ZIARCO

CLINICAL STUDY PROTOCOL

A RANDOMISED, ADAPTIVE DESIGN, DOUBLE-BLIND (3RD PARTY OPEN), PLACEBO CONTROLLED, SEQUENTIAL GROUP STUDY TO DETERMINE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND EFFICACY OF TWICE DAILY APPLICATION OF A TOPICAL ZPL-5212372 (1.0% W/W) OINTMENT ADMINISTERED FOR UP TO 2 WEEKS IN ADULT HEALTHY VOLUNTEERS AND PATIENTS WITH MODERATE TO SEVERE ATOPIC DERMATITIS

Sponsor:	Ziarco Pharma Ltd Innovation House, Discovery Park Ramsgate Road Sandwich Kent CT13 9ND United Kingdom
Principal Investigator	[redacted]
Sponsor Protocol No.:	ZPL521/101 / NCT02795832
EudraCT No.:	2016-000376-26
Study Drug Name:	ZPL-5212372
Development Phase:	Phase 1/2a
Date of Protocol:	28 July 2016
Protocol version:	Final 2.0

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki, and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

Title: A Randomised, Adaptive Design, Double-Blind (3rd Party Open), Placebo Controlled, Sequential Group Study to Determine the Safety, Tolerability, Pharmacokinetics and Efficacy of Twice Daily Application of a Topical ZPL-5212372 (1.0% w/w) Ointment Administered for up to 2 Weeks in Adult Healthy Volunteers and Patients with Moderate to Severe Atopic Dermatitis

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013 and the guidelines on Good Clinical Practice.

[redacted]

[redacted]

Name: [redacted]
Title: [redacted]
Institution: [redacted]

Declaration of the Principal Investigator

Title: A Randomised, Adaptive Design, Double-Blind (3rd Party Open), Placebo Controlled, Sequential Group Study to Determine the Safety, Tolerability, Pharmacokinetics and Efficacy of Twice Daily Application of a Topical ZPL-5212372 (1.0% w/w) Ointment Administered for up to 2 Weeks in Adult Healthy Volunteers and Patients with Moderate to Severe Atopic Dermatitis

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013 and the guidelines on Good Clinical Practice.

Principal Investigator

[redacted] _____ [redacted]

Name: [redacted]
Institution: [redacted]

Date

Declaration of the Investigator

Title: A Randomised, Adaptive Design, Double-Blind (3rd Party Open), Placebo Controlled, Sequential Group Study to Determine the Safety, Tolerability, Pharmacokinetics and Efficacy of Twice Daily Application of a Topical ZPL-5212372 (1.0% w/w) Ointment Administered for up to 2 Weeks in Adult Healthy Volunteers and Patients with Moderate to Severe Atopic Dermatitis

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, Case Report Forms and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IEC, except where necessary to eliminate an immediate hazard to the subjects.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible investigator of the local study center

[redacted]

[redacted]

Name: [redacted] Date

Title: [redacted]

Institution: [redacted]

PROTOCOL SYNOPSIS

Title:	**A Randomised, Adaptive Design, Double-Blind (3rd Party Open), Placebo Controlled, Sequential Group Study to Determine the Safety, Tolerability, Pharmacokinetics and Efficacy of Twice Daily Application of a Topical ZPL-5212372 (1.0% w/w) Ointment Administered for up to 2 Weeks in Adult Healthy Volunteers and Patients with Moderate to Severe Atopic Dermatitis**
Sponsor Study No:	ZPL521/101
Compound:	ZPL-5212372
Phase:	Phase 1/2a
Sponsor:	Ziarco Pharma Ltd
Principal Investigator:	[redacted]

Objective(s):

Primary

Cohorts 1 and 2

To assess the safety and tolerability, local and systemic, of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 1 week, to healthy subjects and patients with moderate to severe atopic dermatitis (AD).

Cohort 3

To evaluate the efficacy of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 2 weeks, to patients with moderate to severe AD.

Secondary

Cohorts 1 and 2

To investigate the systemic pharmacokinetics of ZPL-5212372 following single and multiple dose topical applications of a 1.0% (w/w) concentration of ZPL-5212372 ointment, administered twice daily, for 1 week, to healthy subjects and patients with moderate to severe AD.

Cohort 3

To assess the safety and tolerability, local and systemic, of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 2 weeks, to patients with moderate to severe AD.

To investigate the trough concentrations of ZPL-5212372 in patients with moderate to severe AD following twice daily applications of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL 5212372).

Exploratory
Cohorts 1 to 3
To explore the subjects perception of the Ziarco development ointment formulation using the Ziarco Ointment Questionnaire.

Design: This is a randomised, adaptive design, double-blind (3rd party open), placebo controlled, sequential group study to determine the safety, tolerability, PK and efficacy of twice daily application of topical ZPL-5212372 (1.0% w/w) ointment administered as a 2:1 ratio for active and placebo groups. This study will consist of 3 sequential cohorts each separated by formal safety reviews.

All subjects (both healthy volunteers and AD patients) will be required to attend a screening visit(s) to confirm suitability to enter the study. In order to be eligible AD patients must have moderate-severe AD, defined as:

- Eczema Area and Severity Index (EASI) of ≥9 and ≤48
- Body surface area involvement (BSA) of between 10% and 40%
- Investigator Global Assessment Score (IGA) of at least 3 (moderate disease)
- Active disease for at least 6 months at the time of randomisation

All AD patients will be required to switch to twice daily application of study emollient (instead of their usual emollient) for at least 7 days prior to randomisation (Day 1).

Subjects will attend the clinic on Day 1. If all inclusion/exclusion criteria are met subjects will be randomised to receive either topical 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment twice daily, for either 7 days (Cohorts 1 and 2) or 14 days (Cohort 3).

Cohort 1 will consist of 12 healthy adult volunteers, dosed twice daily for 7 days as in-patients. An initial sentinel group (n=3) will have study ointment applied over 10% BSA. After 48 hours of dosing (i.e. 4 applications) the safety data will be reviewed. Provided that the study ointments are well tolerated the remainder of the cohort (n=9) may start dosing with coverage of 40% BSA with study ointments. Assessments will be conducted on a daily basis as described in Sections 6 & 7. Subjects will remain in the clinic until Day 9 assessments are complete.

Cohort 2 will consist of 12 moderate to severe AD patients, dosed twice daily for 7 days as in-patients. An initial sentinel group (n=3) will have study ointment applied over 10% BSA. After 48 hours of dosing (i.e. 4 applications) the safety data will be reviewed. Provided that the study ointments are well tolerated the remainder of the cohort (n=9) may start

dosing with coverage of 40% BSA with study ointments. Assessments will be conducted on a daily basis as described in Sections 6 & 7. Patients will remain in the clinic until Day 9 assessments are complete.

Cohort 3 will consist of 30 moderate to severe AD patients, dosed twice daily for 14 days as out-patients. Up to a maximum of 50% BSA of AD lesioned skin may be treated with study ointments. Patients will return to the clinic following their Baseline visit (Day 1) on Day 5, Day 8, Day 10 and Day 15 for the assessments described in Sections 6 & 7.

All subjects (both healthy volunteers and AD patients) will be required to have a follow up visit 7-14 days following their last application of study ointment.

Treatment: Each subject will be treated with 1.0% (w/w) ZPL-5212372 ointment or matching placebo, topically applied to the skin twice daily.

Number of Subjects:

Cohort 1
Sufficient subjects will be screened to achieve approximately 12 randomised and evaluable subjects.

Cohort 2
Sufficient patients will be screened to achieve approximately 12 randomised and evaluable patients.

Cohort 3
It is anticipated that approximately 45 patients will be screened to achieve approximately 30 randomised and evaluable patients.

Population:

Cohort 1
Healthy volunteer adult subjects (18 to 55 years).

Cohorts 2 and 3
Adult subjects (18 to 65 years) with moderate to severe atopic dermatitis.

Efficacy Endpoints:

Cohort 3 Only
Primary Endpoints:
Percentage change from baseline in the Eczema Area and Severity Index (EASI) score
Secondary Endpoints:
Change from baseline in the Numerical Rating Score (NRS) for Pruritus (Worst Itch) over 24 hours
Change from baseline in the NRS for Sleep Disturbance
Change from baseline in Duration of Itching
Change from baseline in Verbal Rating Score (VRS) for Pruritus
Change from baseline in BSA
Reduction in Investigators Global Assessment (IGA) Score (subject will be deemed to have had a response with a reduction from baseline of at least 2 points)
Patient Global Impression of Change (PGIC)

Safety Endpoints:	Cohort 1 Adverse events, local tolerance assessments, supine vital signs (blood pressure (BP) and pulse rate), 12-lead ECG, laboratory safety tests (clinical chemistry, haematology and urinalysis). Cohorts 2 and 3 Adverse events, supine vital signs (BP and pulse rate), 12-lead ECG, laboratory safety tests (clinical chemistry, haematology and urinalysis).
Exploratory Endpoints:	Cohorts 1 to 3 Subject responses to Ziarco Ointment Questionnaire.
Pharmaco-kinetic Endpoints:	Cohorts 1 and 2 ZPL-5212372 plasma concentrations Day 1 (sd): AUC_{τ}, C_{max} and T_{max} ZPL-5212372 plasma concentrations Day 7 (ss): AUC_{last}, AUC_{τ}, C_{max}, T_{max}, and $t_{½}$. Observed accumulation ratios based on AUC_{τ} and C_{max} Cohort 3 Trough Plasma ZPL-5212372 concentrations
Statistical Methods:	Separate randomisation codes will be generated for each cohort. The randomisation for Cohort 3 will be stratified by EASI score (EASI ≤20 and EASI ≥20). All statistical testing for Cohort 3 efficacy data will be at the 5% level of significance (1-sided) and all point estimates for the comparison between treatment groups will be accompanied by 2-sided 90% confidence intervals. No adjustment will be made for multiplicity due to multiple endpoints/comparisons. No statistical testing will be performed with the data from Cohorts 1 and 2. For change from baseline endpoints baseline is defined as the last measurement available prior to the start of dosing on Day 1. Separate tables, listings and figures will be generated for each cohort and within each cohort endpoints will be summarized by treatment group and visit. Safety data (adverse events, vital signs, ECG and laboratory data) will be summarized by treatment group and visit (where applicable) using all subjects in the Safety Analysis Set – defined as all randomised subjects who received at least one dose of study treatment. The incidence of treatment emergent adverse events will be tabulated regardless of severity and by maximum severity. Those events regarded as possibly treatment related by the investigator will be summarized separately. Changes from baseline in laboratory parameters, vital signs and ECG data will be summarized in addition to absolute values. ZPL-5212372 concentrations (all cohorts) and parameters (Cohorts 1 and 2 only) will be summarized using all subjects in the Pharmacokinetic Set -

defined as all ZPL-5212372 treated subjects in the Full Analysis Set who have at least one ZPL-5212372 concentration recorded. The ZPL-5212372 pharmacokinetic parameters AUC_{inf}, AUC_{last} and $t_{½}$ will be calculated using non-compartmental methods. C_{max} and T_{max} will be determined directly from the data.

Individual profile and median plots will also be plotted. Median profiles for Cohorts 1 and 2 will be plotted by day.

Though some efficacy assessments are included for Cohort 2 the data will only be listed and no formal comparisons will be made between the treatment groups. Consequently efficacy will only be assessed for Cohort 3.

The Full Analysis Set (FAS) used for efficacy analyses will include all randomized subjects who received at least one dose of study treatment. A Per Protocol (PP) Analysis Set for a sensitivity analysis of the primary efficacy endpoint will include those subjects in the FAS who have not deviated from the protocol in such a way to influence their efficacy assessment.

The primary efficacy endpoint is the percentage change from baseline to Week 2 in the EASI score. The percentage changes from baseline in the EASI score at Week 2 will be summarized and analysed by ANCOVA. The difference between treatment groups in the percentage changes from baseline will be estimated, along with its 90% confidence interval. Baseline will be included as a continuous covariate in addition to treatment.

The proportion of subjects who achieve an EASI-50 response (50% reduction in EASI score from baseline) at Week 2 will be compared between the treatment groups. Group proportions and the difference between these proportions will be calculated along with the corresponding 90% confidence interval. These data will also be analysed using logistic regression, including treatment and baseline. The odds ratio and accompanying 90% confidence interval will be presented. The proportion of subjects who achieve an EASI-75 response (75% reduction in EASI) and the proportion of subjects who are responders for IGA score (reduction from baseline of at least 2 points) at Week 2, will be analysed using the same analyses described for the EASI-50 response.

The changes from baseline in body surface area, pruritus NRS score for worst itch, NRS score for sleep disturbance and duration of itching at Week 2 will be analysed using the same analyses described for the primary analysis. The changes from baseline in VRS score will be summarized using categorical shift tables. The PGIC score will be summarized and analysed using logistic regression, presenting odds ratios and associated 90% confidence intervals for the comparison between treatment groups.

Responses to the Ziarco ointment questionnaire will be summarized.

LIST OF STUDY PERSONNEL

Sponsor	[redacted] Tel: [redacted] [redacted] Tel: [redacted]
Principal or Coordinating Investigator	[redacted] Tel: [redacted]
Serious Adverse Event Reporting	[redacted] Tel: Email : [redacted] Fax :
Clinical Supplies	[redacted] Telephone: [redacted] Fax:

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AD	Atopic Dermatitis
ADR	Adverse Drug Reaction
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of Covariance
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
AUC	Area Under the Curve (inf; last; t)
BID	Twice daily
BSA	Body surface area
CK	Creatine Kinase
Cmax	Maximum observed concentration
CMO	Chief Medical Officer
COX	Cyclo-oxygenase
$cPLA_2\alpha$	Cytosolic phospholipase $A_2\alpha$
CRA	Clinical Research Associate
CRF	Case report form
CS	Corticosteroids
CRU	Clinical research unit
DPI	Dry powder inhaler
EASI	Eczema Area and Severity Index
ECG	Electrocardiogram
ECLIA	Electro-chemiluminescence immunoassay
EDC	Electronic data capture
EMA	European Medicines Agency
FAS	Full analysis set
FBC	Full blood count
FDA	Food and Drug Administration
FIM	First in man
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transpeptidase
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HBcAb	Hepatitis B core Antibody
HBsAg	Hepatitis B surface Antigen
hCG	Human chorionic gonadotropin
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic pituitary-adrenal
IC_{50}	Inhibitory concentration
ICD	Informed consent document
ICH	International Council on Harmonization
IEC	Independent Ethics Committee
IGA	Investigator Global Assessment
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
IWRS	Integrated Web-based Response System
Kg	Kilogram

5-LO	5-lipoxygenase
LFT	Liver function tests
LT	Leukotrienes
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mm Hg	Millimeters of Mercury
mL	Milliliters
nM	Nanomole
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NRS	Numerical Rating Scale
NSAID	Non-steroidal anti-inflammatory drugs
PAF	Platelet activating factor
PDF	Portable Document Format
PG	Prostaglandins
PGIC	Patient Global Impression of Change
PI	Principal investigator
PK	Pharmacokineticμ
PMA	Phorbol 12-myristate 13-acetate
PP	Per protocol
QTcF	Fridericia's corrected QTc interval
QoL	Quality of Life
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
[redacted]	[redacted]
T½	Half life
TB	Total bilirubin
TC	Topical corticosteroids
TCI	Topical calcineurin inhibitors
TNCB	2,4,6-Trinitrochlorobenzene
T_{max}	Time at which the C_{max} is observed
TXA_2	Thromboxane A_2
μg	Microgram
ULN	Upper Limit of Normal
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
VRS	Verbal Rating Scale
WBC	White Blood cells
WOCBP	Women of child bearing potential
w/w	Weight/weight

1 INTRODUCTION

1.1 Indication

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease characterised by impaired epidermal barrier function, inflammatory infiltration, extensive pruritus and a clinical course defined by symptomatic flares and remissions. It is characterised by poorly defined erythema with oedema, vesiculation, and weeping in the acute stage and skin thickening (lichenification) in the chronic stage.[1,2] These irritating symptoms result in a severely reduced quality of life (QOL). In particular, itching (pruritus) that continues throughout the day and worsens at night causes sleep loss and impacts everyday activities and psychosocial wellbeing.[3] AD is commonly associated with other atopic and inflammatory disorders, such as asthma and allergic rhinitis.

The disease occurs in all races and geographic locations, with the highest incidence in urban areas and developed countries.[4,5] The prevalence of AD has at least doubled in industrial countries during the last three decades. Between 15% to 30% of children and 1.4% to 10.2% of adults are affected by AD.[5,6,7,8] The majority of adults present with a chronic, recurrent course of the disease.[9]

There are currently two models explaining the pathogenesis of AD. The predominant model describes AD as a result of impaired epidermal barrier function secondary to inherent structural and functional abnormalities of the skin. The disease evolves from the outside in, as a result of the abnormalities in the epidermal barrier.[10] The second more traditional model describes AD primarily as a disorder of the immune system. In this model, environmental factors cause Langerhans cells, T-cells, and immune effector cells to modulate an inflammatory response.

Clinical features of adult AD include diffuse lesions with an underlying background of erythema, skin dryness, intense pruritus, and sleep disturbances. The face is usually involved and a brown macular ring around the neck from localized deposition of amyloid is common. Xerosis is prominent and lichenification may be present. Visible skin lesions affect social interactions, psychological adjustment, work success, sexual relationships and overall quality of life.[11] Presentation of major mood disturbances such as anxiety, depression, mood changes, self-esteem or stigmatization are prevalent in adult AD patients.[12,13] Spouses of patients with AD also may feel the psychological burden of their partners' severe skin disease and can experience depressive or anxious symptoms.[14]

Topical corticosteroids (TC) have been the mainstay of treatment for AD for several decades. Well known side-effects of TC treatment include skin thinning, and possible hypothalamic pituitary-adrenal (HPA) axis suppression.[15,16] An alternative to TC are topical calcineurin inhibitors (TCIs). TCIs have been approved for the treatment of AD since 2000, the compounds work by inhibiting calcineurin, thus preventing the dephosphorylation activity of phosphatase and the production and release of inflammatory cytokines and T-cell proliferation. In January of 2006, the United States Food and Drug Administration (FDA) issued a black box warning on these compounds mentioning possible concerns about the potential of increased long term malignancy risk due to systemic immunosuppression and recommended that TCIs not be used as first-line therapy for AD.[17]

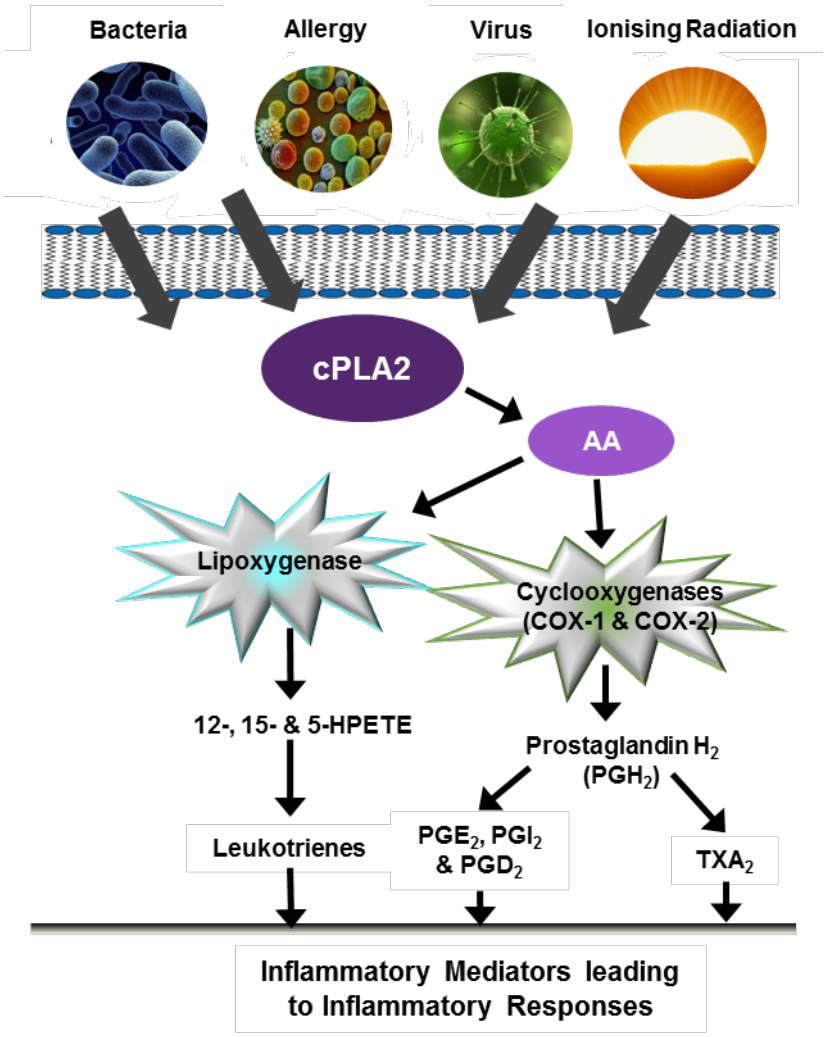
This poses a significant healthcare challenge in treating patients with moderate to severe AD and who have chronic symptoms. Therefore there is a need for new treatment options in AD

that safely and effectively target both the inflammatory and pruritic components of this condition.

1.2 Rationale for cPLA$_2$ Inhibitors

Cytosolic phospholipase A$_2\alpha$ (cPLA$_2\alpha$) releases arachidonic acid from phospholipid membrane and is the rate-limiting enzyme in the biosynthesis of chemical lipid mediators, including various prostaglandins (PGs), leukotrienes (LTs), thromboxaneA$_2$ (TXA$_2$) and platelet activating factor (PAF) as shown in Figure 1.

Figure 1 - Arachidonic Acid Cascade: A Role for cPLA2α in Skin Pathophysiology



It is well documented that PGs, LTs and TXA$_2$ play important roles in inflammation, including recruitment of inflammatory cells, promotion of oedema by increasing vascular permeability and permitting leakage of plasma to the extravascular space.[18] In AD, the levels of eicosanoids such as LTB$_4$ and PGE$_2$ are elevated in skin lesions, as compared to non-lesional skin in patients.[19] PGE$_2$ has been shown to induce vasodilation and itch in patients with AD.[20] In addition, LTB$_4$ and cysteinyl leukotrienes are involved in keratinocyte hyperproliferation, an important process in AD.[21]

Selective inhibitors of $cPLA_2\alpha$ with distinct chemical structures have demonstrated significant anti-inflammatory activity in a variety of skin inflammation models. In a chronic mouse model of phorbol 12-myristate 13-acetate (PMA)-induced dermatitis, topical application of the $cPLA_2\alpha$ inhibitor BMS-229724 significantly reduced oedema and neutrophil infiltration with efficacy comparable to topically-applied betamethasone.[22] In a 21 day 2,4,6-trinitrochlorobenzene (TNCB)-induced mouse ear inflammation model, the selective $cPLA_2\alpha$ inhibitor RSC-3388 significantly inhibited oedema and inflammatory cytokine generation with efficacy comparable to the calcineurin inhibitor tacrolimus.[23] The $cPLA_2\alpha$ knockout mouse is highly resistant to inflammatory challenges and displayed significant reduction in both early and late phase allergen-induced cutaneous responses. ZPL-5212372 is a highly potent and selective inhibitor of $cPLA_2\alpha$ that prevents the biosynthesis of these pro-inflammatory mediators. Ziarco plans to study ZPL-5212372 in a topical formulation for the treatment of patients with AD.

1.3 ZPL-5212372 Background

ZPL-5212372 was formerly known as PF-05212372 and was being developed by Pfizer as an inhaled treatment for asthma, prior to its acquisition by Ziarco Pharma Limited in October 2012.

In vitro studies have shown ZPL-5212372 to be a potent (low nanomolar) inhibitor of $cPLA_2\alpha$ in recombinant enzyme assays. These assays also demonstrated the selectivity of the molecule as it failed to inhibit the closely related $cPLA_{2\zeta}$ isoform or the physiologically related cyclo-oxygenase (COX) 1 and 2. ZPL-5212372 inhibited release of downstream prostaglandins, leukotrienes and thromboxanes in rat and human whole blood assays with 50% inhibitory concentrations (IC_{50}) ranging from 2.7 nM to 19.0 nM. The inhibition of multiple lipid mediators in these primary human cell systems supports the assumption that ZPL-5212372 will block the COX-1, COX-2 and 5-lipoxygenase (5-LO) pathways in man.

Topically administered ZPL-5212372 was effective in blocking skin inflammation induced by PMA in the mouse ear oedema model. As ZPL-5212372 was originally developed for the asthma indications, *in vivo* studies have examined the effects of ZPL-5212372 inhibition of $cPLA_2\alpha$ by inhaled delivery in a variety of lung inflammation models including mouse and sheep models of allergic asthma. Taken together, the clear anti-inflammatory effects demonstrated by ZPL-5212372 in models of skin and lung inflammation support the potential of ZPL-5212372 as a therapeutic agent to treat inflammatory skin diseases.

Pre-clinically there were no safety pharmacology findings of clinical relevance with ZPL-5212372 and the results of genotoxicity studies indicate that ZPL-5212372 does not present a genotoxic risk to humans. In addition, ZPL-5212372 did not induce local toleration reactions *in vitro* in bovine eyes or sensitisation *in vivo* in guinea-pig skin either topically or intradermally. ZPL-5212372 was well tolerated in toxicology studies up to 2 weeks in duration in rats with once daily subcutaneous (SC) systemic administration and with twice daily topical administration to mini-pigs (0.5 mL/kg of vehicle or 0.3%, 1.0% or 3.0% (w/w) ZPL-5212372 ointment applied twice daily (BID) to 10% BSA). No safety findings were identified in either the 2 week rat SC or topical mini-pig studies, hence the NOAELs are 50 mg/kg/day and 30 mg/kg/day (3.0% w/w ZPL-5212372 ointment), respectively. In the rat following 50 mg/kg/day the C_{max} plasma exposures at Day 13 were 5520 and 1740 ng/mL and the AUC_{0-t} values were 44900 and 31200 ng.h/mL in males and females, respectively. In

the mini-pig following 30 mg/kg/day the C_{max} plasma exposures at Day 14 were 14.2 and 44.7 ng/mL and the AUC_{0-t} values were 394 and 908 ng.h/mL in males and females, respectively.

To date, one Phase 1 clinical study has been conducted in 24 male healthy volunteers. In this study ZPL-5212372 was administered by dry powder inhaler (DPI) as part of the original asthma indication development program, supported by the 2 week inhalation toxicology studies. This study assessed the safety, toleration, plasma and urine pharmacokinetics and urine pharmacodynamics of ZPL-5212372 following single-dose inhalation administration of 50 μg –10 mg of ZPL-5212372. Inhaled doses of ZPL-5212372 up to 10 mg were well tolerated. There were no deaths, serious adverse effects (SAEs) or severe adverse effects (AEs) associated with dosing of any treatments and no clinically significant changes in laboratory safety tests, vital signs or electrocardiograms (ECGs) were observed. There is no evidence at the present time of any dose relationship for AEs reported. Plasma concentration-time profiles of ZPL-5212372 indicate that systemic absorption following single inhaled dose administration is rapid (median T_{max} 0.5 hours) with low systemic exposure (10 mg dose: C_{max} 0.32 ng/mL and AUC_{0-last} 1.32 ng.h/mL). Exposure increased dose-proportionally between the 5 and 10 mg dose groups.

Full details of the pre-clinical and clinical data for ZPL-5212372 can be found in the single reference safety document, which for this study is the current ZPL-5212372 Investigator Brochure.

1.4 Study Rationale

This study will be a randomised, adaptive design, double blind (3rd party open), placebo controlled, sequential group study in both healthy volunteers and patients with moderate to severe AD. This study will be divided into 3 separate, sequential cohorts:

- Cohort 1 will assess safety, toleration and pharmacokinetics (PK) with intensive monitoring as in-patients over 7 days of dosing in healthy volunteers.
- Cohort 2 will assess safety, toleration and PK with intensive monitoring as in-patients over 7 days of dosing in moderate to severe AD patients.
- Cohort 3 will assess efficacy, safety, toleration and PK as out-patients over 14 days of dosing in moderate to severe AD patients.

Subjects will receive 1.0% (w/w) ZPL-5212372 or matched placebo ointment topically, twice daily.

In total approximately 54 subjects (12 subjects each for Cohorts 1 and 2; 30 subjects for Cohort 3) will be enrolled into the study and subjects will be allocated active and placebo treatments in a 2:1 ratio in all 3 cohorts.

To mitigate risk to participants in this study the cohorts will be recruited sequentially with progression through the cohorts dependent on adequate safety and PK data from the previous cohort. A decision process will be followed for each safety review and the findings at each review will be documented. Cohort 1, with healthy volunteers, will assess the safety, local toleration and plasma exposure with dosing twice daily for 7 days on normal skin. This cohort will start with a sentinel (n=3) group who will have study ointment applied to 10% BSA BID for 7 days. If well tolerated, following safety assessments 48 hours after the first

application (i.e. 4 applications of study ointment), the remaining subjects (n=9) will begin dosing at 40% BSA, BID for 7 days. If after a formal safety review of the Cohort 1 safety and PK data, the data indicate that the application of ointment can be considered safe and well tolerated, dosing of Cohort 2 will commence. Cohort 2 will recruit moderate to severe AD patients in order to investigate safety, toleration and exposure following application of study ointment to AD diseased skin. Cohort 2 will also include a sentinel (n=3) group who will only have study ointment applied to 10% BSA BID for 7 days. Again if after 48 hours, after the first application of ointment, dosing is well tolerated in the sentinel group, the remainder of the cohort (n=9) will start with 40% BSA treated BID for 7 days with study ointment. Subjects in Cohorts 1 and 2 will remain in the clinical research unit (CRU) for the duration of dosing and until 48 hours after the last study treatment to enable close monitoring. This will provide safety, toleration and PK information from both healthy and AD diseased skin treated with ZPL-5212372. Subjects will only be recruited into Cohort 3 when the safety data from Cohorts 1 and 2 have been formally reviewed by the Safety Review Team.

Cohort 3 (n=30) will recruit moderate to severe AD patients, who will be treated twice daily for 14 days as out-patients returning to the CRU for study assessments on days 5, 8, 10 and 15. Cohort 3 patients will be treated with study ointment up to 50% BSA to their AD lesioned skin. The primary objective of Cohort 3 will be to assess the efficacy of ZPL-5212372 over matched placebo ointment, in addition to obtaining 14 days safety and toleration data. Trough PK levels of ZPL-5212372 will also be collected. This risk averse sequential adaptive design is being used as this will be the first time that ZPL-5212372 will be administered topically to human skin. From the data reviews and ongoing learning in the CRU, protocol defined adaptions may be made to the study between cohorts to minimize any risks to participants. All adaptions outlined in this protocol are constrained by clear boundaries and stopping criteria. All modifications to the in-life phase of the study will be documented and approved by Ziarco's Chief Medical Officer (CMO) and the Principal Investigator (PI).

No rescue medication will be provided in this study for AD patients due to the short duration of dosing (7 days for Cohort 2 and 14 days for Cohort 3). Use of rescue medication in Cohort 3 could potentially impact the efficacy data collected. The severity of AD patients included in the study will be limited to moderate to severe patients with an Ezcema Area and Severity Index (EASI) score of ≤48 as AD patients with AD of a greater severity may not be able to tolerate the conditions in the study without rescue medication. AD patients will be supplied with study emollient to be started 7 days prior to randomisation and to continue throughout their participation in the study. AD patients in Cohort 2 will be resident in the CRU for the duration of treatment and will therefore have close contact with clinical staff to discuss any issues relating to their AD. Patients in Cohort 3 will regularly attend the CRU during treatment for study assessments and will be encouraged to contact the CRU between visits if they feel their AD is deteriorating.

Placebo-controlled trials have been routinely utilised to test new molecular entities in the treatment of dermatological diseases, including AD. This trial is designed to show neither 1.0% (w/w) ZPL-5212372 ointment or placebo has harmful effects when applied to human skin. In addition Cohort 3 is designed to demonstrate efficacy of ZPL-5212372 over placebo. Placebo has been selected as the comparator in this study in order to establish the magnitude of change in clinical endpoints that occur spontaneously during the treatment period. Ethical analysis and international ethical guidance permit the use of placebo controls in randomised trials when there are compelling methodological reasons, and withholding treatment does not

pose a risk of serious harm to participants.[24] The 2:1 randomisation has been selected to increase the number of subjects participating in the active arm, whilst including sufficient control subjects to make valid statistical comparisons.

The 2 week maximum dosing duration has been selected based on the duration of completed toxicology studies, but is considered adequate to provide an indication of efficacy in this patient population.

As AD affects males and females equally, women of child bearing potential (WOCBP) who are using highly effective methods of contraception will be included in this study as they represent an important section of the affected subjects. The inclusion of WOCBP is consistent with both the recommendations of the Clinical Trials Facilitation Group (Clinical Trials Facilitation Group, 2014)[25] following the Rome September 2014 meeting and also the European Medicines Agency (EMA) M3(R2) guidance. M3(R2) states:
"In all ICH regions, WOCBP can be included in repeated-dose Phase I and II trials before conduct of the female fertility study since an evaluation of the female reproductive organs is performed in the repeated-dose toxicity studies".

1.5 Dose Rationale

The dose selected for this study is a 1.0% (w/w) ZPL-5212372 topical ointment, to be applied twice daily for up to 2 weeks.

The 1.0% (w/w) concentration of ZPL-5212372 has been selected to be 3 fold lower than the maximum concentration applied twice daily to mini-pigs for 14 days with no local toleration or systemic toxicity issues. Currently there is no predicted efficacious dose level for topically administered ZPL-5212372 in humans. At the present time the only human plasma exposure data for ZPL-5212372 is from the inhaled first in man (FIM) study conducted by Pfizer when ZPL-5212372 was being developed as a potential treatment for asthma. Systemic exposures of ZPL-5212372 following a single 10 mg inhaled dose resulted in an $AUC_{0\text{-}12}$ 1.32 ng.h/mL and C_{max} 0.32 ng/mL. The human systemic exposures following topical administration are also expected to be low and this is further supported by the much lower systemic exposures seen in mini-pig following topical administration compared to the systemic exposures in rats seen following SC administration of ZPL-5212372. In addition the NOAELs in the 14 day repeat dose studies in both the rat (SC) and mini-pig (topically) were the highest dose levels administered. PK data, in addition to safety data, will be collected in the initial clinical study with topically administered ZPL-5212372, hence both systemic exposure and safety can be carefully monitored. Topical application of ZPL-5212372 to humans is expected to give low systemic exposure and plasma exposures will be monitored on an on-going basis to avoid systemic plasma exposures exceeding the mean $AUC_{0\text{-}t}$ value of 13550 ng.h/mL (the mean NOEL (15 mg/kg/day) systemic exposure seen in the rat after SC dosing for 13 days). Exposures in humans following a topical dose of 1.0% (w/w) ZPL-5212372 ointment are not expected to exceed the exposures seen in the mini-pig following the 30 mg/kg/day dose (mean $AUC_{0\text{-}t}$ 651 ng.h/mL).

Pre-clinical studies indicate that ZPL-5212372 has a low potential for drug-drug interactions as either victim or perpetrator. ZPL-5212372 is primarily hepatically cleared. ZPL-5212372 is highly bound to plasma protein, yet with very high rates of unbound clearance hence circulating systemic drug levels are low, ensuring high systemic safety from topical delivery. In addition the inhibitory effect of ZPL-5212372 has been shown to be reversible.

The combination of high potency, rapid clearance of unbound drug and hence low systemic burden makes ZPL-5212372 an ideal compound for topical delivery for the treatment of moderate to severe AD, both in terms of efficacy as well as systemic safety. The selected dose has been shown to have an adequate safety profile and be well tolerated and is not expected to exceed exposures associated with the NOAEL in preclinical toxicology studies.

1.6 Risk-Benefit Assessment

Benefits

There is preclinical data demonstrating that cPLA$_2\alpha$ inhibition should be effective in relieving skin inflammation and pruritus, both of which are involved in AD. Selective inhibitors of cPLA$_2\alpha$ with distinct chemical structures have demonstrated significant anti-inflammatory activity in a variety of skin inflammation models.[22,23] cPLA$_2\alpha$ knockout mice are highly resistant to inflammatory challenges and displayed significant reduction in both early and late phase allergen-induced cutaneous responses. ZPL-5212372 is a selective cPLA$_2\alpha$ inhibitor which in non-clinical pharmacology studies has been shown to potently inhibit a number of inflammatory-mediated processes both in the skin and lung. Hence it is believed that ZPL-5212372 has the potential to reduce the incidence and severity of both inflammation and pruritus in AD patients.

Risks

Previously ZPL-5212372 has been administered to healthy volunteers as single inhaled doses up to 10 mg with no safety or toleration issues. Adverse events were mild to moderate in severity and there have been no serious adverse events.

Pre-clinically there were no safety pharmacology findings of clinical relevance with ZPL-5212372 and the results of genotoxicity studies indicate that ZPL-5212372 does not present a genotoxic risk to humans. In addition, ZPL-5212372 did not induce local toleration reactions *in vitro* in bovine eyes or *in vivo* sensitisation in guinea-pig skin either topically or intra-dermally. ZPL-5212372 was well tolerated in toxicology studies up to 2 weeks in duration in rats with once daily subcutaneous (SC) systemic administration and with twice daily topical administration to mini-pigs (0.5 mL/kg of vehicle or 0.3%, 1.0% or 3.0% (w/w) ZPL-5212372 ointment applied BID to 10% BSA). No effects were identified in either the 2 week rat SC or topical mini-pig studies hence the NOAELs are 50 mg/kg/day and 30 mg/kg/day (3.0% w/w ZPL-5212372 ointment), respectively. In the rat following 50 mg/kg/day the C_{max} plasma exposures at Day 13 were 5520 and 1740 ng/mL and the AUC_{0-t} values were 44900 and 31200 ng.h/mL in males and females, respectively. In the mini-pig following 30 mg/kg/day the C_{max} plasma exposures at Day 14 were 14.2 and 44.7 ng/mL and the AUC_{0-t} values were 394 and 908 ng.h/mL in males and females, respectively.

In accordance with the latest European regulatory discussions and guidelines as outlined above, WOCBP are eligible for the study provided they are using a highly effective form of contraception. A serum pregnancy test will be performed at screening. In addition, regular urine pregnancy tests will be carried out on all WOCBP at randomisation and continuing until the follow up visit is complete, 7 to 14 days after application of the last dose of study ointment.

Conclusion

In conclusion, the risk-benefit profile of ZPL-5212372 supports the continued development of this compound and confirms that there is minimal risk associated with the evaluation of this compound in an AD patient population. However, as this is the first topical administration of ZPL-5212372 the study will be conducted initially in a cohort of healthy volunteer subjects followed by an in-house cohort of patients with moderate to severe AD prior to treating a cohort of moderate to severe AD patients as out-patients.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives

Cohorts 1 and 2

To assess the safety and tolerability, local and systemic, of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 1 week, to healthy subjects and patients with moderate to severe AD.

Cohort 3

To evaluate the efficacy of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 2 weeks, to patients with moderate to severe AD.

2.2 Secondary Objectives

Cohorts 1 and 2

To investigate the systemic pharmacokinetics of ZPL-5212372 following single and multiple dose topical applications of a 1.0% (w/w) concentration of ZPL-5212372 ointment, administered twice daily, for 1 week, to healthy subjects and patients with moderate to severe AD.

Cohort 3

To assess the safety and tolerability, local and systemic, of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL-5212372) twice daily, for 2 weeks, to patients with moderate to severe AD.

To investigate the trough concentrations of ZPL-5212372 in patients with moderate to severe AD following twice daily applications of ZPL-5212372 administered as a topical ointment (containing 1.0% (w/w) concentration of ZPL 5212372).

2.3 Exploratory Objectives

Cohorts 1 to 3

To explore the subjects perception of the Ziarco development ointment formulation using the Ziarco Ointment Questionnaire.

2.4 Endpoints

Cohort 1 (Healthy Volunteers)

Primary Endpoints: Safety

Adverse events, local tolerance assessments, supine vital signs (BP and pulse rate), 12-lead ECG, laboratory safety tests (clinical chemistry, haematology and urinalysis).

Secondary Endpoints: Pharmacokinetics

ZPL-5212372 plasma concentrations Day 1 (sd): AUC_{τ}, C_{max} and T_{max}

ZPL-5212372 plasma concentrations Day 7 (ss): AUC_{last}, AUC_{τ}, C_{max}, T_{max}, and $t_{½}$.

Observed accumulation ratios based on AUC_{τ} and C_{max}

Exploratory Endpoints:
Subject responses to Ziarco Ointment Questionnaire.

Cohort 2 (Moderate to Severe AD patients)

Primary Endpoints: Safety

Adverse events, supine vital signs (BP and pulse rate), 12-lead ECG, laboratory safety tests (clinical chemistry, haematology and urinalysis).

Secondary Endpoints: Pharmacokinetics

ZPL-5212372 plasma concentrations Day 1 (sd): AUC_{τ}, C_{max} and T_{max}

ZPL-5212372 plasma concentrations Day 7 (ss): AUC_{last}, AUC_{τ}, C_{max}, T_{max}, and $t_{½}$.

Observed accumulation ratios based on AUC_{τ} and C_{max}

Exploratory Endpoints:
Patient responses to Ziarco Ointment Questionnaire.

Cohort 3 (Moderate to Severe AD patients)

Primary Endpoints: Efficacy
Percentage change from baseline in the Eczema Area and Severity Index (EASI) score

Secondary Endpoints: Efficacy

Change from baseline in the Numerical Rating Score (NRS) for Pruritus (Worst Itch) over 24 hours

Change from baseline in the NRS for Sleep Disturbance

Change from baseline in Duration of Itching

Change from baseline in Verbal Rating Score (VRS) for Pruritus

Change from baseline in BSA

Reduction in Investigators Global Assessment (IGA) Score (subject will be deemed to have had a response with a reduction from baseline of at least 2 points)

Patient Global Impression of Change (PGIC)

Secondary Endpoints: Safety

Adverse events, supine vital signs (BP and pulse rate), 12-lead ECG, laboratory safety tests (clinical chemistry, haematology and urinalysis).

Secondary Endpoints: Pharmacokinetics

Trough Plasma ZPL-5212372 concentrations

Exploratory Endpoints:
Patient responses to Ziarco Ointment Questionnaire.

3 OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

This is a randomised, adaptive design, double-blind (3rd party open), placebo controlled, sequential group study to determine the safety, tolerability, PK and efficacy of twice daily application of topical ZPL-5212372 (1.0% w/w) ointment administered for up to 2 weeks in adult healthy volunteers and in patients with moderate to severe atopic dermatitis.

This study will consist of 3 sequential cohorts each separated by formal safety reviews. The study will recruit cohorts sequentially and use sentinel subjects (n=3) in Cohorts 1 and 2 to minimize the risk to subjects and make it possible to modify specific features of the study dependent on emerging data; however the flexibility of the adaptive features will be constrained by clear boundaries/stopping criteria. The main features of each cohort are summarized in Table 1 below:

Table 1 - Summary of cohorts with overview of study

	Cohort 1	**Cohort 2**	**Cohort 3**
Subject Population	Healthy adults	Moderate to severe AD patients	Moderate to severe AD patients
In-patients/Out-patients	In-patients	In-patients	Out-patients
Total Number of subjects	12	12	30
Ratio Active:Placebo	2:1	2:1	2:1
Dosing Duration	7 days	7 days	14 days
Follow-up Visit (after last dose of study medication)	7-14 days	7-14 days	7-14 days

Progression from Cohort 1 to Cohort 3 will be dependent on the demonstration of adequate safety within the protocol defined boundaries. In Cohorts 1 and 2, complete data sets from a minimum of 4 active and 2 placebo subjects with 40% BSA treated with study ointment will need to be reviewed by the Safety Review Team in order to decide whether to proceed as planned to the next cohort. In the event that it is decided not to proceed to the next cohort, the reviewers may decide to modify the study within the boundaries of the adaptive features and continue, to submit a substantial protocol amendment or suspend the study. In addition,

in order to ensure subject safety, Cohorts 1 and 2 will be split to have an initial sentinel group which will precede the remaining subjects in the cohort. The sentinel group will consist of 3 subjects (2:1 Active to Placebo) and these subjects will be dosed twice daily, to cover 10% BSA with study ointments for 7 days. Provided that the study ointments are well tolerated after 48 hours (i.e. 4 applications) the remainder of the cohort (9 subjects; 2:1 Active to Placebo) may start dosing with coverage of 40% BSA BID for 7 days. All subjects in Cohorts 1 and 2 will remain in the CRU for the duration of the study. Cohort 3 will have AD affected skin dosed to a maximum of 50% BSA. A 50% BSA has been selected as the maximum BSA to be dosed to ensure that the total BSA of AD affected skin is not excessively different from the maximum area of treated skin in Cohorts 1 and 2 (40% BSA). It also accounts for the fact that AD patients total BSA will vary over time due to flare ups of the disease for a multitude of reasons which cannot be controlled within the study, including due to withdrawal from their normal medication prior to randomisation.

All subjects will have a screening visit to confirm suitability to enter the study, within 28 days of Day 1. AD patients in Cohorts 2 and 3 will be required to switch from their usual emollient to using the study emollient twice daily for at least 7 days prior to Day 1 and throughout their participation in the study. Dosing will commence on Day 1 and continue for 7 or 14 days, depending on which cohort subjects are allocated to. Subjects in Cohorts 1 and 2 will remain in the unit until Day 9 (48 hours after the last application of study ointment). Subjects in Cohort 3 will have their first application of ointment in the unit and continue dosing at home, returning to the unit for assessments on Days 5, 8, 10 and 15 (after the last ointment application at home on the evening of Day 14).

All subjects will be required to attend the CRU for a follow-up visit 7-14 days after the last application of study ointment. AD patients may recommence use with other topical or systemic treatments for their AD, if required, from either discharge from the CRU (Cohort 2) or the end of dosing (Cohort 3).

This protocol will be adaptive and designed to enable knowledge gained from the previous cohort to be applied to subsequent cohorts. Changes made will be within the boundaries of the adaptive elements with clear control mechanisms and guidance for staying within these boundaries.

A summary of the study design is shown in Figures 2-5.

Figure 2 - Overall Study Design

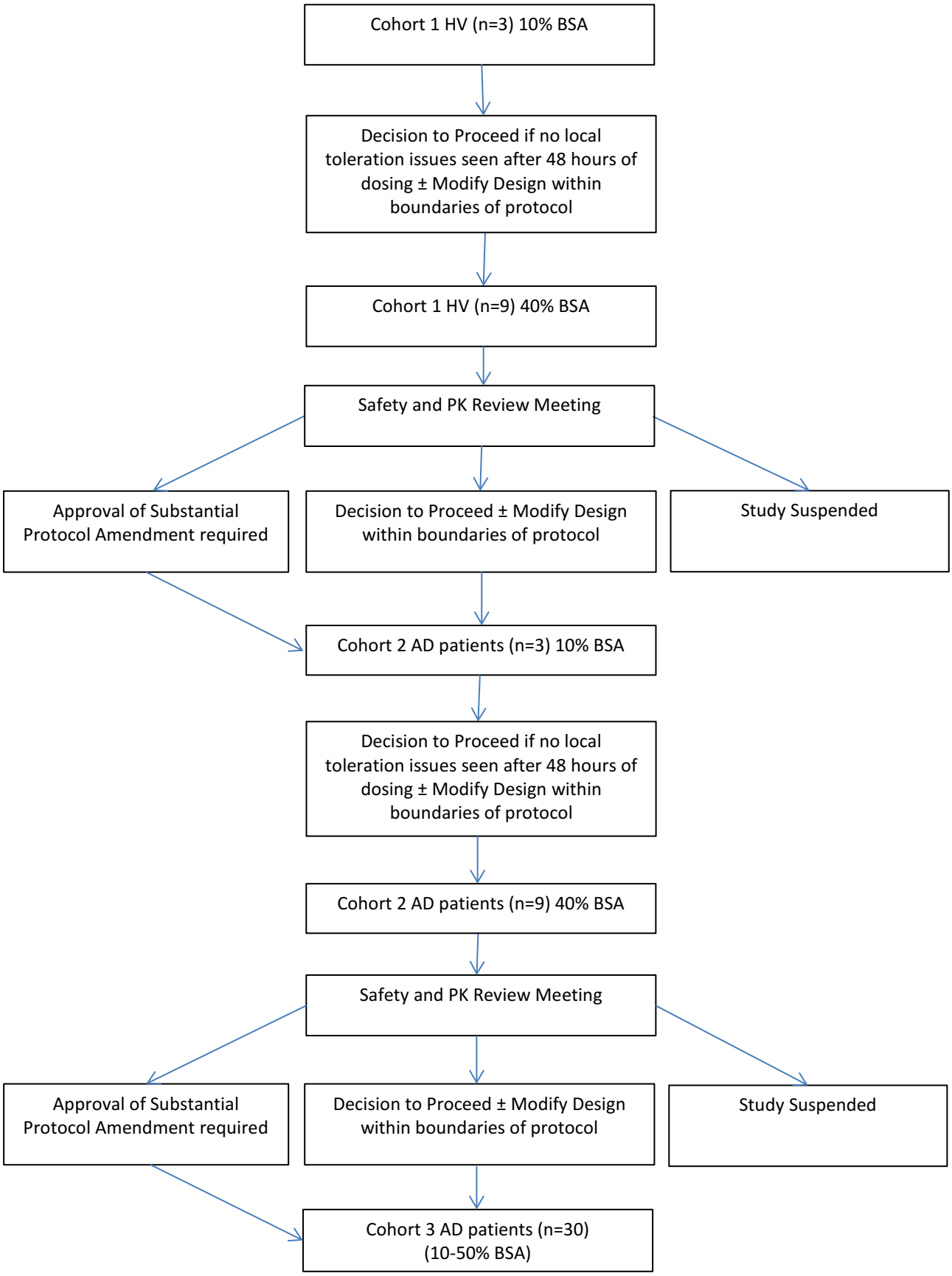


Figure 3 - Study Design Cohort 1 (HV)

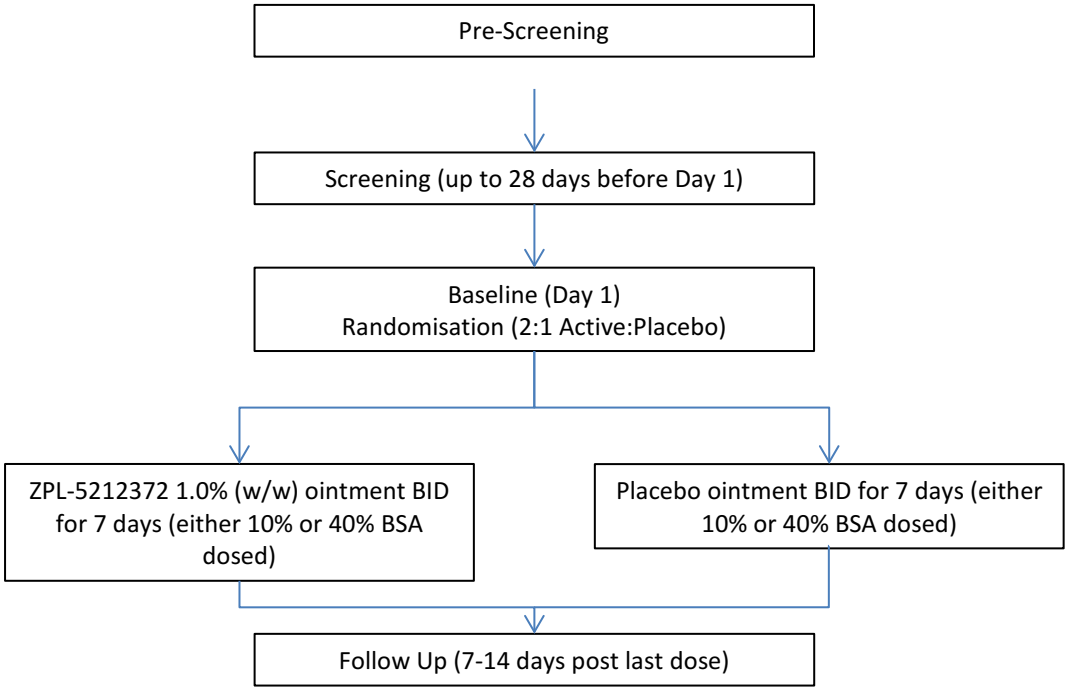


Figure 4 - Study Design Cohort 2 (AD Patients)

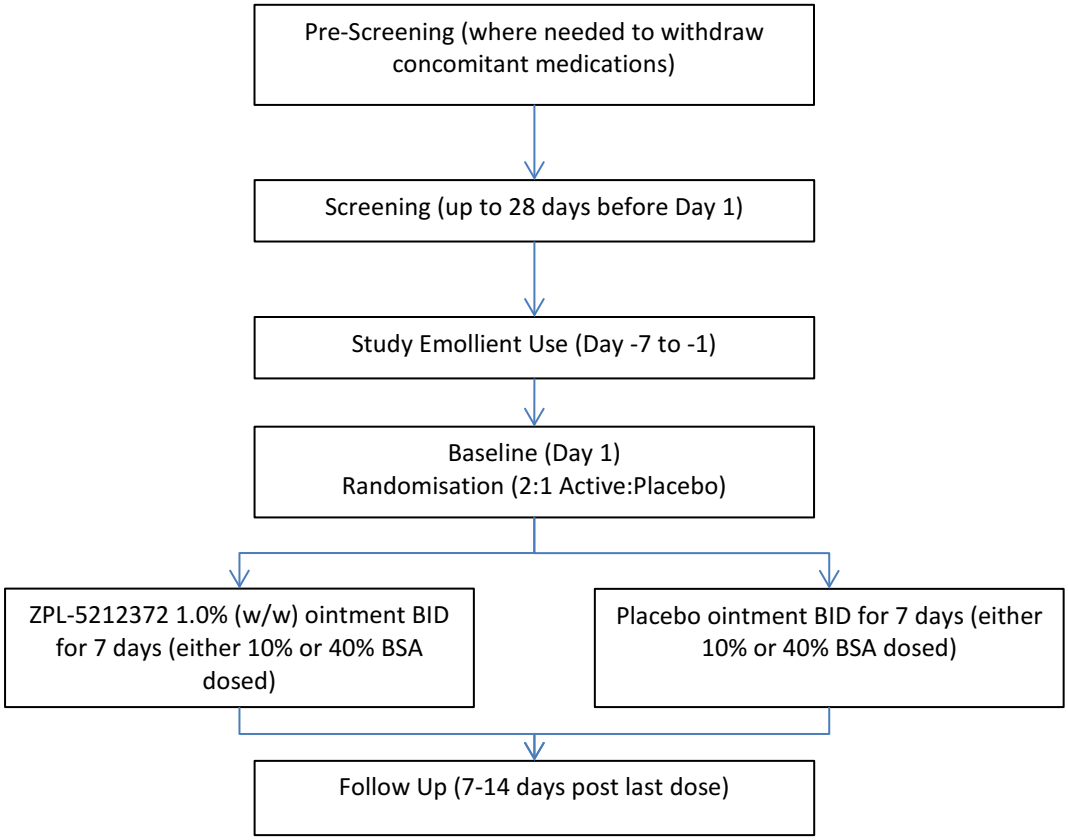
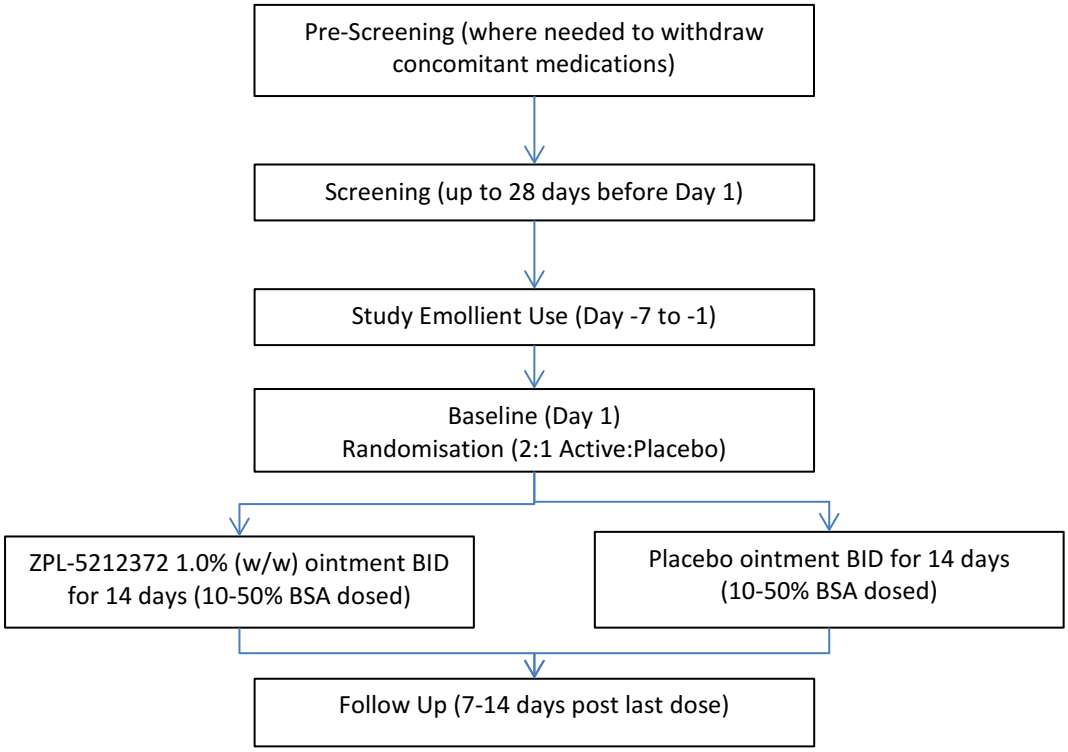


Figure 5 - Study Design Cohort 3 (AD Patients)



A study schedule is provided in Tables 9-11 schedule of activities.

3.2 Adaptive Categories and Features

The adaptive categories and individual features of this study along with the limits (boundaries) which define the maximal risk and minimum safety requirements associated with the features are described in Tables 2 to 5 below.

Table 2 - Investigational Medicinal Product/Dose

Adaptive study design category	Adaptive Features	Boundaries
Dosing Regimen	1. Dosing regimen may be adapted in accordance with PK, safety, tolerability - local and systemic data (as applicable) collected up to the decision making time-point. 2. Permissibility of dosing regimen adaption within and/or between cohorts The term dosing regimen includes (1) the frequency of dosing, (2) the percentage BSA covered and (3) the duration of dosing.	1. Maximum starting percent BSA dosed will be 10% BSA for Cohorts 1 & 2. 2. Maximum total BSA dosed will be 40% BSA for Cohorts 1 & 2. 3. Minimum percent BSA dosed will be 10% BSA for Cohort 3. 4. Maximum total BSA dosed will be 50% BSA for Cohort 3. 5. Minimum dosing frequency will be once daily dosing. 6. Maximum dosing frequency will be twice daily dosing. 7. Maximum duration of dosing will be 14 consecutive days.
Study Ointment Application	1. The volume of ointment applied to each subject may vary e.g. based on the condition of their skin, body size and gender. 2. Subjects will have the same volume of ointment applied throughout the duration of the study. Any changes to a subjects recommended volume of study ointment will be documented.	1. The minimum amount of study ointment to adequately and evenly cover the dosing area should be used for each subject. 2. The minimum volume of study ointment that can be applied for each application is approximately 0.25 mL per 2% BSA covered i.e. 1.25 mL ointment for 10% BSA coverage; 5 mL for 40% BSA coverage & 6.25 mL for 50% BSA coverage. 3. The maximum volume of study ointment that can be applied for each application is approximately 1 mL per 2% BSA covered i.e. 5 mL ointment for 10% BSA coverage; 20 mL for 40% BSA coverage & 25 mL for 50% BSA coverage. 4. For Cohorts 1 and 2 the minimum amount of study ointment should be used for the first application. If there is not enough ointment to cover the dosing area additional study ointment can be applied (up to the maximum 1 mL per 2% BSA). 5. For Cohort 3 the minimum amount of study ointment should be used for the first application in the CRU. If there is not enough ointment to cover the dosing area additional study ointment can be applied (up to the maximum 1 mL per 2% BSA).
Study Emollient Application	1. Cohorts 2 and 3 only. Adaptable use of different methods of application for study ointment and study emollient 2. Cohort 3 only. Alternate emollients other than those defined as ‘study emollients’	1. Use of study ointment before or after study emollient administration 2. Duration of time between study ointment and study emollient applications between 5 and 30 minutes. 3. Stop the use of study emollient on areas treated with study ointment.

	may be used.	4. Stop the use of study emollient in the event of any hypersensitivity reactions caused by emollient. 5. Change the emollient used from 'study emollient'due to any potential or actual hypersensitivity reactions with the approval of the Ziarco CMO (Cohort 3 only).

Table 3 - Timing/Scheduling

Adaptive study design category	Adaptive Features	Boundaries
Visits	1. The number and timing of the study in-life visits may be adjusted for Cohort 3. Excluding any unscheduled visits for subject safety.	1. Minimum of 3 visits (excluding unscheduled visits) 2. Maximum of 5 visits (excluding unscheduled visits) 3. Visit windows may vary by ± 1 day
	2. Cohort 3 maybe subjected to short in-patient stay at beginning of treatment	1. Maximum of 3 days in-patient stay to mitigate any local toleration issues and ensure appropriate dosing by patients

Table 4 - Study Participants

Adaptive study design category	Adaptive Features	Boundaries
Subject number	1. The number of subjects in Cohorts 1 and 2 may be decreased as there is no formal sample sizing for Cohorts 1 and 2.	1. Upon completion of a cohort the minimum safety data requirements for progression between cohorts is complete datasets for 4 active and 2 placebo subjects treated with 40% BSA coverage of ointments. 2. Minimum and maximum evaluable subjects for Cohorts 1 and 2 will be 6 and 12. 3. Ratio of 2 active :1 placebo will be used throughout the study.

Table 5 - Assessments

Adaptive study design category	Adaptive Features	Boundaries
Assessments	1. Safety/tolerability samples and assessments (such as dermal scoring, safety laboratory, vital signs, ECG's etc).	1. No changes to the timing and frequency of non-safety assessments is allowed. 2. Timing and number of safety assessments may change based on clinical need.
	2. PK samples: Timing and number of PK samples may change based on the emerging PK profile.	1. There will not be an increase in the number of PK samples taken but fewer samples may be taken. 2. Timing of samples may be changed dependent on the emerging plasma concentration data.

As the study progresses and safety, toleration and PK data become available the adaptive features of the study may need modifying to improve subject safety and reduce associated risks. Any decisions to modify any adaptive features within the boundaries described in the protocol will be documented and approved (signed) by the CMO prior to any implementation of the modifications. If the protocol requires changes which are outside the boundaries described in the protocol then a protocol amendment will be written. Any associated study documents e.g. informed consent document (ICD), will be updated and re-approved as appropriate.

3.3 Termination or Suspension of Study

The study will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the study.

- New information or other evaluation regarding the safety or efficacy of the study medication that indicates a change in the known risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.

3.4 Termination or Suspension of Investigational Sites

A study site may be terminated prematurely or suspended if the site (including the investigator) is found in significant violation of GCP, the protocol or the contractual agreement or is unable to ensure adequate performance of the study.

In the event that the sponsor, the IEC or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

3.5 Progression From Sentinel Group To Main Cohort

Cohorts 1 and 2 both contain an initial sentinel group (n=3) who will only have 10% BSA treated with study ointment BID for 7 days. After 48 hours (i.e. 4 consecutive applications) of study dosing, if no local toleration (Cohort 1 healthy volunteers) or no persistent dermal AE's (Cohort 2 AD patients) are recorded the remaining n=9 participants in the cohort may start study treatment with 40% BSA being dosed BID for 7 days. The investigator will review all the sentinel group's blinded safety data and the investigator's findings and recommendation will be communicated to Ziarco by email. Based on the investigators findings Ziarco will, either confirm via email that the remainder of the cohort may commence dosing to 40% BSA or, if there is any concern from either the investigator or the Ziarco clinical team, a teleconference will be arranged and the data from the sentinel group will be discussed. The outcome and decision of the meeting will be documented and the minutes will be signed by both the investigator and the Ziarco CMO.

3.6 Safety Review Meeting

Blinded review of subject safety data (AEs, local toleration assessments, ECGs, laboratory safety tests and vital signs) and pharmacokinetic data will be performed by the Safety Review

Team in a formal safety review meeting at the end of Cohorts 1 and 2 to identify any potential safety signals and decide whether progression of the study to the next cohort is appropriate. There will be sufficient time between cohorts to allow for a thorough review of data and decision-making. The decision will be documented.

During the study, subject safety will be assessed through physical examination, vital signs, ECGs, local toleration assessments (Cohort 1 only), safety clinical laboratory tests and adverse event monitoring.

Continuation to the next cohort will proceed unless study drug-related intolerance or AEs are identified through safety monitoring. Progression to Cohort 2 will not proceed if 5 or more Cohort 1 subjects, dosed over 40% BSA, have persistent (on at least 3 consecutive occasions) local toleration reactions of ≥ 5 total score in the modified Draize local toleration assessment. Progression to Cohort 3 will not proceed if 5 or more Cohort 2 AD patients, dosed over 40% BSA, report persistent (on at least 3 consecutive occasions) skin related AE's potentially linked to study ointment application as opposed to changes in their normal pattern of AD disease. This includes if a patients' AD worsens beyond tolerability.

The maximum human plasma exposure level has been set at 13550 ng.hr/mL which is the no observed effect level (NOEL) exposure in rats following subcutaneous systemic dosing ($AUC_{0\text{-}t}$). Progression to the next cohort will not proceed if the mean area under the concentration-time curve (AUCτ) PK data in the previous cohort exceeds 13550 ng.hr/mL or if any of the safety stopping criteria are met.

Further details on the operation of the Safety Review Meetings will be defined in a separate procedures document.

3.7 Stopping Criteria

3.7.1 Cohort Stopping Criteria

Formal safety review meetings will be held following each cohort to assess the safety, toleration and pharmacokinetic data collected and decide whether to proceed to the next cohort.

- PK Stopping Criteria: Progression to the next cohort will not proceed if the mean AUCτ from existing cohort data exceeds 13550 ng.h/mL.
- Skin Toleration Stopping Criteria: Progression to Cohort 2 will not proceed if 5 or more Cohort 1 subjects, dosed over 40% BSA, have persistent (on at least 3 consecutive occasions) local toleration reactions of ≥ 5 total score in the Draize local toleration assessment. Progression to Cohort 3 will not proceed if 5 or more Cohort 2 AD patients, dosed over 40% BSA, report persistent (on at least 3 consecutive occasions) skin related AE's potentially linked to study ointment application as opposed to changes in their normal pattern of AD disease. This includes if a patients' AD worsens beyond tolerability.
- Safety Stopping Criteria: Dose escalation will not proceed if any of the following occur in a ZPL-5212372-treated subject:
  - Severe adverse reaction(s) indicating study drug-related intolerance.

- Clinically significant ECG abnormalities (such as arrhythmias) or effects on other vital signs indicating dose-related intolerance.
- Clinically significant laboratory abnormalities (see Appendix 1 and 2) indicating dose-related intolerance.
- Other findings that, at the joint discretion of the CMO and investigator, indicate dose escalation should be halted.

If any of the above occurs, prompt medical treatment should be provided as appropriate and adverse events promptly reported to the Sponsor via telephone or email.

After satisfactory review of the available pharmacokinetic, safety, and tolerability data progression to the next cohort will occur if the administration to the previous cohort was well tolerated. Adaptive features may be discussed and modifications made based on the safety/PK or learnings from the cohort. Any modifications within the boundaries specified in the protocol will not require a protocol amendment, but will be documented in the minutes of the safety review meeting.

3.7.2 Individual Stopping Criteria

- If a healthy volunteer subject has a persistent (on at least 3 consecutive occasions) grade 3 or greater score in erythema, oedema or desquamation, application of study ointment will be discontinued and only safety and PK evaluations will be performed for the remaining scheduled study visits until the AE is resolved.

- If, in the opinion of the investigator, a patient's AD worsens beyond tolerability the patient will have their study medication discontinued and will be followed up until the worsening of their AD has resolved and is under control.

3.8 Planned Sample Size and Number of Study Centers

For Cohorts 1 and 2 it is envisaged that sufficient subjects will be screened to achieve approximately 12 randomised and evaluable subjects (i.e. sufficient subjects completing study treatment to meet the requirements of the data review before proceeding to the next cohort). Recruitment for Cohorts 1 and 2 will be from a single centre in the United Kingdom.

For Cohort 3 it is envisaged that approximately 45 subjects will be screened to achieve approximately 30 randomised and evaluable subjects. Recruitment for Cohort 3 will be from across approximately 4 centers in the United Kingdom. See Section 8.9 for a discussion of sample size.

4 STUDY POPULATION

Upon screening, each subject will receive a 3 digit subject number which will be used throughout the study. Randomisation will occur on Day 1, after all screening procedures have been performed and eligibility for the study confirmed.

Any subjects who terminate their study participation for any reason, regardless of whether study drug was applied or not, will retain their subject number.

Subjects will be randomised on a 2:1 basis to topical 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment applied twice daily. For the randomisation of subjects, the investigator will use an Integrated Web-based Response System (IWRS). The IWRS will use a computer-generated randomisation schedule to assign subjects to a treatment sequence. For Cohort 3 the randomisation will be stratified based on baseline EASI score (either EASI ≤20 or EASI >20) to ensure an even distribution of EASI and associated BSA affected across both treatment groups.

To fulfil the study objectives it is essential that appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. Subject eligibility should be reviewed and documented by an appropriate member of the site study team. The study population will consist of a cohort of adult healthy volunteers and 2 cohorts of adult patients with moderate to severe AD.

4.1 Inclusion Criteria: Cohort 1

Healthy volunteer subjects for Cohort 1 must meet all of the following inclusion criteria to be eligible for inclusion in the study

1 Healthy males or females, aged between 18 and 55 years, inclusive (healthy is defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, 12-lead ECG and clinical laboratory tests).

2 Females should be either of non-child-bearing potential (as specified in Section 4.7) or must agree to use highly effective methods of contraception (as specified in Section 4.7). Males with partners who are WOCBP must also use contraception (as specified in Section 4.7)

3 Body weight of ≥ 50 kg.

4 Body Mass Index of ≤34.9 kg/m^2.

5 Subjects must have a Fitzpatrick Skin Type[26] of between I to III to be included in the study.

6 Willing and able to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

7 Evidence of a personally signed and dated informed consent form, indicating that the subject has been informed of all pertinent aspects of the study.

4.2 Inclusion Criteria: Cohorts 2 and 3

Moderate to severe AD patients for Cohorts 2 and 3 must meet all of the following inclusion criteria to be eligible for inclusion in the study

1 Males and females aged 18-65 years inclusive with physician documented history or diagnosis of atopic dermatitis for at least 6 months prior to screening. AD should be diagnosed by the Eichenfield revised criteria of Hanifin and Rajka.[1]

2 Females should be either of non-child-bearing potential (as specified in Section 4.7) or must agree to use highly effective methods of contraception (as specified in Section 4.7).

Males with partners who are WOCBP must also use contraception (as specified in Section 4.7)

3 Body weight of ≥ 50 kg.

4 Body Mass Index of ≤34.9 kg/m^2.

5 Eczema Area and Severity Index (EASI) of ≥9 and ≤48 at Screening and an EASI of ≥12 and ≤48 at Day 1.

6 An Investigator's Global Assessment (IGA) score ≥ 3 at both Screening and Day 1.

7 Atopic dermatitis affecting between ≥10 to ≤40% BSA at Screening and ≥10% to ≤50% BSA on Day 1.

8 Patients must be willing to stop applying their daily emollients and instead use the study emollient twice daily from at least 7 days prior to randomisation and throughout their participation in the study.

9 Willing and able to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

10 Evidence of a personally signed and dated informed consent form, indicating that the subject has been informed of all pertinent aspects of the study.

4.3 Exclusion Criteria: Cohort 1

Healthy volunteer subjects for Cohort 1 presenting with any of the following will not be included in the study:

1 Evidence or history of clinically significant haematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing).

2 Have tattoos covering areas of skin to be dosed with study ointment.

3 Subjects who are hirsute in areas of skin to be dosed with study ointment.

4 Subjects who are unwilling to stop hair removal by any means (including shaving, waxing or depilatory creams) to skin areas to be dosed with study ointment for 2 weeks prior to randomisation and throughout the duration of the study.

5 Have concomitant skin disease or infection (e.g. acne, impetigo) or presence of skin comorbidities in the study area to be dosed that may interfere with study assessments.

6 Hypersensitivity to any excipients in the study ointment formulation, study emollient [redacted] or study shower cream [redacted]

7 Hypersensitivity to any laundry detergents.

8 Use of a tanning booth/parlour/sunbathing (including excessive exposure to sunlight) or use of tanning products within 4 weeks before start of the study and for the duration of their participation in the study.

9 History of sensitivity to nonsteroidal anti-inflammatory drug(s) (NSAIDs).

10 Clinically significant cardiac disease or ECG abnormalities, including:

- Predominant heart rhythm other than normal sinus rhythm
- AV block greater than first degree
- Resting pulse rate >100 or <50 bpm
- Electrocardiographic evidence or a history of myocardial infarction
- Electrocardiographic evidence of conduction system abnormality
- Subjects with pre-randomisation evidence of QTc prolongation (defined as >450 msec).

The PI should decide whether ECG abnormalities other than those listed are clinically significant and should exclude the subject from enrolment.

11 Subjects with a personal or family history of prolonged QTc interval.

12 Clinically significant liver function tests (LFTs; AST, ALT, total Bilirubin) >1.5 X upper limit of normal (ULN). Subjects with LFTs >1.5 X ULN and <2 X ULN may be retested once.

13 Positive HIV, HBsAg and HBcAb result or positive anti-hepatitis C virus serology (as determined by multi-antigen EIA)

14 Pregnant and nursing females.

15 Donation or intent to donate blood, or blood components during the study or within one month after completion of the study.

16 Subjects who have received treatment with an investigational drug within 3 months prior to screening.

17 Subjects with a known history (of at least 2 years) of drug abuse.

18 A positive urine drug screen.

19 Subjects who are smokers (including subjects who use nicotine substitutes).

20 History of regular alcohol consumption exceeding 21 drinks/week in males and 14 drinks/week in females (1 drink = 150 mL of wine or 360 mL of beer or 45 mL of spirits) within 6 months of screening.

21 Subjects using potent CYP3A4 inhibitors (i.e, cimetidine, clarithromycin, cyclosporine, danazol, dexamethasone, diethyldithiocarbamate, erythromycin, fluconazole, fluoxetine, grapefruit juice, ketoconazole, metronidazole, norfloxacin, omeprazole, ranitidine, sertraline, valproic acid)

22 Inability to comprehend or unwilling to follow the study requirements including restrictions on treatments, attendance at out-subject clinic visits and participation in laboratory testing as called for by the protocol.

23 Any other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator in discussion with the Ziarco CMO, would make the subject inappropriate for entry into this study.

4.4 Exclusion Criteria: Cohorts 2 and 3

Moderate to severe AD patients presenting with any of the following will not be included in the study:

1 AD of such severity (EASI >48) that the subject could not comply with the demands of the study and/or the subject is not a suitable candidate for a placebo-controlled study.

2 Have concomitant skin disease or infection (e.g. acne, impetigo) or presence of skin comorbidities in the study area to be dosed that may interfere with study assessments.

3 Patients who are hirsute in areas of skin to be dosed with study ointment.

4 Patients who are unwilling to stop hair removal by any means (including shaving, waxing or depilatory creams) to skin areas to be dosed with study ointment for 2 weeks prior to randomisation and throughout the duration of the study.

5 Hypersensitivity to any excipients in the study ointment formulation, study emollient or study shower cream

6 Hypersensitivity to any laundry detergents (Cohort 2 only).

7 Have received phototherapy (e.g. UVA, UVB or PUVA therapy), or systemic therapy (e.g. immunosuppressants [such as cyclosporine, azathioprine, methotrexate], cytostatics) known or suspected to have an effect on AD, within 4 weeks of the start of the study. All other biologics should not have been used within 3 months of the start of study.

8 Have received systemic corticosteroids (e.g. oral, intravenous, intraarticular, rectal) within 4 weeks of the start of the study. Subjects on a stable maintenance dose (over the preceding 3 months) of inhaled or intranasal CS may participate.

9 Patients treated with oral antihistamines or topical calcineurin inhibitors or topical steroids within 7 days of starting study; intranasal antihistamines for the treatment of allergic rhinitis are acceptable.

10 Use of a tanning booth/parlour/sunbathing (including excessive exposure to sunlight) or use of tanning products within 4 weeks before start of the study and for the duration of their participation in the study.

11 Have used antiseptic treatments (e.g. bleach baths, potassium permanganate etc.) within 4 weeks before the start of the study. (Patients who have recently used antiseptic treatment

may be rescreened at a later date if they wish to participate in the study and agree to stop antiseptic treatment.)

12 History of sensitivity to nonsteroidal anti-inflammatory drug(s) (NSAIDs).

13 Patients who have evidence of significant concomitant clinical disease that could interfere with the conduct or safety of this study, based upon a complete medical history, full physical examination, and a 12-lead resting ECG and laboratory safety tests. For example the presence of any other acute and/or unstable clinical disease, other than AD, such as:

- Poorly controlled Type I or Type II diabetes.
- Seizure disorder or epilepsy.
- Cerebrovascular accident.
- Active or latent infection (eg, hepatitis).
- Cancer (other than cutaneous basal cell) in the last 5 years.
- Congestive heart failure.
- Coronary artery disease.
- Renal insufficiency and/or serum creatinine >1.5 X upper limit of normal (ULN). Subjects with serum Creatinine <2 X ULN may be retested once.
- A major surgical operation during the 30 days prior to screening.

Stable well-controlled chronic conditions such as controlled hypertension [excluding those on ß-blockers, calcium channel blockers (Class I or II)], thyroid disease, well-controlled Type 1 or Type 2 diabetes, hypercholesterolemia, gastroesophageal reflux, or depression under control with medication (other than tricyclic antidepressants), are acceptable provided the symptoms and medications would not be predicted to compromise safety or interfere with the tests and interpretations of this study.

14 Clinically significant cardiac disease or ECG abnormalities, including:

- Predominant heart rhythm other than normal sinus rhythm
- AV block greater than first degree
- Resting pulse rate >100 or <50 bpm
- Electrocardiographic evidence or a history of myocardial infarction
- Electrocardiographic evidence of conduction system abnormality
- Subjects with pre-randomisation evidence of QTc prolongation (defined as >450 msec).

The PI should decide whether ECG abnormalities other than those listed are clinically significant and should exclude the subject from enrolment.

15 Patients with a personal or family history of prolonged QTc interval.

16 Clinically significant liver function tests (LFTs; AST, ALT, total Bilirubin) Subjects with LFTs >1.5 X ULN and <2 X ULN may be retested once.

17 Positive HIV, HBsAg and HBcAb result or positive anti-hepatitis C virus serology (as determined by multi-antigen EIA)

18 Pregnant and nursing females.

19 Donation or intent to donate blood, or blood components during the study or within one month after completion of the study.

20 Patients who have received treatment with an investigational drug within 3 months prior to screening.

21 Patients with a known history (of at least 2 years) of drug abuse.

22 A positive urine drug screen.

23 Patients who are smokers (including patients who use nicotine substitutes). (Cohort 2 only)

24 History of regular alcohol consumption exceeding 21 drinks/week in males and 14 drinks/week in females (1 drink = 150 mL of wine or 360 mL of beer or 45 mL of spirits) within 6 months of screening.

25 Patients using potent CYP3A4 inhibitors (i.e, cimetidine, clarithromycin, cyclosporine, danazol, dexamethasone, diethyldithiocarbamate, erythromycin, fluconazole, fluoxetine, grapefruit juice, ketoconazole, metronidazole, norfloxacin, omeprazole, ranitidine, sertraline, valproic acid

26 26 Inability to comprehend or unwilling to follow the study requirements including restrictions on treatments use of study emollient, attendance at out-subject clinic visits and participation in laboratory testing as called for by the protocol.

27 Any other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator in discussion with the Ziarco CMO, would make the subject inappropriate for entry into this study.

4.5 Life Style Guidelines: Cohorts 1 and 2

The following life-style guidelines affect all subjects in Cohorts 1 and 2:

Meals and Dietary Restrictions

Water may be consumed without restriction. Non-caffeinated drinks (except grapefruit or grapefruit-related citrus fruit juices - see below) may be consumed with meals and the evening snack.

Subjects will be fasted from midnight.

Breakfast will be provided approximately 1 hour before the morning application except on Days 1 and Day 7 when no food will be provided until after the 4 hours post dose assessments have been completed.

Lunch will be provided approximately 4 hours after the morning application.

Dinner will be provided approximately 9 to 10 hours after the morning application.

An evening snack will be permitted.

Subjects will not be allowed to eat or drink grapefruit or grapefruit-related citrus fruits (eg, Seville oranges, pomelos) from 7 days prior to the first dose of study medication until collection of the final pharmacokinetic blood sample.

While in the CRU, the total daily nutritional composition should be approximately 50% carbohydrate, 35% fat and 15% protein. The daily caloric intake per subject should not exceed approximately 3200 kcal.

Alcohol and Caffeine

Subjects will abstain from alcohol for 24 hours prior to admission to the CRU and continue abstaining from alcohol until collection of the final pharmacokinetic sample. Subjects may undergo an alcohol breath test at the discretion of the investigator.

Subjects will abstain from caffeine-containing products for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample.

Activity

Subjects will abstain from unaccustomed strenuous exercise (e.g., heavy lifting, weight training, calisthenics, aerobics) for at least 48 hours prior to each blood collection for clinical laboratory tests. Regular routine exercise and walking at a normal pace will be permitted.

Subjects should have a normal day time awake, night time asleep cycle throughout the study.

Showering

Subjects will briefly shower (approximately 5 minutes) once in the morning at least 30 minutes prior to the first application of study ointment using [redacted]

Clothing

Subjects will be provided with cotton T-shirts and trousers to wear during the study.

4.6 Life Style Guidelines: Cohort 3

Patients in Cohort 3 will be recommended to use old clothes or test the ointment on a small, unobtrusive area of clothing as this is a developmental formulation which has not been tested for compatibility with different types of fabric.

4.7 Contraceptive Requirements

Contraceptive requirement for the study have been defined using the recent recommendations of the Clinical Trials Facilitation Group, Rome September 2014 meeting and also the EMA M3(R2) guidance.[25]

Females

Females of child-bearing potential must agree to use highly effective methods of contraception from screening to 60 days after the last study dose. Highly effective methods are:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation administered by oral or intravaginal route
- oral, injectable or implantable progestogen-only hormonal contraception associated with inhibition of ovulation
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner
- true sexual abstinence, where this is in line with the usual lifestyle of the subject; periodic (calendar, ovulation, symptothermal or post-ovulation) abstinence and withdrawal are not acceptable methods of contraception.

Females are considered to be of non-child-bearing potential if they fulfill at least one of the following criteria:

- Postmenopausal, defined as 45 to 65 years of age and amenorrheic for at least 2 years PLUS have a serum follicle stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal women, at screening.
- have undergone a documented hysterectomy and/or bilateral oophorectomy.

Males with partners who are WOCBP

Males with partners who are WOCBP must agree to use contraception from the start of dosing study ointment until 120 days after the last dose of study ointment.

4.8 Subject Withdrawal and Replacement

Subjects will be discontinued from the investigational product and potentially from the study in the following circumstances:

1 Enrollment in other clinical studies involving investigational products or enrollment in other types of clinical research judged not to be scientifically or medically compatible with this study.

2 The subject requests to be discontinued from the study.

3 Use of prohibited concomitant medications.

4 Sponsor decision to stop the study or to stop the subject's participation in the study for medical, safety, pharmacokinetic or regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practices (GCP).

5 A severe or serious adverse event occurs that the investigator considers is related to study drug.

6 Any criteria for laboratory safety or vital sign values of Potential Clinical Concern (Appendix 1).

7 Any criteria for Hepatic Safety (Appendix 2).

8 Any criteria for ECG Values of Potential Clinical Concern (Appendix 3).

Subjects may have their study drug discontinued if they have abnormal laboratory results which are considered to be clinically significant as assessed by the investigator. This decision should be made in the best interest of the subject and follow up should be conducted using standard clinical practices. Subjects may also be temporarily discontinued from study drug while AEs and/or laboratory test abnormalities are being investigated and/or at the request of the sponsor's CMO. The investigator should discuss with Ziarco's CMO any subject for whom temporary or permanent discontinuation of study drug is being proposed. Subjects who discontinue early from the study should be asked to complete all the efficacy and safety assessments scheduled for the end of study assessment.

Subjects may withdraw from the study at any time without penalty and for any reason without prejudice to their future medical care. In all cases, the reason(s) for withdrawal, including the primary reason, must be recorded on the case report form (CRF). If a subject is prematurely withdrawn from the study for any reason, the investigator must make every effort to perform the evaluations described for the early termination/follow up visit (in clinic) and a further follow up contact (either by telephone or in clinic) 7 to 14 days later. Subjects who withdraw due to lack of efficacy (Cohort 3) or adverse events which may be attributed to double blind treatment will not be replaced.

Cohort 3 patients who withdraw due to personal reasons, poor compliance, other protocol deviations or are lost to follow up may be replaced until at least 30 evaluable patients have completed the study.

4.9 Temporary Discontinuations

A subject who misses one or more doses due to personal circumstances, or has an AE (not related to study drug), may be allowed to restart dosing after discussion between the investigator and CMO.

5 STUDY TREATMENT

5.1 Allocation to Treatment

All subjects will be allocated a unique 3-digit subject number, by the IWRS, that will be recorded in the CRF and on all subject related study documentation. The IWRS will use a computer generated randomisation schedule to assign subjects to a treatment sequence on a 2:1 basis and will stratify subjects in Cohort 3 based on the EASI score to ensure an even distribution of severities across the treatment groups.

5.2 Breaking the Blind

This study is double-blind third party open i.e. the study will be subject- and investigator-blinded. Details of personnel who will be able to review unblinded subjects' data during study will be held in a separate document by Ziarco.

At the initiation of the study, the study site will be instructed on the method for breaking the blind. The study blind should not be broken except in a medical emergency, where knowledge of the study drug received would affect the treatment of the emergency or obtaining this information is a regulatory requirement. If time permits, the investigator should notify the Sponsor prior to contacting IWRS. All calls resulting in an unblinding event will be reported by the IWRS to the Sponsor.

If the blind is broken, the date, time and reason must be recorded in the subject's CRF Blood specimens will be obtained from all subjects for pharmacokinetic analysis to maintain the study blind at the investigator site. The study monitor will remain blinded to treatment until the conclusion of the study. To minimize the potential for bias, treatment randomisation information will be kept confidential by Ziarco personnel and will not be released to the investigator, investigator site personnel, or the study monitor until the conclusion of the study.

ZPL-5212372 will be provided as single dose strength of a 1.0% (w/w) concentration of ointment. Placebo will be provided as visually matched ointment. All study drugs will be supplied in identical pots and will be similar in colour and appearance, thereby maintaining double-blind conditions for the subject and investigator.

5.3 Drug Supplies

[redacted] to all Cohort 1 and 2 subjects, for use when showering each morning whilst resident in the CRU.

[redacted] will be supplied as study emollient to all AD patients (Cohorts 2 and 3 only) for use prior to randomisation and throughout the study by MAC.

[redacted] will be supplied as an alternate study emollient to any AD patients (Cohort 3 only) who have a potential (or known) hypersensitivity reaction to [redacted] for use prior to randomisation and throughout the study by MAC.

[redacted] and supplied with a certificate of analysis.

Matching placebo ointment will be manufactured by [redacted] and supplied with a certificate of analysis.

ZPL-5212372 and placebo will be administered as a topical ointment. Both ointments contain the same excipients; placebo ointment has been manufactured in the same way except for the addition of 1.0% (w/w) ZPL-5212372.

5.4 Treatment Packaging and Labelling

Ointment will be packaged in [redacted] and will be labelled and released in accordance with the Clinical Trials Directive 2001/20/EC and GMP Directive 2003/94/EC for Investigational Medicinal Products. Each jar will contain 100 g of ointment. Supplies will be identified by

the batch number, expiry date and a unique 5-digit jar code which will be used by the IWRS to identify the content and allocate jars to study subjects.

5.5 Dispensing

[redacted]

For Cohort 3 study medication will be supplied to the subject as:

- Randomisation Visit (Day 1): 4 glass jars containing either 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment, plus an additional glass jar containing 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment, to be used as spare medication, in the event of late visits or lost/damaged container, to ensure continuous dosing.

- On Visit 3 (Day 8): empty or partially used glass jars containing either 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment will be replaced as required to enable dosing till the end of the study based on the patients usage. If required the spare ointment supply will also be replaced as necessary.

Cohort 3 patients will be supplied with an adequate quantity (including some spares) of [redacted] and sealable plastic bags to store and return used [redacted] for disposal at the CRU. In addition, patients will be supplied with an adequate quantity of disposable gloves to allow carers to apply the study ointment onto hard to reach areas of their body as required.

Cohort 3 patients will be instructed by the unit staff to only open and use one jar of study ointment at a time.

5.6 Drug Storage

Study medication should be stored at 15 to 25°C, in a securely locked area, accessible to authorised personnel only in the CRU Pharmacy. For Cohort 3 patients study ointment should be stored at 15 to 25°C (i.e. room temperature) and kept out of the reach of children. Ointment remaining more than 14 days after opening should not be used.

5.7 Administration

Subjects in Cohorts 1 and 2 will have twice daily applications of either 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment approximately 12 hours apart. The morning dose will be administered between 07:00 to 10:00 hours and the evening dose approximately 12 hours later between 19:00 – 22:00 hours. Study ointment will be dispensed from the pharmacy (one jar per subject) to clinical staff. Clinical staff will consult the subjects 'body map' to confirm whether 10 or 40% BSA will have application of ointment. For 10% BSA approximately [redacted] and for 40% BSA approximately [redacted] by clinic staff at the subject's bedside. If the initial volume of ointment is inadequate to cover the dosing area, additional ointment maybe applied up to a maximum of 1 mL/2% BSA (i.e. 5 mL for 10% BSA or 20 mL for 40% BSA).

Study Ointment Administration

- Healthy volunteer subjects in Cohort 1 in the 10% BSA sentinel group will have study ointment applied by clinic staff directly onto their skin [redacted] onto the top half of their back (see Appendix 4 for example body map). Subjects in the 40% BSA group will have study ointment applied by clinic staff directly onto their skin [redacted] onto their back and the fronts and backs of their upper legs (see Appendix 5 for example body map). Clinical staff will gently spread the study ointment over the entire dosing area with a gloved hand to ensure a thin, even coverage of ointment. No study emollient will be applied to Cohort 1 healthy volunteers to ensure local toleration of the study ointment can be fully assessed.

- AD patients in Cohort 2 in the 10% BSA sentinel group will have study ointment applied by clinic staff directly onto 10% of their AD affected skin [redacted] In some patients this will be all their AD affected skin and in others it may only be a portion of their AD affected skin (see Appendix 6 for example body map). Patients in the 40% BSA group will have study ointment applied by clinic staff directly onto all their AD affected skin. In some patients this will be all their AD affected skin and in others it maybe their total AD affected skin does not cover 40% BSA hence additional non-AD lesion skin will also need to be covered (see Appendix 7 for example body map). Individual patient body maps will need to be followed on each dosing occasion. Clinical staff will gently spread the study ointment over the entire dosing area with a gloved hand to ensure a thin, even coverage of ointments. After 15 minutes the study emollient will be applied by the patient to the same areas treated with the study ointment and any additional body areas the patient routinely applies emollient to. Patients will need to wash their hands immediately after application of study emollient in case of contamination with study ointment.

- AD patients in Cohort 3 will apply twice daily (approximately 12 hours apart) applications of either 1.0% (w/w) ZPL-5212372 ointment or matching placebo ointment at home. At the patients randomisation visit the extent of their AD will be assessed and a recommended amount (approximately 1.25 to 5 mL/10% BSA) of ointment for use on their AD affected skin will be recorded on their individual body map (see Appendix 8 for example body map). Patients will be instructed not to use ointment on non-AD affected skin. Study ointment will be dispensed from the pharmacy (three jars per subject) to clinical staff. The first application of study ointment will be applied by the patient in the clinic under the supervision and direction of the clinical staff i.e. the amount to apply, body area to cover using the body map as a guide and how to [redacted] will be explained, as this will vary from patient to patient. Study emollient will be applied at least 15 minutes AFTER application of study ointment to all study ointment treated areas and any additional body areas the patient usually applies emollient to. Thereafter patients will be advised to dispense and apply the study ointment and emollient in the same manner for the remainder of the study at home. Patients will be supplied with a copy of their individual body map and dosing instructions. Patients will record their study ointment and emollient applications on a paper dosing diary which they will bring to the clinic on visits for compliance assessments by the clinic staff (this information will not be databased). On study visit days, patients should NOT apply their morning application of study ointment and

emollient. Instead they should bring their remaining study ointment and emollient to the clinic and the study ointment and emollient will be applied in the clinic after the visits study assessments have been performed under the supervision of clinic staff.

In all cases, once recorded the areas of skin to be treated with study ointment will be treated consistently for the duration of the study regardless of any changes in the size or extent of AD affected skin areas during the study period.

Regardless of the location of an AD patient's lesioned skin **no study ointment will be applied to areas on the face or scalp above the jaw line**.

Emollients

The emollient [redacted] will be supplied for this study for AD patients in Cohorts 2 and 3. In addition an alternate emollient will be available for Cohort 3 patients who have a potential for any hypersensitivity skin reactions to [redacted] Any emollients and bathing products used by the patient should be documented at screening. Patient emollients may be used up until 7 days prior to Day 1 and then patients must stop using their current emollient and switch to using the study emollient in the same manner as their previous emollient. From randomisation patients must use study emollient at least 15 minutes after application of study ointment. Patients should use study emollient on all skin areas which have been treated with study ointment and in addition any areas which the patient would treat as part of their normal skin care routine. Cohort 2 patients must continue using study emollient twice daily until discharge from the CRU on Day 9 and Cohort 3 patients must use study emollient twice daily until they have completed their visit on Day 15.

Shower Cream

[redacted]
subjects, for use when showering each morning whilst resident in the CRU.

5.8 Excessive Pharmacological Effects

No mechanistically specific antidotes for ZPL-5212372 are available and standard supportive measures should be used in the case of excessive pharmacological effects.

5.9 Drug Accountability

The investigator is responsible for maintaining accurate study drug accountability records throughout the study.

The IWRS will be used to allocate jars of study drug to all study subjects. In addition, details of jars dispensed to patients and applications of study medication will be documented in accountability forms, the CRF and the patient diaries.

Cohort 1 and 2 (in-patients): Jars of study ointment allocated by the IWRS will be documented on the accountability log. Jars will be weighed and the weight recorded. Clinical staff, and the checker, will need to confirm and document in the CRF that both the correct volume (mL) of ointment and the correct jar of ointment is used on each dosing occasion. Empty jars will be weighed and the weight recorded and at the end of dosing any partially used jars will be weighed and the weight recorded. At the end of the subject's participation

in the study the approximate total amount of study ointment used by the subject over 7 days will then be calculated. Each dose administered will also be documented in the CRF.

Cohort 3 (out-patients): Jars of study ointment allocated by the IWRS and will be weighed, at the time of dispensing, and the weight recorded on the accountability log. The initial dose applied in the clinic will be taken from one of the patient's allocated jars and the application documented in the CRF. Clinical staff, and the checker, will need to confirm and document in the CRF that both the correct volume (mL) of ointment and the correct jar of ointment is used on the initial dosing occasion. The patient must bring all study medication (including empty containers) to the clinic at each visit, so that compliance can be confirmed. At the end of the study all jars allocated to the patient will be weighed and the weight recorded on the drug accountability log and used to calculate the approximate total amount of study ointment used by the patient over the 14 days treatment period.

Any supplies, including placebo remaining at the end of study will be returned to the Sponsor or their representative or destroyed locally on behalf of the Sponsor and the destruction fully documented.

5.10 Compliance (Cohort 3 only)

At each clinic visit patient compliance with the study ointment regimen will be monitored by recording any empty containers and weighing any partially used containers and by reviewing the patients dosing diary. Patients failing to take 2 or more doses in a 7 day period will be reminded of the importance of complying with the dosing requirements. If a patient consistently fails to take their medication, the investigator should ask the patient about compliance and following this discussion should alert the Ziarco CMO and study monitor and a decision will be made as to whether the subject should be withdrawn for non-compliance.

5.11 Concomitant Medications

Any medication the subject takes other than the study drug is considered a concomitant medication. All concomitant medications must be recorded in the CRF. The following information must be recorded in the CRF for each concomitant medication: generic name, route of administration, start date, stop date and indication.

At screening, subjects will be asked what concomitant medications they are currently taking. At each subsequent study visit, subjects will be asked what medications they have taken since the last clinic visit.

Any medications taken prior to the start of dosing will be documented as prior medications. Any medications taken after the first dose of study medication will be documented as concomitant medications.

The type of emollient or bathing products used by the AD patients (Cohorts 2 and 3) should be captured during screening. For AD patients study emollient will be used from 7 days prior to dosing and throughout the remainder of the study until either discharge from the CRU on Day 9 (Cohort 2) or following their visit on Day 15 (Cohort 3).

AD patients may not use the medications and treatments listed in Table 6 within the indicated time interval prior to the start of dosing and these medications must be withheld for the duration of the study, and where possible until completion of the follow up visit. However AD patients may commence use with other topical or systemic treatments for their AD if required from either discharge from the CRU (Cohort 2) or the end of dosing (Cohort 3).

Table 6 - Prohibited Medications for Cohort 2 & 3 AD patients

Medication	Time interval prior to Day 1
Biologic treatments for AD	3 months
UVA, UVB or PUVA therapy	4 weeks
Oral calcineurin inhibitors and immunosuppressants (e.g. cyclosporine, azathioprine, methotrexate)	4 weeks
Systemic corticosteroids (all potencies)	4 weeks
Oral antihistamines and leukotriene inhibitors and tricyclic antidepressants	1 week
Topical steroids (any potency)	1 week
Topical calcineurin inhibitors (tacrolimus, pimecrolimus)	1 week
Cytostatics known or suspected to have an effect on AD	4 weeks
Allergen Immunotherapy	4 weeks
Investigational drug	3 months from start of screening
Potent CYP3A4 inhibitors (e.g. cimetidine, clarithromycin, cyclosporine, danazol, dexamethasone, diethyldithiocarbamate, erythromycin, fluconazole, fluoxetine, grapefruit juice, ketoconazole, metronidazole, norfloxacin, omeprazole, ranitidine, sertraline, valproic acid)	1 week

Oral antibiotics are allowed for up to 2 weeks for disease-associated superficial skin infections, but subjects should have completed any course prior to the start of Day 1. Note that active skin infection is an exclusion criterion.

The following concomitant procedures are prohibited during study participation:

- Elective major surgical procedures
- Use of artificial tanning treatments or products

6 STUDY CONDUCT

This is a multicenter study in the United Kingdom, Cohorts 1 and 2 will be conducted at a single site and Cohort 3 will be conducted at up to 4 study sites.

Schedules of assessments are presented in Section 6.7 Tables 9 to 11. Details of study assessments are provided in Section 7.

Cohort 3 visits should occur on the scheduled day ± 1 day, except screening and follow-up, where some flexibility is allowed. All times should be recorded using the 24-hour clock (e.g., 23:20 not 11:20 pm).

6.1 Screening

All subjects will be given both a written and verbal description of the study. Subjects should be given adequate time to think about whether they want to participate and to ask questions. The investigator (or an appropriate delegate at the investigator site) will obtain written consent from each subject prior to commencing any study related activities or assessments. Following consent, all subjects will undergo screening in the 28 days prior to the start of the study (Day 1), to ensure that they meet the selection criteria for the study.

At screening all subjects will be allocated a unique 3-digit subject number by the IWRS, which will be recorded in the CRF and used for the duration of the study.

The following procedures will be performed:

- Subject demography
- Complete medical history (including medication [prescription and non-prescription drugs/treatments, topical products, vitamins and dietary supplements taken in the last 4 weeks], alcohol and tobacco use)
- Full physical examination (including height and weight)
- Fitzpatrick skin type assessment (for Cohort 1 only must be I to III for a subject to be eligible)
- Duplicate vital signs (supine blood pressure and pulse rate)
- Single 12-lead ECG for Cohorts 1 and 2. Triplicate 12-lead ECG for Cohort 3.
- Breath alcohol testing
- Blood and urine samples for:
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood for Hepatitis B/C
    - HIV serology
    - Serum for Hb1Ac
    - Serum FSH test (post-menopausal females only)
    - Serum pregnancy test (pre-menopausal women only)
    - Urine drug screen
- EASI, IGA and BSA assessment for Cohorts 2 and 3 only (AD patients)

Provided all inclusion and exclusion criteria are met the subject may start the wash out period (if applicable).

To prepare for study participation, subjects will be reminded of the study restrictions and prohibited concomitant medications. AD patients will be provided with study emollient and reminded to start using study emollient twice daily at least 7 days prior to Day 1 and throughout the study.

6.2 Cohort 1 (Healthy Volunteers)

Cohort 1 will be split with a lead (n=3) sentinel group which will only have 10% BSA treated with study ointments. If after 48 hours of treatment (4 applications) the study ointments are well tolerated then the remaining subjects of the cohort (n=9) may start treatment with 40% BSA being treated with study ointments. Apart from this difference in percentage BSA treated all subjects in Cohort 1 will have the following assessments conducted in the designated timeframe.

Subjects will be admitted to the Unit on the morning of Day 1 or the evening before (if required) and will remain in the Unit until Day 9 following collection of the 48 hours post dose blood sample.

6.2.1 Day 1 assessments

On Day 1 the following assessments will be carried out **prior** to randomisation and dosing:

- Review of concomitant medications, including emollient use (may be conducted the evening before)
- Review changes in the subject's medical history since screening (may be conducted the evening before)
- Brief physical examination (may be conducted the evening before)
- Completion of body map of skin areas to record the skin area to be treated with study ointments (may be conducted the evening before)
- Breath alcohol testing (may be conducted the evening before)
- Triplicate 12-lead ECG
- Duplicate vital signs (supine blood pressure and pulse rate)
- Assessment of skin area to be treated for any abnormalities e.g. erythema
- Review of baseline adverse events
- Obtain blood and urine samples:
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
    - Blood sample for baseline reference. Serum to be stored frozen at the MAC laboratory through to completion of the study for possible use as a baseline reference should additional safety laboratory tests or markers of disease activity be indicated.
    - Urine for pregnancy testing (pre-menopausal women only) (may be conducted the evening before)
    - Urine drug screen (may be conducted the evening before)

Provided the subject meets all the study entry criteria they will be randomised to treatment. The IWRS will allocate jars of study ointment to be dispensed to the subject

and the appropriate study ointment either 1.0% (w/w) ZPL-5212372 or matched placebo will be [redacted] by the unit staff, as described in section 5.7, immediately prior to administration. Particular care must be taken to avoid contamination of the area to be used for blood sampling.

After dosing the following assessments will be carried out at 1, 2, 4, 8 and 12 hours after the morning application:

- Duplicate supine blood pressure and pulse rate
- Triplicate 12-lead ECG
- Blood sample for pharmacokinetic analysis of ZPL-5212372
- Local toleration assessment (12 hours post dose only)

Following the 12 hour blood sample and assessment of local toleration, the second application of study ointment will be administered by the clinical staff. After dosing the following assessments will be carried out at 13, 16 and 24 hours after the first application of ointment:

- Duplicate supine blood pressure and pulse rate (no collection at 16 hours)
- Triplicate12-lead ECG (no collection at 16 hours)
- Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”

6.2.2 Days 2 to 6 Assessments

On Days 2 to 6 inclusive, subjects will be dosed at 12 hourly intervals (twice per day) and the following assessments will be carried out prior to each study ointment application:

- Duplicate supine blood pressure and pulse rate
- Triplicate 12-lead ECG
- Local toleration assessment
- Obtain blood and urine samples for
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis) - Day 4 prior to first application only
    - Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”

6.2.3 Day 7 Assessments

On Day 7 the following assessments will be carried out pre-dose and 1, 2, 4, 8, 12 and 16 hours after the final application of study ointment on the morning of Day 7:

- Duplicate supine blood pressure and pulse rate (no collection at 16 hours)
- Triplicate 12-lead ECG (no collection at 16 hours)

- Local toleration assessment (immediately prior to final administration of study ointment only)
- Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"

6.2.4 Day 8 Assessments

On Day 8 the following assessments will be carried out at 24 and 36 hours after the final application of study ointment on the morning of Day 7:

- Duplicate supine blood pressure and pulse rate
- Triplicate 12-lead ECG
- Blood sample for pharmacokinetic analysis of ZPL-5212372
- Local toleration assessment (at 24 hours after final administration of study ointment only)
- Completion of Ziarco Ointment Questionnaire
- Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"

6.2.5 Day 9 Assessments

On Day 9 the following assessments will be carried out at 48 hours after the final application of study ointment on the morning of Day 7:

- Brief physical exam
- Local toleration assessment
- Duplicate supine blood pressure and pulse rate
- Triplicate 12-lead ECG
- Obtain blood and urine samples for
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
- Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"

On Day 9 after the 48 hour post dose assessments have been completed subjects may leave the Unit at the discretion of the supervising clinical staff and will be asked to return for a follow up visit 7-14 days following the final application of study ointment.

6.3 Cohort 2 (AD Patients)

Cohort 2 will be split with a lead (n=3) sentinel group which will only have 10% BSA treated with study ointments. If after 48 hours of treatment (4 applications) the study ointments are well tolerated then the remaining patients in the cohort (n=9) may start treatment with up to 40% BSA being treated with study ointments. Apart from this difference in percentage BSA treated all subjects in Cohort 2 will have the following assessments conducted in the designated timeframe.

Patients will attend the Unit on the morning of Day 1 or the evening before (if required) and will remain in the Unit until Day 9 following the 48 hours post dose blood sample.

6.3.1 Day 1 assessments

On Day 1 the following assessments will be carried out **prior** to randomisation and dosing:

- EASI, IGA and BSA assessments. For inclusion in the study, an EASI score of between 12 and 48, an IGA score of ≥3 and BSA affected by AD of ≥10% and ≤ 50% must be recorded (may be conducted the evening before)
- Review of concomitant medications (may be conducted the evening before)
- Review changes in the subject's medical history since screening (may be conducted the evening before)
- Brief physical examination (may be conducted the evening before)
- Completion of body map of skin areas affected by AD and identification and recording of skin area to be treated (may be conducted the evening before)
- Breath alcohol testing (may be conducted the evening before)
- Triplicate12-lead ECG
- Duplicate vital signs (supine blood pressure and pulse rate)
- Review of baseline adverse events
- Obtain blood and urine samples:
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
    - Blood sample for baseline reference. Serum to be stored frozen at the MAC laboratory through to completion of the study for possible use as a baseline reference should additional safety laboratory tests or markers of disease activity be indicated.
    - Urine for pregnancy testing in pre-menopausal women only (may be conducted the evening before)
    - Urine drug screen (may be conducted the evening before)

Provided the patient meets all the study entry criteria they will be randomised to treatment. The IWRS will allocate jars of study ointment to be dispensed to the patient and the appropriate study ointment either 1.0% (w/w) ZPL-5212372 or matched placebo will be [redacted] by the unit staff, as described in section 5.7, immediately prior to administration. Particular care must be taken to avoid contamination of the area to be used for blood sampling. At least 15 minutes after study ointment application study emollient should be applied.

After dosing the following assessments will be carried out at 1, 2, 4, 8 and 12 hours after the after the morning application:

- Duplicate supine blood pressure and pulse rate
- Triplicate 12-lead ECG
- Blood sample for pharmacokinetic analysis of ZPL-5212372

Following the 12 hour blood sample the second application of study ointment and emollient will be administered by the clinical staff. After dosing the following assessments will be carried out at 13, 16 and 24 hours after the first application of ointment:

- Duplicate supine blood pressure and pulse rate (no collection at 16 hours)
- Triplicate 12-lead ECG (no collection at 16 hours)
- Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

6.3.2 Day 2 to 6 assessments

On Days 2 to 6 inclusive subjects will be dosed at 12 hourly intervals (twice per day) and the following assessments will be carried out prior to each study ointment application:

- Duplicate supine blood pressure and pulse rate
- Obtain blood and urine samples for
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis) - Day 4 prior to first application only
    - Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

6.3.3 Day 7 assessments

On Day 7 the following assessments will be carried out pre-dose and 1, 2, 4, 8, 12 and 16 hours after the final application of study ointment on the morning of Day 7:

- Duplicate supine blood pressure and pulse rate (no collection at 16 hours)
- Triplicate 12-lead ECG (no collection at 16 hours)
- Blood sample for pharmacokinetic analysis of ZPL-5212372
- EASI, IGA and BSA assessments (on a single occasion only)

Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

6.3.4 Day 8 assessments

On Day 8 the following assessments will be carried out at 24 and 36 hours after the final application of study ointment on the morning of Day 7:

- Duplicate supine blood pressure and pulse rate
- Blood sample for pharmacokinetic analysis of ZPL-5212372
- Triplicate 12-lead ECG (24 hours post dose only)Completion of Ziarco Ointment Questionnaire

Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

6.3.5 Day 9 assessments

On Day 9 the following assessments will be carried out at 48 hours after the final application of study ointment on the morning of Day 7:

- Brief physical exam
- Duplicate supine blood pressure and pulse rate
- Obtain blood and urine samples for
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372

Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

On Day 9 after the 48 hour post dose assessments have been completed patients may leave the Unit at the discretion of the supervising clinical staff and will be asked to return for a follow up visit 7-14 days following the final application of study ointment.

6.4 Cohort 3 (AD Patients)

6.4.1 Baseline Visit (Day 1)

AD patients will attend the Unit on the morning of Day 1 when the following assessments will be carried out **prior** to randomisation and dosing:

- EASI, BSA and IGA assessments. For inclusion in the study, an EASI score of between 12 and 48, an IGA score of ≥3 and BSA affected by AD of ≥10% and ≤50% must be recorded.
- Completion of the Ziarco Itch Questionnaire
- Review of concomitant medications
- Review changes in the subject's medical history since screening
- Brief physical examination
- Duplicate vital signs (supine blood pressure and pulse rate)
- Review of baseline adverse events
- Completion of body map of skin areas affected by AD and identification and recording of skin area to be treated
- Breath alcohol testing
- Obtain blood and urine samples:
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
    - Blood sample for baseline reference. Serum to be stored frozen at the MAC through to completion of the study for possible use as a baseline reference should additional safety laboratory tests or markers of disease activity be indicated.
    - Urine for pregnancy testing (pre-menopausal women only)

- Urine drug screen

Provided the patient meets all the study entry criteria they will be randomised to treatment. The IWRS will allocate jars of study ointment to be dispensed to the patient and the appropriate study ointment either 1.0% (w/w) ZPL-5212372 or matched placebo. The patient will be shown how to dispense the study ointment and administer the study ointment by the clinical staff. At least 15 minutes after study ointment application study emollient should be applied under clinical staff's supervision.

Patients may then leave the Unit at the discretion of the supervising clinical staff after being provided with enough study medication and study emollient for at least one week.

Patients will be reminded of the study restrictions and also instructed on study medication dosing requirements and concomitant medications use and study emollient use.

6.4.2 Visits 2 to 4 (Days 5, 8 and 10)

Patients will attend the Unit on Days 5, 8 and 10. On these days the patient will be asked not to apply the morning dose of ointment or study emollient at home, but to apply it at the clinic under instruction of the clinic staff after the following assessments have been carried out:

- Review study medication compliance
- Duplicate supine blood pressure and pulse rate pre-dose
- EASI, BSA and IGA assessments.
- Completion of the Ziarco Itch Questionnaire
- Blood sample for pharmacokinetic analysis of ZPL-5212372.
- Review concomitant medications, including emollient use
- Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

Once all pre-dose activities have been completed the patient will apply both study ointment and study emollient under supervision of clinical staff, provided the dose was not already taken prior to the visit. If the patient has applied the morning dose of ointment prior to the visit then the approximate time of dosing should be recorded on the CRF.

Patients may then leave the Unit at the discretion of the supervising clinical staff after being provided with enough study medication and emollient, for the following week. Subjects will be reminded of the study restrictions and concomitant medications use and study emollient use.

6.4.3 Visit 5 Day 15 Or Early Termination

Patients will have applied their last study ointment application on the evening of Day 14, the day prior to the Day 15 clinic visit. Patients will attend the Unit on Day 15 when the following assessments will be carried out:

- Brief physical examination
- Duplicate supine blood pressure and pulse rate

- EASI, BSA and IGA assessments
- Completion of the Ziarco Itch Questionnaire
- Completion of the PGIC Questionnaire
- Completion of the Ziarco Ointment Questionnaire
- Safety laboratory tests (haematology, clinical chemistry and urinalysis)
- Blood sample for pharmacokinetic analysis of ZPL-5212372 (the date and time of the last dose of study drug should be recorded in the CRF)
- Collect study medication (including emollient)
- Drug compliance check
- Review concomitant medications, including emollient usage
- Assess symptoms by spontaneous reporting of adverse events and by asking the patients to respond to a non-leading question such as "How do you feel?"

Patients may then leave the Unit at the discretion of the supervising clinical staff and will be asked to return for a follow up visit 7-14 days following the final application of study ointment.

6.5 Follow Up visit

Subjects will return to the Unit 7-14 days following the last application of study ointment for a follow up visit. The assessments conducted at the follow up visit will vary depending in which cohort the subject participated. Table 7 summarises the assessments to be conducted for each cohort:

Table 7 - Follow up visit assessments for each cohort

Assessment	Cohort 1 (Healthy Volunteers)	Cohort 2 (AD Patients)	Cohort 3 (AD Patients)
Brief physical examination	Yes	Yes	Yes
Single 12-lead ECG	Yes	Yes	Yes
Duplicate supine blood pressure and pulse rate.	Yes	Yes	Yes
Safety laboratory tests (haematology, clinical chemistry and urinalysis)	Yes	Yes	Yes
Urine pregnancy testing (pre-menopausal women only)	Yes	Yes	Yes
EASI, BSA and IGA assessments	No	Yes	Yes
Ziarco Itch Questionnaire	No	No	Yes
Review concomitant medications, (including emollient usage if applicable)	Yes	Yes	Yes
Review of adverse events	Yes	Yes	Yes

6.6 Early Termination Assessments

Subjects who discontinue early from the study should, if possible, have early termination assessments performed. For patients in Cohort 3 they should have an early termination visit take place as soon as possible after the patient stops taking study drug. The observations and procedures summarized in Table 8 should be performed. The assessments conducted will vary depending in which cohort the subject participated. For the completion of questionnaires, the subject should be requested to answer with respect to how they were on their last dosing day.

Table 8 - Early termination visit assessments for each cohort

Assessment	Cohort 1 (Healthy Volunteers)	Cohort 2 (AD Patients)	Cohort 3 (AD Patients)
Brief physical examination	Yes	Yes	Yes
Single 12-lead ECG	Yes	Yes	No
Duplicate supine blood pressure and pulse rate.	Yes	Yes	Yes
Safety laboratory tests (haematology, clinical chemistry and urinalysis)	Yes	Yes	Yes
Urine pregnancy testing (pre-menopausal women only)	No	No	Yes
Blood sample for pharmacokinetic analysis of ZPL-5212372	Yes	Yes	Yes
Assessment of local toleration	Yes	No	No
EASI, BSA and IGA assessments	No	Yes	Yes
Ziarco Itch Questionnaire	No	No	Yes
PGIC	No	No	Yes
Ziarco Ointment Questionnaire	No	No	Yes
Review concomitant medications, (including emollient usage if applicable)	Yes	Yes	Yes
Review of adverse events	Yes	Yes	Yes

After the early termination visit, the subject should be instructed to return for the follow up visit which should be scheduled for 7-14 days after their last application of study ointment.

6.7 Schedule of Activities

Table 9 - Schedule of activities for Cohort 1 (Healthy Volunteers)

Activities	Screening[1]	Day 1	Day 2 to Day 6	Day 7	Day 8	Day 9/ Early Termination	Follow up 7-14 days post last dose
In-patient in CRU		X[2]----------	----------	----------	----------	----------X	
Informed Consent	X						
Demographics	X						
Medical history	X	X[3]					
Physical exam[4] (inc ht/wt)	X	X				X	X
12-lead ECG[5]	X	X	X	X	X	X	X
Vital signs (BP and PR)[6]	X	X	X	X	X	X	X
Breath alcohol and drug screen	X	X					
Pregnancy Test[7]	X	X					X
Serum FSH[8]	X						
Safety labs (inc urinalysis)	X	X[9]	X[10]			X	X
Hepatitis B/C, HbA1c, HIV	X						
Fitzpatrick skin type assessment	X						
Body Map Recorded		X					
PK blood sample[11]		X	X	X	X	X	
Assessment of local toleration[12]		X	X	X	X	X	
Ointment Questionnaire					X		
Randomisation		X					
Dosing[13]		X----------	----------	----------X			
Concomitant & prior medications	X----------	----------	----------	----------	----------	----------	----------X
AE collection and review[12]	X----------	----------	----------	----------	----------	----------	----------X

1 Screening may occur over more than 1 day.
2 Subjects may come into the CRU on the evening before and some pre-dose activities may be completed.
3 Review changes in the subject's medical history since screening.
4 Full Physical examination at Screening, a brief examination on all other occasions (guided by AEs, but to include, as a minimum, general appearance, heart, lungs and skin).
5 Single ECG assessments at screening, follow up and early termination (if applicable). Triplicate ECG assessments will be performed at pre-dose, 1, 2, 4, 8, 12, 13 (Day 1 only) and 24 hours post-morning dose on Day 1 and Day 7, prior to each dose on Days 2 to 6, and at 36 hours (Day 8) and at 48 hours (Day 9) post last dose.
6 Duplicate, supine only, vital sign assessments will be performed at Screening, pre-dose, 1, 2, 4, 8, 12, 13 (Day 1 only) and 24 hours post-morning dose on Day 1 and Day 7, prior to each dose on Days 2 to 6, and at 36 hours (Day 8) and at 48 hours (Day 9) post last dose, follow up and at early termination (if applicable).
7 Serum pregnancy test at Screening, urine at all other specified time points; for women of child-bearing potential only.
8 Serum FSH testing for post-menopausal females 45-60 years only.
9 An additional blood sample will be taken at Baseline and stored frozen at MAC, as a reference sample, in case extra tests are required.
10 An additional safety lab sample will be taken on Day 4.
11 Blood samples for analysis of ZPL-5212372 will be taken prior to dosing and at 1, 2, 4, 8, 12, 13 (Day 1 only), 16 and 24 hours post-morning dose on Day 1 and Day 7, prior to each dose on Days 2 to 6, and at 36 hours (Day 8) and at 48 hours (Day 9) post last dose.
12 Assessment of local toleration on treated skin will be conducted prior to each dosing occasion and at 24and 48 hours post last dose.
13 Dosing twice daily, 12 hours apart from Days 1 to 6, the last application of study medication will be the morning of Day 7.
14 Adverse events will be collected from the signing of the consent form until the follow up visit. SAE's collected up to 30 days post last dose.

Table 10 - Schedule of activities for Cohort 2 (Moderate to Severe AD Patients)

Activities	Screening[1]	Washout Period[2]	Day 1	Day 2 to Day 6	Day 7	Day 8	Day 9/ Early Termination	Follow up 7-14 days post last dose
In-patient in CRU			X[3]----------	----------	----------	----------	----------X	
Informed Consent	X							
Demographics	X							
Medical history	X		X[4]					
Physical exam[5] (incl ht/wt)	X		X				X	X
12-lead ECG[6]	X		X		X		X	X
Vital signs (BP and PR)[7]	X		X	X	X	X	X	X
Breath alcohol and drug screen	X		X					
Pregnancy Test[8]	X		X					X
Serum FSH[9]	X							
Safety labs (inc urinalysis)	X		X[10]	X[11]			X	X
Hepatitis B/C, HbA1c, HIV	X							
Fitzpatrick skin type assessment	X							
Study emollient use[12]		X----------	----------	----------	----------	----------	----------X	
Body Map Recorded			X					
PK blood sample[13]			X	X	X	X	X	
EASI/IGA/BSA[14]	X		X		X			X
Ointment Questionnaire						X		
Randomisation			X					
Dosing[15]			X----------	----------	----------X			
Concomitant & prior medications	X----------	----------	----------	----------	----------	----------	----------	----------X
AE collection and review[16]	X----------	----------	----------	----------	----------	----------	----------	----------X

1 Screening may occur over more than 1 day.
2 Washout period from AD medication of between 1 week and 1 month dependent on each patient's current AD medication. Study Emollient should be started 7 days before Day 1.
3 Patients may come into the CRU on the evening before and some pre-dose activities may be completed.
4 Review changes in the subject's medical history since screening.
5 Full Physical examination at Screening, a brief examination on all other occasions (guided by AEs, but to include, as a minimum, general appearance, heart, lungs and skin).
6 Single ECG assessment at screening, follow up and early termination (if applicable). Triplicate ECG assessments will be performed at pre-dose, 1, 2, 4, 8, 12, 13 (Day 1 only) and 24 hours post-morning dose on Day 1 and Day 7.
7 Duplicate, supine only, vital sign assessments will be performed at Screening, pre-dose, 1, 2, 4, 8, 12, 13 (Day 1 only) and 24 hours post-morning dose on Day 1 and Day 7, prior to each dose on Days 2 to 6, and at 36 hours (Day 8) and at 48 hours (Day 9) post last dose and at follow up.
8 Serum pregnancy test at Screening, urine at all other specified time points; for women of child-bearing potential only.
9 Serum FSH testing for post-menopausal females 45-60 years only.
10 An additional blood sample will be taken at Baseline and stored frozen at the MAC, as a reference sample, in case extra tests are required.
11 An additional safety lab sample will be taken on Day 4.
12 Study emollient to be used from day -7 onwards until discharge from the CRU on day 9.
13 Blood samples for analysis of ZPL-5212372 will be taken prior to dosing and at 1, 2, 4, 8, 12, 13 (Day 1 only), 16 and 24 hours post-morning dose on Day 1 and Day 7, prior to each dose on Days 2 to 6, and at 36 hours (Day 8) and at 48 hours (Day 9) post last dose.
14 EASI/IGA/BSA assessed at screening and prior to randomisation to ensure inclusion criteria are met. Assessed again at the end of the study (or early termination) to document severity and extent of AD following study medication.
15 Dosing twice daily, 12 hours apart from Days 1 to 6, the last application of study medication will be the morning of Day 7.
16 Adverse events will be collected from the signing of the consent form until the follow up visit. SAE's collected up to 30 days post last dose.

Table 11 - Schedule of activities for Cohort 3 (Moderate to Severe AD Patients)

Activities	Screening[1]	Washout Period[2]	Baseline Visit 1 (Day 1)	Visit 2 (Day 5)	Visit 3 (Day 8)	Visit 4 (Day 10)	Visit 5 (Day 15)/ Early Termination	Follow up 7-14 days post last dose
Informed Consent	X							
Demographics	X							
Medical history	X		X[3]					
Physical exam[4] (incl ht/wt)	X		X				X	X
12-lead ECG[5]	X							X
Vital signs (BP and PR)[6]	X		X	X	X	X	X	X
Breath alcohol and drug screen	X		X					
Pregnancy Test[7]	X		X				X	X
Serum FSH[8]	X							
Safety labs (inc urinalysis)	X		X[9]				X	X
Hepatitis B/C, HbA1c, HIV	X							
Fitzpatrick skin type assessment	X							
Study emollient use[10]		X----------						----X
Body Map Recorded			X					
PK blood sample[11]			X	X	X	X	X	
EASI/BSA/IGA	X		X	X	X	X	X	X
Ziarco Itch Questionnaire[12]			X	X	X	X	X	X
PGIC							X	
Ointment Questionnaire							X	
Randomisation			X					
Dosing[13]			X----------			----X		
Concomitant & prior medications	X----------							----X
AE collection and review[13]	X----------							----X

1. Screening may occur over more than 1 day.
2. Washout period from AD medication of between 1 week and 1 month dependent on each patient's current AD medication. Study Emollient should be started 7 days before Day 1.
3. Review changes in the patient's medical history since screening.
4. Full Physical examination at Screening, a brief examination at all other visits (guided by AEs, but to include, as a minimum, general appearance, heart, lungs and skin).
5. A triplicate ECG assessment will be performed at Screening and a single ECG at Follow Up.
6. Duplicate, supine only, vital sign assessments to be performed at Screening and pre-dose on days 1, 5, 8, 10, 15 and follow-up.
7. Serum pregnancy test at Screening, urine at all other specified time points; for women of child-bearing potential only.
8. Serum FSH testing for post-menopausal females 45-60 years only.
9. An additional blood sample will be taken at Baseline and stored frozen at the central lab, as a reference sample, in case extra tests are required.
10. Study emollient to be used from day -7 onwards until completion of visit on day 15.
11. Blood samples for analysis of ZPL-5212372 will be taken prior to dosing on days 1, 5, 8 and 10 and an approximately 12 hours post last dose sample will be taken on Day 15.
12. Pruritus NRS, VRS, duration and impact of itch on sleep disturbance for the previous 24 hour period at clinic visits (Days 1, 5, 8, 10 and 15) by clinical staff asking the patients to complete the Ziarco Itch Questionnaire (Appendix 9).
13. To be dosed topically twice daily at home except on study visit days when the morning dose will be applied in the clinic after all other study assessments have been completed. Medication should be applied in the morning (preferably after showering) and approximately 12 hours later in the evening. Study medication should be applied approximately 15 minutes prior to using the Study Emollient. Subjects will apply their last dose of study medication at home in the evening of day 14 prior to their visit to the clinic on day 15.
14. Adverse events will be collected from the signing of the consent form until the follow up visit. SAE's collected up to 30 days post last dose.

7 ASSESSMENTS

Where time-points coincide, assessments should be taken in the following order:

- 12-lead ECG, vital signs and then blood for PK closest to the required time (± 10% from nominal time)

7.1 Physical Examination

All physical examinations will be carried out by trained medical personnel. At screening the subject will have a full physical examination, to include an assessment of head, neck, heart, lungs, abdomen, skin, peripheral circulation, joints and general appearance. Any abnormalities should be recorded in the study CRF. At all other visits subjects will have a brief physical examination, which should include general appearance, heart, lungs and skin. Other systems examined should be determined by clinical findings and any adverse events reported.

7.2 Vital Signs Measurements

Blood pressure (BP, systolic and diastolic mmHg) and pulse (beats per minute) will be measured in duplicate (approximately 2 minutes between measurements).

All measurements will be assessed with the subject in a supine position. Subjects should be supine for at least 5 minutes before taking the measurement. Blood pressure will be measured from the same arm throughout the study and recorded to the nearest mmHg. Ideally this will be the subject's dominant arm unless this arm is affected by AD and study ointment application could pose a potential contamination issue. To minimise any contamination issue the blood pressure cuff should be wiped clean with an alcohol wipe between subjects.

Pulse should be measured in the brachial/radial artery for at least 30 seconds.

7.3 Electrocardiogram (ECG) Recording

Single or triplicate (3 recordings over a 10 minute period) ECG recordings will be measured (see Section 6.7 schedule of activities for details). Measurements will be taken after the subject has rested for at least 10 minutes in a supine position using a 12-lead ECG machine.

To ensure safety of the subjects, a qualified individual at the investigator site will make comparisons to the baseline measurements. If the QTcF is ≥60 msec from baseline; or an absolute QTcF value is ≥500 msec for any ECG, the ECG intervals should be inspected carefully to ensure that the RR interval has been recorded correctly, and a single ECG will be repeated at 5-minute intervals up to 3 times to confirm any increased QT interval values. If the values are consistently high, the subject will be withdrawn from the study, allowed to return home (so long as no other clinical findings preclude this) and will be instructed to return for the follow up visit 7-14 days later.

In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads are placed in the same positions each time in order to achieve precise ECG recordings. If a machine-read QTcF value is prolonged, as defined above, repeat measurements may

not be necessary if a qualified physician's interpretation determines that the QTcF values are acceptable for the individual.

7.4 Fitzpatrick Skin Type Assessment

All subjects will have their skin type assessed using the Fitzpatrick Skin Type assessment[26] and recorded in the CRF at screening. For Cohort 1 subjects only, subjects must have a Skin Type of between I to III to meet the inclusion criteria for the study.

Fitzpatrick skin type classification:

Type I – White; very fair; red or blonde hair; blue eyes; freckles (Always burns, never tans)

Type II – White; fair; red or blonde hair; blue, hazel or green eyes (Usually burns, tans with difficulty)

Type III – Cream white; fair with any eye or hair colour (Sometimes mild burn, gradually tans)

Type IV – Light brown (Rarely burns, tans with ease)

Type V – Dark brown (Very rarely burns, tans very easily)

Type VI – Black (Never burns, tans very easily)

7.5 Assessment of Local Toleration

For Cohort 1 subjects only. All subjects will have skin area to be dosed assessed for local toleration using a modified Draize Score[28] prior to application of study ointment and until discharge from the CRU.

Erythema and Eschar Formation	**Grade**
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema Formation	**Grade**
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

Desquamation	**Grade**
No desquamation	0
Mild desquamation (dry skin)	1
Moderate desquamation (flaky skin)	2
Severe desquamation (skin cracking)	3

For each assessment the grading value for erythema and eschar formation, oedema formation and desquamation will be recorded and a total of the three gradings will also be recorded. Individual subject stopping criteria will be based on a score of ≥ 3 for any of the parameters on 3 consecutive occasions. Cohort 1 stopping criteria will be based on a total score of ≥ 5 on 3 consecutive occasions in 5 subjects dosed over a 40% BSA.

7.6 Skin Area Body Map

For Cohort 1 healthy volunteer subjects: schematic body map (Front and Back views) will be used on Day 1 prior to randomisation to map the area of skin to be treated with study ointments to ensure consistency of the percentage body area to be treated.

For Cohort 2 and 3 AD patients: the areas of skin affected by AD and the approximate percentage of BSA affected by AD will be recorded on a schematic body map (Front and Back views) on Day 1 prior to randomisation for each patient. The body map will also be used to indicate the area of skin which will be dosed for the duration of the study (Appendix 10). For Cohort 2 patients, depending on whether they are in the 10% BSA treated sentinel group or the remaining 40% BSA treated group, it may be not all their AD lesioned skin will treated with study ointment (See Appendix 6 for an example) or that skin which is not currently affected by AD lesions will need to be treated with study ointment (see Appendix 7 for an example). However in all cases, once recorded the areas of skin to be treated with study ointment will be treated consistently for the duration of the study regardless of any changes in the size or extent of AD affected skin areas during the study period.

7.7 Eczema Area and Severity Index (EASI)

For Cohort 2 and 3 AD patients only. The Eczema Area and Severity Index (EASI) [27] (see Appendix 11) is a validated tool used to measure the severity and extent of atopic eczema. The total score incorporates the extent of body regions affected and the intensity of a representative area of eczema. The approximate percentages affected by eczema are calculated for each region.

The EASI score will be used to determine eligibility for the study and any changes in AD severity/extent following study medication (Cohort 2) as well as assessing efficacy (Cohort 3 only).

The investigator will be provided with further detail of how to calculate the EASI score at the site initiation visits.

EASI must be ≥9 and ≤48 at screening and ≥12 and ≤48 on Day 1 for the patient to be eligible for inclusion in the study.

7.8 Body Surface Area (BSA)

For Cohorts 2 and 3 AD patients only. Assessment of the percentage of a patient's body surface area affected by AD will be made by best estimates (see Appendix 12 for guidance) of the Investigator and recorded on the individual patients body map. Affected BSA must be ≥ 10% and ≤40% at screening and must be ≥ 10% and ≤50% on Day 1 for the patient to be randomised.

7.9 Investigator Global Assessment (IGA)

For Cohorts 2 and 3 AD patients only. An overall assessment of the severity of AD will be made, by the investigator, using the Investigator Global Assessment at each visit. IGA scores take values on a 5-point scale from 0-4, where 0=clear to 4=severe disease.

0=clear (no inflammatory signs of AD),
1=almost clear (just perceptible erythema, and just perceptible papulation/infiltration)
2=mild disease (mild erythema, and mild papulation/infiltration)
3=moderate disease (moderate erythema, and moderate papulation/infiltration)
4=severe disease (severe erythema, and severe papulation/infiltration)

At screening and Day 1 this must be ≥ 3 for the patient to be eligible.

7.10 Ziarco Itch Questionnaire

For Cohort 3 AD patients only. Ziarco has developed an Itch Questionnaire to capture both Numerical and Verbal rating Scales for Pruritus and Sleep Disturbance (Appendix 9).

Pruritus NRS, VRS, duration of itch and impact of itch on sleep disturbance for the previous 24 hour period will be assessed on clinic visits by clinical staff asking the patients to complete the Ziarco Itch Questionnaire.

7.10.1 Numerical Rating Scale (NRS) for Pruritus (Itching)

The Pruritus NRS is an assessment tool that will be used to assess the subject's worst itch as a result of AD in the previous 24 hours.

They will be asked the following question:

> *On a scale of 0 (No Itching) to 10 (Itching as bad as you can imagine), please rate the WORST itching that you felt over the last 24 hours.*

7.10.2 Duration of Itching

Patients will be asked the following question to determine their duration of itching:

> *Over the last 24 hours approximately how many hours, if any, did you itch?*

7.10.3 Sleep Disturbance

Patients will be asked the following question to determine the level of sleep disturbance due to itching:

> *On a scale of 0 (No sleep disturbance) to 10 (Awake all night), please rate how much your sleep was disturbed by itch last night.*

7.10.4 Verbal Rating Score

Patients will be asked to rate their itch over the last 24 hours using a list of adjectives describing different levels of symptom intensity:

Over the last 24 hours how would you rate your itch?

no itch
mild
moderate
severe

7.11 Patient Global Impression of Change (PGIC)

For Cohort 3 AD patients only. At the end of treatment (Day 15) or early termination visit, the patient will be asked to rate their degree of improvement (or worsening) of their AD compared to before the start of treatment with study drug, using a 7-point scale, standardized PGIC.

Since the start of the study (dosing), my overall status is:

1 □ Very Much Improved

2 □ Much Improved

3 □ Minimally Improved

4 □ No change

5 □ Minimally Worse

6 □ Much Worse

7 □ Very Much Worse

7.12 Ziarco Ointment Questionnaire

At the end of the treatment phase each subject will be asked a series of questions about their impressions of the study ointment they have used. Information regarding the ointment will be used to learn the practicalities of handling the ointment in a clinical setting for subsequent cohorts and clinical development. The full list of questions contained in the Ziarco Ointment Questionnaire can be found in Appendix 13.

7.13 Pharmacokinetic Blood Samples

Blood samples for determination of ZPL-5212372 concentrations will be collected either via a direct venepuncture or via an indwelling cannula whichever is more appropriate and will reduce potential contamination from application of topical study medication.

A 5.0 mL sample, to provide a minimum of 2 mL plasma for pharmacokinetic analysis, will be collected into an appropriately labeled tube containing lithium heparin. Blood samples will be centrifuged within 1 hour of collection at 1700 g and 4°C for 10 minutes. Plasma will be transferred to appropriate labelled screw-capped polypropylene tubes and stored at approximately -20°C within 1 hour of collection.

Plasma samples will be transferred to the bioanalytical laboratory on dry ice to maintain frozen conditions. Shipment details for the analytical laboratory will be provided to the sites prior to shipping any samples.

Samples will be analysed using a validated analytical method. After completion of the study plasma samples may also be analysed for metabolites and further evaluation of the assay performance.

7.14 Clinical Laboratory Safety Tests

Blood and urine samples will be collected for laboratory safety tests for all cohorts at the times detailed in Section 6.

Details of the urine and blood sampling procedures and subsequent storage and shipment can be found in the ZPL-5212372 TDL Trials Laboratory manual. Sites will be provided with lab kits from TDL Trials laboratory. Details of parameters to be tested are listed in Table 12.

Table 12 – Safety Laboratory parameters

Biochemistry	**Haematology**
Sodium	Red cell count
Potassium	Haemoglobin
Calcium	Packed cell volume (hematocrit)
Urea	Mean cell volume (MCV)
Uric acid	Mean cell haemoglobin (MCH)
Creatinine	Mean cell haemoglobin concentration (MCHC)
Glucose	Platelet count
Triglycerides	White cell count
Total Bilirubin	Neutrophils
Total protein	Lymphocytes
Albumin	Monocytes
Alkaline phosphatase	Eosinophils
Aspartate transferase (AST)	Basophils
Alanine transferase (ALT)	
Gamma glutamyl transferase (GGT)	
Total cholesterol	
hCG (screening, WOCBP only)	
FSH (screening, post-menopausal females only)	
Serological Markers (Screening only) Hepatitis B surface antigen (HBsAg) Hepatitis B core antibody (HbcAb) Anti-hepatitis C virus serology (by ECLIA) Human immunodeficiency virus (HIV) Glycosylated haemoglobin (HbA1c)	
Urinalysis pH, protein, glucose, ketone and blood Any significant abnormalities in the urinalysis should be investigated via microscopy and the findings reported as a comment in the CRF. Pregnancy testing (to be measured at the clinic, using kits supplied) Drug Screen - Cocaine, cannabinoids, opiates, barbiturates, benzodiazepines, methadone and amphetamines (Screening and Day 1 only)	
Breath Alcohol Test Screening and Day 1 only	

An extra blood sample (10 mL) will be taken pre-dose on Day 1 and the serum will be stored frozen at the central laboratory as a reference sample in the event that further tests or re-tests are required.

7.15 Blood Volume

The approximate volume of blood to be taken from each subject during the study is detailed in Table 13. Additional blood may be required for repeats of safety laboratory tests.

Table 13 - Approximate Blood Volume Required for Study

Test	Number of Samples	Volume (mL)	Total (mL)
Haematology	5	5	25
Biochemistry	5	5	25
Hep B/C/HbA1c/HIV	1	5	5
PK samples (Cohorts 1 and 2)	28	5	140
PK samples (Cohort 3)	5	5	25
Baseline serum sample	1	10	10
Total volume for Cohorts 1/2			205
Total volume for Cohort 3			90

7.16 Safety

7.16.1 Adverse Events

7.16.1.1 Definitions

An AE (or adverse event) is any untoward medical event that occurs in a subject and does not necessarily have a causal relationship with study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the product.

A treatment-emergent AE will be defined as an AE that begins or that worsens in severity after at least one dose of study drug (ZPL-5212372 or placebo ointment) has been administered.

An adverse drug reaction (ADR) is any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

An unexpected adverse reaction is an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in the Investigator's Brochure.

All AEs, including intercurrent illnesses, occurring during the study will be documented in the CRF. Concomitant illnesses, which existed prior to entry into the study, will not be considered AEs unless they worsen during the treatment period. Pre-existing conditions will be recorded in the CRF as Medical History.

7.16.1.2 Assessment of Adverse Event

Each AE will be assessed by the investigator or delegated physician with regard to the following categories.

Seriousness
A serious AE (SAE) (or serious ADR or unexpected serious adverse reaction) is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  This means that the subject is at risk of death at the time of the event; it does not mean that the event hypothetically might have caused death if it were more severe.
- Requires or prolongs subject hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event(s) that may not be immediately life-threatening or result in death or hospitalization but that may jeopardize the subject or require intervention to prevent one of the above outcomes. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

Note: Cases of potential drug-induced liver injury as assessed by laboratory test values ("Hy's Law Cases") are also reportable. If a subject develops abnormal values in aspartate transaminase (AST) or alanine transaminase (ALT) or both, concurrent with abnormal elevations in total bilirubin and no other known cause of liver injury, that event would be classified as a Hy's Law Case. In this clinical study, the term SAE will be understood to also include Hy's Law Cases.

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate.

Severity
The intensity of each AE must be assessed by the investigator using one of the following categories, and recorded in the CRF:

- Mild: An AE that does not interfere with usual activities;
- Moderate: An AE that interferes with usual activities;
- Severe: An AE that prevents usual activities

Causality
The investigator will assess the causality / relationship between the study drug and the AE and record that assessment in the CRF. Causality will be shown as Probably related, Possibly related, Not related or Related.

7.16.1.3 Recording Adverse Events

Adverse event reporting will extend from signing the informed consent until completion of final visit/follow-up telephone call. Adverse events occurring after the

end of the study must be reported if the investigator considers there is a causal relationship with the study drug. All AEs, regardless of the relationship to study drug, will be recorded in the CRF.

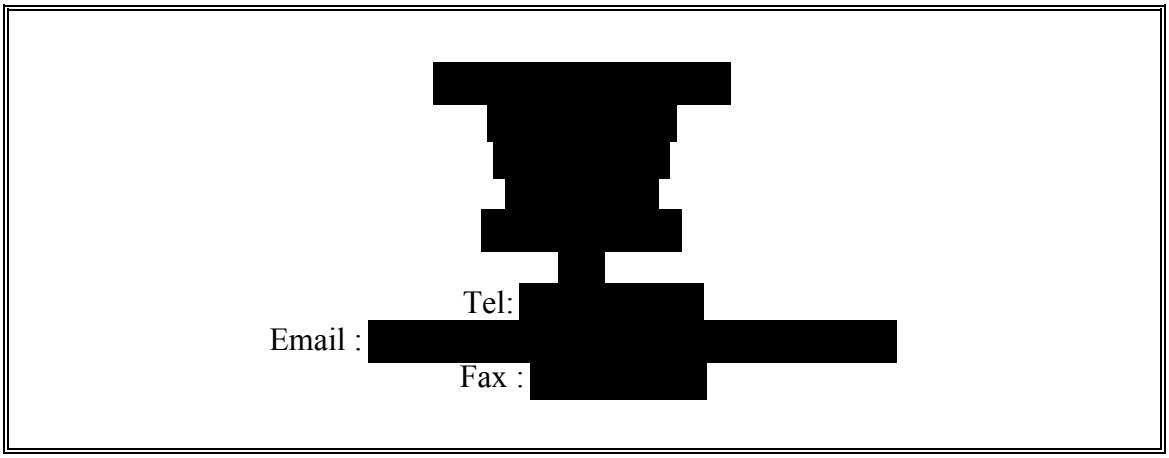
All AE reports should contain a brief description of the event, date of onset, date of resolution, intensity, treatment required, relationship to study drug, action taken with the study drug, outcome, and whether the event is classified as serious.

7.16.1.4 Reporting Serious Adverse Events

All SAEs that occur during the study and up to 30 days after receiving the last dose of study drug, whether considered to be associated with the study drug or not, must be reported within 24 hours by telephone or fax to the study pharmacovigilance team. The minimum information required for an initial report is:

- Sender of report (name, address of investigator)
- Subject identification (screening/randomisation number, initials, NOT subject name)
- Protocol number
- Description of SAE
- Causality assessment, if possible

However, as far as possible all points on the SAE form should be covered in the initial report and the completed SAE form faxed to the pharmacovigilance team (details below). The completed SAE should be sent to the address below and Ziarco Chief Medical Officer informed by email. In addition, the event must be documented in the CRF.



After receipt of the initial report, the pharmacovigilance team will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. will be responsible for all information processing and reporting according to local legal requirements.

7.16.1.5 Follow-up of Adverse Events

All AEs experienced by a subject, irrespective of the suspected causality, will be monitored until the event has resolved, any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the investigator and the Ziarco Chief Medical Officer, until there is a satisfactory explanation for the changes observed, or until the subject is lost to follow-up.

8 STATISTICAL METHODS

Before the study database is locked, a statistical analysis plan (SAP) will be finalized providing detailed methods for the analyses outlined below.

Any deviations from the planned analyses will be described and justified in the final clinical study report.

Separate tables, listings and figures will be generated for each cohort.

8.1 Study Subjects

8.1.1 Randomisation

Separate randomisation codes will be generated for each cohort. Computer generated pseudo random codes will assign subjects to treatment.

The randomisation for Cohort 3 will be stratified by EASI score (EASI ≤20 and EASI >20).

8.1.2 Disposition of Subjects

The number and percentage of subjects entering and completing each phase of the study will be presented for each treatment group. Reasons for withdrawal pre- and post-randomisation will also be summarized.

8.1.3 Protocol Deviations

Deviations from the protocol, including violations of inclusion/exclusion criteria, will be categorized as “minor” or “major” in cooperation with the Sponsor. Deviations will be assessed prior to the study database being locked.

8.1.4 Analysis Populations

Full Analysis Set:	All randomised subjects who received at least one dose of study treatment
Safety Set:	All randomised subjects who received at least one dose of study treatment
Per Protocol Set: (Cohort 3 only)	All subjects in the Full Analysis Set who have not violated any inclusion or exclusion criteria and/or deviated from the protocol, in a way that could influence their efficacy assessment. Precise reasons for excluding subjects from the population will be fully defined and documented prior to locking the study database.
Pharmacokinetic Set:	All ZPL-5212372 treated subjects in the Full Analysis

Set who have at least one ZPL-5212372 concentration recorded (including concentrations below the limit of quantification)

The Full Analysis Set (FAS) will be used in the analyses of efficacy endpoints.

A secondary analysis of the primary endpoint for Cohort 3 may also be performed based upon the Per Protocol (PP) Set.

All safety and pharmacokinetic analyses will be based upon the Safety and Pharmacokinetic sets respectively.

8.2 General Considerations

All statistical testing for Cohort 3 data will be at the 5% level of significance (1-sided) and all point estimates for the comparison between treatment groups will be accompanied by 2-sided 90% confidence intervals. No adjustment will be made for multiplicity due to multiple endpoints/comparisons. No statistical testing will be performed with the data from Cohorts 1 and 2.

All endpoints will be summarized by treatment group and visit. Continuous data will be summarized using descriptive statistics (e.g. mean and standard deviation) and categorical data will be summarized using frequency tables (counts and percentages).

For change from baseline endpoints baseline is defined as the last measurement available prior to the start of dosing on Day 1.

All possible efforts will be made to ensure that subjects complete all the required assessments. As missing data may potentially bias the outcome of the statistical analyses and the subsequent estimation of the magnitude of the treatment effect, different strategies will be applied to provide a balanced assessment of treatment efficacy. The handling of missing data for each endpoint and sensitivity analyses will be detailed in the statistical analysis plan prior to the study database being locked.

Any outliers that are detected during the review of the data will be investigated. Methods for dealing with outliers will be defined in the SAP or in an addendum to the SAP.

8.3 Demographics, Baseline Characteristics and Concomitant Medications

Demographics, baseline characteristics (Cohorts 2 and 3 only), medical history and concomitant medication data will be summarized for each treatment group and overall within each cohort.

8.4 Treatment Compliance

As Cohorts 1 and 2 will receive their study ointment as in-patients non-compliance will be reported by the site staff through protocol deviations. For Cohort 3 compliance will be assessed through reviewing the patient diary and the amount of study ointment used.

8.5 Efficacy Analyses

As Cohort 1 includes healthy volunteers no efficacy data will be collected.

Though some efficacy assessments are included for Cohort 2 the data will only be listed and no formal comparisons will be made between the treatment groups.

Consequently efficacy will only be assessed for Cohort 3. Hence the following analyses apply to Cohort 3 data.

8.5.1 Primary Endpoint

The primary efficacy endpoint is the percentage change from baseline to Week 2 in the EASI score.

The primary analysis will be performed using the FAS. A secondary analysis of the primary endpoint may be conducted using the PP analysis set, if sufficiently different to the FAS, to examine the robustness of the primary analysis results.

The percentage changes from baseline in the EASI score at Week 2 will be summarized by treatment group and analysed by ANCOVA. The difference between treatment groups in the percentage changes from baseline will be estimated, along with its 90% confidence interval. Baseline will be included as a continuous covariate in addition to treatment. This analysis will be repeated for the sensitivity analyses where different approaches will be adopted for handling missing data.

Sub-groups will include the levels of the stratification factor. Other sub-groups will be specified in the SAP.

8.5.2 Secondary Endpoints

The proportion of subjects who achieve an EASI-50 response (50% reduction in EASI score from baseline) at Week 2 will be compared between the treatment groups. Group proportions and the difference between these proportions will be calculated along with the corresponding 90% confidence interval. These data will also be analysed using logistic regression, including treatment and baseline. The odds ratio and accompanying 90% confidence interval will be presented.

The proportion of subjects who achieve an EASI-75 response (75% reduction in EASI) and the proportion of subjects who are responders for IGA score (reduction from baseline of at least 2 points) at Week 2, will be analysed using the same analyses described for the EASI-50 response. The changes from baseline in IGA score will also be summarized using categorical shift tables.

The changes from baseline in body surface area, pruritus NRS score for worst itch, NRS score for sleep disturbance and duration of itching at Week 2 will be analysed using the same analyses described for the primary analysis.

The changes from baseline in VRS score will be summarized using categorical shift tables.

The PGIC score will be summarized and analysed using logistic regression, presenting odds ratios and associated 90% confidence intervals for the comparison between treatment groups.

Secondary endpoints will be analysed using the Full Analysis Set.

8.5.3 Exploratory Endpoints

Responses to the Ziarco ointment questionnaire will be summarized by treatment group.

8.6 Safety Analyses

Safety data will be summarized by treatment group for each cohort using all subjects in the Safety Analysis Set.

No imputation will be used for handling missing data, with the exception of conservative approaches taken for missing adverse event information (e.g. intensity). Details of such conventions will be documented in the SAP prior to the study database being locked.

Adverse events will be coded using the MedDRA dictionary. The incidence of all treatment emergent adverse events will be tabulated by treatment group and by treatment group and severity. Those events regarded as possibly treatment related by the investigator will be summarized separately.

Changes from baseline in laboratory parameters, vital signs and ECG data will be summarized by visit for each treatment group.

For Cohort 1 only, local toleration assessed by the Draize score, will be summarized by visit for each treatment group. The changes from baseline in Draize score will also be summarized using categorical shift tables.

8.7 Pharmacokinetic Analyses

ZPL-5212372 concentrations (all cohorts) and parameters (Cohorts 1 and 2 only) will be summarised for each cohort using all subjects in the Pharmacokinetic Set.

Individual profile and median plots will also be plotted. Median profiles for Cohorts 1 and 2 will be plotted by day.

The ZPL-5212372 pharmacokinetic parameters AUC_{τ}, AUC_{last} and $t_{½}$ will be generated using non-compartmental methods. C_{max} and T_{max} will be determined directly from the data. Where possible, the apparent terminal elimination phase rate constant (k_{el}) will be calculated by linear regression of the log-linear plasma concentration-time curve. Only those time points judged to describe the terminal log-linear decline will be used in the regression. The apparent terminal elimination half-life ($t_{½}$) will be calculated as follows:

$t_{½} = \ln 2/k_{el}$

8.8 Interim Analyses

The study will have data reviews conducted between each cohort (see section 3).

8.9 Determination of Sample Size

The sample sizes for Cohorts 1 and 2 are not based on formal statistical testing. Their sample sizes are considered sufficient to obtain suitable safety, tolerability and PK data prior to starting Cohort 3.

For Cohort 3 the sample size of 30 subjects has been determined on the basis of comparing the mean ZPL-5212372 Week 2 percentage change from baseline in the EASI score to that for placebo. With 20 subjects in the ZPL-5212372 group and 10 subjects in the placebo group, there is 80% power of obtaining a statistically significant result, assuming that there is an improvement of at least 30% with ZPL-5212372 compared to placebo and a standard deviation for the percentage changes from baseline between subjects of 30%. This is based on the analyses described and a 1-sided test at the 5% level of significance.

9 ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1 Data Quality Assurance

The Sponsor (or its delegate) will conduct a site visit to verify the qualifications of each investigator, inspect the site facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded on the CRFs for this study must be consistent with the subjects' source documentation (i.e., medical records).

9.2 Case Report Forms and Source Documentation

All data obtained during this study should be entered in the CRFs promptly. All source documents from which CRF entries are derived should be placed in the subject's medical records. Measurements for which source documents are usually available include laboratory assessments, and ECG recordings.

The original CRFs for each subject will be checked against source documents at the study site by the study monitor.

After review by the study monitor, completed CRFs will be marked as complete and verified. [redacted] Data Management will review the CRFs within the electronic data capture (EDC) system. Where data is discrepant, Data Management will raise queries for the site to resolve within the EDC.

9.3 Access to Source Data

During the course of the study, a monitor will make site visits to review protocol compliance, compare CRFs and individual subject's medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. CRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

Checking of the CRFs for completeness and clarity, and cross-checking with source documents will be required to monitor the progress of the study. The investigator should be available at each monitoring visit to answer any queries. Moreover, regulatory authorities of certain countries, IECs, and/or the Sponsor's Clinical Quality Assurance Group may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The investigator assures the Sponsor of the necessary support at all times.

9.4 Data Processing

The site will be supplied with the following data collection tool: a web browser address for EDC that has been fully validated and conforms to 21CFR11 requirements. The trained investigator site staff will enter the data required by the protocol into the CRFs from source documents (e.g., medical records and study-specific data capture tools as needed) using the [redacted] supplied data collection tool. All information in the CRFs must be traceable to these source documents. Data recorded directly into the CRFs will be defined before study start and the CRFs will be considered the source data. Clinical Research Associates and a Data Manager will review CRFs entered by investigational staff for completeness and accuracy. Automatic quality programs check for data discrepancies in the CRFs and the resulting queries will be notified to the investigational site using an electronic data query process within the EDC. Designated investigator site staff are required to respond to queries and make any necessary changes to the data. Details of the data correction process will be specified in the Data Management Plan. After database lock, the investigator will receive a CD-ROM of the subject CRFs (PDF) for archiving at the investigational site.

A validated, electronic database will be employed from the EDC system. An audit trail of all changes to this database, including the date, reason for the data change and who made the change, will be maintained within the same database. The audit trail will be part of the archived data at the end of the study.

9.5 Archiving Study Records

Adequate records as required by ICH GCP and FDA CFR, will be maintained for the study. This will include subject medical records, investigator logs, CRFs, laboratory reports, work sheets, signed SICFs, drug dispensing records, adverse experience reports, information regarding subjects' discontinuation and electronic data. Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the final discontinuation of clinical development of the investigational product. These documents should be retained for a longer period. However, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9.6 Good Clinical Practice

The procedures set out in this study protocol are designed to ensure that the Sponsor and investigator abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH), and of the Declaration of Helsinki (2013). The study also will be carried out in keeping with local legal requirements.

9.7 Confidentiality

All study findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on CRFs and other documents submitted to [redacted] by their subject number and/or birth year, not by name. Documents not to be submitted to [redacted] that identify the subject (e.g., the signed informed consent) must be maintained in confidence by the investigator.

9.8 Informed Consent

Before each subject is admitted to the study, informed consent will be obtained from the subject (or his/her legally authorized representative) according to the regulatory and legal requirements of the participating country (i.e., the Declaration of Helsinki, ICH GCP, and other applicable local regulations). This consent form must be dated and retained by the investigator as part of the study records. The investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the CRF.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC, and signed by all subjects subsequently enrolled in the study as well as those currently enrolled in the study.

9.9 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IEC/Competent Authority approval prior to implementation (if appropriate). Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

9.10 Duration of the Study

The planned duration of the study for each subject will be approximately 6 to 8 weeks (assuming 4 weeks screening, 1-2 weeks treatment [Cohort 1 and 2: 1 week of treatment or Cohort 3: 2 weeks of treatment] and 1-2 weeks follow-up). The study will close when all subjects have completed the final follow-up visit.

9.11 Premature Termination of the Study

If the investigator or the Sponsor becomes aware of conditions or events that suggest a possible hazard to subjects if the study continues, the study may be terminated after appropriate consultation between the relevant parties. The study may also be terminated early at the Sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study;
- Failure to enroll subjects at an acceptable rate;
- A decision on the part of the Sponsor to suspend or discontinue development of the drug.

9.12 Liability and Insurance

The Sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the Sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this study are governed by the applicable law.

The Sponsor will arrange for subjects participating in this study to be insured against financial loss due to personal injury caused by the pharmaceutical products being tested or by medical steps taken in the course of the study.

9.13 Publication Policy

By signing the study protocol, the investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

After study completion, data from the entire study will be considered for reporting at a scientific meeting and for publication in a scientific journal. Ziarco will coordinate these activities and will work with the Principal Investigator to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted and other related issues. Authorship will be based on criteria stipulated by leading clinical journals (e.g. contribution to one or more areas of study design, data analysis and interpretation, manuscript preparation and review, etc.).

An investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance.

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11 APPENDICES

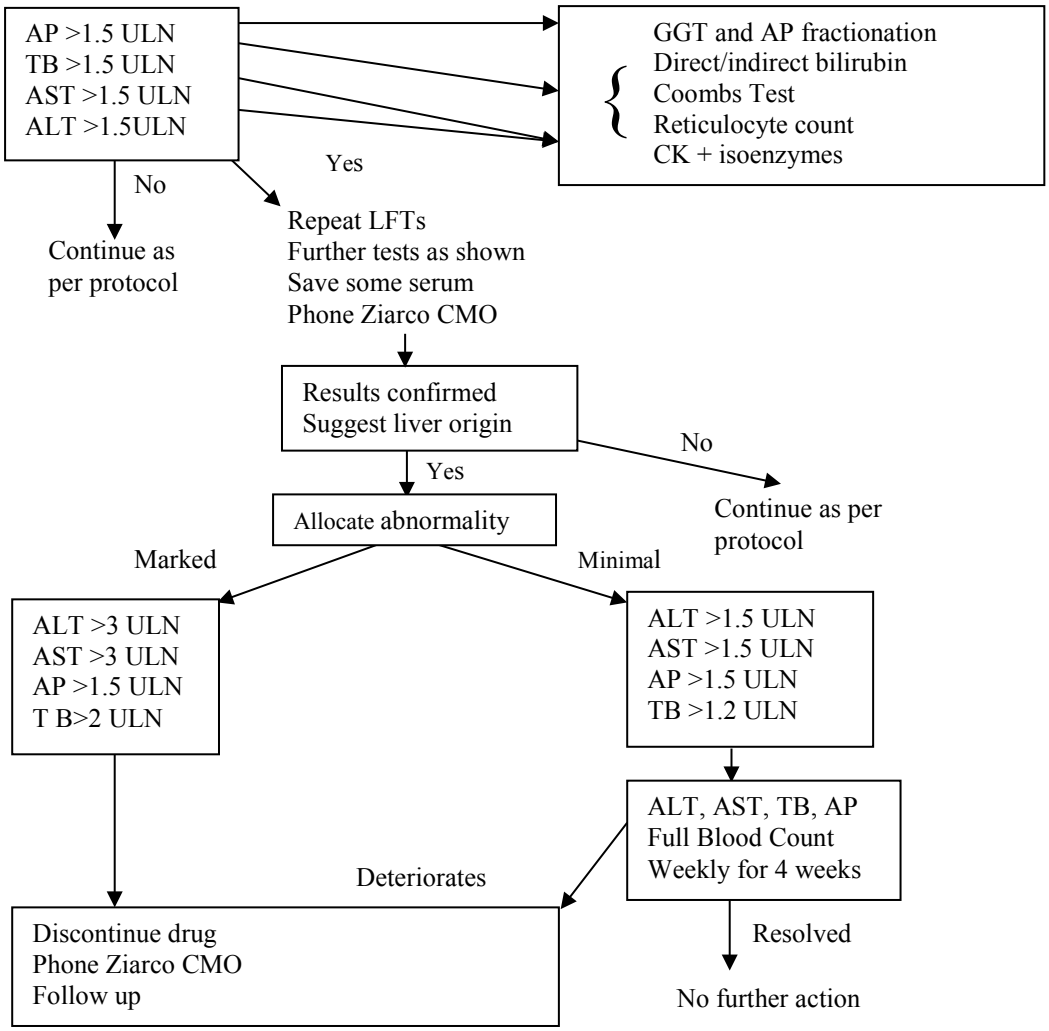
APPENDIX 1 – CRITERIA FOR SAFETY VALUES OF POTENTIAL CLINICAL CONCERN

Haematology	
Hemoglobin	<0.8 x baseline
Hematocrit	<0.8 x baseline
WBC	<2.5 or >17.5 x 10^3/mm^3
Platelets	<75 or >700 x 10^3/mm^3
Chemistry	
Total bilirubin	>1.5 times the upper limit of the reference range
AST	>3 times upper limit of the reference range
ALT	>3 times upper limit of the reference range
GGT	>3 times upper limit of the reference range
Alk Phosphatase	>3 times upper limit of the reference range
Creatinine	>1.3 times upper limit of the reference range
Urea	>1.3 times upper limit of the reference range
Glucose, fasting	<0.6 or >1.5 times the limits of the reference range
Uric acid	>1.2 times upper limit of the reference range
Sodium	<0.95 or >1.05 times the limits of the reference range
Potassium	<0.9 or >1.1 times the limits of the reference range
Calcium	<0.9 or >1.1 times the limits of the reference range
Albumin	<0.8 or >1.2 times the limits of the reference range
Total protein	<0.8 or >1.2 times the limits of the reference range
Urinalysis	
Urine WBC	≥6/HPF
Urine RBC	≥6/HPF
Vital Signs	
Pulse Rate	Supine/Sitting: <40 or >120 bpm Erect: <40 or >140 bpm
Blood Pressure	Systolic ≥30 mm Hg change from baseline in same posture Systolic <90 mm Hg Diastolic ≥20 mm Hg change from baseline in same posture Diastolic <50 mm Hg

APPENDIX 2 – HEPATIC SAFETY

Baseline Liver Function Test (LFT) Requirements
Before including a subject in the study they must have:
Negative HBsAg, HBcAb and anti-hepatitis C virus serology
No clinical evidence of hepatic disease

During the study - if



Follow up for marked abnormality
HBsAg, HBcAb, anti-HAV (IgM and IgG), AST, ALT
AP (including isoenzymes), TB (direct and indirect), one stage prothrombin time, serum proteins and, if clinically warranted, cytomegalovirus, toxoplasma and heterophile antibody titres. LFTs and FBC repeated WEEKLY until they return either to baseline or to a level deemed acceptable by the investigator and the Ziarco CMO.

APPENDIX 3 – ECG VALUES OF POTENTIAL CLINICAL CONCERN

Interval Durations of Potential Clinical Concern

PR Interval	≥300 msec; ≥25% increase when baseline >200 msec; Increase ≥50% when baseline ≤200 msec
QRS Interval	≥200 msec; ≥25% increase when baseline >100 msec ≥50% increase when baseline ≤100 msec
QT/QTcF Interval	≥500 msec; an increase of ≥60 msec from baseline

Rhythms of Potential Clinical Concern

-Asymptomatic Marked Sinus Bradycardia (rate <35 bpm)

-Asymptomatic Supraventricular Couplets, Atrial Bigeminy lasting >30 seconds

-Asymptomatic Ventricular Couplets, Ventricular Bigeminy lasting >30 seconds

-Asymptomatic Type I Second Degree (Wenckebach) AV Block of >30 seconds duration

-Asymptomatic Frequent Premature Ventricular Complexes (PVCs) (≥200/24 hours)

-Asymptomatic Frequent Premature Atrial Complexes (PACs) (≥240/24 hours)

Adverse Experiences

-Symptomatic Marked Sinus Bradycardia (rate <40 bpm)

-Asymptomatic Sinus Pause >3 seconds without an escape beat

-Asymptomatic Atrial Flutter or Fibrillation, subcategorized by ventricular response rate: controlled = rate <120 bpm; rapid = rate >120 bpm

-Asymptomatic Supraventricular Tachycardia ≥3 beats (rate >120 bpm)

-Asymptomatic Nonsustained Ventricular Rhythms ≥3 beats, but duration of <30 seconds, including idioventricular rhythm (rate <40 bpm), accelerated idioventricular rhythm (40< x<100) and monomorphic/polymorphic ventricular tachycardia >100 bpm (such as Torsade des pointes)

-Asymptomatic Type II Second Degree (Mobitz) AV Block

-Asymptomatic Complete (third degree) Heart Block

Serious Adverse Experiences

-Sustained Ventricular Arrhythmias (>30 seconds duration)

-Ventricular Fibrillation

-At the discretion of the investigator, any arrhythmia classified as an adverse experience

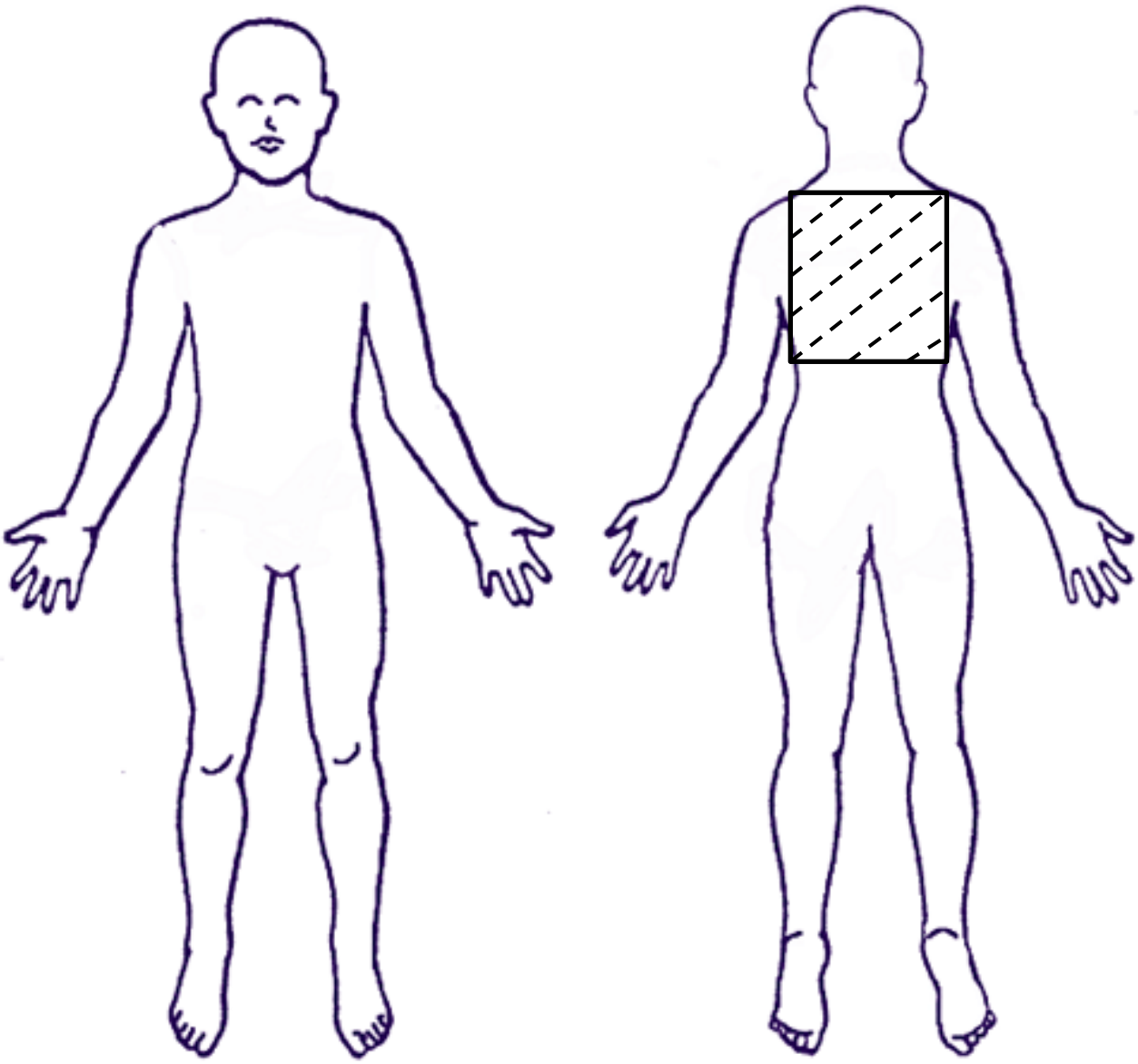
APPENDIX 4 – COHORT 1 10% BSA TREATMENT BODY MAP EXAMPLE

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Cohort 1 (HV) – 10% BSA Treatment Example

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD_____0_____ %
BSA Treated with Study Ointment ____10_____ %



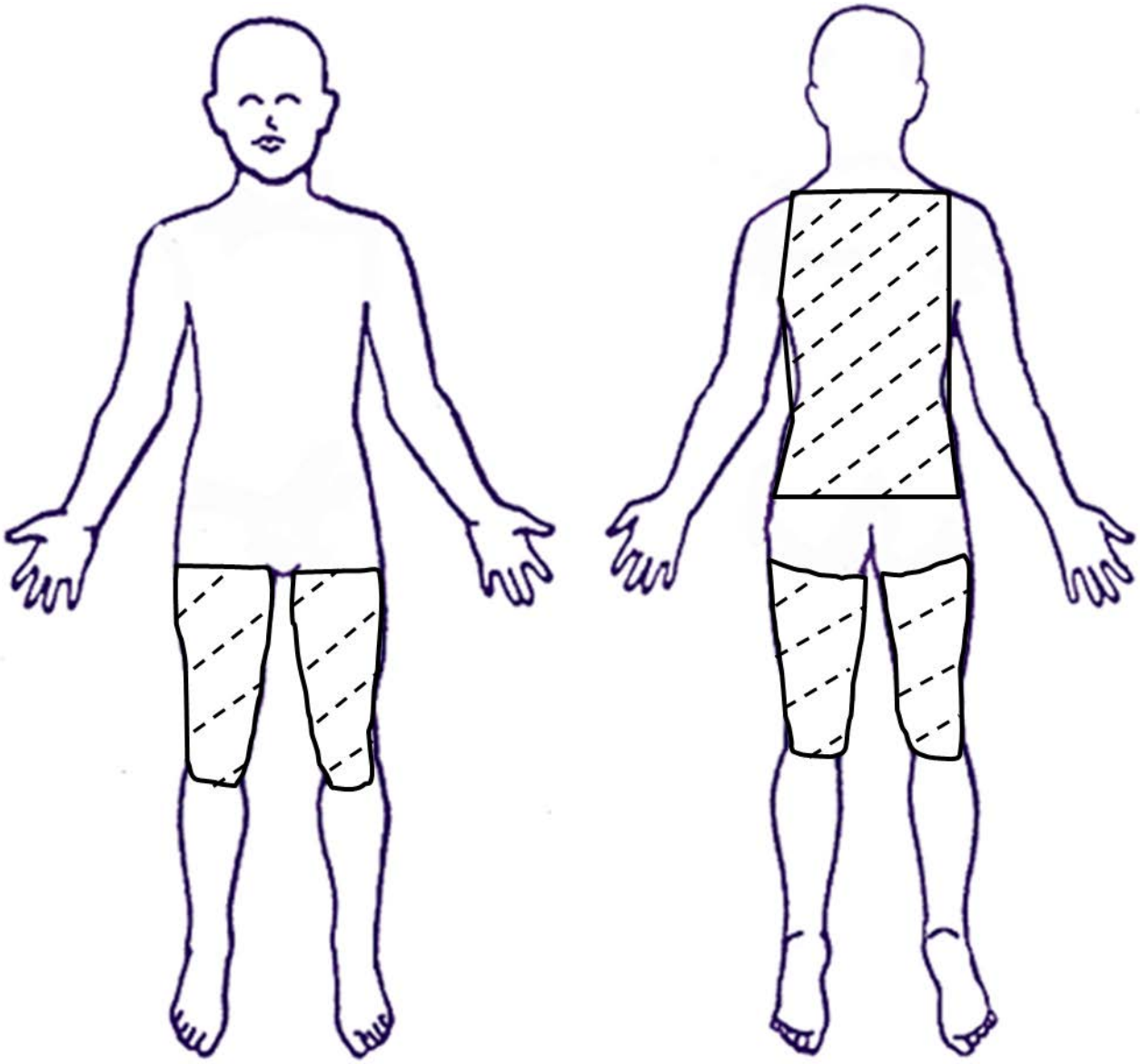
APPENDIX 5 – COHORT 1 40% BSA TREATMENT BODY MAP EXAMPLE

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Cohort 1 (HV) – 40% BSA Treatment Example

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD_____0_____ %
BSA Treated with Study Ointment ____40_____ %



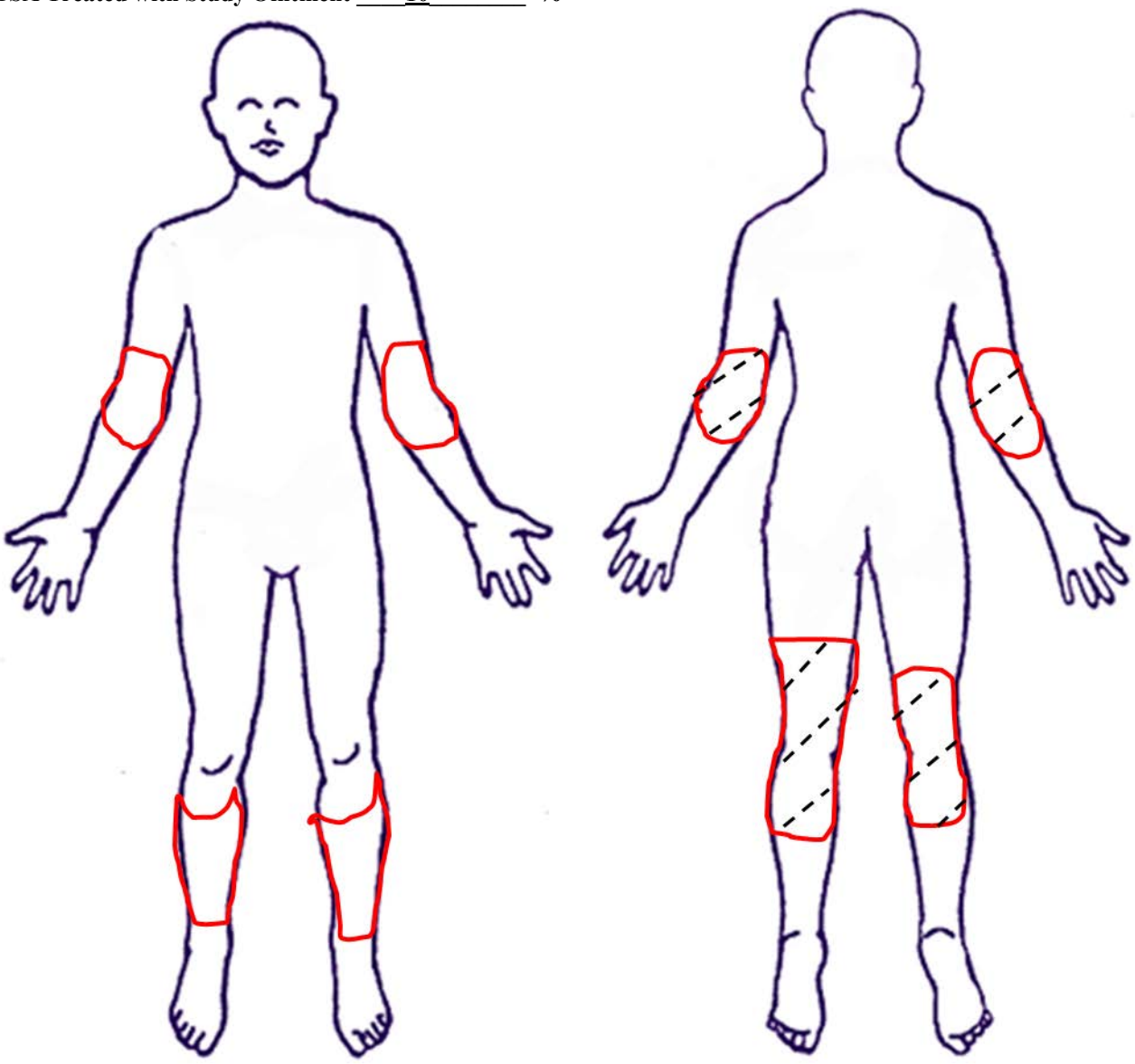
APPENDIX 6 – COHORT 2 10% BSA TREATMENT BODY MAP EXAMPLE

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Cohort 2 (AD Patients) – 10% BSA Treatment Example

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD_____20_____ %
BSA Treated with Study Ointment ____10_____ %



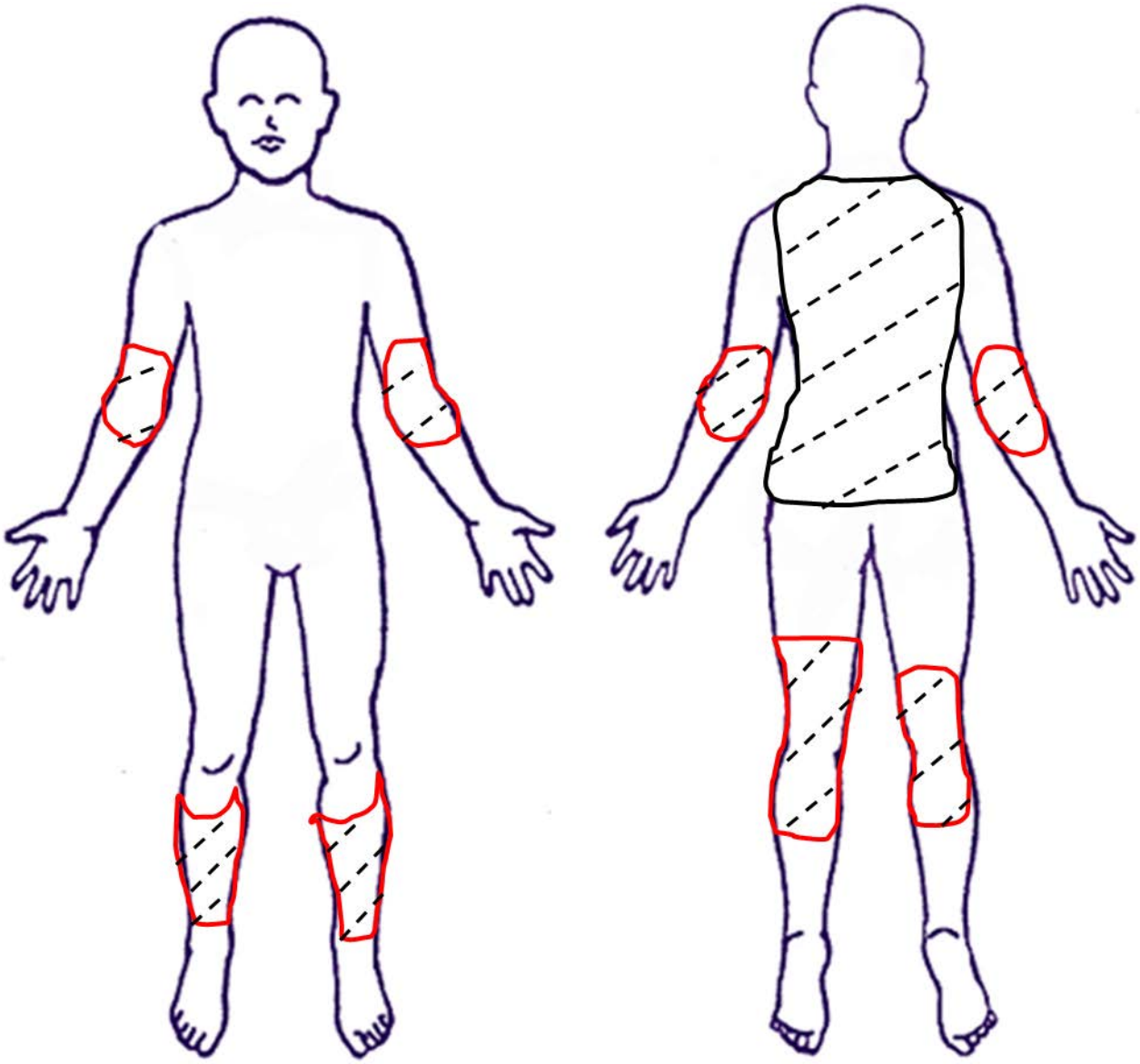
APPENDIX 7 – COHORT 2 40% BSA TREATMENT BODY MAP EXAMPLE

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Cohort 2 (AD Patients) – 40% BSA Treatment Example

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD_____20_____ %
BSA Treated with Study Ointment ____40_____ **%**



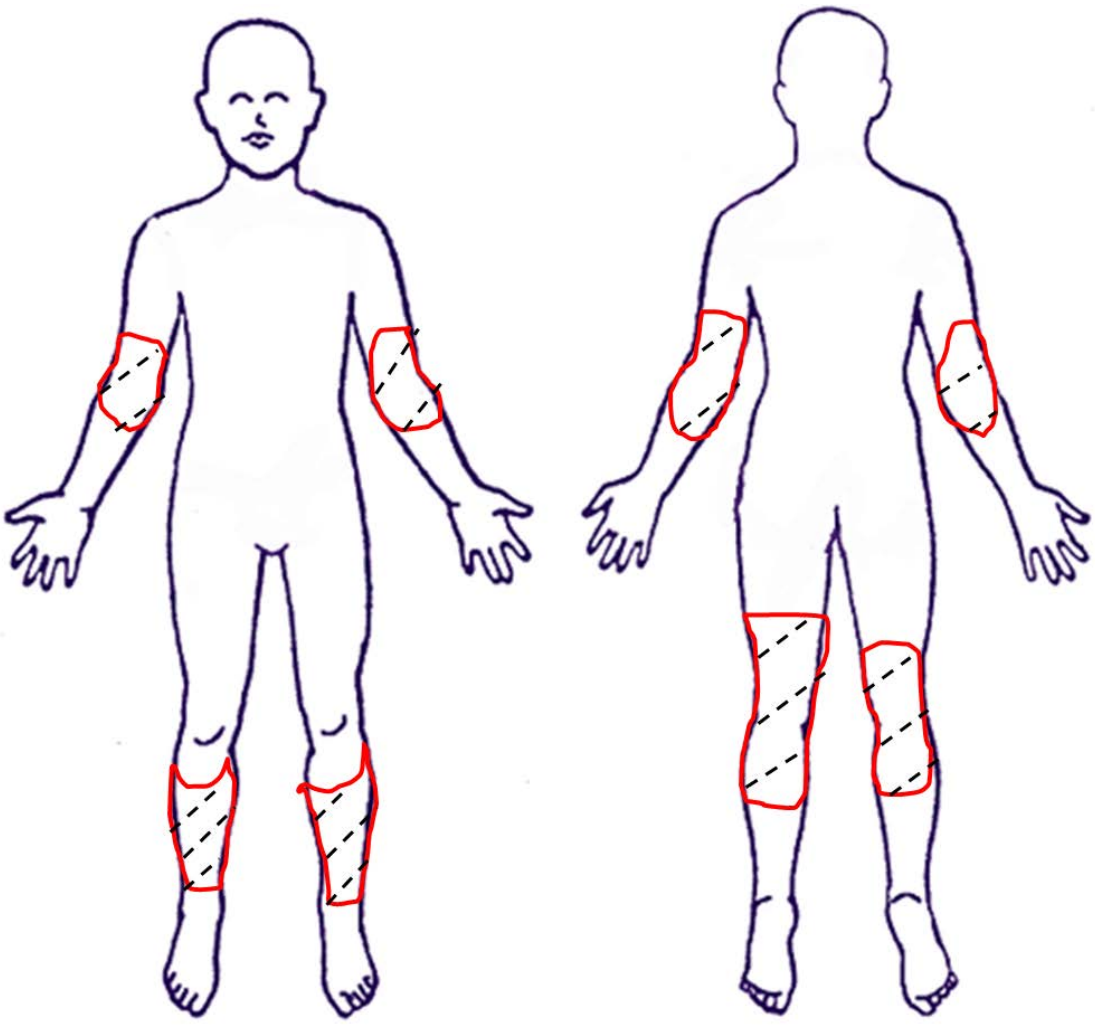
APPENDIX 8 – COHORT 3 BODY MAP EXAMPLE

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Cohort 3 (AD Patients) –Example

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD____20_____ %
BSA Treated with Study Ointment ____20_____ %



APPENDIX 9 – ZIARCO ITCH QUESTIONNAIRE

1. On a scale of 0 (No Itching) to 10 (Itching as bad as you can imagine), please rate the WORST itching that you felt over the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Itching										Itching as bad as you can imagine

2. Over the last 24 hours approximately how many hours, if any, did you itch? ___ hours

3. On a scale of 0 (No sleep disturbance) to 10 (Awake all night), please rate how much your sleep was disturbed by itch last night.

0	1	2	3	4	5	6	7	8	9	10
No sleep Disturbance										Awake all night

4. Over the last 24 hours how would you rate your itch?

No itch
Mild
Moderate
Severe

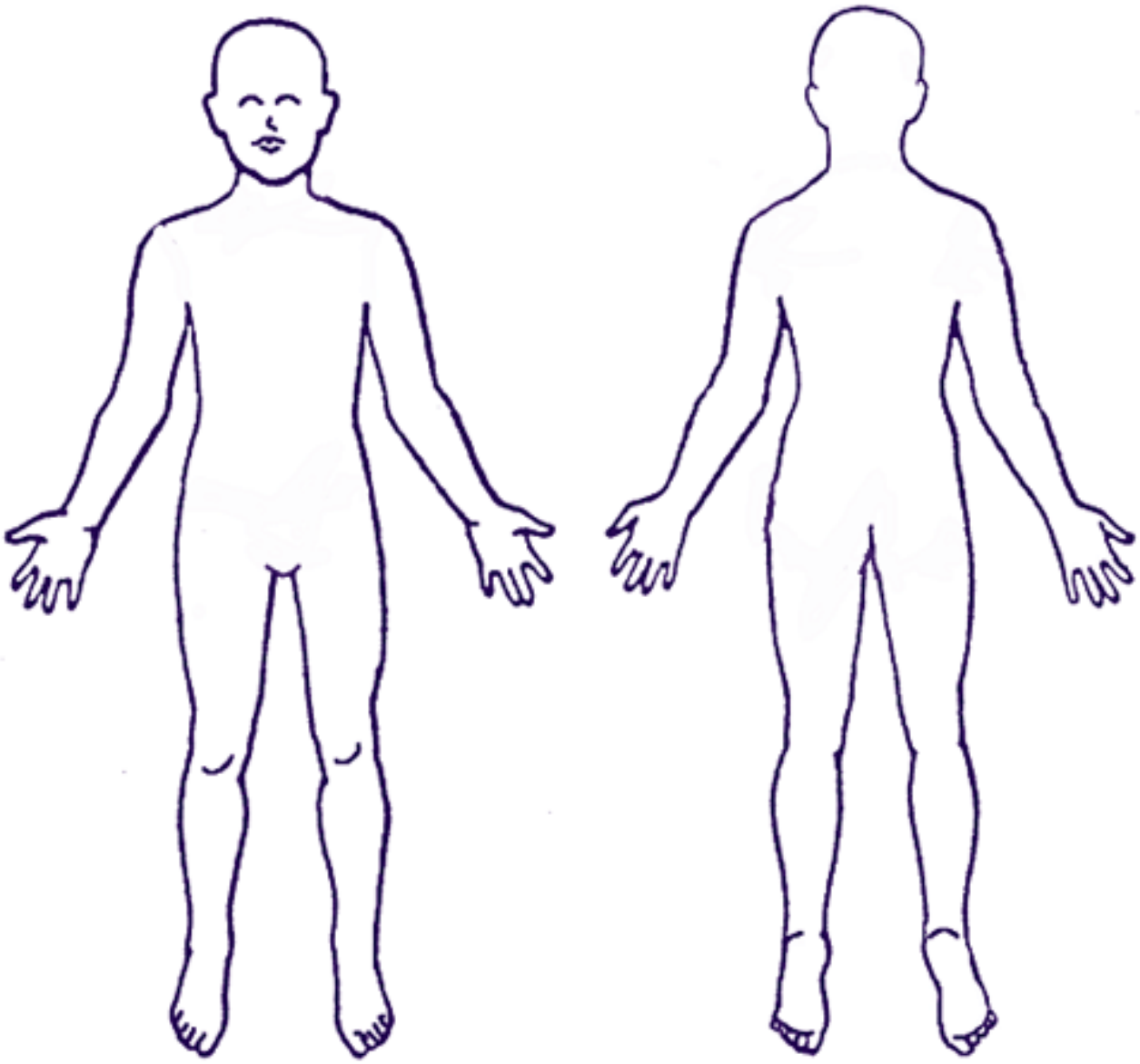
APPENDIX 10 – BODY MAP

Site:		**Subject Initials:**	
Date:		**Subject Number:**	

Please use the diagram below to mark the approximate location and size of the areas affected by atopic dermatitis in RED and record the areas to be treated with Study Ointment with cross hatches in BLACK. Record the estimated BSA affected by atopic dermatitis (if applicable) and also the estimated BSA to be treated with Study Ointment (to nearest whole number).

BSA Affected by AD_____ %

BSA Treated with Study Ointment _____ %



APPENDIX 11 - EASI

An EASI score is a tool used to measure the severity and extent of atopic eczema (Eczema Area and Severity Index).

The body is divided into 4 sections of total skin area: head including neck (10%); arms including extremities (20%); trunk (30%) and legs including extremities (40%). Each area is scored and the 4 scores are combined into the final EASI

Within each area, the severities of eczema are determined for four clinical signs: **erythema (E), oedema/papulation (O), excoriation (X)** and **lichenification (L)**. Severity parameters are measured on a scale of 0 to 3, from none to severe. Half scores may be used.

Score	Grade
0	None
1	Mild
2	Moderate
3	Severe

For guidance, the following definitions may be used:

Severity	Erythema	Oedema/Papulation	Excoriation	Lichenification
None	No evidence of erythema	No evidence of oedema/papulation	No evidence of excoriation	No evidence of lichenification
Mild	Faintly detectable, pink	Barely perceptible elevation	Scant, superficial excoriations	Slight thickening of the skin with skin markings minimally exaggerated
Moderate	Clearly distinguishable dull red	Clearly perceptible elevation but not prominent	Many superficial and /or some deeper excoriations	Clearly thickened skin with exaggerated skin markings and/or some prurigo nodules
Severe	Deep dark or fiery bright red	Prominent elevation	Diffuse extensive superficial and /or many deep excoriations	Prominent skin thickening with exaggerated skin markings creating deep furrows and/or many prurigo nodules

For each section, the percentage area of skin involved with eczema is estimated and transformed into an **extent** grade from 0 to 6:

- 0% of involved area, grade: **0**
- 1-9% of involved area, grade: **1**
- 10-29% of involved area, grade: **2**
- 30-49% of involved area, grade: **3**
- 50-69% of involved area, grade: **4**
- 70-89% of involved area, grade: **5**

- 90-100% of involved area, grade: **6**

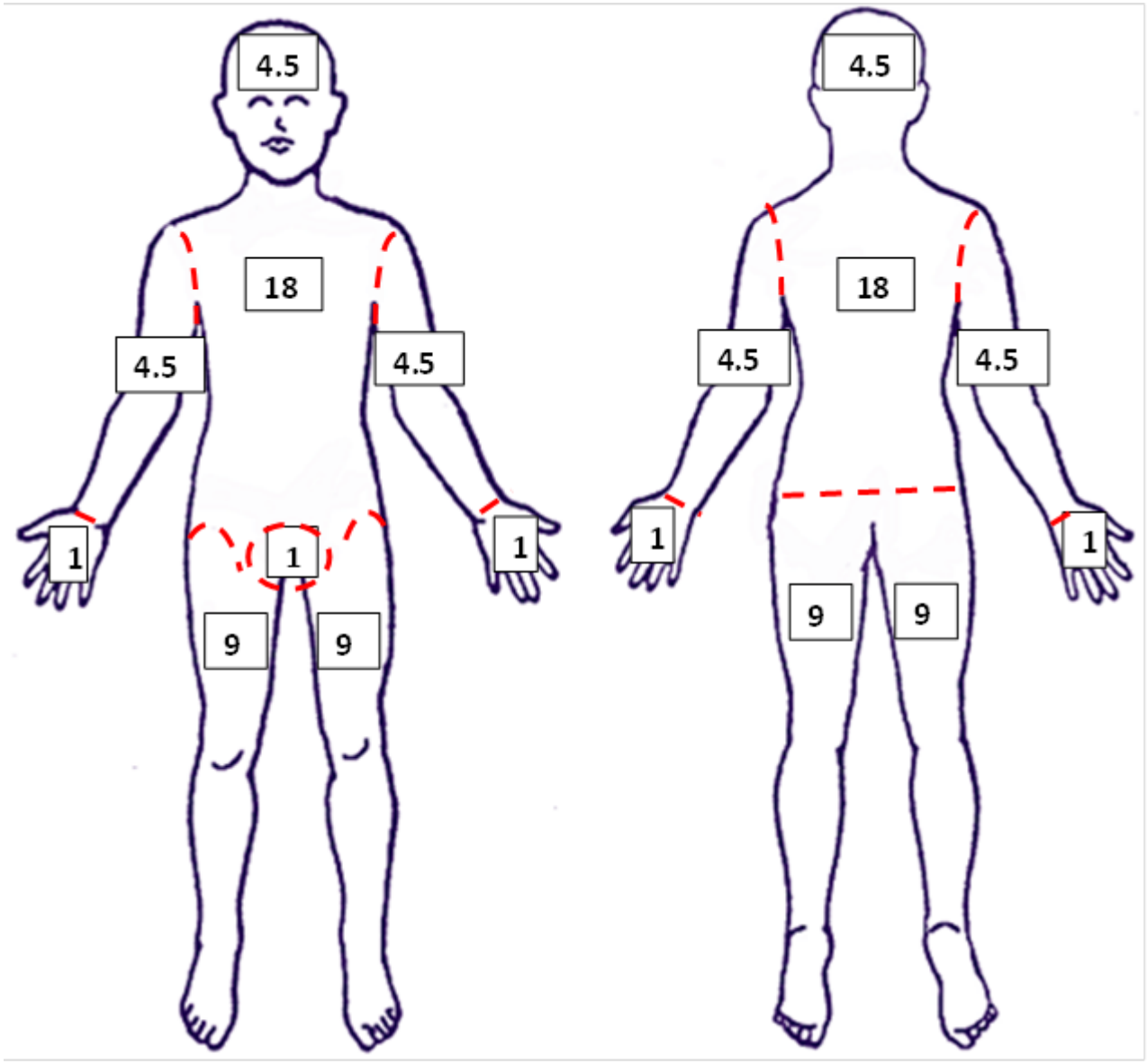
The sum of all four severity parameters is calculated for each section of skin and then multiplied by the weight of the respective section (0.1 for head, 0.2 for arms, 0.3 for trunk and 0.4 for legs). This value is then multiplied by the extent score for that body area.

The EASI score is calculated using the following formula:
$EASI = [0.1 \times (E_{head} + O_{head} + X_{head} + L_{head}) \times Extent_{head}] + [0.2 \times E_{arms} + O_{arms} + X_{arms} + L_{arms}) \times Extent_{arms}] + [0.3 \times (E_{trunk} + O_{trunk} + X_{trunk} + L_{trunk}) \times Extent_{trunk}] + [0.4 \times (E_{legs} + O_{legs} + X_{legs} + L_{legs}) \times Extent_{legs}]$

The EASI value can range from 0-72

Body Region	**Erythema (E) (0-3)**	**Oedema/ papulation (O) (0-3)**	**Excoriation (X) (0-3)**	**Lichenification (L) (0-3)**	**Extent Grade for Region (0-6)**	**Multiplier**	**Score per Body Region**
Head	(+	+	+	)	x	x 0.1	=
Arms	(+	+	+	)	x	x 0.2	=
Trunk	(+	+	+	)	x	x 0.3	=
Legs	(+	+	+	)	x	x 0.4	=
The final EASI score is the sum of the 4 region scores:							

APPENDIX 12 – % BODY SURFACE AREA GUIDANCE



APPENDIX 13 – ZIARCO OINTMENT QUESTIONNAIRE

1. Did you find the study ointment easier to apply than your usual topical treatment or emollients?

   □ Yes □ No □ No Difference

2. On a scale of 1 to 5 where 1 is Very Good and 5 is Very Poor how would you rate the study ointment for the following:

   Texture?
   □ 1 Very Good □ 2 Good □ 3 Indifferent □ 4 Poor □ 5 Very Poor

   Smell?
   □ 1 Very Good □ 2 Good □ 3 Indifferent □ 4 Poor □ 5 Very Poor

   Absorption into your skin?
   □ 1 Very Good □ 2 Good □ 3 Indifferent □ 4 Poor □ 5 Very Poor

3. Would you be happy to wear your own clothes after application of the ointment if your clothes were in contact with the skin which had been treated with study ointment?

   □ Yes □ No □ Don't Know

4. Any other thoughts or feedback on the study ointment?

APPENDIX 14 – PROTOCOL AMENDMENT 1 CHANGES

Protocol Final version 2.0 / 28 July 2016 contains protocol amendment 1 changes

Summary of Changes

Rationale for Substantial Amendment 1: The purpose of this amendment is to document a change to the study emollient for Cohort 3 patients as a consequence of unforeseen hypersensitivity reactions to the [redacted] study emollient by some patients in Cohort 2.

Listing of changes made

Section 3.2 Adaptive Categories and Features Table 2 Study Emollient Application
Additional text
Adaptive features: Cohort 3 only. Alternate emollients other than those defined as 'study emollients' may be used.
Boundaries: Stop the use of study emollient in the event of any hypersensitivity reactions caused by emollient.
Change the emollient used from 'study emollient' due to any potential or actual hypersensitivity reactions with the approval of the Ziarco CMO (Cohort 3 only).

Section 4.2 Inclusion Criteria: Cohorts 2 and 3 Criteria 8
Original text
[redacted] daily from at least 7 days prior to randomisation and throughout their participation in the study.

New Text
Patients must be willing to stop applying their daily emollients and instead use the study
emollient twice daily from at least 7 days prior to randomisation and throughout their participation in the study.

Section 4.4 Exclusion Criteria: Cohorts 2 and 3 Criteria 5
Original text
Hypersensitivity to any excipients in the study ointment formulation, study emollient [redacted] or study shower cream [redacted]

New Text
Hypersensitivity to any excipients in the study ointment formulation, study emollient or study shower cream.

Section 5.3 Drug Supplies
Additional text
Epaderm 3 in 1 ointment manufactured by [redacted] will be supplied as an alternate study emollient to any AD patients (Cohort 3 only) who have a potential (or known) hypersensitivity reaction to [redacted] for use prior to randomisation and throughout the study by MAC.

Section 5.7 Administration Emollients

Original text

The emollient [redacted] will be supplied for this study for AD patients in Cohorts 2 and 3. Any emollients and bathing products used by the patient should be documented at screening. Patient emollients may be used up until 7 days prior to Day 1 and then patients must stop using their current emollient and switch to using the study emollient [redacted] in the same manner as their previous emollient. From randomisation patients must use study emollient at least 15 minutes after application of study ointment. Patients should use study emollient on all skin areas which have been treated with study ointment and in addition any areas which the patient would treat as part of their normal skin care routine. Cohort 2 patients must continue using study emollient twice daily until discharge from the CRU on Day 9 and Cohort 3 patients must use study emollient twice daily until they have completed their visit on Day 15.

New Text

The emollient [redacted] will be supplied for this study for AD patients in Cohorts 2 and 3. In addition an alternate emollient will be available for Cohort 3 patients who have a potential for any hypersensitivity skin reactions to [redacted] Any emollients and bathing products used by the patient should be documented at screening. Patient emollients may be used up until 7 days prior to Day 1 and then patients must stop using their current emollient and switch to using the study emollient in the same manner as their previous emollient. From randomisation patients must use study emollient at least 15 minutes after application of study ointment. Patients should use study emollient on all skin areas which have been treated with study ointment and in addition any areas which the patient would treat as part of their normal skin care routine. Cohort 2 patients must continue using study emollient twice daily until discharge from the CRU on Day 9 and Cohort 3 patients must use study emollient twice daily until they have completed their visit on Day 15.

Rationale for Non-Substantial Amendment: will be made to Exclusion criteria 23 which excludes smokers from the study to only apply this exclusion to the Cohort 2 in-patient cohort rather than the Cohort 3 the out-patient cohort.

Listing of changes made

Section 4.4 Exclusion Criteria: Cohorts 2 and 3 Criteria 23

Original text

Patients who are smokers (including patients who use nicotine substitutes).

New Text

Patients who are smokers (including patients who use nicotine substitutes). (Cohort 2 only)

Additional minor corrections/clarifications

List of abbreviations and definitions of terms

Original text

EIA	Elisa immunoassay

New Text
ECLIA Electro-chemiluminescence immunoassay
Reason
An oversight in the original protocol resulted in the wrong method assay for hepatitis C serology being listed. This updates the abbreviation.

Section 4.4 Exclusion Criteria: Cohorts 2 and 3 Criteria 24 onwards
Numbering of exclusion criteria corrected to remove duplication of number 24.

Section 6.1 Screening
Original text
- Blood and urine samples for:
- Safety laboratory tests (haematology and clinical chemistry)

New Text
- Blood and urine samples for:
- Safety laboratory tests (haematology, clinical chemistry and urinalysis)

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.2.1 Day 1 assessments
Original text
- Obtain blood and urine samples:
- Safety laboratory tests (haematology and clinical chemistry)

New Text
- Obtain blood and urine samples:
- Safety laboratory tests (haematology, clinical chemistry and urinalysis)

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.2.2 Days 2 to 6 Assessments
Original text
- Obtain blood and urine samples for:
- Safety laboratory tests (Day 4 prior to first application only)

New Text
- Obtain blood and urine samples for
- Safety laboratory tests (haematology, clinical chemistry and urinalysis) - Day 4 prior to first application only

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.2.5 Day 9 Assessments
Original text

- Obtain blood and urine samples for:
- Safety laboratory tests

New Text

- Obtain blood and urine samples for
- Safety laboratory tests (haematology, clinical chemistry and urinalysis)

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.3.1 Day 1 assessments
Original text

- Obtain blood and urine samples:
- Safety laboratory tests (haematology and clinical chemistry)

New Text

- Obtain blood and urine samples:
- Safety laboratory tests (haematology, clinical chemistry and urinalysis)

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.3.2 Day 2 to 6 assessments
Original text

- Obtain blood and urine samples for:
- Safety laboratory tests (Day 4 prior to first application only)

New Text

- Obtain blood and urine samples for
- Safety laboratory tests (haematology, clinical chemistry and urinalysis) - Day 4 prior to first application only

Reason
To ensure consistency on safety laboratory tests being conducted with Section 6.7 Schedule of Activities

Section 6.3.4 Day 8 assessments
Original text

- Duplicate supine blood pressure and pulse rate
- Blood sample for pharmacokinetic analysis of ZPL-5212372
- Completion of Ziarco Ointment Questionnaire

New Text

- Duplicate supine blood pressure and pulse rate

- Blood sample for pharmacokinetic analysis of ZPL-5212372
- Triplicate 12-lead ECG (24 hours post dose only)
- Completion of Ziarco Ointment Questionnaire

Reason
To ensure consistency on ECGs being conducted with Section 6.7 Schedule of Activities

Section 6.4.1 Baseline Visit (Day 1)
Original text

- Completion of body map of skin areas affected by AD and identification and recording of skin area to be treated
- Obtain blood and urine samples:
    - Safety laboratory tests (haematology and clinical chemistry)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
    - Blood sample for baseline reference. Serum to be stored frozen at the MAC through to completion of the study for possible use as a baseline reference should additional safety laboratory tests or markers of disease activity be indicated.
    - Urine for pregnancy testing (pre-menopausal women only)

New Text

- Completion of body map of skin areas affected by AD and identification and recording of skin area to be treated
- Breath alcohol testing
- Obtain blood and urine samples:
    - Safety laboratory tests (haematology, clinical chemistry and urinalysis)
    - Blood sample for pharmacokinetic analysis of ZPL-5212372
    - Blood sample for baseline reference. Serum to be stored frozen at the MAC through to completion of the study for possible use as a baseline reference should additional safety laboratory tests or markers of disease activity be indicated.
    - Urine for pregnancy testing (pre-menopausal women only)
    - Urine drug screen

Reason
To ensure consistency on ECGs being conducted with Section 6.7 Schedule of Activities

Section 6.7 Schedule of Activities Table 9 Footnote 12
Original text
Assessment of local toleration on treated skin will be conducted prior to each dosing occasion and at 24, 36 and 48 hours post last dose.

New Text

Assessment of local toleration on treated skin will be conducted prior to each dosing occasion and at 24 and 48 hours post last dose.

Reason
To ensure consistency on local toleration assessments being conducted with Section 6.2.4

Section 6.7 Schedule of Activities Table 10 Footnote 12
Original text
Study emollient to be used from day -6 onwards until discharge from the CRU on day 9.

New Text
Study emollient to be used from day -7 onwards until discharge from the CRU on day 9.

Reason
To ensure consistency with the rest of the protocol.

Section 6.7 Schedule of Activities Table 11 Footnote 10
Original text
Study emollient to be used from day -6 onwards until day 14 final application of ointment.

New Text
Study emollient to be used from day -7 onwards until completion of visit on day 15.

Reason
To ensure consistency with the rest of the protocol.

Section 7.13 Pharmacokinetic Blood Samples
Original text
A 5.0 mL sample, to provide a minimum of 2 mL plasma for pharmacokinetic analysis, will be collected into an appropriately labeled tube containing lithium heparin. Blood samples will be centrifuged within 15 minutes of collection at 1700 g and 4°C for 10 minutes. Plasma will be transferred to appropriate labelled screw-capped polypropylene tubes and stored at approximately -20°C within 1 hour of collection.
Plasma samples will be transferred to the bioanalytical laboratory on dry ice to maintain frozen conditions. A sample handling document and shipment details for the analytical laboratory will be provided to the sites at study initiation.
Samples will be analyzed using a validated analytical method. After completion of the study plasma samples may also be analyzed for metabolites and further evaluation of the assay performance.
New Text
A 5.0 mL sample, to provide a minimum of 2 mL plasma for pharmacokinetic analysis, will be collected into an appropriately labeled tube containing lithium heparin. Blood samples will be centrifuged within 1 hour of collection at 1700 g and 4°C for 10 minutes. Plasma will be transferred to appropriate labelled screw-capped polypropylene tubes and stored at approximately -20°C within 1 hour of collection.

Plasma samples will be transferred to the bioanalytical laboratory on dry ice to maintain frozen conditions. Shipment details for the analytical laboratory will be provided to the sites prior to shipping any samples.
Samples will be analysed using a validated analytical method. After completion of the study plasma samples may also be analysed for metabolites and further evaluation of the assay performance.
Reason
Updated information regarding centrifugation post clinical assay validation and correction of minor typographical errors.

Section 7.14 Clinical Laboratory Safety Tests Table 12
Original text
Anti-hepatitis C virus serology (by multi-antigen EIA)
New Text
Anti-hepatitis C virus serology (by ECLIA)
Reason
Update a minor error in original protocol.

Section 7.16.1.2 Assessment of Adverse Event Causality
Original text
The investigator will assess the causality / relationship between the study drug and the AE and record that assessment in the CRF. Causality will be shown as Probably related, Possibly related, or Not related.
New Text
The investigator will assess the causality / relationship between the study drug and the AE and record that assessment in the CRF. Causality will be shown as Probably related, Possibly related, Not related or Related.
Reason
Update a minor error or omission in original protocol and for consistency with SAE form.

Section 7.16.1.4 Reporting Serious Adverse Events
Original text
The original SAE should be sent to the address below and Ziarco Chief Medical Officer informed by email. In addition, the event must be documented in the CRF.
New Text
The completed SAE should be sent to the address below and Ziarco Chief Medical Officer informed by email. In addition, the event must be documented in the CRF.
Reason
Updated for clarity to ensure no delays in reporting SAE's as emailed or faxed copies are acceptable to ensure reporting timelines are met.

Section 7.16.1.4 Reporting Serious Adverse Events
Original text
After receipt of the initial report, the pharmacovigilance team will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. [redacted] will be responsible for all information processing and reporting according to local legal requirements. Where necessary, investigators will inform the authorities in their own countries.
New Text

After receipt of the initial report, the pharmacovigilance team will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. [redacted] will be responsible for all information processing and reporting according to local legal requirements.
Reason
Updated for clarity to ensure no confusion on regulatory reporting responsibilities.

Section 7.16.1.4 Reporting Serious Adverse Events
Original text
After receipt of the initial report, the pharmacovigilance team will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. [redacted] will be responsible for all information processing and reporting according to local legal requirements. Where necessary, investigators will inform the authorities in their own countries.
New Text
After receipt of the initial report, the pharmacovigilance team will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. [redacted] will be responsible for all information processing and reporting according to local legal requirements.
Reason
Updated for clarity and consistency with SAE reporting plan to ensure no confusion on regulatory reporting responsibilities.

Section 8.1.1. Randomisation
Original text
The randomisation for Cohort 3 will be stratified by EASI score (EASI ≤20 and EASI ≥20).
New Text
The randomisation for Cohort 3 will be stratified by EASI score (EASI ≤20 and EASI >20).
Reason
Correct a mistake in the original protocol.

Section Appendix 11 - EASI
Original Text
Within each area, the severities of eczema are determined for four clinical signs: erythema (E), oedema/papulation (O), excoriation (X) and lichenification (L). Severity parameters are measured on a scale of 0 to 3, from none to severe.
New Text
Within each area, the severities of eczema are determined for four clinical signs: erythema (E), oedema/papulation (O), excoriation (X) and lichenification (L). Severity parameters are measured on a scale of 0 to 3, from none to severe. Half scores may be used.
Reason
To highlight the fact that half scores can be used in the calculation of the EASI and for consistency with the IWRS system where the EASI data is collected for the study.