±2) days after the end of test phase (Day 2). SAEs will be monitored until the

Investigator and Sponsor agree that the event is satisfactorily resolved; telephone contact or visit according to the investigator's discretion.

3.2 Study duration

Clinical trial participation for each subject is of 2 days plus the screening phase and a possible follow-up phase.

3.3 Study schedule

Table 1 –Study flow chart

Trial Phases:	**Screening**	**Test**		**Follow-up(2)**
Trial Days :	**Day-15 to Day-1**	**Day1**	**Day2**	**Day15 (±2days)**
Informed Consent Form	☑			
Demographics/Medical history	☑			
Previous treatments/procedures	☑			
Inclusion/exclusion criteria	☑	☑		
Selection of test sites		☑		
Physical exam, vital signs	☑			
Urine pregnancy test(1)	☑	☑		
Screening test for blanching response	☑			
Randomization		☑		
Local tolerance(2)			☑	☑
Application of IMPs		☑		
Product excess removal(3)			☑	
Measurement of skin blanching by 2 trained evaluators(4)			☑	
Concomitant treatments	☑	☑	☑	☑
Adverse events	☑	☑	☑	☑
End of treatment			☑	
End of study				☑

1: For female subject of childbearing potential. Result must be negative to continue the trial.

2: For subjects with a local tolerability scores >0 at Day 2 or if a non-serious adverse event classified as reasonably or possibly related to the investigational products or an SAE is ongoing once a subject has completed the test phase (Day 2), a follow-up visit/contact (telephone contact or visit according to the investigator's discretion) will take place up to 14 (±2) days after the end of test phase (Day 2). AEs and SAEs assessed as reasonably or possibly related to the trial medication must be followed until resolved or until the medical condition of the subject is stable.

3: 16 hours +/- 30 min after IMP application

4: 2 hours +/- 30 min after IMP removal

3.4 Population characteristics

Approximately 60 subjects will be pretested in order to have 36 subjects randomized and at least 30 subjects completed.

3.5 Study treatments

Table 2 – Study treatments

	Investigational product 1	**Investigational product 2**	**Reference product 1**	**Reference product 2**	**Reference product 3**	**Reference product 4**	**Reference product 5**
Trade Name or Equivalent	MC2-01	MC2-01 vehicle	Clobex® (Galderma Labs LP)	Betamethasone Dipropionate (Augmented) (Taro Pharmaceuticals)	Triamcinolone Acetonide (Ascend Laboratories LLC)	Locoid® (Precision Dermatology)	Desowen® (Galderma Labs LP)
Name of Drug Substance	Calcipotriol + Betamethasone dipropionate	NA	Clobetasol propionate	Betamethasone dipropionate	Triamcinolone acetonide	Hydrocortisone butyrate	Desonide
Pharmaceutical Form	cream	cream	lotion	cream	cream	cream	cream
Concentration	0.005% + 0.064%	NA	0.05%	0.064% w/w (equivalent to 0.05% betamethasone)	0.1%	0.1%	0.05%
Package	60 g aluminium tubes	60 g aluminium tubes	Bottle 59ml	15 g aluminium tubes	30 g tube	30 g aluminium tube	60 g tube
Storage conditions	Store between 2-8°C between treatment days – May be store at room temperature below 25°C at the day of treatment application		Store below 30°C	Do not store above 25°C	Store at 20-25°C	Do not store above 25°C. Do not refrigerate or freeze	Store between 2° and 30°C
Dosage (per site)	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL
Route	Topical (Cutaneous to the skin)						
Dose Regimen	A single application of each IMP						
Duration of administration	Contact duration of 16 hours (+/- 30 min)						
Location of Treated Area	Area of 2.2 cm diameter						

4 STUDY EVALUATION

4.1 Visual assessment of skin blanching

Visual assessment of blanching will be made independently by two trained readers on Day 2 (2 hours after removal of products, following 16 hours application time) according to the following scale:

Table 3 - Skin blanching visual scale

Score*	Description
0	No change in skin color
1	Slight (barely visible) blanching
2	Obvious blanching
3	Intense blanching
4	Blanching judged to be maximal

*Intermediate scores (half units) may be used when needed (e.g. 0.5 for a doubtful blanching).

Two visual score values (one per trained evaluator) will be recorded for each test site.

4.2 Assessment of local tolerability

At Day 2, the (sub)investigator will assess each test site for local tolerability according to the scale in Table 4.

Table 4 - Local tolerability scale

Score*	Description
0	No reaction
0.5	Only slight erythema
1	Only erythema
2	Erythema with papules or oedema
3	Erythema, oedema with papules, oedema with vesicle
4	Blisters
IR	Irritant reaction including: miliaria (sweat rash), follicular pustules, burn-like reactions, dry scales

Subjects with a local tolerability score >0 will be re-assessed at a follow-up visit.

Cutaneous reactions scored as local tolerability will be reported as local Adverse Events (AEs).

4.3 Safety assessment

A safety assessment will be conducted for all subjects at the screening visit (from the Informed Consent signature) and every subsequent visit. The safety parameters are AEs, to be recorded as specified in the protocol, global cutaneous tolerance assessments, physical examination and vital signs assessments (Screening visit only).

AEs will be reported individually on an ongoing basis.

All clinical medical events, whether observed by the Investigator or reported by the Subject will be considered as adverse events and recorded on the appropriate Adverse Event form. Assessment of severity and causality will be based on specific definitions.

5 STATISTICAL METHODS

5.1 Trial Population

5.1.1 Definition

A detailed counting of subjects included or excluded from analysis will be performed prior to any data review and analysis. All protocol deviations will be reported.

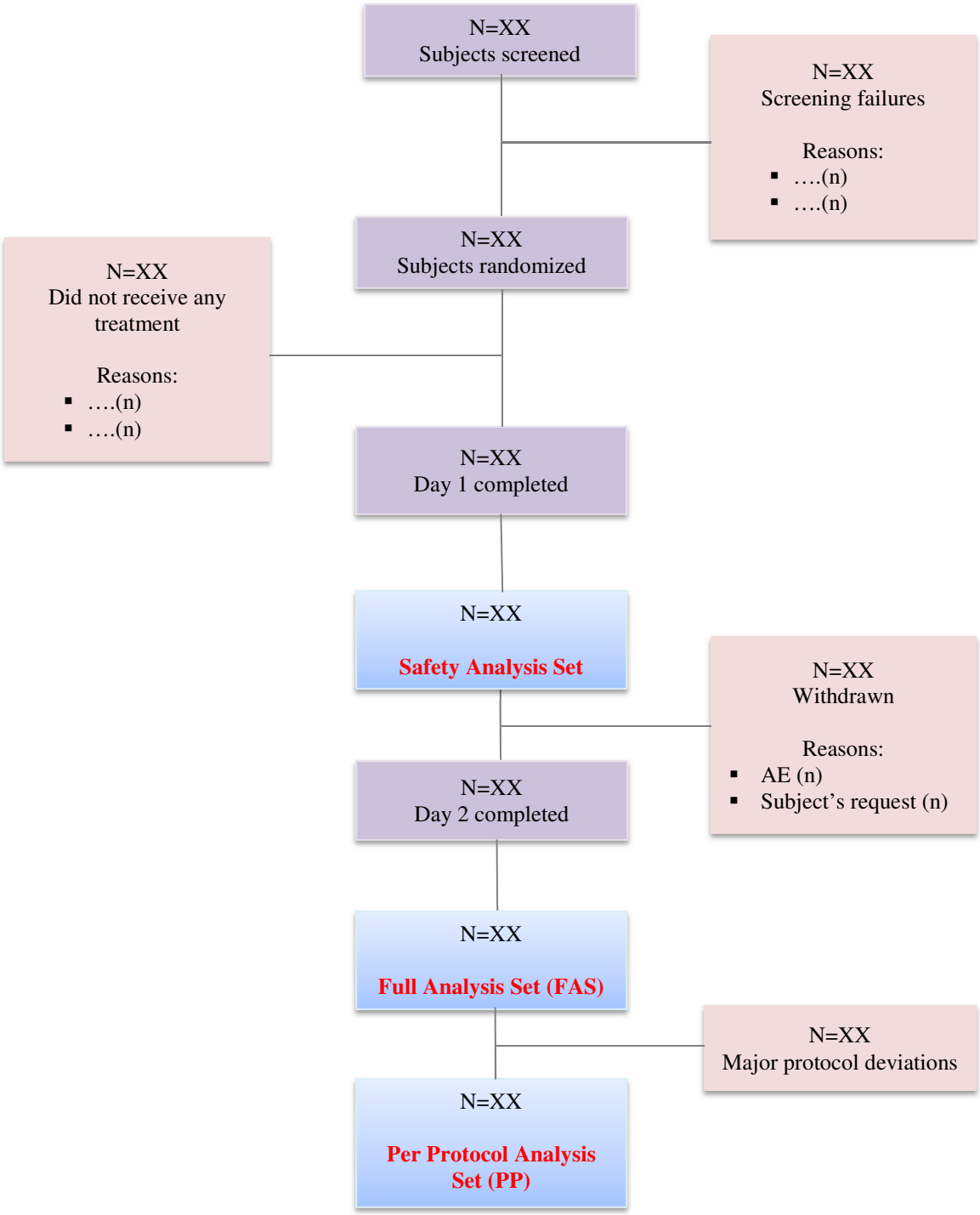
All randomized subjects who receive any trial medication and who contribute any efficacy data in the trial will comprise the **full analysis set** (FAS) and will be analyzed for efficacy.

A **per protocol analysis set** (PP) will be defined by exclusion of subjects from the FAS, who do not fulfil all of the inclusion criteria or who meet any of the exclusion criteria. Further subjects and/or subject data may be excluded from the per protocol analysis when agreed upon on the basis of the actual data obtained as evaluated before breaking the randomization code.

All subjects who receive any treatment with trial medication and for whom the presence or confirmed absence of AEs is available will be included in the **safety analysis set** and analyzed for safety.

The following chart will be completed to describe the different populations:

Figure 1: Disposition of subjects



A data blind review will be performed after the database lock and before breaking the randomization code. A listing of all protocol deviations will be provided, including the type (major/minor) and their consequences in terms of analysis, according to the decisions made by the reviewers.

A major protocol deviation is any event likely to biase significantly the interpretation of the efficacy results and especially the primary ones. Then, this type of deviation will lead to subject exclusion from the PP population. All other protocol deviations will be considered as minor.

A priori the following deviations will be considered as major:

- Deviations in inclusion and non-inclusion criteria at screening,
- Exclusion criteria during the study (pregnancy, health problems, protocol deviation / violation, consent withdrawal, intake of any product or treatment able to interfere with study results, onset of an intolerance to the study treatments),
- Evaluation of primary criterion non-available.

Other elements will be detailed to detect possible deviations:

- Consistency of the randomization scheme,
- Premature withdrawals from study,
- Medical and surgical history,
- Previous and concomitant treatments,
- Adverse events,
- Deviations in dates of visits.

Number and percentage of subjects having at least one minor protocol deviation will be presented by type and deviation class. Major deviations will be listed.

5.1.2 Baseline characteristics

Summary statistics of subject characteristics (age, sex, skin type, height, weight and BMI) and of baseline characteristics (hormonal status, concurrent diagnoses and concomitant medication at baseline as well as screening test for skin blanching) will be tabulated.

The results will be presented as follows:

		Full Analysis Set (N=XX)
Quantitative variable	N Mean±SD Median (Min,Max)	
Qualitative variable	N Modality 1 Modality 2 Modality n	 N (%) N (%) N (%)

5.2 . Analysis of pharmacodynamic effect (blanching)

5.2.1 Primary pharmacodynamic criterion

The analysed variable will be the mean between the two readers at each evaluation time.

The results will be expressed by the mean values obtained at each assessment time by product/site.

MC2-01 cream will be compared with Clobex® lotion, Betamethasone Dipropionate (Augmented) cream, Triamcinolone Acetonide cream, Locoid® cream, DesOwen® cream and MC2-01 cream vehicle in terms of visual score of skin blanching 2 hours after a 16 hours application, using an ANOVA with subjects and treatments as factors.

Some minor changes in the protocol statistical methods have been decided and reported in the SAP to improve the data analysis of the primary criterion.

The protocol planned that In case of a significant treatment effect, estimates of treatment effect and 95% confidence interval of differences between reference products and MC2-01 cream will be calculated from the model without correction for multiplicity in the primary analysis and that correction for multiplicity using Dunnett's test will be used as secondary analysis. In case of significant deviation to the normal assumption, a non-parametric approach with Kruskall-Wallis test for the overall product effect and Wilcoxon's Sign Rank Test for the pairwise comparisons will be used

The present Statistical Analysis Plan states that In case of a significant treatment effect, the comparisons between reference products and MC2-01 cream will be done from the model with correction for multiplicity using Dunnett's test directly in the primary analysis. For each fitted model, residual plots will be used to check the model assumptions. If there is an obvious deviation to these assumptions, the analysis will be done on the rank-transformed data.

The data will be also presented graphically by treatment using boxplot of visual assessment of skin blanching.

The results will be presented as follows:

	MC2-01 cream (n=XX)	Clobex® lotion (n=XX)	Betamethasone Dipropionate (Augmented) cream (n=XX)	Triamcinolone Acetonide cream (n=XX)	Locoid® cream (n=XX)	Desowen® cream (n=XX)	MC2-01 cream vehicle (n=XX)
N **Mean±SD** **Median** **(Min,Max)**							
p-value[a] (treatment comparison versus MC2-01 cream)	-						

a: method used for multiple comparisons

5.2.2 Secondary pharmacodynamic criterion

Non Applicable

5.3 Analysis of local tolerance / Systemic safety

5.3.1 Local safety

The local tolerance score will be summarized using frequency and percentage by trial product. The worst score will also be summarized.

The results will be presented as follows:

	MC2-01 cream (n=XX)	Clobex® lotion (n=XX)	Betamethasone Dipropionate (Augmented) cream (n=XX)	Triamcinolone Acetonide cream (n=XX)	Locoid® cream (n=XX)	Desowen® cream (n=XX)	MC2-01 cream vehicle (n=XX)
N **Modality 1** **Modality 2** **Modality n**							

5.3.2 Adverse events (AEs)

An overview of all AEs occurred during the trial will be presented as follows:

	Safety Population (N=XX)	
	n events	n (%) subjects
All AEs		
Related AEs		
All cutaneous AEs		
Related cutaneous AEs		
All serious AEs		
Related serious AEs		
Severe AEs		
Related severe AEs		
AEs of special interest		
AEs leading to discontinuation		
Related AEs leading to discontinuation		
Deaths		

Adverse events will be coded during the course of the trial and will be in accordance with the current version of the MedDRA dictionary. The adverse events will be tabulated by Preferred Terms and System Organ Class.

The number of subjects experiencing each type of adverse event (according to MedDRA Preferred Terms and System Organ Class) will be tabulated regardless of the number of times each adverse event is reported by each subject.

Adverse events where the investigator has not excluded a causal relationship to trial medication (i.e. not described relationship as "no reasonable possibility", adverse drug reactions (ADR)) will be evaluated separately. A table or a description of these ADRs will be produced depending on the number of ADRs. As with adverse events, the number of subjects affected, not the number of events, will be considered.

The causal relationship of adverse events to trial medication and the intensity of adverse drug reactions will be tabulated or described. Where there are several recordings of causal relationship and intensity for the same event, causal relationship will be taken from the last report of the event (since that is when the investigator will be in possession of most information and so best able to judge causal relationship) and intensity will be taken as the worst ever recording. Adverse events observed on the application areas will be tabulated or described by treatment.

5.4 General principles

All tests will be two-sided and the 0.05 probability level will be chosen to declare significance. The analysis will be performed using the R-Software version 3.3.2

5.4.1 Handling of missing values

It was assumed that only very few skin blanching scores would be missing for the primary endpoint. Drop-outs and missing values will be accounted for by the definition of the trial

analysis sets prior to unblinding. No particular treatment will be done on missing values. In case of missing data concerning the primary pharmacodynamic primary criterion, the subject will be excluded from the analysis.

A last observation carried forward (LOCF) approach is not relevant as the study involves a single pharmacodynamic endpoint.

5.4.2 Data presentation and graphics

Continuous variables will be summarized using mean, median, minimum (Min), maximum (Max) and standard deviation (SD). Categorical variables will be summarized by counts (N) and percentages (%) for each response category.

Subject disposition, demographic variables, baseline characteristics and concomitant treatments will be summarized for all randomized subjects.

The number of decimals presented will refer to the original values. Min and Max will have as many decimals as the original values.

The tables will be structured with a column for each study treatment and will be annotated with the total population size relevant to that treatment.

AEs will be tabulated in frequency tables by System Organ Class (SOC) and Preferred Term (PT) based on the MedDRA dictionary.